UNIVERSIDADE FEDERAL DE SÃO CARLOS
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ANÁLISE DA OCORRÊNCIA, DISTRIBUIÇÃO, RIQUEZA E ENDEMICIDADE DE MAMÍFEROS DE MÉDIO E GRANDE PORTE NA MATA ATLÂNTICA BRASILEIRA, COM ÊNFASE NO CENTRO DE ENDEMISMO PERNAMBUCO E NAS ABORDAGENS NÃO INVASIVAS

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Tese apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais (PPGERN) da Universidade Federal de São Carlos, para obtenção do título de Doutora em Ciências.

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Orientador: Prof. Dr. Pedro Manoel Galetti Junior

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The greatest threat to our planet is the belief that someone else will save it.

— Robert Swan

RESUMO

A Mata Atlântica brasileira (MAB) é globalmente reconhecida como um *hotspost* de biodiversidade, abrigando alta riqueza e endemicidade de espécies ao mesmo tempo que sofre intensa ameaça antrópica. Nesse bioma são reconhecidos três centros de refúgios climáticos do Pleistoceno: São Paulo, Bahia e Pernambuco. Entretanto, as rápidas mudanças climáticas contemporâneas associadas a outras atividades antrópicas reduzem a área de distribuição das espécies, reduzindo riqueza e podendo resultar na extinção de muitas delas. Para entender a respostas dos mamíferos de médio e grande porte da MAB as mudanças climáticas, construímos modelos de distribuição de espécies e comparamos a riqueza e endemicidade para o passado (21000 anos atrás), presente e futuro (2081-2100). Também examinamos a mudança potencial na distribuição entre o presente e o futuro nos refúgios da MAB. Para entender a história evolutiva das comunidades, calculamos a diversidade filogenética de cada refúgio. Observamos padrões de riqueza e endemicidade contemporâneas congruentes com as áreas dos refúgios do Pleistoceno. A diversidade filogenética revelou diferentes histórias evolutivas em cada refúgio e a presença de espécies filogeneticamente distantes na área de menor riqueza (refúgio Pernambuco), indicando que a diversidade ecológica pode estar sendo preservada. Nossos resultados destacam os impactos generalizados e negativos das mudanças climáticas na distribuição dos mamíferos. A elaboração dos modelos de distribuição de espécies exige dados precisos e confiáveis de identificação e ocorrência das espécies. Muitos mamíferos, em especial os carnívoros, ocorrem em baixas densidades, o que dificulta sua detecção e estudo. Através de uma revisão sistemática da literatura exploramos como o DNA fecal, uma técnica de amostragem não invasiva, tem sido utilizada para estudar espécies de carnívoros consideradas raras. Investigamos quais aplicações e técnicas têm sido empregadas, o esforço amostral necessário e a eficácia do DNA fecal na detecção de carnívoros raros. Compilamos os dados de 250 artigos e observamos que o DNA fecal é uma fonte valiosa de material genético para determinar espécies, identidade individual e sexo, entre outras várias aplicações. Verificou-se que o DNA fecal foi mais e igualmente eficaz na detecção do número de indivíduos e espécies presentes, respectivamente, em comparação com métodos alternativos. Dentre os três refúgios da MAB, o Centro de Endemismo Pernambuco (CEP) ou refúgio Pernambuco merece destaque por sua menor área, escassos estudos e intensa ameaça antrópica, resultando na perda, fragmentação e degradação de habitat. Dessa forma, é fundamental determinar a eficiência dos métodos de amostragem para produzir levantamentos rápidos e confiáveis. Comparamos cinco métodos de amostragem não invasivos para avaliar a comunidade de mamíferos de médio porte em um fragmento de floresta imerso em uma matriz de cana-de-açúcar. Identificamos treze espécies e discutimos os resultados com relação ao grupo de espécies detectadas, a taxa de sucesso de detecção proporcional e a latência na deteção de cada metodologia. Expandimos a identificação molecular de fezes para seis áreas importantes do CEP no estado de Alagoas em busca de canídeos e felinos nativos. Identificamos duas espécies: *Leopardus tigrinus* e *Cedocyon thous*. Os resultados apontam para a utilidade da amostragem por meio do DNA fecal para detecção de espécies raras, como *L. tigrinus,* mas também reforçam o processo de defaunação na região. Os achados em cada um destes capítulos são relevantes para a conservação dos mamíferos na Mata Atlântica brasileira, lançando luz sobre estratégias e ações que podem ser adotadas para preservar a diversidade biológica deste ecossistema.

Palavras-chave: antropização; comparação de métodos; ecologia molecular; efeito de tamanho; floresta tropical; Maxent

ABSTRACT

The Brazilian Atlantic Forest (BAF) is globally recognized as a biodiversity hotspot, harboring high species richness and endemicity while suffering from intense anthropogenic threat. Three Pleistocene climate refuge centers are recognized in this biome: São Paulo, Bahia and Pernambuco. However, rapid contemporary climate change associated with other anthropogenic activities reduces species distribution area, impacting species richness, and may result in the extinctions of many species. To understand the responses of medium and large mammals of the BAF to climate change, we built species distribution models and compared species richness and endemicity for the past (21 000 years ago), present and future (2081-2100). We also examined the potential change in distribution between the present and the future in each of the BAF refugia. To understand the evolutionary history of the communities, we calculated the phylogenetic diversity of each refuge. We observed patterns of contemporary richness and areas of endemism congruent with the areas of the Pleistocene refugia. Phylogenetic diversity revealed different evolutionary histories in each refuge and the presence of phylogenetically distant species in the area of lowest richness (Pernambuco refuge), indicating that ecological diversity may be preserved. Our results highlight the widespread and negative impacts of climate change on mammal distribution. The species distribution modeling approach requires accurate and reliable species identification and occurrence data. Many mammal species, especially carnivores, occur at low densities, which makes them difficult to detect and study. Through a systematic literature review we explored how fecal DNA, a noninvasive sampling approach, has been used to study carnivore species considered rare. We investigated the applications and techniques that have been employed, the sampling effort required and the efficacy of fecal DNA in detecting rare carnivores. We compiled data from 250 papers and found that fecal DNA is a valuable source of genetic material for determining species, individual identity, and sex, among other applications. Fecal DNA was found to be more and equally efficient in determining the number of individuals and species present, respectively, compared to alternative methods. Among the three BAF refugia, the Pernambuco Endemism Center (PEC) or Pernambuco refuge deserves to be highlighted for its smaller area, scarce studies, and intense anthropogenic threat, resulting in habitat loss, fragmentation, and degradation. Therefore, it is essential to determine the efficiency of sampling methods to produce rapid and reliable assessments. We compared five noninvasive survey methods to assess the medium-sized mammal community in a forest fragment immersed in a sugarcane matrix. We identified thirteen species and discussed the results in relation to the group of species detected, the proportional detection success and the latency to first detection of each method. We expanded the molecular identification of feces to six important areas of the PEC in the state of Alagoas searching for native Canidae and Felidae species. We identified two species: *Leopardus tigrinus* and *Cedocyon thous*. The results show the usefulness of fecal DNA sampling for detecting rare species, such as *L. tigrinus*, but also reinforce the process of defaunation in the region. The findings in each of these chapters are relevant to the conservation of mammals in the Brazilian Atlantic Forest, shedding light on strategies and actions that can be taken to preserve the biological diversity of this ecosystem.

Keywords: anthropization; effect size; tropical forest; Maxent; method comparison; molecular ecology

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LISTA DE ABREVIAÇÕES E SIGLAS

ARCA-CEP – Avaliação, Recuperação e Conservação da Fauna Ameaçada de Extinção do Centro de Endemismo Pernambuco
CEP – Centro de Endemismo Pernambuco
CEUA – Comissão de Ética no Uso de Animais
CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico
dNTP – Desoxirribonucleotídeos Fosfatados
ESEC – Estação Ecológica
FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo
GHG – *Greenhouse gases*
ha – hectare
IPCC – *Intergovernmental Panel on Climate Change*
IUCN – *International Union for Conservation of Nature and Natural Resources*
LGM – *Last Glacial Maximum*
LTD – *Latency to initial detection*
MNTD – *Mean nearest taxon distance*
MPD – *Mean pairwise distance*
mM – milimolar
mL – mililitro
ng – nanograma
pb – par de bases
PCR – *Polymerase chain reaction*
PEC – *Pernambuco Endemism Center*
PIB – Produto interno bruto
PD – *Phylogenetic diversity*
PDS – *Proportional detection success*
qPCR – *Quantitative polymerase chain reaction*
q.s.p – quantidade suficiente para
RPPN – Reserva Particular do Patrimônio Natural
SDM – *Species distribution model*

SISBIO – Sistema de Autorização e Informação em Biodiversidade
SSP – *Shared Socioeconomic Pathways*
SR – *Species richness*
WE – *Weighted endemism*
12S – rRNA 12S
16S – rRNA 16S
µL – microlitro
µM – micromolar

SUMÁRIO

APRESENTAÇÃO

A presente tese tem quatro principais elementos norteadores de seus capítulos: o bioma Mata Atlântica, com especial foco no Centro de Endemismo Pernambuco; os mamíferos de médio e grande porte; as técnicas de amostragem não invasivas; e a biologia da conservação. A introdução geral é organizada de forma a explorar esses quatro assuntos sequencialmente. O presente trabalho está inserido no âmbito do projeto temático “Avaliação, Recuperação e Conservação da Fauna Ameaçada de Extinção do Centro de Endemismo Pernambuco (ARCA – CEP)” (FAPESP, Processo nº 2017/23548-2). Em acordo com as diretrizes e objetivos do projeto ARCA-CEP, essa tese visa aumentar o conhecimento sobre a distribuição e ocorrência de mamíferos na Mata Atlântica brasileira, com foco no Centro de Endemismo e nas abordagens não invasivas, fornecendo ferramentas para estudá-los, potencialmente fomentando ações de conservação. A tese está dividida em quatro capítulos, que serão apresentados a seguir.

O primeiro capítulo, submetido para revista *Biological Conservation*, compreende a análise da distribuição, riqueza e diversidade filogenética dos mamíferos de médio e grande porte da Mata Atlântica brasileira. Para isso, analisamos separadamente as três áreas de refúgios paleoclimáticos da Mata Atlântica: São Paulo, Bahia e Pernambuco. Utilizando pontos de ocorrência das espécies disponíveis em bancos de dados, construímos modelos de distribuição de espécies com o uso do algoritmo Maxent. Analisamos a riqueza e endemicidade modeladas para todo o bioma e comparamos os resultados entre refúgios. Também calculamos os índices de diversidade filogenética contemporâneos para a comunidade de mamíferos presente em cada refúgio, a fim de levar em consideração suas respectivas histórias evolutivas. Considerando que as áreas de refúgios paleoclimáticos da Mata Atlântica foram propostas baseado nas respostas de comunidades frente as mudanças climáticas que ocorreram durante o Pleistoceno, também modelamos a respostas dos mamíferos, em termos de distribuição, riqueza e endemicidade, frente as mudanças climáticas preditas para os anos de 2081 a 2100. Como a projeção das mudanças climáticas futuras depende de diversos fatores geofísicos e antrópicos, como a tendência nas emissões de gases do efeito estufa, também avaliamos a resposta dos mamíferos a diferentes projeções de mudanças climáticas. Por fim, discutimos as implicações para a conservação das espécies e enfatizamos a necessidade de medidas de mitigação para proteção da biodiversidade contra as mudanças climáticas.

Dados de ocorrência (identificação e localização) acurados, como os necessários para os modelos de distribuição das espécies, são fundamentais para fomentar ações de conservação. Tendo em vista essa necessidade e a natureza elusiva de muitos mamíferos, em especial os carnívoros, a identificação molecular a partir de amostras biológicas obtidas de forma não invasiva é uma importante fonte de informação de ocorrência das espécies. A partir do DNA de amostras não invasivas, como as fezes, outros dados necessários para fomentar ações de conservação também podem ser obtidos, como a identificação de indivíduos, suas relações de parentesco e parâmetros demográficos. Dessa forma, o segundo capítulo dessa tese compreende uma revisão sistemática da literatura sobre o uso do DNA obtido a partir de amostras fecais para o estudo de carnívoros considerados raros. Nesse capítulo objetivamos entender como o DNA obtido a partir das fezes tem sido utilizado para estudar carnívoros que ocorrem em baixas densidades, quais as principais técnicas empregadas (métodos de coleta, preservação e procedimentos laboratoriais), e quais as principais aplicações do DNA fecal. Para avaliar a eficácia do DNA fecal em identificar indivíduos e espécies, também reunimos artigos que compararam a identificação molecular das fezes com metodologias alternativas. Os resultados são discutidos de forma a fomentar futuros estudos que objetivem utilizar essas ferramentas para estudar carnívoros raros. Esse capítulo será submetido para a revista *Conservation Genetics*.

Tendo em vista a falta de estudos para o CEP e a necessidade de produzirmos dados de forma rápida e acurada para fins de conservação das espécies, concentramos nossos esforços em uma Reserva Particular do Patrimônio Natural (RPPN Mata do Cedro, Rio Largo/AL) para realizar o levantamento total da comunidade de mamíferos de médio e grande porte. Neste trabalho, amostramos os mamíferos por cinco metodologias não invasivas (armadilhamento fotográfico e de pelo, DNA fecal, avistamentos em transectos lineares e a coleta oportunista de carcaças) e comparamos os resultados obtidos entre elas, levando em consideração o esforço amostral. A RPPN Mata do Cedro foi escolhida por ser um fragmento representativo dos remanescentes de Mata Atlântica no nordeste brasileiro, em termos de tamanho, composição florística e de matriz, e por ser o primeiro local de reintrodução do mutum-de-Alagoas (*Pauxi mitu*), uma ave endêmica do CEP e extinta na natureza. Também discutimos se as espécies de mamíferos detectadas podem representar uma ameaça à reintrodução do mutum-de-Alagoas e outras espécies de aves. Esse capítulo será submetido para a revista *Wildlife Research*.

Por fim, os resultados do capítulo II reforçam a utilidade do DNA fecal na identificação de carnívoros elusivos, em especial das famílias Canidae e Felidae devido ao seu comportamento de marcar território em locais proeminentes, como trilhas. Dessa forma, realizamos um estudo em larga escala para a identificação de canídeos e felinos nativos na Mata Atlântica no estado de Alagoas. Delimitamos e percorremos 44 transectos amostrais no interior e ao redor de seis fragmentos importantes para a conservação da Mata Atlântica no nordeste brasileiro em busca de fezes. As fezes encontradas foram submetidas à identificação molecular e três espécies dos grupos focais foram identificadas: duas nativas (*Cerdocyon thous* e *Leopardus tigrinus*) e o cão doméstico. Discutimos os resultados levando em consideração os registros publicados até o momento. Esse trabalho compreende a primeira tentativa bem-sucedida de amostragem de mamíferos por meio do DNA fecal no CEP e apontam para o potencial dessa metodologia para a obtenção de dados genéticos das espécies nativas detectadas.

Finalizamos a tese apresentando as considerações finais somando os resultados dos quatro capítulos apresentados.

1 INTRODUÇÃO GERAL

1.1. MATA ATLÂNTICA BRASILEIRA

Durante as oscilações climáticas do Pleistoceno, a Mata Atlântica apresentou períodos de conexão e isolamento com outras florestas sul-americanas, mais especificamente, com a Amazônia e a floresta dos Andes (SOBRAL-SOUZA; LIMA-RIBEIRO; SOLFERINI, 2015). Essa dinâmica florestal deu origem a hipótese do modelo de paleorrefúgios climáticos que propõe que o isolamento de espécies em fragmentos florestais (refúgios) durante períodos mais frios, como o Último Máximo Glacial (LGM – aproximadamente 21 000 anos atrás) (BEHLING, 2002) teria levado a diferentes processos de especiação e extinção em cada uma das regiões de Mata Atlântica isoladas entre si (CARNAVAL; MORITZ, 2008; MORITZ et al., 2000). Contudo, quando as condições climáticas eram favoráveis, os fragmentos de Mata Atlântica expandiam, se conectavam e trocavam indivíduos e espécies entre si e com as outras florestas (CABANNE et al., 2008; SOBRAL-SOUZA; LIMA-RIBEIRO; SOLFERINI, 2015). O termo refúgio denota habitats de dimensões que operam em escalas evolutivas e facilitam a sobrevivência da biodiversidade sob condições ambientais variáveis por uma grande escala temporal (KEPPEL et al., 2012). Dessa forma, a dinâmica florestal de expansão e contração em resposta ao clima teria moldado os padrões de biodiversidade do presente. As áreas de refúgio foram definidas com base nas respostas da floresta frente as mudanças climáticas passadas, por meio de paleomodelagens, e reforçadas por dados filogeográficos (CARNAVAL et al., 2009; CARNAVAL; MORITZ, 2008; MARTINS, 2011). Apesar de fornecer suporte para a distribuição e diversidade genética de várias espécies (CABANNE et al., 2008; FITZPATRICK et al., 2009), essa hipótese não explica totalmente a distribuição de todas as espécies testadas (THOMÉ et al., 2014). Contudo, a existência de outros eventos de diversificação na complexa história da Mata Atlântica não invalida os eventos acontecidos durante o Pleistoceno que resultaram nas regiões atualmente conhecidas como refúgios paleoclimáticos (SILVA et al., 2012).

Dessa forma, dentro da Mata Atlântica foram definidas três áreas de refúgios: São Paulo, que compreende a porção da Mata Atlântica ao sul do Rio Doce; Bahia, localizada entre o Rio Doce e o Rio São Francisco; e Pernambuco, ao norte do Rio São Francisco. Esses rios representam uma barreira física para muitas espécies, sendo utilizados como limites para as áreas de refúgio e atuando como barreiras para a distribuição de espécies de diversos grupos taxonômicos (SILVA et al., 2012).

Devido a sua complexa história e biodiversidade atual, a Mata Atlântica brasileira é considerada um dos *hotspots* de biodiversidade e uma prioridade para a conservação das espécies (MYERS et al., 2000). Nesse sentido clássico proposto por Myers et al. nos anos 2000, um *hotspot* é definido como uma área que concentra grande endemicidade de espécies e alta ameaça devido a perda de habitat. Originalmente a Mata Atlântica cobria cerca de 1 350 000 km^2 do território brasileiro, estendendo-se de 4º a 32ºS e apresentando elevações que variam do nível do mar até aproximadamente 2900 metros de altitude (RIBEIRO et al., 2009). Esse bioma abriga impressionantes 2645 espécies de tetrápodes, sendo 1025 aves, 719 anfíbios, 517 répteis e 384 mamíferos (FIGUEIREDO et al., 2021). Além disso, a região da Mata Atlântica brasileira tem papéis econômicos e sociais fundamentais no país. Em seu território é gerado 80% do produto interno bruto (PIB) e vivem aproximadamente 70% da população brasileira (PINTO; METZGER; SPAROVEK, 2022), sendo também provedora de insubstituíveis serviços ecossistêmicos (JOLY; METZGER; TABARELLI, 2014; TEEB, 2010).

A Mata Atlântica tem um longo histórico de ocupação humana. Povos indígenas já utilizavam e modificavam esse bioma antes da colonização europeia do Brasil, tendo tido, inclusive, importante papel na extinção da megafauna (CIONE; TONNI; SOIBELZON, 2009; DENEVAN, 1992; SOLÓRZANO; BRASIL; OLIVEIRA, 2021). Contudo, a alteração e remoção da cobertura florestal começou a ocorrer de forma mais significativa com a chegada de colonizadores europeus, tendo início com o ciclo de extração de pau-brasil (*Paubrasilia echinata*), posteriormente sendo pautada no desflorestamento para o plantio de *commodities* como cana-de-açúcar e café e, em seguida, para a produção de energia e urbanização (BARRETO, 2013; SOLÓRZANO; BRASIL; OLIVEIRA, 2021). Além de perda de cobertura florestal, o desflorestamento resultou em intensa fragmentação, sendo a maioria dos fragmentos remanescentes menores de 50 hectares e ilhados em uma matriz composta de agricultura e pecuária (RIBEIRO et al., 2009). As estimativas de cobertura vegetal remanescente da Mata Atlântica brasileira variam de acordo com o método de levantamento utilizado, restando atualmente entre 11,4% e 28% da cobertura original e apenas cerca de 9% estaria protegido em áreas de conservação legalmente estabelecidas (REZENDE et al., 2018; RIBEIRO et al., 2009). Outros fatores ainda ameaçam os remanescentes, como a caça, extrativismo vegetal ilegal, invasão por espécies exóticas e as mudanças climáticas (BROADBENT et al., 2008; CULLEN et al., 2000; SCARANO; CEOTTO, 2015; TABARELLI; CARDOSO DA SILVA; GASCON, 2004).

1.1.1. Centro de Endemismo Pernambuco

Dentre os três refúgios da Mata Atlântica propostos, é chamado Centro de Endemismo Pernambuco (CEP) a região de Mata Atlântica que se estende ao norte do Rio São Francisco pelos estados da Alagoas, Pernambuco, Paraíba e Rio Grande do Norte. Devido à grande amplitude latitudinal da Mata Atlântica e falta de conexão do CEP com outros biomas florestais, essa região apresenta impressionante diversidade de espécies (NEMÉSIO; SANTOS JUNIOR, 2014; OLMOS, 2005; RODA, 2003) e alto número de espécies endêmicas para diversos grupos taxonômicos (BATISTA et al., 2021; DALAPICOLLA et al., 2021; FIALHO et al., 2014; NETO; TABARELLI, 2002; PEIXOTO; CARAMASCHI; FREIRE, 2003; PEREIRA et al., 2014; SILVA; SOUSA; CASTELLETTI, 2004; SILVEIRA; OLMOS; LONG, 2003). Por exemplo, dois terços de todas as espécies de aves da Mata Atlântica ocorrem no CEP (RODA, 2003) e dentre os 160 táxons de aves brasileiras ameaçadas de extinção, 22% são endêmicos ao CEP (OLMOS, 2005). Ainda, algumas das espécies endêmicas só foram descobertas nessa década, como o mamífero coandu-mirim (*Coendou speratus*) (PONTES et al., 2013), com o aumento do número de estudos na região (BARNETT; BUZZETTI, 2014; FARIA; MELO, 2011), demonstrando que a região deve abrigar uma biodiversidade ainda não conhecida.

Paralelamente à alta riqueza e endemicidade, o CEP encontra-se intensamente fragmentado e ameaçado (DA SILVA; TABARELLI, 2000). Distribuído na forma de uma estreita faixa de floresta, a área original do CEP cobria cerca de 56 000 km^2, mas hoje apenas entre 5,6 e 12,5% de floresta ainda permanece (MINISTÉRIO DO MEIO AMBIENTE, 2005; RIBEIRO et al., 2009). Cerca de 60% de toda a floresta remanescente encontra-se a menos de 100 metros de qualquer borda e aproximadamente apenas 1% está em áreas protegidas por lei (RIBEIRO et al., 2009). Ao contrário do observado nas regiões Sul e Sudeste do Brasil, onde ainda é possível encontrar algumas grandes extensões da floresta remanescente, como o contínuo da Serra do Mar (BROWN JR; BROWN, 1992), a mata do nordeste brasileiro encontra-se mais severamente fragmentada, sendo 48% dos fragmentos remanescentes menores que 10 hectares (RANTA et al., 1998) e a maior parte das unidades de conservação menores que 1000 hectares (NETO; TABARELLI, 2002). No Estado de Alagoas, onde os trabalhos de campo do presente projeto foram conduzidos existem apenas 11 unidades de conservação de proteção integral implementadas, com tamanho médio de 845 ha e que representam cerca de 0,2% do território total do estado (NETO; TABARELLI, 2002).

O CEP sofreu diversos ciclos de desmatamento resultando em uma matriz predominantemente composta por cana-de-açúcar, pois o processo de desflorestamento para seu plantio foi mais intenso nessa região (BARRETO, 2013; LINS-E-SILVA; FERREIRA; RODAL, 2021). O ápice desse processo ocorreu com o programa PROALCOOL que foi lançado em 1975 pelo governo brasileiro e objetivava o aumento da produção de etanol como substituto para a gasolina (CALVALCANTI, 1992). Com isso, a paisagem se tornou dominada pela monocultura de cana-de-açúcar e os últimos grandes remanescentes de Mata Atlântica foram reduzidos (TABARELLI et al., 2010). De acordo com Silva e Tabarelli (2000), grande parte da composição de plantas lenhosas do CEP pode se extinguir em breve como resultado da extinção de espécies de vertebrados frugívoros responsáveis pela dispersão de sementes. Uma dessas espécies, o mutum-de-Alagoas (*Pauxi mitu*), um Cracidae endêmico do CEP, foi extinto na natureza no final dos anos 80, mas sobrevive em cativeiro graças à um programa de reprodução *ex-situ* (PEREIRA et al., 2014; SILVEIRA; OLMOS; LONG, 2004).

O Centro de Endemismo Pernambuco é, dessa forma, a região de Mata Atlântica considerada mais ameaçada pela perda e fragmentação de habitat decorrente da atividade antrópica e, ao mesmo tempo, a região com menos estudos para todo o bioma (DA SILVA; MENDES PONTES, 2008; DA SILVA; TABARELLI, 2000; DE SOUZA; LANGGUTH; GIMENZES, 2004; GARBINO et al., 2018). Essas perturbações antropogênicas podem levar a extinções locais imediatas, resultando na perda de processos ecológicos, em comunidades menos abundantes, com menor riqueza, menor diversidade, com maior variabilidade na distribuição espacial das espécies e maior número de espécies invasoras e generalistas (BECA et al., 2017; BELLO et al., 2016; GIBSON et al., 2011; PARDINI et al., 2005).

1.2. MAMÍFEROS DE MÉDIO E GRANDE PORTE

O Brasil é considerados por muitos autores o país da região Neotropical que apresenta a maior riqueza de mamíferos, com a Mata Atlântica abrigando a segunda maior riqueza de mamíferos dentre os biomas brasileiros (COSTA et al., 2005). Existem cerca de 321 espécies de mamíferos em toda a extensão da Mata Atlântica, incluindo espécies de pequeno, médio e grande porte (GRAIPEL; CHEREM; CARMIGNOTTO, 2017). Apesar de já existir uma extensa literatura destinada a entender a taxonomia e distribuição das espécies, ainda estamos longe de esgotar todas as lacunas do conhecimento (GRAIPEL; CHEREM; CARMIGNOTTO, 2017; QUINTELA; DA ROSA; FEIJÓ, 2020).

Os mamíferos estão presentes em todos os níveis da cadeia trófica, apresentando complexas relações entre si, com outras espécies e com o ambiente (O'FARRILL; GALETTI; CAMPOS-ARCEIZ, 2013; ROEMER; GOMPPER; VAN VALKENBURGH, 2009; TURKINGTON, 2009) e fornecendo importantes serviços ecossistêmicos (DAVIDSON; DETLING; BROWN, 2012; KUNZ et al., 2011; RIPPLE et al., 2014). Contudo, a maioria dos mamíferos em praticamente todos os ambientes, mas principalmente os de médio e grande porte nos trópicos, encontram-se em severo declínio populacional devido a perda, fragmentação e degradação de habitat, caça, tráfico, doenças, espécies invasoras e mudanças climáticas (BOGONI et al., 2018; DIRZO et al., 2014; GALETTI et al., 2021). O processo de redução populacional podendo levar a extinção local, regional ou funcional de vertebrados de médio e grande porte causado pela ação antrópica é chamado de defaunação (DIRZO et al., 2014). Vertebrados de grande porte são particularmente afetados por fatores intrínsecos, como maior tempo geracional, baixa taxa de fecundidade e de crescimento e altos requerimentos energéticos (CARDILLO et al., 2005), mas também por fatores externos, como a caça preferencial para consumo de carne, tráfico, obtenção de produtos "medicinais" e como forma de retaliação (ALVES, 2009; CULLEN et al., 2000; DE CARVALHO; MORATO, 2013; JEROZOLIMSKI; PERES, 2003). Esse processo já tem sido observado na Mata Atlântica brasileira, contudo a magnitude da defaunação é difícil de ser estimada (GALETTI et al., 2016, 2021).

1.2.1. Mamíferos do Centro de Endemismo Pernambuco

No Centro de Endemismo Pernambuco ocorrem cerca de 36% de todas as espécies de mamíferos terrestres da Mata Atlântica, incluindo pequenos, médios e grandes, sendo a maior parte composta por pequenos mamíferos (FEIJÓ et al., 2021; FIGUEIREDO et al., 2021; QUINTELA; DA ROSA; FEIJÓ, 2020). Baseado em dados morfológicos, genéticos e de distribuição das espécies, estudos indicam a existência de pelo menos cinco mamíferos de médio porte endêmicas ao CEP: *Coendou prehensilis* (MENEZES et al., 2021), *Coendou speratus* (PONTES et al., 2013), *Sylvilagus brasiliensis* (RUEDAS et al., 2017), *Dasyprocta iacki* (FEIJÓ;

LANGGUTH, 2013) e *Sapajus flavius* (VALENÇA-MONTENEGRO et al., 2021). No entanto, são poucos os levantamentos da comunidade de mamíferos realizados na região até o momento (DA SILVA; MENDES PONTES, 2008; GARBINO et al., 2018; MENDES PONTES et al., 2016; SÁ-NETO et al., 2013). Ainda, esse conhecimento está desproporcionalmente distribuído entre os estados brasileiros que compõe o CEP, sendo o estado de Alagoas proporcionalmente menos estudado (FEIJÓ et al., 2021). Feijó et al. (2021) estimam que mais de 50% da área do CEP ainda não foi inventariada. Mais preocupante é o fato de não haver consenso entre os levantamentos já realizados sobre a composição atual de mamíferos da região. Pontes et al. (2016) compilaram uma lista de 43 espécies de mamíferos de médio e grande porte que ocorriam no CEP no passado, a partir da literatura produzida desde a época primeiros colonizadores europeus do século 16 até a literatura contemporânea, considerando inclusive espécies descritas pelos autores no século 21. Baseado nesses dados e na amostragem contemporânea de fragmentos florestais do CEP por meio do avistamento em transectos lineares, os autores apontam para a extinção de 21 das 43 espécies do CEP. Contudo, os autores também apontam para a defasagem da literatura contemporânea, por exemplo, a espécie *Cuniculus paca* que ocorre atualmente amplamente na região é citada na literatura antiga, mas não na literatura contemporânea. Considerando o cenário mais pessimista em que a redução de tamanho e perda dos fragmentos de floresta continue nos mesmos níveis do que os vivenciados até o momento, Pontes et al. (2016) apontam para a uma possível extrema simplificação e redução da comunidade, restando apenas quatro espécies de mamíferos de médio porte: *Callithrix jacchus*, *Guerlinguetus alphonsei*, *Bradypus variegatus* e *Sapajus flavius*.

O número de espécies atualmente extintas no CEP é veementemente contestado por Garbino et al. (2018), que propõem que apenas sete das 21 espécies propostas por Pontes et al. (2016) estão localmente extintas. Garbino et al. (2018) discutem que oito espécies nunca teriam ocorrido no CEP (falsas presenças), enquanto outras cinco ainda estariam presentes (falsas ausências). Apesar da discordância entre esses autores e de poucos estudos conduzidos na região até o momento, a maior parte deles já indica um marcante processo de defaunação, resultando na confirmação da extinção local de todos os mamíferos de grande porte (*Puma concolor*, *Panthera onca*, *Tapirus terrestris*, *Myrmecophaga tridactyla*), com exceção da capivara (*Hydrochoreus hydrochaeris*) (ASFORA; PONTES, 2009; BELTRÃO et al., 2019; DA SILVA; MENDES PONTES, 2008; FEIJÓ et al., 2021). Sendo assim, a biodiversidade do CEP está seriamente ameaçada e esforços em prol tanto da descrição da diversidade de espécies existentes quanto visando ações para a conservação dessas espécies são necessários.

1.3. AMOSTRAGEM NÃO INVASIVA

A amostragem não invasiva pode ser descrita como qualquer método de amostragem que não envolva a observação direta ou o manuseio do animal, dessa forma, teoricamente não interferindo em seu comportamento ou cansando-lhe estresse (LONG et al., 2012; TABERLET; LUIKART, 1999). Nos últimos trinta anos, o termo amostragem não invasiva tem sido aplicado com mais frequência à coleta de amostras biológicas de animais selvagens para obtenção de matéria genético sem a captura ou manipulação do animal (TABERLET; LUIKART, 1999). Os tipos de amostras não invasivas incluem fezes, pelos, urina, pelotas regurgitadas de coruja, folhas e frutos mastigados contendo saliva, e até mesmo placentas deixadas em locais de parto (LEFORT et al., 2022; LOBO et al., 2015; RODGERS; JANEČKA, 2013; VALTONEN et al., 2015; WAITS; PAETKAU, 2005). Entre a miríade de métodos não invasivos aplicados para o estudo de populações de animais selvagens, a amostragem genética não invasiva (NGS – *noninvasive genetic sampling*) de fezes foi introduzida em 1992, quando a presença de ursos-marrons (*Ursus arctos*) foi avaliada na Itália a partir do DNA de amostras fecais (HÖSS et al., 1992). A partir de então, as amostras fecais tem sido utilizadas como fonte de material genético para identificação da espécie, individual e do sexo dos indivíduos depositores das fezes e como uma alternativa não invasiva para amostras biológicas obtidas a partir da captura dos indivíduos (PIGGOTT; TAYLOR, 2003).

1.3.1. Identificação molecular a partir de amostras fecais

A amostragem não invasiva de fezes, associada à identificação molecular, tornou-se uma importante ferramenta em pesquisas em ecologia, manejo, conservação e análise forense da vida silvestre (BEJA-PEREIRA et al., 2009). Apesar de amostras biológicas não invasivas geralmente apresentarem DNA degradado pela ação do tempo e pela mistura do DNA da espécie depositora com o material genético de presas ingeridas e do ambiente (GOOSSENS; BRUFORD, 2009), a amostragem não invasiva associada a identificação molecular apresenta muitas vantagens para o monitoramento da biodiversidade (HAJIBABAEI et al., 2007; LAGUARDIA et al., 2015). Primeiro, por necessitar de pequenos fragmentos de vestígios que contenham células, como pelos ou fezes, que permitem a identificação da espécie e do indivíduo; segundo por não necessitar da captura dos indivíduos sendo, dessa forma, menos estressante aos animais; terceiro, por propiciar uma identificação que não é influenciada por caracteres fenotípicos e que podem gerar dúvidas de identificação (TABERLET, 1996; TABERLET et al., 1997). Essa abordagem não necessita ser empregada de forma a substituir outras metodologias, mas vem para corroborar resultados de outras técnicas, tornando-as mais confiáveis (ALBERTS et al., 2017).

Devido à alta variação interespecífica e baixa variação intraespecífica, sequências de pequenas regiões do DNA mitocondrial, também conhecidas como "*mini barcodings*", são utilizadas como identificadoras de espécies a partir de amostras degradadas com o uso de marcadores moleculares específicos (CHAVES et al., 2012; FARRELL; ROMAN; SUNQUIST, 2000; RODRÍGUEZ-CASTRO et al., 2020; VALENTINI; POMPANON; TABERLET, 2009).

1.4. BIOLOGIA DA CONSERVAÇÃO

O campo da Biologia da Conservação surgiu no ano de 1978 quando Michel Soulé e Bruce Wilcox organizaram a primeira "*International Conference on Conservation Biology*", dada a crescente preocupação com o aumento da ameaça de extinção das espécies (SOULÉ; WILCOX, 1980). Soulé (1985) definiu o campo da biologia da conservação em relação ao campo da biologia geral como uma ciência análoga ao que a guerra é para as ciências políticas, ou seja, uma "disciplina de crise". Essa disciplina engloba conceitos e metodologias de Ecologia e Genética, entre outros campos da biologia, com o objetivo de fornecer ferramentas para a preservação da biodiversidade (SOULÉ, 1985). Dada a natureza de "disciplina em crise", biólogos da conservação perceberam que precisavam de métodos de obtenção de informação rápidos, eficientes e relevantes para o planejamento de estratégias para a conservação, sendo a genética uma das mais poderosas ferramentas (HAUFFE; SBORDONI, 2009; SOULÉ; SIMBERLOFF, 1986). Apesar do imenso potencial da genética de auxiliar em questões de conservação, as metodologias genéticas só se tornaram mais utilizadas com o desenvolvimento de técnicas mais precisas, rápidas, baratas e não invasivas (GOOSSENS; BRUFORD, 2009). Um problema crítico da biologia da conservação é prever a distribuição geográfica das espécies. Para proteger uma espécie, primeiramente é preciso saber qual é a espécie observada e em quais ambientes há os requisitos necessários para sua sobrevivência. As estimativas de extinção de espécies continuam tão relevantes e preocupantes quanto as estimativas dos anos 1990, sendo necessárias ferramentas que auxiliem na sua conservação (COWIE; BOUCHET; FONTAINE, 2022; LIU et al., 2022; SMITH et al., 1993).

1.4.1. Modelos de distribuição de espécies como uma ferramenta para a conservação

Modelos de distribuição de espécies (SDM, *species distribution models*, em inglês) são ferramentas tem sido amplamente utilizadas para entender aspectos da distribuição das espécies voltados para fins de conservação (ELITH et al., 2011; FERRAZ et al., 2012; FRANKLIN, 2013; RODRÍGUEZ et al., 2007). Dada a escassez de dados sobre a distribuição e abundância de espécies, os modelos permitem a extrapolação de relativamente poucos pontos de ocorrência para toda a distribuição potencial das espécies (CAYUELA et al., 2009; RODRÍGUEZ et al., 2007). As avaliações de risco de extinção da *International Union for Conservation of Nature* (IUCN), por exemplo, tem como uma das categorias de avaliação a área de ocorrência das espécies, que pode ser beneficiada e refinada pelo uso de SDMs (PAPEŞ; GAUBERT, 2007; SYFERT et al., 2014)

Os modelos de distribuição de espécies baseiam principalmente na teoria de nicho, na qual a distribuição e abundância das espécies é mediada por processos ecológicos, e nas ciências de informação geográfica e sensoriamento remoto (FRANKLIN, 2013). Modelos de distribuição de espécies associam pontos de ocorrência das espécies (observações georreferenciadas) com múltiplas variáveis ambientais das localidades de ocorrência, por meio de uma variedade de métodos estatísticos (ELITH; LEATHWICK, 2009). Os algoritmos atualmente disponíveis variam com relação ao tipo de dado de ocorrência que necessitam (ELITH et al., 2006). Muitos modelos, como o caso do algoritmo Maxent, são chamados *presence-only,* ou seja, necessitam apenas de dados de presença das espécies, por exemplo, os disponíveis em bancos de dados ou coleções biológicas (ELITH et al., 2011). Esses modelos podem ser extrapolados para diferentes cenários (no espaço e no tempo) afim de projetar a possível distribuição das espécies frente a uma diferente condição ambiental (ELITH; LEATHWICK, 2009). Contudo, um gargalo que pode enviesar a predição da distribuição das espécies é justamente a defasagem no número de registros das espécies focais (FEELEY; SILMAN, 2011).

Alguns trabalhos aplicando modelagens de distribuição de espécies para fins de conservação já foram realizados para espécies da Mata Atlântica brasileira (e.g., DE SOUZA; PREVEDELLO, 2019; OLIVEIRA et al., 2022; SOBRAL-SOUZA et al., 2018). Dada a natureza climática das variáveis ambientais que geralmente são utilizadas para construir os modelos de distribuição de espécies, especial foco tem se dado a trabalhos que mensuraram o efeito das mudanças climáticas contemporâneas na distribuição de espécies. Esse processo já foi estudado em mariposas (FERRO et al., 2014), aves (DE SOUZA et al., 2011), anuros (SOARES DE OLIVEIRA et al., 2016), mamíferos piscívoros (BOGONI; TAGLIARI, 2021), e na comunidade de plantas (WILSON et al., 2021), entre outros. De forma geral, os estudos apontam para os efeitos negativos das mudanças climáticas contemporâneas na distribuição das espécies. Contudo, nenhum trabalho ainda avaliou a resposta a nível de comunidade de mamíferos para toda a extensão do bioma.

2 OBJETIVOS

2.1. OBJETIVO GERAL

O objetivo geral desta tese é analisar os padrões de distribuição, riqueza e endemicidade de mamíferos de médio e grande porte na Mata Atlântica brasileira, com especial foco no Centro de Endemismo Pernambuco, explorando o potencial das metodologias de amostragem não invasivas.

2.2. OBJETIVOS ESPECÍFICOS

Capítulo I

A partir da construção de modelos de distribuição de espécies de mamíferos de médio e grande porte na Mata Atlântica brasileira, nós objetivamos 1) determinar a riqueza e endemicidade dessas espécies para o presente e passado (21 mil anos atrás) dentro dos três refúgios paleoclimáticos; 2) estimar mudanças na área de distribuição das espécies e 3) nos padrões de riqueza e endemismo dos refúgios em resposta as mudanças climáticas preditas para 2081-2100. Ao incorporar medidas de diversidade filogenética objetivamos 4) entender a história evolutiva da comunidade de espécies presentes nos refúgios. Ao combinar todos os resultados, pretendemos 5) estimar locais onde a biodiversidade estaria mais ameaçada nos futuros cenários possíveis de mudanças climáticas, sendo prioridades para ações de conservação e mitigação.

Capítulo II

Através de uma revisão sistemática da literatura, pretendemos compilar a riqueza de experiências de vários grupos de pesquisa acumulada durante as últimas três décadas sobre o uso do DNA fecal para o estudo de carnívoros raros. Nós buscamos responder as seguintes questões: 1) Quais são as aplicações das amostras de DNA derivadas de fezes? 2) Quais são as técnicas usadas na genética baseada em amostras fecais? 3) Qual é o esforço de amostragem necessário para detectar espécies raras de carnívoros? E 4) qual é a eficiência do DNA fecal na detecção de espécies raras em comparação com outros métodos? Objetivamos também apresentar quais as direções que têm sido propostas para futuras pesquisas sobre carnívoros raros com o uso do DNA fecal.

Capítulo III

Neste capítulo objetivamos caracterizar a comunidade de mamíferos de médio porte na Reserva Particular do Patrimônio Natural Mata do Cedro (Rio Largo/AL), com especial interesse em avaliar a presença de carnívoros (nativos e domésticos) que poderiam impactar a reintrodução do mutum-de-Alagoas. Também visamos avaliar a eficácia dos vários métodos dos amostragem não invasivos empregados: armadilhamento fotográfico, armadilhamento de pelos, avistamento em transectos lineares, amostragem de fezes associada à identificação molecular e encontro oportunista de carcaças e ossos.

Capítulo IV

Neste capítulo objetivamos avaliar a presença de canídeos e felinos nativos no Centro de Endemismo Pernambuco no estado de Alagoas por meio da identificação molecular das fezes deixadas no ambiente e, assim, ajudar a alcançar um panorama mais completo sobre a ocorrência dessas espécies.

3 CAPÍTULO I – MODELING RESPONSES OF BRAZILIAN ATLANTIC FOREST MAMMALS ASSEMBLE TO CLIMATE CHANGE

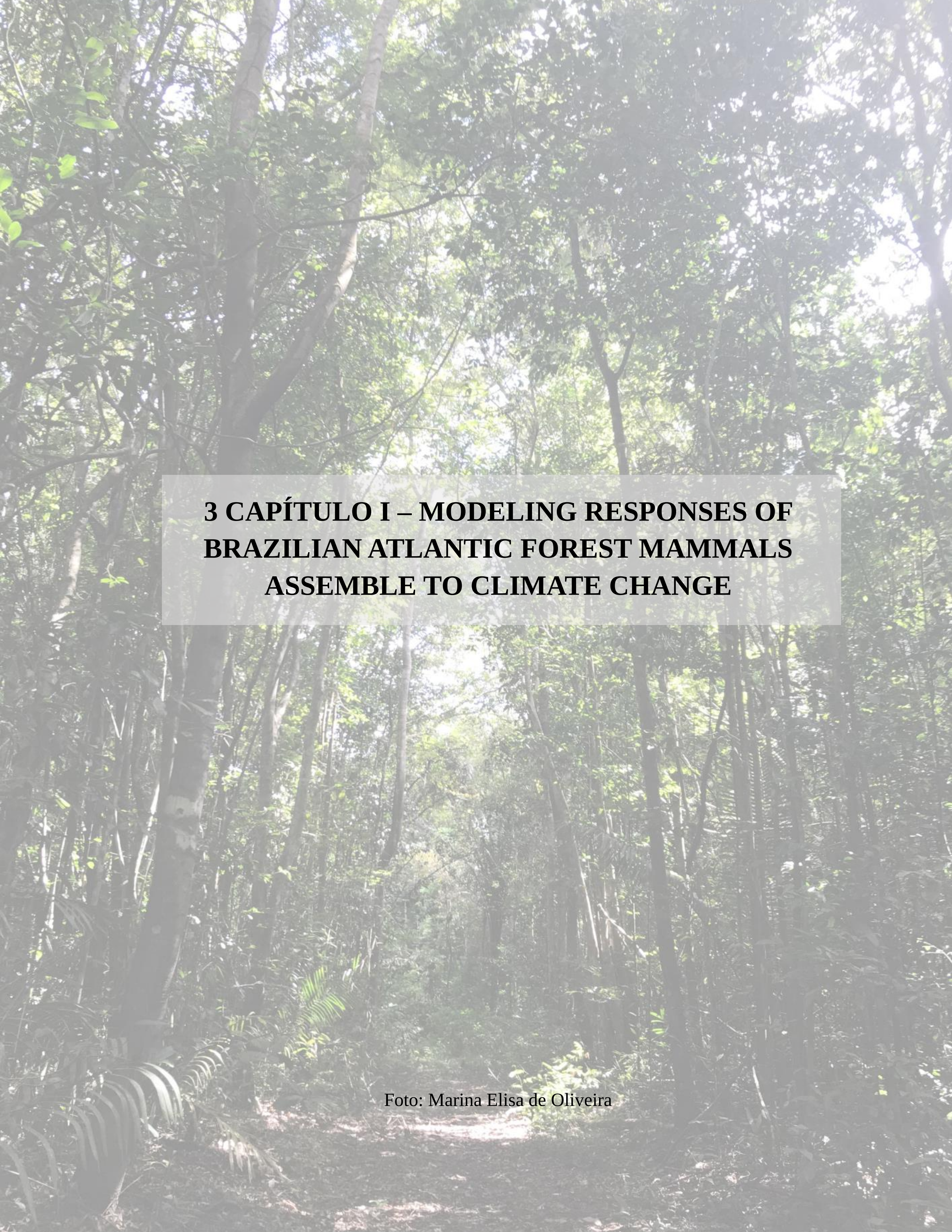


Foto: Marina Elisa de Oliveira

Modeling responses of Brazilian Atlantic Forest mammals assemble to climate change

Abstract

Biodiversity hotspots, such as the Brazilian Atlantic Forest (BAF), are expected to be more affected by climate change. Three distinct areas of past climatic refugia occur in the BAF: Pernambuco, Bahia, and São Paulo, that will likely be impacted differently by climate change. To estimate the distribution of mammal assemblages in response to climate change, we built individual species distribution models for 68 medium and large size mammals of the BAF under past (21 kya), current and future projected climatic conditions. We evaluated changes in predicted species distribution, richness and endemism. To understand the evolutionary history of these regions, we also calculated current phylogenetic diversity. We observed high mammal richness in refugia Bahia and São Paulo, and centers of endemism congruent with refugia areas. Phylogenetic diversity revealed different evolutionary histories within each refugium, and that ecological diversity may be preserved even in areas of lower species richness (Pernambuco refugium). Most species contracted its original distribution, and endemicity varied under all future scenarios, being most impacted by a higher increase in mean annual temperature. It was projected that up to 32% of species will lose 95% of their climatic spaces and up to 73% of species will lose half of it by 2100. Our major results highlight the widespread and negative impacts of climate change on mammal distribution. Biodiversity loss due to climate change can be buffered by both reducing deforestation and prompting restoration projects. Yet, we reinforce that the action window to achieve this goal is closing rapidly.

Keywords: conservation; macroecology; maxent; Neotropical; SDM; South America

3.1. INTRODUCTION

For the next decades, climate change is likely to be the principal driver of species range changes (NEWBOLD, 2018). Although species have responded to climate changes during their evolutionary history (HAWKINS et al., 2007; THEODORIDIS et al., 2020), the current speed of changes coupled with the addictive effects of other anthropogenic activities lead to immediate threat to the persistence of species in their current distribution (NEWBOLD, 2018; SANDEL et al., 2011; THOMAS et al., 2004). Many terrestrial and aquatic species have already been negatively impacted by climate change and many more are vulnerable to its long-term effects (MACLEAN; WILSON, 2011; PARMESAN, 2006; THOMAS et al., 2004). Maximum temperature increase followed by mean precipitation change are the most important determinants in the degree of biodiversity response to the effects of habitat loss and fragmentation (MANTYKA-PRINGLE; MARTIN; RHODES, 2012). Current mean global temperature has increased by approximately 1ºC since 1880 and projections point to at least 2ºC more and up to 4.5ºC of increase by 2100 (LENSSEN et al., 2019; RAFTERY et al., 2017). Notwithstanding, highly biodiverse areas are expected to be more affected by climate change and its synergistic effects (SEGAN; MURRAY; WATSON, 2016). Although fine-scale climatic variation plays an important role in accumulation of diversity and potentially buffers biodiversity from the effects of climatic change, many hotspots have been climatically stable through large-scale evolutionary time, making them more vulnerable to rapid large-scale and human-induced climate change (TREW; MACLEAN, 2021). Hence, understanding species' responses to future climate change in highly biodiversity areas should be a top conservation priority (TREW; MACLEAN, 2021; WILLIG, 2003).

The Brazilian Atlantic Forest (BAF) biome is considered one of the world’s hotspots of biodiversity (MYERS et al., 2000). Besides recent and historic climate change, this biome has endured constant human disturbances since the European colonization of Brazil, causing an overwhelming decline of vegetation cover that currently reaches 28% of its original area, of which only 9% is within strictly protected areas (REZENDE et al., 2018). Despite this alarming scenario, the Atlantic Forest sustains impressive richness and endemism (MORELLATO; HADDAD, 2000; SILVA; SOUSA; CASTELLETTI, 2004).

Species richness and endemism are important metrics to evaluate the biodiversity of different biomes and ecosystems, however these metrics are not always congruent with the spatial distribution of other aspects of diversity (SAFI et al., 2011). The evolutionary relationship among taxa can be reflected in phylogenetic diversity, which in turn can be a proxy of character or function diversity in an ecosystem (FAITH, 1992; FOREST et al., 2007; WEBB et al., 2002). Understanding which and how species clades have evolved and are currently structured in a community can enhance our comprehension of species vulnerability to global changes and guide conservation practices (MACE; GITTLEMAN; PURVIS, 2003; SECHREST et al., 2002). Thus, integrating measures of biogeographic distribution and phylogenetic diversity is vital for conservation purposes at biogeographical scales (CADOTTE; JONATHAN, 2010; COSTION et al., 2015).

Carnaval and Moritz (2008) proposed two historically climatic stable regions within the BAF, Pernambuco and Bahia refugia, separated by the São Francisco River, and a third climatically unstable region south of the Doce River, latter denoted São Paulo refugium (CARNAVAL et al., 2009). These three refugia areas were also reinforced by phylogeographical data (MARTINS, 2011). Keppel et al. (2012) defined the classical view of refugia as “habitats that components of biodiversity retreat to, persist in and can potentially expand from under changing environmental conditions”. Although refugia dynamics are often complex and involve species-specific responses to environmental variables throughout space and time, these climatically stable regions may represent protection for species under rapid future climate change (KEPPEL et al., 2012). Different degrees of change in higher mean annual temperature and average precipitation are forecasted for different parts of the Atlantic Forest biome (ANJOS et al., 2021; IPCC, 2021). This will likely affect the biodiversity of Atlantic Forest refugia differently. Furthermore, Pernambuco, Bahia and São Paulo have heterogeneous human occupation and deforestation trajectories. For example, the northeastern Atlantic Forest of Pernambuco refugium, also known as an endemism center (Pernambuco Endemism Center - PEC), is much more human-degraded than Bahia and São Paulo, this latter retaining the largest continuous forest remnants within the BAF (RIBEIRO et al., 2009). Thus, mapping areas that are able to sustain high species richness and endemism in light of current and future threats can aid the prioritization of areas for conservation (CEBALLOS; EHRLICH, 2006; JENKINS; PIMM; JOPPA, 2013).

In this paper, we built species distribution models (SDM) to 1) determine current and past (21 000 years ago) richness and endemicity of medium and large mammals across the BAF refugia areas. Mammals are a valuable group for understanding broad-scale responses to environmental change for several reasons: public databases have comprehensive records of mammal occurrences (SANTOS et al., 2020b), they are present in different trophic levels within communities (ROEMER; GOMPPER; VAN VALKENBURGH, 2009; TURKINGTON, 2009), and are charismatic species that can pose as umbrella species for the overall conservation of habitats (BRANTON; RICHARDSON, 2011). By modeling species distribution under different possible scenarios of future climate change we also aimed 2) to measure changes (expansion or contraction) in the predicted areas of species distribution per refugia and 3) to determine the changes in species richness and endemism in response to climate changes considering multiple future predictions. Although these metrics are fundamental to assess the threat status of biodiversity, other components of diversity must be considered to address conservation issues (FERGNANI; RUGGIERO, 2015). By incorporating phylogenetic analysis, we aimed at 4) estimating current phylogenetic diversity within each refugia area to understand the evolutionary history of these regions (SECHREST et al., 2002). Finally, by combining all results, we intended to 5) estimate locations where biodiversity would be most threatened under future climatic scenarios and, consequently, be a priority for conservation.

We expected to find current high mammal species richness in the southern Atlantic Forest, as similarly reported for other taxonomic groups (e.g., amphibians - LEMES et al., 2014; birds - VALE et al., 2018; butterflies - SANTOS et al., 2020) because of the larger forest patches of São Paulo and Bahia refugia. We also expected to find LGM species richness congruent with past Atlantic Forest distribution (i.e., higher in Bahia and Pernambuco refugia, when compared to the more climatic unstable São Paulo refugium). However, species richness and endemism are not always congruent (ORME et al., 2005), thus we do not expect to observe spatial congruence between these two metrics for any time period. We also expected that future scenarios predicting higher temperature increase will cause larger reduction in species distribution area. Among the refugia, we expect the Pernambuco to be among the refugia centers that will be most adversely affected by climate change, because of its smaller territory and history of severe local extinction events of mammals (GARBINO et al., 2018; MENDES PONTES et al., 2016). Based on the principle that species present in stable areas accumulate genetic divergence through time (CARNAVAL et al., 2014), we expect that the historical stability of Pernambuco and Bahia refugia (CARNAVAL; MORITZ, 2008) reveals higher phylogenetic diversity than São Paulo refugium, based on metrics that are not strongly correlated to species richness.

3. 2. MATERIAL AND METHODS

3.2.1. Species distribution modeling

To better represent the extent of mammal’s presence records used in the SDM approach, we restricted the analysis to the integrative limit of the BAF proposed by Muylaert et al. (2018) and used the two large rivers São Francisco River and Doce River as boundaries for the refugia (Figure 1.1A), as proposed by Carnaval and Moritz (2008).

We first compiled a list of all terrestrial medium and large mammal species (>0.5kg, hereinafter referred to as mammals) inhabiting the BAF based on species distribution maps available at the IUCN (International Union for Conservation of Nature and Natural Resources) assessments, the Red Book of Brazilian Fauna Threatened of Extinction (INSTITUTO CHICO MENDES DE CONSERVAÇÃO DA BIODIVERSIDADE, 2018) and the Extinction Risk Assessments published by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, Ministério do Meio Ambiente). The final list comprised 89 species (Table S1), for which presence records were obtained – in January 10th 2023 – from the online databases: Global Biodiversity Information Facility (GBIF; www.gbif.org), SpeciesLink project (splink.cria.org.br), Biodiversity Extinction Risk Assessment System (SALVE/ICMBio; www.salve.icmbio.gov.br); and the published datasets: CULOT et al., (2018); DE OLIVEIRA et al., (2022); NAGY-REIS et al., (2020); SANTOS et al., (2019); SOUZA et al., (2019) (see Table S2).

We eliminated records collected before 1980, that reported more than 5000 meters of imprecision, with latitudinal and longitudinal precision of less than three decimal degrees, duplicated records and those with geographic position obscured to protect endangered species. We established the 1980 date threshold to ensure that species records coincide temporarily with environmental variables and because older records may be inaccurate due to limited GPS technology (KUMAR; MOORE, 2002). We also removed roadkill and interview records, given that roadkill data may bias the models towards areas of higher road density and interview records cannot be assessed to confirm its spatial and taxonomic accuracy. The remaining records were reprojected to DATUM WGS84 and cropped to the extent of the BAF in ArcGIS 10.5 software (ESRI, 2011).

Near current (1970-2000) and projected future (2081-2100) bioclimatic variables were obtained from the WorldClim 2.1 database (www.worldclim.org; FICK; HIJMANS, 2017) and past (21 000 years ago, referring to the Last Glacial Maximum) bioclimatic variables were obtained from the PaleoClim database (www.paleoclimchelsea-climate.org; BROWN et al., 2018; KARGER et al., 2023). All variables were obtained at the 2.5 arc-min resolution (~5km) and cropped to the extent of the study area. To reduce autocorrelation between bioclimatic variables, we calculated Pearson's correlation coefficient for the 19 bioclimatic variables available for the near current period, maintaining only variables with a correlation ≤0.7. We thus maintained the following: mean diurnal range (mean of monthly (maximum temperature – minimum temperature)), isothermality, maximum temperature of warmest month, minimum temperature of coldest month, mean temperature of wettest quarter, annual precipitation, precipitation of wettest month, precipitation of warmest quarter and precipitation of coldest quarter. We also included a continuous elevation layer at the same 5 km resolution from the EarthEnv database (www.earthenv.org; AMATULLI et al., 2018) and a categorical land cover layer. Land cover (30 meters resolution) was obtained from the MapBiomas Project – collection 6 (www.mapbiomas.org; SOUZA et al., 2020) and resampled by the NEAREST method to match the resolution of bioclimatic variables. We reclassified land cover into 6 classes: forest, non-forest natural formation, farming, non-vegetated area, water and non-observed, based on Level 1 classifications of MapBiomas. The best models for current conditions were projected onto LGM and future climate scenarios. We used current cover land to build the distribution models for the future, projecting scenarios with only climate as future changes. LGM models were built only with climatic variables and elevation.

Given the uncertainties in the magnitude of future climate changes and their impact on mammal biodiversity, we estimated future distribution under two trends in greenhouse gasses emissions (GHG). Recent studies have suggested that an increase in temperature of less than 2°C is unlikely (RAFTERY et al., 2017), thus we modeled future species distribution under moderate and high-emission rates, referred to Shared Socioeconomic Pathways (SSP) 2-4.5 and 5-8.5, respectively (IPCC, 2021). The SSP5-8.5 scenario follows the current emission trend with no modification in emission policies, where economic growth relies on fossil fuels and lifestyles demand high energy consumption. The SSP2-4.5 is an intermediate scenario where emissions do not reach zero but begin to decline by 2050 (RIAHI et al., 2017). To simulate varying future changes in mean annual precipitation and mean annual temperature, we selected four different General Circulation Models (GCM) from the CMPI6 (Coupled Model Intercomparison Project) for the period of 2081 to 2100, compared to the baseline period of 1981-2010 (GUTIÉRREZ et al., 2021). These GCMs represent different scenarios of future climate conditions, including low increase (INM-CM4-8), moderate increase (MRI-ESM2-0), high increase (CanESM5), and annual fluctuations (MIROC6) of mean annual temperature and variations in mean annual precipitation (Table S3). With this approach, we obtained eight distinct future scenarios.

We applied the maximum entropy algorithm (Maxent; PHILLIPS et al., 2006) as implemented in SDMtoolbox 2.4 (BROWN; BENNETT; FRENCH, 2017) to build distribution models for each species. To ensure spatial independence between occurrence points and reduce model overfitting, we used SDMToolbox to reduce species occurrence to a single point per species within a 5-km radius (BROWN et al., 2017). Individual models were generated for 68 mammal species for which more than 15 occurrence records were available (Table S1). We tested different combinations of feature classes, depending on the number of records used to build individual SDM (set at AUTO), and applied eight regularization multipliers (0.5, 1, 1.5, 2, 2.5, 3, 4, 5; BROWN et al., 2017). Thus, the use of SDMToolbox improved the SDM performance by optimizing model parameters through tuning experiments (BROWN et al., 2017).

Model fit was evaluated by the lowest omission rate, higher area under the curve (AUC) and lowest model complexity, in this order. Continuous suitability maps were transformed into binary maps using the minimum training presence threshold. To limit overprediction, the final models were pruned with a 100 km buffer around the minimum convex polygon of the occurrence points for each species.

3.2.2. Richness, endemicity and distribution changes

The best binary model generated for each species was used to estimate species richness and weighted endemism (WE). Species richness is the sum of unique species per cell, in the same spatial resolution as species distribution maps (2.5 arc-min or approximately 0.0416º). Weighted endemism is calculated as $WE=\Sigma\ 1/C$, where C is the number of grid cells (0.5º resolution) that each species is present in. WE emphasizes cells that have more range-restricted species by attributing them to higher WE values (CRISP et al., 2001). Change in the distribution area was calculated as the percentage of difference between present and future predicted distribution area.

Species distribution change was calculated per species for all GCM under both shared-socioeconomic pathways.

3.2.3. Phylogenetic diversity

To estimate the phylogenetic diversity within Brazilian Atlantic Forest refugia, we used a near-complete phylogeny of the class Mammalia (5911 species), based on a 31-gene supermatrix (UPHAM; ESSELSTYN; JETZ, 2019) from the Vertlife database (www.vertlife.org/phylosubsets). Among the 89 species that occur in the BAF (Table S1), 85 species were found in the database, accounting for 95.5% of our species dataset. Subsets of 10 000 randomly selected plausible trees were obtained from the dataset "Mammals birth-death node-dated completed trees" (UPHAM et al., 2019). The maximum credibility tree was created with TreeAnnotator v2.6.0 utility in BEAST (BOUCKAERT et al., 2019) and this tree was used in all downstream analyses. The default settings were used in TreeAnnotator (burn in percentage 25, posterior probability limit 0.0), and common ancestor heights were applied for the node heights.

For the three refugia the phylogenetic diversity was estimated based on six metrics: Faith's phylogenetic diversity (PD_{Faith}; FAITH, 1992), mean pairwise distance (MPD; WEBB, 2000) and mean nearest taxon distance (MNTD; WEBB et al., 2002) and their equivalents standardized for species richness, ses.PD_{Faith}, ses.MPD and ses.MNTD. All metrics were estimated using the Picante package (Phylocom integration, community analyses, null-models, traits and evolution; KEMBEL et al., 2010) in the R environment (R CORE TEAM, 2022). These three metrics explore different aspects of phylogenetic diversity. PD_{Faith} is defined based on cladistic information, i.e., it is the sum of the length of all branches in a phylogenetic tree of a taxa set from the entire community. This metric is usually strongly correlated with species richness (TUCKER et al., 2017), thus we also tested if our dataset shows this positive correlation by applying a linear regression between these two metrics. MPD and MNTD use a distance matrix as a measure of phylogenetic relatedness and represent the evolutionary diversity in communities and are less dependent on species richness. MPD is based on the average pairwise distance between all species, being more sensitive to basal tree patterns. High values of this metric indicate that species from the taxa set (refugia mammals) belong to a wide range of clades, while low values represent phylogenetic clustering. MNTD focuses on the average pairwise distance between closely related species, being more sensitive to terminal branch patterns. High MNTD value suggest that closely related species do not co-occur in the taxa set, consequently low values indicate that they do. For the standardized effect size (ses) calculations, we used the phylogeny pool null model, which maintains the number of species of each refugium as a constant, but randomly shuffles species identity (HARDY, 2008). We generated 1000 null communities and compared them with the observed community. Values of observed PD_{Faith}, MPD and MNTD greater than expected given the species richness return positive values of $ses.PD_{Faith}$, ses.MPD and ses.MNTD, respectively, and are representative of overdispersion, whereas negative values are indicative of observed values lower than expected, and are representative of clustering (WEBB et al., 2002).

3.3. RESULTS

After filtering occurrence points, we used a total of 12 091 unique localities distributed across 68 species to perform the individual SDMs (177 unique localities per species on average, SD = 204). The number of localities per species varied from 15 to 1061 (Table S1). Overall, species distribution models exhibited good model fit (mean omission rate = 0.034, mean AUC > 0.7, Table S1). High species richness was observed south of Doce River (São Paulo refugium) and between Doce and São Francisco rivers (Bahia refugium) (Figure 1.1B). These areas with high species richness showed high climatic suitability for up to 53 species of medium and large mammals. Among the three refugia, Pernambuco showed the lowest species richness (up to 31 species). Other areas of low species richness include the western portion of Bahia refugia, an area surrounded by the Cerrado biome, and the southernmost portion of São Paulo refugium, which is in contact with southern Brazilian savannah formation (i.e., Pampas). In the LGM model, suitable climate conditions for mammal assemblies were primarily concentrated in the central portion of Bahia refugium, in the coastal area of Pernambuco and in a few patches along the coastline of São Paulo refugia. In all future scenarios, we observed varying degrees of climatic suitability reduction for future species richness, with more pronounced changes in the GCM CanESM5 (high maximum mean temperature change and high decrease in mean precipitation) in both GHG emission trends, but more pronounced in the higher emission trend (Figure 1.1C, Figure S1).

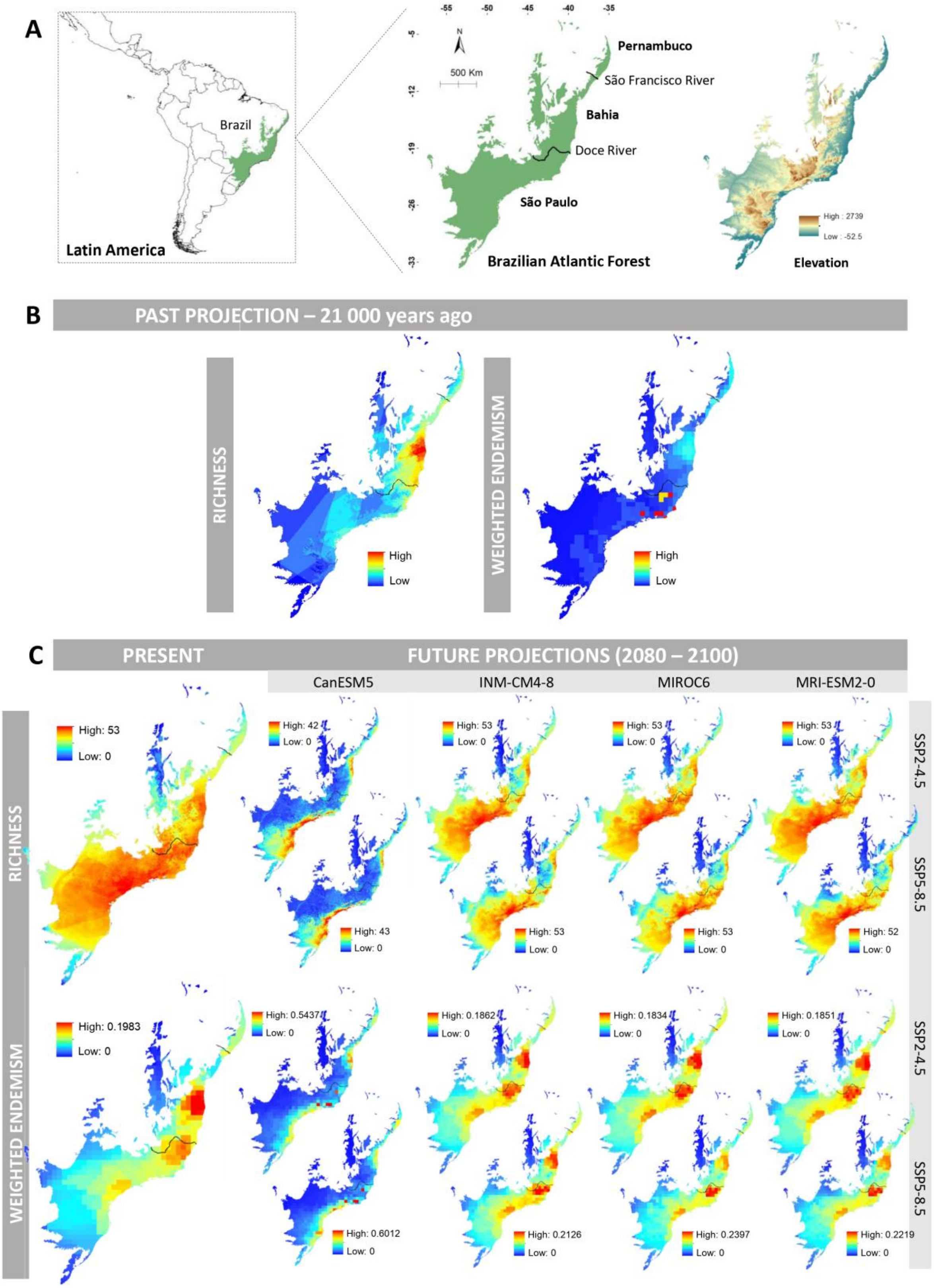
We observed four separated areas in the weighted endemism analysis for the present, corresponding to the central and the most northern regions of São Paulo refugium, central Bahia and almost the entire Pernambuco refugium. In the LGM model, Bahia and Pernambuco exhibit one endemic center each, while São Paulo presented a few isolated 0.5º cells with high endemism values. The value of weighted endemism slightly decreases in most future scenarios in the SSP2-4.5 emission trend and increases in all scenarios of the SSP5-8.5, reaching its maximum in the hottest and driest scenario (CanESM5-SSP5-8.5). In this scenario we observe a shift in the São Paulo core endemism area towards the south. For all other CGM in SSP5-8.5 different degrees of endemic areas contraction are foreseen.

Species showed the same direction (contraction or expansion) of distribution change in both SSP (2-4.5 and 5-8.5), differing in the intensity of the response (Table 1.1, Figure 1.2, Figure S2). In Figure 1.2 we display only the results under the SSP2-4.5 (intermediate GHG emission), and results for the SSP5-8.5 emission trend are presented in Figure S2. Most species contracted their distribution area and most responded in the same direction to all four models.

Table 1.1 – Mean percentage and standard deviation of distribution change per refugium in the eight scenarios modeled. Number of species modeled per refugium within parenthesis.

Scenario		**Pernambuco (30)**	**Bahia (56)**	**São Paulo (62)**
CanESM5	**SSP2-4.5**	-52.8% (±35%)	-65.5% (±42.5%)	-61% (±37.5)
	SSP5-8.5	-59.7% (±34.4%)	-68.2% (±46.2%)	-68.8% (±30.3%)
INM-CM4-8	**SSP2-4.5**	-13.7% (±16.3%)	-27.8% (±22.7%)	-11.5% (±17.4%)
	SSP5-8.5	-25.4% (±24.4%)	-37.8% (±34.1%)	-22.4% (±27.2%)
MIROC6	**SSP2-4.5**	-26% (±24.1%)	-18.4% (±26.1%)	-10.4% (±16.4)
	SSP5-8.5	-48.2% (±31%)	-35.8% (±39.6%)	-21.1% (±27.2%)
MRI	**SSP2-4.5**	-18.8% (± 21.8%)	-15.2% (±27.6%)	-9.4% (±15.7)
	SSP5-8.5	-39.4% (±31.7%)	-27.7% (±45.3%)	-20.4% (±25.5%)

Figure 1.1 – Location and elevation of the Brazilian Atlantic Forest and the three Pleistocene refugia (A), pattern of species richness and endemism for past projection (B), present and projected future scenarios (C) of medium and large mammals in the Brazilian Atlantic Forest



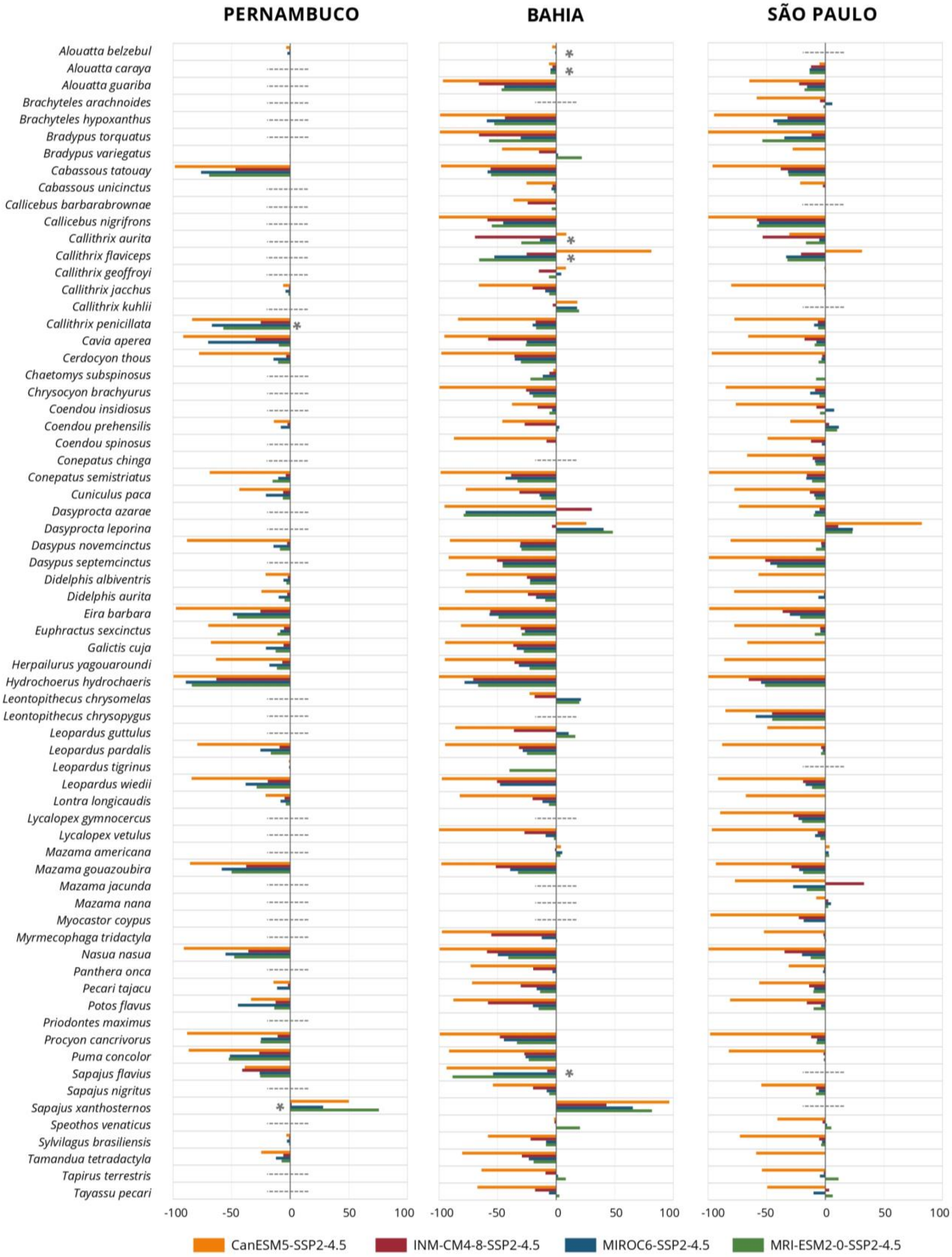
Up to eighteen species in Bahia refugium, twelve in São Paulo and three in Pernambuco showed over 95% distribution contraction in the hottest and driest future scenario (CanESM5; Figure 1.2). These species represent 32%, 19% and 10% of all species modeled for each area, respectively. Considering the other three GCM, no species showed contractions superior to 88%. In at least one scenario 16 out of 30 (53%), 41 out of 56 (73%) and 45 out of 62 (72%) species will lose over half of their climatic spaces within Pernambuco, Bahia and São Paulo refugia, respectively (Figure 1.2). The species Brown-throated Sloth (*Bradypus variegatus*), Geoffroy's Marmoset (*Callithrix geoffroyi*), Wied's Marmoset (*Callithrix kuhlli*), Azara's Agouti (*Dasyprocta azarae*), Red-rumped Agouti (*Dasyprocta leporina*), Golden-headed Lion Tamarin (*Lentopithecus chrysomelas*), Southern Tiger Cat (*Leopardus guttulus*), Red Brocket (Mazama americana), Buff-headed Capuchin (*Sapajus xanthosternos*), Bush Dog (*Speothos venaticus*) and Lowland Tapir (*Tapirus terrestris*) in Bahia refugium; Southern Muriqui (*Brachyteles arachnoides*), Buffy-headed Marmoset (*Callithrix flaviceps*), Bahia Porcupine (*Coendou insidiosus*), Brazilian Porcupine (*Coendou prehensilis*), *D. leporina,* Small Red Brocket (*Mazama jacunda*), *S. venaticus, T. terrestris* and White-lipped Peccary (*Tayassu pecari*) in São Paulo refugium and no species in Pernambuco refugium were predicted to expand their distribution in at least 5% in one or more future scenario. All remaining species were modeled to either contract or exhibit no change in their range distribution in response to future climate change scenarios (Figure 1.2). Mean change in species distribution was more negative for the shared socio-economic pathway 5-8.5 than for SSP2-4.5 (Table 1.1).

We estimated an ultrametric phylogenetic tree for medium and large mammals from Atlantic Forest refugia based on 31 markers (39,099 base pairs - UPHAM et al., 2019) for 85 species, 65 of which were present in SDM analyses (Table S1). Most of the relationships among species showed high posterior probabilities, mainly in the deep nodes (Figure S2). For phylogenetic diversity (PD_{Faith}), São Paulo refugium showed the highest diversity (1594.42, Table 1.2) and Pernambuco refugium the lowest (1340.91, Table 1.2). This metric exhibited a significant positive relationship for species richness ($r = 0.999$, $p = 0.045$). For MPD, Pernambuco and Bahia refugia showed the higher phylogenetic diversity (163.58 and 156.03, respectively) compared to São Paulo refugium (153.84, Table 1.2). The MNTD values were low in Bahia and São Paulo refugia (19.28 and 16.5, respectively), while Pernambuco showed the highest value for this metric (40.81). The standardized effect size for PD_{Faith} and MNTD did not show values that significantly differ from the null model for São Paulo and Bahia refugia, whereas Pernambuco refugium showed a phylogenetic overdispersion pattern for ses.PD_{Faith}. For ses.MPD metric, all refugia have a mammal community that tends to be phylogenetically overdispersed (Table S4).

Table 1.2 – Values of species richness (SR) and the three phylogenetic diversity metrics (PD_{Faith}, MPD and MNTD) for medium and large-sized Atlantic Forest mammals. PD_{Faith}: Faith's phylogenetic diversity; MPD: mean pairwise distance; MNTD: mean nearest taxon distance.

	SR	**PD_{Faith}**	**MPD**	**MNTD**
Pernambuco	35	1340.91	163.58	40.81
Bahia	62	1548.37	156.03	19.28
São Paulo	71	1594.42	153.84	16.50

Figure 1.2 – Percentage of predicted changes in distribution of mammal species analyzed per refugia in the four GCM under the SSP2-4.5



3.4. DISCUSSION

Patterns of current medium and large mammal richness are similar to what was found for other animal communities in the BAF (FERRO et al., 2014; LEMES et al., 2014; SANTOS et al., 2020), where high species richness is seen south of the Doce River. Although the region of São Paulo refugium was climatically more unstable over time (CARNAVAL; MORITZ, 2008), today it hosts the largest and most continuous Atlantic Forest remnant (e.g., Serra do Mar, FUNDAÇÃO SOS MATA ALTÂNTICA, 2020). Larger regions usually facilitate a greater variety of species, also known as the species-area relationship (SCHOENER, 1976). For example, animals with large home ranges and high resource requirements, as the Jaguar (*Panthera onca*), are still present in São Paulo refugium, more specifically in the Serra do Mar region, whilst this species is already extinct from Pernambuco (GARBINO et al., 2018; MENDES PONTES et al., 2016) and in reduced numbers in the Bahia refugium (BEISIEGEL et al., 2012; CANALE et al., 2012). Pernambuco refugium is one of the most degraded forest regions of the world, due to the long-lasting effects of fragmentation, poaching, land use change and urbanization (BARRETO, 2013; BOGONI et al., 2018). The biodiversity in this area resists on the 2% of original forest cover remaining, but it is still modeled as an area of intermediate richness for mammals in the BAF.

Regarding LGM modeling, it is important to highlight two premises to this prediction: first is that we are assuming species tolerance to climatic conditions are the same as current. The second is that since we used current presence records to build SDM, species that are locally extinct in Pernambuco refugium or other localities were not included as present in this area (GARBINO et al., 2018; MENDES PONTES et al., 2016). The absence of records for these species may have underestimated LGM richness in Pernambuco refugium. If species physiological boundaries have not changed or changed only slightly over time, modeling present species niche constraints onto past environmental conditions can provide insight into past refugia (FLØJGAARD et al., 2009). Although forest dynamics during LGM have been debated (ARRUDA et al., 2018; LEITE et al., 2016), we did not extend the distribution models onto the continental shelf and thus we are only discussing species distribution onto the current area of the BAF.

The results for LGM mammal richness and present and past areas of higher endemism reinforce the three refugia areas proposed by Carnaval and Moritz (2008). Studies have reported current areas of endemism for other vertebrate groups that are somewhat compatible with the three refugia areas (COSTA et al., 2010; DALAPICOLLA et al., 2021; SILVA; SOUSA; CASTELLETTI, 2004; VALE et al., 2018), however the areas proposed do not align perfectly with the ones we observed here, which is not surprising given that the number and distribution of species, their specific requirements as well as different approaches can provide different results (AAGESEN; SZUMIK; GOLOBOFF, 2013; DALAPICOLLA et al., 2021). Endemism centers exist because these regions probably present very specific environmental conditions that make species distribution restricted to these areas. In fact, the BAF and more specifically Pernambuco Endemism Center are areas of climatic rarity, which means that there are few climatically homologous areas in proximity to these areas (OHLEMÜLLER et al., 2008).

The largest and most species-rich area, São Paulo refugium, showed higher values of PD_{Faith}, which was expected as this metric is closely related to species richness: the more species, the more branches in the tree, consequently the greater phylogenetic diversity. As we hypothesized, stable areas across time showed high values of phylogenetic diversity not dependent on species richness: Bahia and Pernambuco refugia present high values of MPD, revealing older evolutionary legacies. When looking at the MNTD metric, only Pernambuco has high values, also signaling that closely related species in the phylogeny do not co-occur in this refuge. The low values of PD_{Faith}, and high MPD and MNTD in the Pernambuco community indicate that, although this area is not as rich as other refugia, species present are spread across the various branches of the phylogenetic tree. This could mean that the ecological diversity within this habitat is preserved to some extent in the current species diversity, because functional diversity usually accumulates with phylogenetic age in mammal communities (SAFI et al., 2011).

The phylogenetic structure of the refugia communities was assessed considering alternative metrics either sensitive to basal or terminal branching in the phylogeny. Only the standardized effect size for MPD exhibited a significant and consistent deviation from random expectations. For this metric, all refugia presented a basal phylogenetic overdispersion pattern, suggesting that distantly related species with less niche overlap co-occur (WEBB et al., 2002). This pattern can be a consequence of competition-driven coexistence and it is a common tendency in mammalian assemblages (COOPER; RODRÍGUEZ; PURVIS, 2008). According to Hutchinson (1959), intraspecific competition could play a crucial role in structuring animal communities because species that are ecologically similar will be unable to coexist due to competitive exclusion, thus communities should contain only species distantly related with different niches.

In all future scenarios, we forecasted decreased climatic suitable areas for high species richness in the whole extension of western BAF (Figure S3), as similarly reported for other taxonomic groups such as amphibians (LEMES et al., 2014), moths (FERRO et al., 2014) and primates (LIMA et al., 2019), concentrating richness along the coastline. More drastic changes are seen with higher increases in temperature (CanESM5), when we observe the almost total separation of three high richness areas by mostly unsuitable climatic habitat, in agreement with the three refugia, concentrating richness in lower elevations (Figure 1.1). Endemism areas also responded differently to climate change. Areas either shrunk and/or shifted and endemism values either intensified or slightly reduced. For the most drastic GHG emission trend (SSP5-5.8), in the São Paulo refugium concentration of endemic areas was observed along the coastline and within a few 0.5° cells of high endemicity north of this refugium. Considering the metric used here, high values of future endemism mean that either more range-restricted species or few species with highly restricted ranges or both will be present in these areas (Figure 1.2). Thus, range-restricted species that rely on the unique climate conditions of these localities will be most affected by climate change.

Our results show that the climatic space of most species will be reduced in the next 60 years. To the best of our knowledge, this is the first study that assessed medium and large mammal endemism and richness response to climate change in the Atlantic Forest at a fine resolution. Even a moderate increase in greenhouse gas emissions, as proposed by the SSP2-4.5 will lead to a decrease in the climatic suitability for mammals in the BAF as a whole, reflected in the reduced species distribution and, hence, areas with lower species richness. Drastic increase in mean temperature (CanESM5) will result in more aggressive reductions of climatically suitable areas. In this scenario, besides local extinctions forecasted, 72%, 73% and 53% of all species will lose over half of their climatic spaces in São Paulo, Bahia and Pernambuco, respectively. Reduced suitable area can lead to a reduction in population size, which is of high conservation concern, because small populations can be more vulnerable to the effects of genetic deterioration through genetic drifting and inbreeding, and environmental and demographic stochasticity, being more prone to extinction (FRANKHAM et al., 2002; LYNCH; CONERY; BURGER, 1995; MELBOURNE; HASTINGS, 2008).

Other future scenarios are also concerning. The GCM MIROC6 that foreseen annual fluctuations in both mean change in precipitation (increase or decrease) and in mean annual maximum temperature increase is one of the scenarios that predict the least changes in climatic suitability for species richness. However, climatic fluctuations can cause extreme weather changes and, consequently, stochastic events that affect species distribution and persistence (WOODWARD et al., 2016). These processes are not modeled by the methods applied here but deserve further investigation on how biodiversity will respond to extreme weather events and how to mitigate the effects of such events (see MAXWELL et al., 2019 for a more comprehensive synthesis).

A few species showed expansion in their climatic spaces, which is not surprising given species varying climatic requirements that can lead to responses in alternative directions (BATALHA-FILHO; CABANNE; MIYAKI, 2012). However, it is unlikely that these species will actually expand their distributions due to several reasons. Firstly, the projections of climate impact on species distribution rely on the unrealistic assumption that land cover will not change over time. Climate change and anthropogenic factors will likely change future land cover by decreasing habitat suitability for forests and increasing anthropogenic landscapes (DE SANTANA; DELGADO; SCHIAVETTI, 2020; LEÃO et al., 2021). The effects of climate change will likely aggravate the effects of habitat loss and fragmentation (SEGAN; MURRAY; WATSON, 2016), hindering species from dispersing and colonizing new areas. Even protected areas are expected to suffer from increasing fragmentation (DE OLIVEIRA et al., 2020), further intensifying the effects of climate change on species presumably protected within these areas. Moreover, climate change-induced range reduction was also modeled for tree species within the BAF (COLOMBO; JOLY, 2010), which can also affect mammal species that depend on these trees for ecological processes (feeding, shelter, among others). Changes in species interactions under current rates of climate change have already been reported and it is expected to be intensified under more extreme climate conditions (TYLIANAKIS et al., 2008). Overall, these factors collectively suggest that the predicted expansion of species' ranges due to climate change will be severely restricted or non-existent.

Finally, the forecasted areas of high species richness and endemism consider that species maintain their climatic requirements equal to the present, however, species can respond to climate change in ways that were not modeled. Species can either adjust to new climate conditions or change their distribution to areas that are within species tolerance limits (ROOT et al., 2003; VISSER, 2008). Due to the requirements and longevity of medium and large mammals, especially those living in anthropogenic landscapes, distribution shifts and rapid adaptation are unlike options for these species to respond to fast climate changes (HETEM et al., 2014).

3.5. CONSERVATION IMPLICATIONS

All evidence presented here points to the significant impact of future climate change on mammal distribution in a widely recognized biodiversity hotspot. The extent of this impact will largely depend on the degree of temperature increase, as well as on the synergistic effects resulting from land cover change and interactions between species and other biotic and abiotic conditions (NEWBOLD, 2018). Undeniably, climate change poses a major threat to biodiversity, as our study highlights its impacts on mammal distribution, richness and endemism in the BAF. Because patterns of species richness, endemism and threat are not congruent (ORME et al., 2005), determining conservation priorities is not straightforward. Therefore, implementing multiple strategies for mammal conservation is imperative (RIBEIRO; SALES; LOYOLA, 2018).

Areas that have experienced climatic stability over time, such as the Pernambuco refugium, can be more vulnerable to future changes (TREW; MACLEAN, 2021). Thus, this entire refugium should be a conservation priority. Also, the current endemism centers in Bahia and São Paulo refugia coincide with areas of high species richness and should receive attention. Different strategies considering the histories of each refugium will likely be needed to meet conservation goals.

Refugia are defined as areas providing “temporal and/or spatial protection from disturbances”, which include the effects of climate change (KEPPEL et al., 2012). Nonetheless, nature-based solutions play a pivotal role in mitigating future global climate changes (TONETTI et al., 2022). Not only restoration of fragmented areas and reduction in deforestation can help buffer species from the impacts of climate change, but they can also reduce the emission of greenhouse gases by capturing carbon dioxide. These solutions must be addressed now to achieve long-term objectives (GIRARDIN et al., 2021; PÜTZ et al., 2014). There are extensive areas of Atlantic Forest under private ownership that fail to comply with Brazilian legislation and lacking restoration (REZENDE et al., 2018). Addressing this issue could be the initial step towards protecting biodiversity and safeguarding the evolutionary history of the community against the inevitable impacts of climate change (BELLARD et al., 2012).

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4 CAPÍTULO II – FECAL DNA APPLICATIONS FOR RARE CARNIVORE SURVEY: TECHNIQUES, EFFICIENCY, AND FUTURE DIRECTIONS

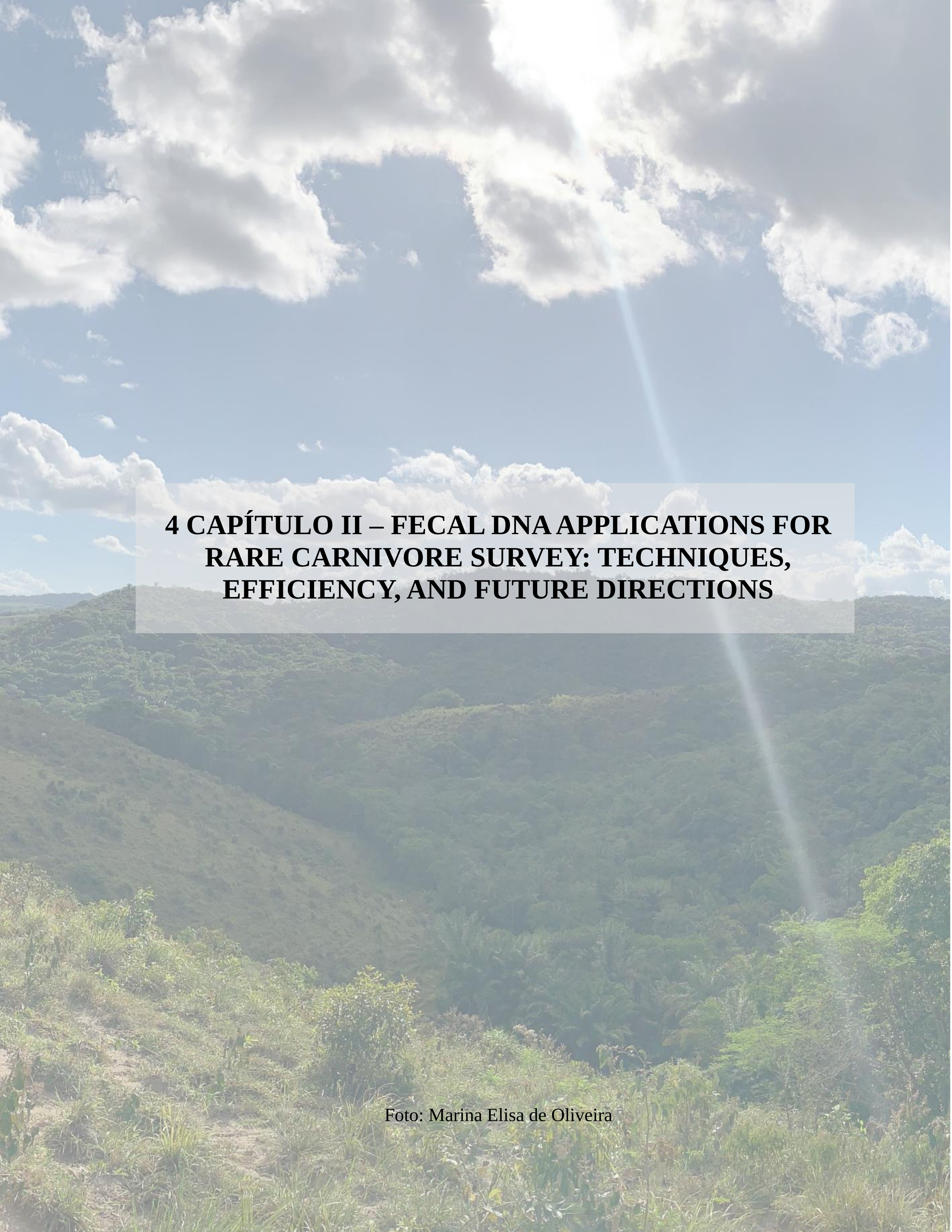


Foto: Marina Elisa de Oliveira

Fecal DNA applications for rare carnivore survey: Techniques, Efficiency, and Future Directions

Abstract

Large and mesocarnivores can occur at low densities due to both natural factors and contemporary human activities. The remote collection of rare carnivore data, particularly biological samples for genetic analyses, is a robust alternative to capture-based data. In this review we focused on rare carnivores to answer: What are the applications and techniques used for fecal-derived DNA samples? What is the sampling effort required to detect target rare carnivore species? How efficient is fecal DNA for detecting rare species compared to other methods? What are the methodological advances in fecal DNA analyses? We compiled the results of 250 papers and found that most studies were conducted in Europe and Asia, encompassing mostly Felidae, Canidae and Mustelidae species. Fecal DNA is most used to determine species, individual identity, and sex, but it is also a reliable source of genetic material for a myriad of applications. In terms of techniques a variety of sample storage and DNA extraction methods have been tested and implemented. In general, studies recommend collecting scats as fresh as possible, storing, and extracting DNA with methods already employed and tested for the focal or similar species, considering logistic constraints and resource availability. Researchers must find a balance between allowing enough time for scats to accumulate in the field, and collecting scats before significant DNA degradation occurs. However, fecal samples show an inherent high variability in amplification success rate. Considering all carnivore families and the collection of scats on transects as the most applied sampling design, the sampling effort to encounter one molecularly identified scat was 2.56 km surveyed. Fecal DNA was found to be more and equally efficient in detecting the number of individuals and species present, respectively, in comparison to alternative methods. The potential for SNPs as population markers is still little explored. This information should guide new studies focusing on rare carnivores, providing a basis for more cost-effective surveys, and improving data quality for carnivore conservation.

Keywords: behavior; individual identification; microsatellites; molecular ecology; predators

4.1. INTRODUCTION

Carnivore species are commonly observed in various habitats. However, certain species are considered rare, occurring at low densities (FRAGOSO et al., 2019). This scarcity may be attributed to their substantial energetic demands and wide-ranging behavior (CARBONE et al., 1999; NOONAN et al., 2023; STONEKING et al., 2002) or influenced by human disturbances (GALETTI et al., 2021). Besides their intrinsic value as components of biodiversity, carnivores play crucial roles in ecosystems as top-down regulators through the direct effects of predation or indirect effects of fear (ESTES et al., 2011), and other complex interactions such as seed dispersal (SARASOLA et al., 2016). Mesocarnivores are usually more abundant and generalists in terms of habitat and feeding resources than large carnivores, and they also play an important role in structuring communities, although not always through the same processes as large carnivores (AVRIN et al., 2023; ROEMER; GOMPPER; VAN VALKENBURGH, 2009). Nonetheless, anthropogenic-driven changes in the environment, such as climate change, habitat destruction and depletion of prey are leading to reductions in large and mesocarnivore populations, endangering the ecosystem roles played by these species (CROOKS, 2002; RIPPLE et al., 2014). Thus, carnivore research and management are pivotal to the conservation of species and ecosystems (TREVES; KARANTH, 2003).

To ensure successful conservation and management of species, accurate knowledge of their occurrence patterns and distribution ranges is crucial, alongside practical data analysis methods (JENKINS; PIMM; JOPPA, 2013; LAMB et al., 2019). Several approaches have been developed to analyze and model field data, allowing researchers to define the threat status of species and propose appropriate conservation actions (CARAVAGGI et al., 2017; HEBBLEWHITE; HAYDON, 2010; PIGGOTT; TAYLOR, 2003; TROLLIET et al., 2014). However, the reliability of the results and inferences made from the data analyzed depend heavily on the initial quality of data collected in the field. Moreover, some species may have restrictions on the type of data that can be collected. For instance, some species cannot be directly handled because they are sensitive and/or protected by law (e.g., BANKS; PIGGOTT, 2022; DÍAZ-SACCO; IZAWA; IMAI, 2014; DUECK; STEFFENS, 2022), and capture-based methods might have negative effects on individuals, potentially impacting data quality (JEWELL, 2013; ROCHA; RAMALHO; MAGNUSSON, 2016). To overcome these difficulties, noninvasive sampling methods have emerged as alternatives to traditional observation-based or capture-based sampling methods (LONG et al., 2012).

Over the past three decades, the term “noninvasive sampling” has been more often applied to the remote collection of DNA samples from wild animals (LONG et al., 2012; TABERLET; WAITS; LUIKART, 1999). Rodgers and Janečka (2013) reviewed the applications and techniques of fecal samples and genetics in Felidae research and conservation. Additionally, a comprehensive overview of how scat detection dogs can be used to survey carnivores, mainly in North America, has been conducted (MACKAY et al., 2012). Piggot and Taylor (2003) provided an overview of potential applications and limitations of remotely collected DNA samples of rare and cryptic species. Surprisingly, despite significant progress in the field since the early 2000s, no systematic review has yet been conducted to compile the wealth of experience accumulated during the last three decades from various research groups towards the conservation of rare carnivores. While a few species of carnivores occur in high densities (e.g., feral cats, LAVERY et al., 2020; REES et al., 2019), our focus was on discovering how fecal DNA is being used to study carnivores that are challenging to study (e.g., elusive behavior, low densities, threatened). In this review we aimed to answer the following questions: 1) What are the applications for fecal-derived DNA samples? 2) What are the techniques used in genetics based on fecal samples? 3) What is the sampling effort required to detect a target rare carnivore species? And 4) how effective is fecal DNA for detecting rare species compared to other methods? We conclude our review by presenting future directions in the use of fecal DNA to survey wildlife populations and the conservation implications of our findings.

4.2. METHODS

4.2.1. Database search and screening

To answer our questions, we conducted a systematic literature search. Strings were chosen based on preliminary trial searches aimed at maximizing encounter rate with papers related to the main subject of this review while minimizing encounters with papers focused on medicine, parasitology, microbiology, and studies not related to carnivores. The search was performed in the Web of Science database (www.webofknowledge.com) using the following strings: (noninvasive OR non-invasive OR "non invasive" OR feces OR fecal OR faecal OR faeces OR scat OR "environmental DNA" OR eDNA) AND (genetic* OR molecular* OR DNA)

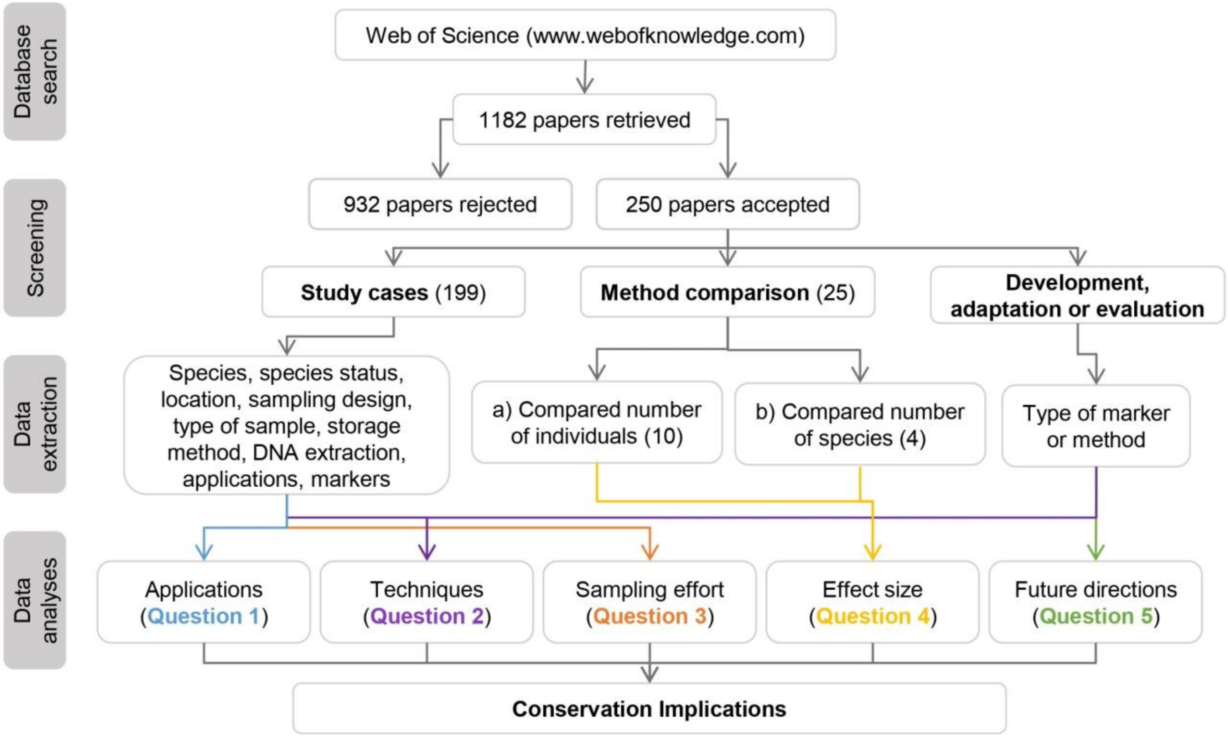
AND (rare OR elusive OR endangered OR threatened OR cryptic OR vulnerable OR endemic) AND (mammal OR species OR wildlife OR animal OR carnivore OR predator) AND NOT (pathogen* OR disease OR Escherichia OR fish OR bird OR reptile OR amphibian OR immun* OR prenatal OR microbio* OR infection OR hormone* OR diet).

We included in this review papers from indexed journals that used, developed, adapted, or evaluated tools to study rare carnivores through sampling of scat and molecular tools. Carnivores were considered rare if classified by the authors as either rare, elusive, endangered, threatened, cryptic or vulnerable in either the title, abstract or keywords. We categorized papers accepted into four groups based on their objectives: case studies; method development, adaptation or evaluation; marker development or adaptation; and comparison of species and individual identification between DNA derived from scats and an alternative method. Some papers were assigned to more than one category if presented multiple objectives. We excluded papers solely focused on examining carnivore diet because this topic warrants dedicated attention and separate analysis. Each article was manually inspected twice, and relevant information was extracted based on the paper's category (Figure 2.1). We included all papers published up to April 17th of 2023, when the search was conducted.

4.2.2. Data extraction

For the study cases, we extracted the following information: the species studied; its threat status according to the International Union for Conservation of Nature and Natural Resources (IUCN) (www.iucnredlist.org, date of access: July 20th of 2023); location of the study; scat sampling design (transects, opportunistic, following tracks or individuals, random sampling of the entire area, grid sampling, using scat detection dogs, other, or combined sampling design); type of biological sample (scats only, noninvasive samples only - scats and hair, noninvasive and “invasive” - blood and tissue - samples, or not informed); sample storage and DNA extraction methods; applications for which fecal DNA was used; the marker used for species identification, if conducted; and amplification success of identification marker, if conducted. For marker development or adaptation, we extracted the type of marker (mitochondrial, microsatellite, SNP, RFLP, qPCR essay, sexual chromosome marker or adaptive locus). For method development, adaptation, or evaluation, we extracted the type of method (sample collection, DNA extraction, PCR protocol, marker selection, genotype analysis, capture and recapture statistics, or other abundance estimates). In studies that compared fecal DNA and one or more alternative methods (e.g., camera trapping, sigh survey, track survey), we recorded the alternative method, sampling effort, and the number of detections (number of individuals or number of species detected) per method.

Figure 2.1 – Flow chart showing the steps followed in the systematic literature review



4.2.3. Data analyses

To answer the first two questions, we assessed the trends regarding species studied, study locations, methods, and applications of fecal DNA by summarizing all information available from the study cases (Figure 2.1). To answer the third question, we calculated the sampling effort necessary to encounter one molecularly identifiable scat from the focal species for studies that employed transects in the sampling design. We calculated sampling effort as the number of molecularly identified scats divided by the total distance traveled (in kilometers) during the entire survey period. We excluded studies that used scat detection dogs, as these can significantly improve scat encounters (BECKER et al., 2017; SMITH et al. 2006). Within our dataset, scats could be identified to species level by amplifying either a mitochondrial DNA fragment or a panel of microsatellite loci of specific size. However, mitochondrial DNA and microsatellites often exhibit different amplification success rates, due to the greater number of copies of mitochondrial DNA within a single cell, the greater number of microsatellite loci needed to individualize samples, amplicon length, among other possible factors (BROQUET; MÉNARD; PETIT, 2007; FORAN, 2006; MURPHY et al., 2007). Hence, we first calculated whether there was a significant difference in the number of identified scats per km traveled between scats identified using mitochondrial and microsatellite markers (Wilcoxon rank-sum test, p-value = 0.113). Then, we conducted a Kruskal-Wallis test with Dunn's post-hoc multi comparisons to compare the number of molecularly identified scats per km between different carnivore families. The statistical tests were chosen based on data distribution normality and performed using the Past 4.04 software (HAMMER; HARPER; RYAN, 2001). For studies that employed both scat sampling and camera trapping simultaneously, we also compared the sampling effort required to obtain a detection of the focal species between these two methods (Figure 2.1).

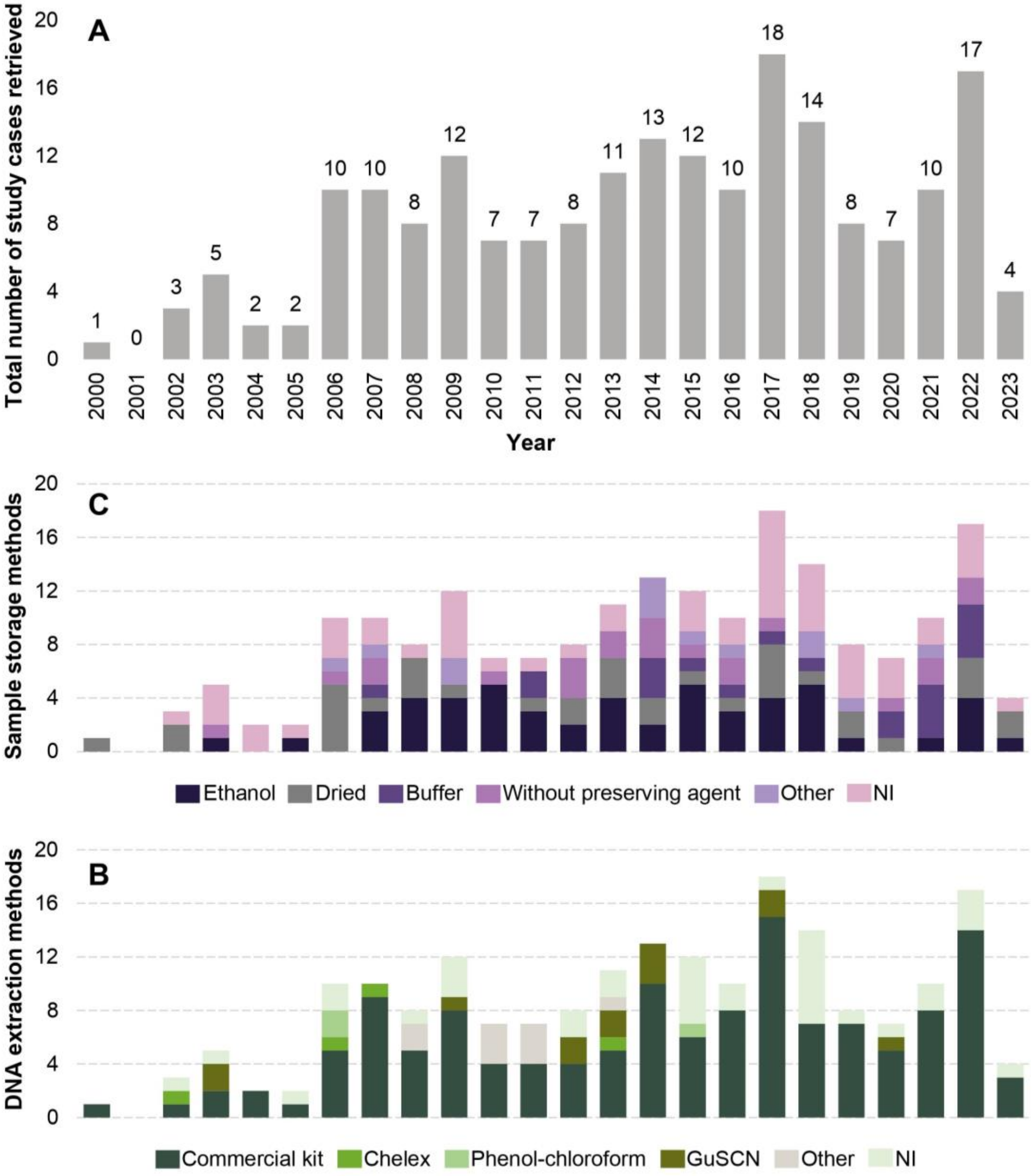
To answer the fourth question, we examined the data set of papers that compared fecal DNA with an alternative survey method. These studies provided information on either a) the number of species or b) the number of individuals detected with each method. We analyzed these two datasets separately using the log risk ratio as the effect size (i.e., measure outcomes and their corresponding sampling variances), using the *metafor* package in the R environment (R CORE TEAM, 2022; VIECHTBAUER, 2010). The log risk ratio was computed as the log of (ai/n1i)/(ci/n2i), where ai and ci are the number of species or individuals not detected by fecal DNA and the alternative method, respectively, n1i and n2i are total number of individuals or species assumed to be present (VIECHTBAUER, 2010). A negative log ratio risk indicates that the risk of not detecting a species or an individual is higher with the alternative method compared to the fecal DNA approach, whereas a positive value indicates the opposite. We employed a random-effect model for model fitting that assumes that the variability in true effect sizes is random, as we were interested in providing an "unconditional inference about a larger set of studies from which the k studies included in the meta-analysis are assumed to be a random sample" (HEDGES; VEVEA, 1998). The log ratio risk and model fitting were conducted using the functions 'escalc' and 'rma', respectively (VIECHTBAUER, 2010). A study could provide more than one effect size, by identifying individuals from different species or by comparing different methods with fecal DNA or by conducting the survey in more than one study area or period. We analyzed whether the significance of the estimated average effect size would differ between individual effect sizes and combined into one per study. We combined studies using the function aggregate of R package *metafor* (VIECHTBAUER, 2010). We adapted scripts provided by Carvalho et al. (2022) (Supplementary Material S1). Lastly, to explore the future directions in the use of fecal DNA, we examined recent trends among studies categorized as study cases and as developing, adapting, or evaluating molecular markers or methods. We present both quantitative and qualitative results for the five questions we sought to respond to and provide illustrative examples gathered from the papers included in this review. We provide all information extracted from papers on Table S5 in a user-friendly format, enabling users to search for any species or topic of interest.

4.3. RESULTS AND DISCUSSION

4.3.1. What are the applications for fecal-derived DNA samples?

Piggot and Taylor (2003) in their review on the potential applications of remotely collected DNA samples to study aspects of rare and cryptic species pointed out the yet little explored potential of noninvasive genetic sampling. This prospect has changed over the last twenty years. We retrieved 1182 papers from the database search, of which 250 fit our criteria of using, developing, or evaluating tools to study rare carnivores through scat sampling and molecular tools. Among the 250 papers accepted, 199 were categorized as case studies, starting in the year 2000 with the leading work of Farrell et al. (2000) that designed and applied a primer set to identify scats from sympatric carnivores in a Neotropical region. The number of studies conducted per year since 2000 has increased, but with great variation between years (Figure 2.2A).

Figure 2.2 – Number of studies that met the inclusion criteria of the review categorized as study cases (n = 199) per published year (A). (B) and (C) show the types and frequency of sample storage and DNA extraction methods, respectively, employed in the study cases per year. For 2023, only papers published up to April 2023 are included. NI – not informed



Within the study cases, we identified a total of 79 species surveyed using fecal DNA, including subspecies and excluding domestic species, spanning the Families: Felidae (33 species), Canidae (15 species), Mustelidae (20 species), Ursidae (7 species), Phocidae (2 species), Ailuridae (one species), and Viverridae (one species, Table S5). Most studies were conducted in Europe (65), followed by Asia (58), North America (42) and South America (24). Oceania and Africa ranked last with six and four studies, respectively (Figure 2.3). In Europe, the most studied species were the Near Threatened Eurasian Otter (*Lutra lutra*) and the Grey Wolf (*Canis lupus*), classified as Least Concern, appearing in 15 studies each. In Asia, the Endangered Tiger (*Panthera tigris*) and its subspecies were the most studied (15 studies in total). In North America, the focus was on the family Canidae, with the Kit Fox (*Vulpes macrotis*) and its subspecies, and the Coyote (*Canis latrans*) appearing in six studies each, followed by the Endangered Red Wolf (*Canis rufus*) in four studies. In South America, the Near Threatened Jaguar (*Panthera onca*) was the most studied species, appearing in 10 studies, sometimes as the only focal species, in other cases accompanied by other felid species and in four studies accompanied by South American Felidae and Canidae species. In Oceania the only species studied among the study cases was the invasive Red Fox *(Vulpes vulpes*). Although only four studies conducted in Africa were included in this review, three different species were studied in three distinct papers, and one study sampled scats from at least four other species (BUSBY et al., 2009).

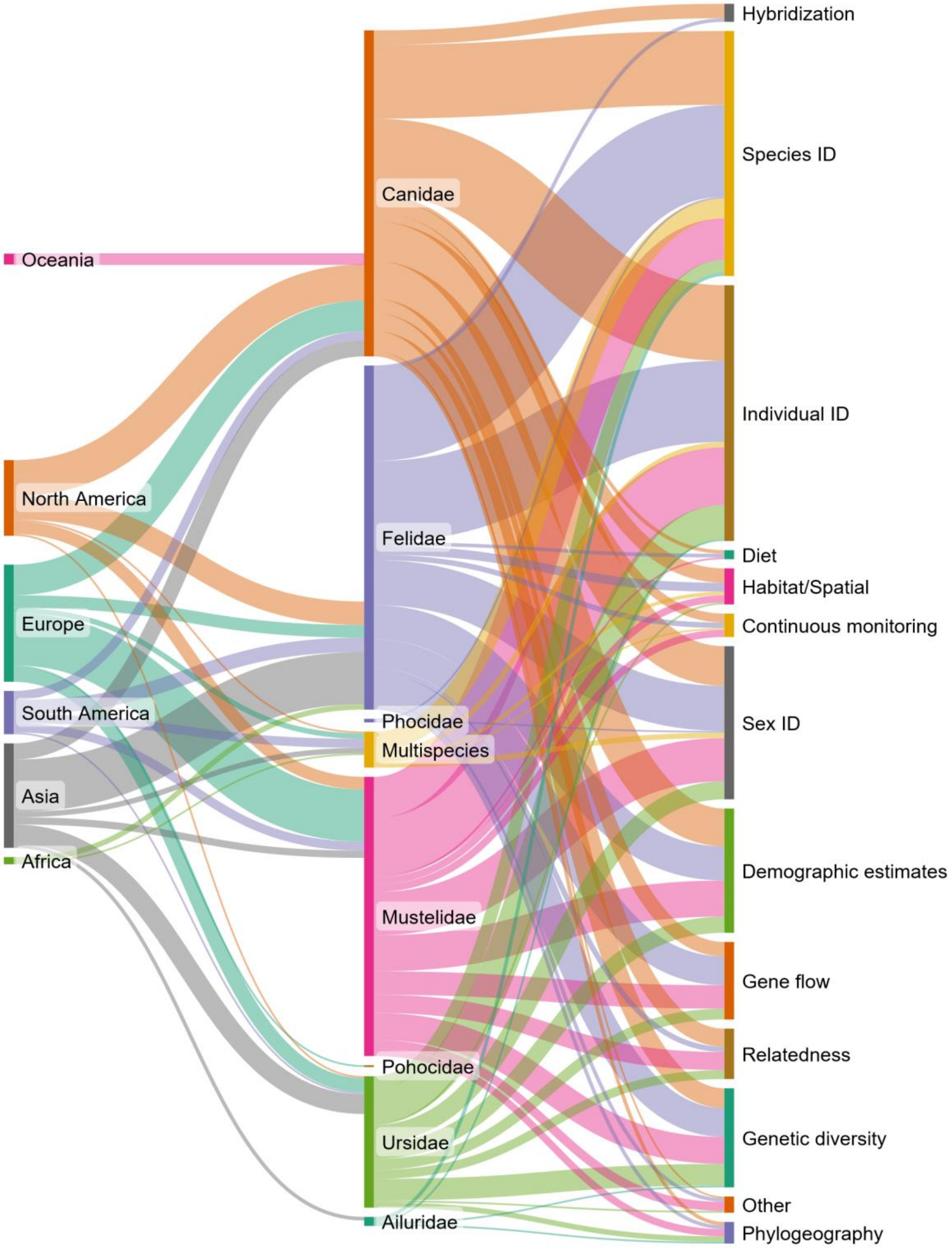
One clear advantage of the use of fecal DNA is the detection of recent presence of rare species with confidence in their identification, owing to the high power of molecular identification and the rapid degradation of DNA from scats (e.g., ARDANI; REZAEI; KABOLI, 2022; CHO et al., 2022; JANEČKA et al., 2008). Piggott and Taylor (2003) divided the use of noninvasively collected DNA samples into two major objectives. The first is to determine species, identity and sex from the individual the sample came from when the direct observation or capture of the species is impossible or inefficient. The second is as an alternative to “traditional" nondestructive DNA samples (i.e., blood and tissue) that depend on the capture of individuals to perform genetic analyses such as relatedness, population genetics and phylogeny or phylogeography. Accordingly, the most common application of fecal DNA among the study cases analyzed here were identification of individuals (in 142 papers – 71% of study cases), followed by species (137 papers – 68%) and sex identification (85 papers – 42%, Figure 2.3). Molecular species confirmation is not always necessary as the scats from the focal species can often be morphologically distinguished from sympatric species (for example, *Ursus arctos* - MOQANAKI et al., 2018 and *L. lutra* - BALESTRIERI et al., 2022; MARTIN et al., 2017); when there are typical defecation behaviors of the focal species (LAMPA et al., 2015; MARTIN et al., 2017; WILSON et al., 2003); when scat detection dogs trained on the focal species’ scat are used (BECKER et al., 2017; MENGÜLLÜOĞLU et al., 2019); or when the focal species is observed to defecate (by camera traps, for example, CANU et al., 2017; MATTIOLI et al., 2018).

However, in cases where species with morphologically similar scats co-occur, misidentification becomes an important factor influencing precise species identification even for experienced field researchers, and specially for rare species (DAVISON et al., 2002; SOLLER; AUSBAND; GUNTHER, 2020).

Molecular identification of scats relies on amplifying and identifying a diagnostic DNA sequence or fragment size specific to the target species. Feces contain epithelial cells from the source species, as well as genetic material from prey species, demanding primers that do not anneal to regions present in prey species. Numerous studies have been dedicated to developing markers for diagnostic regions, considering either the regional carnivore community (FARRELL; ROMAN; SUNQUIST, 2000; MODAVE; MACDONALD; SARRE, 2017; RODRÍGUEZ-CASTRO et al., 2018) or a single species (CRUZ et al., 2019; THATTE; PATEL; RAMAKRISHNAN, 2018). Among the study cases, the most used diagnostic regions were within the Cytochrome c (44 studies), followed by the control or D-loop (42 studies) and the 16S regions (16 studies). For individual identification, microsatellites were the most popular markers (131 studies), whereas Single Nucleotide Polymorphism (SNPs) were used in only six studies. The choice of the best markers is of relevance when designing new studies targeting rare carnivores, as the establishment of tested protocols is pivotal for the effective use of fecal DNA (CHO et al., 2022; MODI et al., 2018; WULTSCH et al., 2015).

Scat samples were the only source of genetic material for 117 study cases, and only samples classified as noninvasive (hair, scats, saliva) were the source in another 80 studies. Thus, fecal DNA alone has been enough as a source of valuable genetic material for several applications, providing a noninvasive alternative to what Piggot and Taylor (2003) described as “traditional” non-destructive DNA samples. The estimation of demographic parameters was assessed in 69 study cases, genetic diversity in 55, relatedness in 28, spatial ecology (e.g., habitat use, home range, distribution) in 20, phylogeography and phylogenetics in 12 and hybridization in 10 (Table S5). Additionally, one study used fecal DNA to track the genetic basis of pseudomelanism in Tigers (*P. tigris* in India, SAGAR et al., 2021), and another conducted hormone analyses to determine pregnancy and age of individuals among other aforementioned applications for *L. lutra* in Germany (KALZ; JEWGENOW; FICKEL, 2006). Although we deliberately excluded papers that focused solely on examining carnivore diet, we included five studies that studied carnivore diet in conjunction with other aspects of carnivore biology. The numbers presented do not sum up the total number of study cases as one study could employ fecal DNA in multiple applications.

Figure 2.3 – Diagram linking the continents where case studies were conducted, Family groups studied and applications of fecal DNA employed in the study cases (n = 199)



4.3.2. What are the techniques used in genetics based on fecal samples?

DNA from scats can have a relatively long life in the field, but at the cost of reduced amplification success (KUBASIEWICZ et al., 2016; STEINMETZ et al., 2021). For pinnipeds, Steinmetz et al. (2021) found suitable DNA material up to four weeks after scat deposition, both in sheltered and exposed environmental conditions. For tigers (*P. tigris*) in India, Reddy et al. (2012) observed progressive DNA degradation when scats are exposed to direct sunlight during a period of 10 days and recommended that sample collection be done in the first three days after scat deposition. For the European Pine Marten (*Martes martes*) in Scotland, the exposure to rainfall increased microsatellite amplification failure (KUBASIEWICZ et al., 2016). A negative correlation between environmental humidity and amplification success was also found for Otter (*L. lutra*) samples (LERONE et al., 2014; SITTENTHALER et al., 2021). For *L. lutra* the type of sample (anal jellies, jelly spraints and spraints) was also important in the success of microsatellite genotyping, as samples containing high proportion of mucus showed higher amplification success (LERONE et al., 2014; SITTENTHALER et al., 2021).

A variety of sample storage methods have been described in the literature (Figure 2.2B). The most common are the storage of scats or fragments of it in 70-100% ethanol (53 study cases), air-dried or stored with desiccant (36), and stored in different commercial or “home-made” buffers (19, Figure 2.2B, Table S5). A few studies tested the efficacy between storage methods, defining amplification success based on the amplification of microsatellite loci. In a study with Neotropical felid scats, samples stored in dimethyl sulfoxide saline solution (DETs buffer) showed significantly higher PCR amplification success than samples stored in 95% ethanol, both at room temperature, but the authors highlighted that other factors, such as the focal species and diet may influence storage efficiency (WULTSCH et al., 2015). In a study with fresh wolf scats, samples stored in 95% ethanol at -20°C and in GUS buffer (composed of guanidine thiocyanate, Tris-HCl, EDTA, and Triton X-100) at room temperature showed equally high amplification success after six months of sample collection (SANTINI et al., 2007). Velli et al. (2019) found no significant difference in amplification success and genotyping errors between scat samples stored in 96% ethanol and cotton swabs in a commercial buffer. These latter authors suggested that scat sampling by cotton swabs is safer (no inflammable substance), more practical during laboratory procedures, and more cost-effective due to reduced transport and shipping costs resulting from the smaller size of cotton swabs than the sampling of scat fragments and storage in ethanol. For the Amur leopard (*Panthera pardus orientalis*), scats stored in plastic bags at -20°C with no preserving agent were successfully genotyped after over four years of storage (CHO et al., 2022). Furthermore, the collector's experience in the sampling process also seems to influence amplification success (VELLI et al., 2019). While aging and storage can affect amplification success, fecal samples inherently show a high variability in amplification success rate (SANTINI et al., 2007; SITTENTHALER et al., 2021). Among the study cases, the reported amplification success for species identification varied from 24.5% (PAGACZ, 2016) to 100% (HARRINGTON; HARRINGTON; MACDONALD, 2008). This information is important because it is often impractical for DNA extraction to be conducted within a short period after scat collection and samples should be preserved as long as possible maintaining as much DNA quality as possible as more analyses can be conducted. Depending on the logistics and the accessibility in the field, it is also often not possible to access freezers or to use storage methods that require large space or are very labor intensive. Thus, researchers must find a balance between allowing enough time for scats to accumulate in the field, considering the low density of carnivores and deposition rates, the specific environmental conditions of the study area, and collecting scats before significant DNA degradation occurs.

In terms of laboratory procedures, there appears to have been considerable standardization over the past three decades. The use of commercial DNA extraction kits is widespread among the study cases included in this review. At least 135 utilized commercial kits and, in the last five years, over 70% of study cases employed it (Figure 2.2C, Table S5). Most of these 135 case studies employed commercial kits tailored specifically to extracted DNA from scats or other degraded samples (95 studies), the most popular ones produced by Qiagen (QIAamp DNA Stool Mini Kit and QIAamp Stool Kit). The remaining study cases either did not inform the extraction method used (35) or used more traditional DNA extraction methods (30 studies), based on phenol-chloroform, chelex or guanidinium thiocyanate (GuSCN), for example (Figure 2.2C, Table S5). Previously, the DNA extraction from scats was a more time-consuming process compared to DNA extraction from hair or tissue samples (PIGGOTT; TAYLOR, 2003). Commercially available DNA extraction kits tailored for fecal DNA are intended to increase DNA yield and amplification success by removing PCR inhibitors, while reducing processing time. However, Bhagavatula and Sigh (2006) tested a commercial kit ("Qiagen Stool kit") and five conventional protocols to extract DNA from Bengal Tiger (*Panthera tigris tigris*) scats and found no difference in the amplification success of five microsatellite loci between three of these methods (the commercial kit, phenol-chloroform and GuSCN). Lerone et al. (2014) tested two different commercial kits and found no significant difference in genotyping success, concluding that less costly alternatives are possible without harming downstream analysis. Although the costs per sample associated with the use of commercial kits often exceed those of traditional protocol-based methods, commercial kits for more "traditional" biological samples (tissue and blood) are comparable in cost to DNA extraction kits designed for soil, scats or forensic analyses (personal observation). In general, studies recommend collecting scats as fresh as possible (within a few days), storing scats, and extracting DNA in methods already employed and tested for the focal or similar species, considering logistic constraints and resource availability.

Following appropriate DNA extraction methods, screening of quality and quantity of fecal DNA can greatly improve cost-effectiveness and reliability of the use of fecal DNA, especially for when it is used as an alternative to more invasive biological samples (CULLINGHAM et al., 2010). The multi-tube approach has been extensively reported and recommended as a standard practice when constructing multiloci microsatellite panels for species (TABERLET; LUIKART, 1999; WAITS; PAETKAU, 2005). Although this practice is indispensable in producing reliable data by accounting for genotyping errors (e.g., false alleles, allelic dropout), it is also time, resource and DNA consuming, especially for more degraded samples (SANTINI et al., 2007; WAITS; PAETKAU, 2005). DNA quality and quantity screening can increase amplification success by discarding samples that fail to provide sufficient DNA material. DNA quality screening can be conducted by amplifying a single diagnostic marker, either a mitochondrial DNA fragment (MOQANAKI et al., 2018; MORIN et al., 2018; TRINCA et al., 2013) or a single microsatellite locus (BELLEMAIN et al., 2005; HEDMARK; ELLEGREN, 2007). DNA quantity can be assessed by qPCR (SRIVATHSA et al., 2021) or by quantifying total DNA extracted with a quantification equipment (GRANROTH-WILDING et al., 2017; SAGAR et al., 2021). Prior to their application, several possible diagnostic markers should be tested to understand their relative success and error rates in experimental situations (KUBASIEWICZ et al., 2016). However, some degree of genotyping errors is to be expected for fecal samples regardless of collection, storage, DNA extraction and quality control protocols (SITTENTHALER et al., 2021).

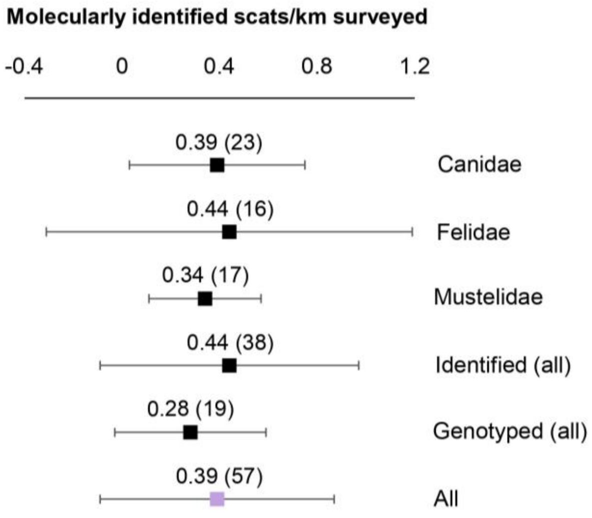
4.3.3. What is the sampling effort required to detect target carnivore species?

To understand the sampling effort required for surveying rare carnivores, it is essential to understand the sampling designs employed among the studies compiled. We classified the sampling design into seven categories: transects, opportunistic, following tracks and signs, random sampling of the entire study area or a subset of it, grid sampling, using scat detection dogs, and others. Studies could also report combined sampling design, such as transects within a grid sampling scheme or sampling the entire study area with the aid of scat detection dogs (Table S5). Each sampling design has its advantages depending on the study aim. For example, systematic sampling in quadrants of a grid-divided study area and/or repeated systematic survey seasons allow for the estimation of demographic parameters via robust capture-recapture calculations (AZIZ et al., 2017; MONDOL et al., 2009). Opportunistic sampling can allow data collection over an extended period and area by relying on a large contingent of non-scientific collaborators, such as hunters, local wildlife agencies, park rangers, and volunteers (BELLEMAIN et al., 2005; CHO et al., 2022; GRANROTH-WILDING et al., 2017). This is particularly valuable for endangered species that often require periodic genetic monitoring (CHO et al., 2022; KOJOLA; HEIKKINEN; HOLMALA, 2018). Transects were the most popular design, being employed in 113 studies. For our classification, transects encompass any existing dirt or paved roads, trails, or linear natural features like water streams or ridgelines, where scats could be found and collected. Opportunistic sample collection was the second most applied scheme, being employed in 36 studies, and was the sole sampling scheme for 11 studies. In 16 studies, tracks and signs were followed until the encounter of fecal samples, which seems to be most useful in snowy environments (SCHWARTZ et al., 2004; SUGIMOTO et al., 2014). Scat detection dogs were employed in 21 studies. Other sources of scats such as known latrines (WILSON et al., 2003), scent marking points (bridges, stones, elevated areas – MEIJER et al., 2008), feeding (COHEN; BAROCAS; GEFFEN, 2013) and kill sites (SANTOSTASI et al., 2021) were utilized in 14 studies (Table S5). Understanding the various possible sampling methods is central in designing a study. However, almost one-quarter of studies (45) did not disclose the sampling design, thus we urge that scientists disclose methodological details to ensure reproducibility.

Considering only the most employed sampling design (transects), we found 26 studies that disclaimed information on total sampling effort (kilometers traveled) and total number of molecularly identified scats (Table S6). The Kruskall-wallis test showed no significant difference between family medians. Considering all carnivore families, studies found on average 0.39 (SD ± 0.48) identified scats per kilometer surveyed or one identified scat every 2.56 km (SD ± 2.08 km) surveyed (Figure 2.4). This straightforward metric (number of scats per km traveled) can be used to estimate relative abundance when conducting surveys over consecutive seasons and individual identification is not possible (VELANDO et al., 2017).

For five studies that simultaneously employed scat sampling through transects and camera trapping, the number of molecularly identified scats per km was on average 0.265 (SD ± 0.33), whereas the number of photographic captures per camera trap day was on average 0.04 (SD ± 0.02; Table S6). These values correspond approximately to an average sampling effort of 3.8 km of transect traveled and 25 camera-trap days for one focal species detection. Focal species for these five studies were *P. tigris*, *Felis silvestris silvestris*, *C. lupus* and *M. martes*. We acknowledge that other variables can impact the number of molecularly identified scats, such as species abundance, diet, behavior, age of fecal sample, length of amplicon, among others. However, these results can help guide the design of more effective scat surveys, either conducted alone or in combination with camera trapping.

Figure 2.4 - Mean number and standard deviation of molecularly identified scat per kilometer of transect surveyed. Number of data used to calculate mean and standard deviation in parentheses. Identified (all) and Genotyped (all) indicate scats identified by amplification of mitochondrial and microsatellite markers, respectively, with all Families pooled together; whereas Family names indicate results for both markers pooled together. All data combined is indicated in purple



We introduce a few words of caution when designing scat surveys for molecular analyses. A bias towards higher encounter of male scats can be observed in some populations (*L. lutra*, LAMPA et al., 2015; *P. pardus orientalis*, CHO et al., 2022), but not in others (*L. lutra*, SITTENTHALER et al., 2021). Also, species can show behavioral responses to handling their scats (*L. lutra*, LAMPA et al., 2015) increasing marking behaviors, whereas for other species individuals may vary greatly in marking behavior with less intense markers being harder to detect (*C. lupus*, MARUCCO et al., 2009). Biases in sample collection should be considered, particularly for species that this process is not yet understood. Furthermore, scat detection dogs can greatly increase the detection of rare carnivores. In Angola, scat detection dogs found 50 scats of cheetah (*Acinonyx jubatus*), a species that occur in densities below 2 individuals/100 km^2, whereas spoor count survey conducted only by humans returned no spoor (BECKER et al., 2017). Smith et al., (2006) found almost four times more San Joaquin Kit Foxes (*Vulpes macrotis mutica*) in California (USA) with detection dog-handler teams than with human observers.

4.3.4. How efficient is fecal DNA for detecting rare species compared to other methods?

In total we found 25 studies that aimed comparing species detection by fecal DNA and an alternative method (Table S7). When considering the number of species detected with fecal DNA and an alternative method (four studies), the estimated average log risk ratio was not statistically significant (Table 2.1, Figure 2.5). Which means there is no difference in the number of species detected by fecal DNA and the alternative method employed. The alternative methods for surveying the community of carnivores were camera trapping, morphological identification of scats, track plates, direct observation, and questionnaire survey (Table S7). For the number of individuals detected with fecal DNA and an alternative method (ten studies), the estimated average log risk ratio was statistically significant (-0.5, 95% CI: -0.81 to -0.18 for individual effect sizes and -0.49, 95% CI: -0.89 to -0.08 for combined effect sizes - Table 2.1, Figure 2.5). This result means that the risk of not detecting an individual that is present at a given study area was greater using the alternative method than using fecal DNA. The alternative methods employed for individual identification were camera trapping, direct observation, snow tracking, and hair trapping followed by molecular identification. In both cases (species and individuals), we observed no difference in the significance of the estimated average log risk ratio when effect sizes are combined per study or separated (Table 2.1, Figure 2.5).

Figure 2.5 – Forest plot showing the results of the effect size analysis. A negative log ratio risk indicates that the risk of not detecting a species or an individual is higher with the alternative method compared to the fecal DNA approach, whereas a positive value indicates the opposite. The estimated average log risk ratio is shown at the bottom of each plot. The paper ID is shown on the left and individual effect size and standard error on the right

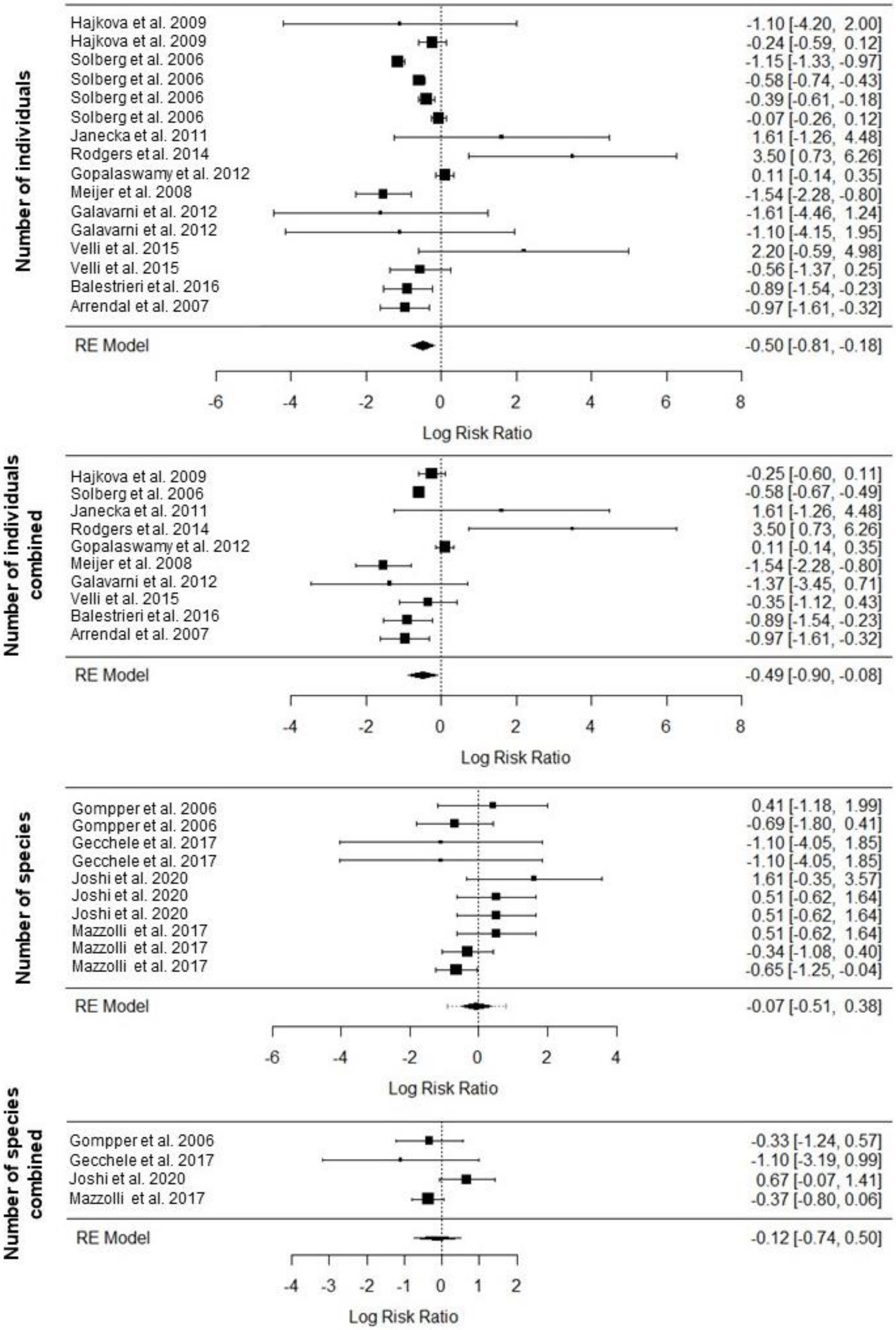


Table 2.1 - Results of random-effects models to assess the efficiency of fecal DNA to detect species and individuals of rare carnivores in comparison to other survey methods. CI (lb) and CI (ub) indicate lower and upper boundaries of the 95% confidence interval. * indicate significant p-values (p<0.05)

	Mean log ratio risk	CI (lb)	CI (ub)	z	p-value
Individuals	-0.50	-0.8138	-0.1774	-3.0522	0.0023*
Individuals combined	-0.49	-0.8955	-0.0832	-2.3613	0.0182*
Species	-0.07	-0.51	0.38	-0.2901	0.7717
Species combined	-0.12	-0.74	0.5	-0.3679	0.7129

By focusing our systematic review on carnivore species considered rare, we were able to thoroughly evaluate several aspects of the set of studies focusing on rare carnivores. However, this restriction may have limited the power of statistical analyses due to the small sample size. Such trade-offs are inherent to systematic reviews (PULLIN; STEWART, 2006). Nonetheless, the random-effect model used here is an "unconditional inference about a larger set of studies from which the k studies included in the meta-analysis are assumed to be a random sample" (HEDGES; VEVEA, 1998). Therefore, we consider the results presented here meaningful in uncovering efficiency of fecal DNA compared to other survey methods. Our results showed that fecal DNA is equally efficient in surveying species composition and more efficient in detecting individuals of rare carnivores. This finding is especially evident for species of the Families Canidae, Felidae, Ursidae and Mustelidae which were the focal species in the studies analyzed (Table S3). Individual identification of scats can provide valuable information for a great number of individuals even considering the challenges imposed by degraded DNA in fecal samples and the need for replicates to account for genotyping errors (HÁJKOVÁ et al., 2009; LÓPEZ-BAO et al., 2020). For example, in a study on *Panthera pardus fusca* in India, only approximately 33% (65 samples) of all scats identified as leopard produced genotypes with nine or more loci (out of 13 loci), however these 65 samples represented 56 unique leopard individuals (BHATT et al., 2020).

4.3.5. What are the methodological advances in fecal DNA analyses?

Sixty-four studies focused on developing or adapting (transferring between species) molecular markers specifically designed for fecal samples. Among these, 25 studies described markers within mitochondrial regions, 12 studies developed or adapted microsatellites, six studies developed SNP loci, five studies developed RFLP, five studies developed qPCR essays, seven studies focused on developing or adapting sex identification markers, and only one study developed a marker for a region within a gene under selection (WAN et al., 2006). The development and use of markers specifically tailored for noninvasive samples is pivotal as the low amplification success for loci with larger amplicon size is commonly described in fecal DNA studies (BROQUET; MÉNARD; PETIT, 2007). Some studies tested the transferability of microsatellite and sex identification markers between different species (MODI et al., 2018; MONDOL et al., 2009, 2012; VITERI; WAITS, 2009), while others designed markers specifically for a focal species (LATORRE-CARDENAS; GUTIÉRREZ-RODRÍGUEZ; RICO, 2020). Queirós et al. (2015) in a study comparing species-specific and transferred microsatellites for the Red Deer (*Cervus elaphus*) recommended using species-specific microsatellites in population genetic inference to avoid ascertainment bias (i.e., markers tend to be more polymorphic for the species they have been designed for). These authors also noted that population differentiation seems to be less affected by the specificity of markers (QUEIRÓS et al., 2015). Despite the extensive use of microsatellites in studies involving individual identification, we did not find any study that compared the biases and efficiency of species-specific and transferred microsatellite loci for rare carnivores. Consequently, we believe this knowledge gap needs to be explored, as the results could impact resource allocation for the study of rare carnivores.

SNP loci offer an alternative to the use of microsatellites. Although SNPs have been much less explored, they have the potential to increase genotyping success by being easier to amplify in degraded samples, resulting in fewer discarded samples due to low genotyping success (SRIVATHSA et al., 2021). Natesh et al. (2019) demonstrated the feasibility of multiplex SNP genotyping of degraded samples (scats, shed hair, and saliva from wild Indian tigers *P. tigris*), a method considered fast and inexpensive by the authors. They estimated implementation cost to be as low as 5 USD per sample when processing several hundred samples (2019 values) after initial development costs. SNPs genotyping errors were found to be similar or lower than microsatellites, and relatedness values obtained from SNP loci were close to the expected based on the known relationship between individuals, suggesting great promise for the study of rare carnivores (KLEINMAN-RUIZ et al., 2017; NATESH et al., 2019). However, SNP discovery in non-model organisms is still a major obstacle, although recent advances in next-generation sequencing (NGS) have greatly facilitated this process (DAVEY et al., 2011). NGS technologies have also facilitated the isolation and characterization of species-specific microsatellite loci (BRANDT et al., 2014; LATORRE-CARDENAS; GUTIÉRREZ-RODRÍGUEZ; RICO, 2020). Both microsatellites and SNPs require several replicates when working with fecal samples and are susceptible to genotyping errors (KLEINMAN-RUIZ et al., 2017; LÓPEZ-BAO et al., 2020). Comprehensive cost-benefit assessments, encompassing financial costs, genotyping success, and scalability in different countries are not yet available, as implementation costs can vary greatly depending on the country and the technologies employed (KLEINMAN-RUIZ et al., 2017; LÓPEZ-BAO et al., 2020; SRIVATHSA et al., 2021; TYAGI et al., 2022).

Fifty-four studies developed, adapted, or evaluated methods specifically designed for fecal samples. Among these, 20 addressed pre-laboratorial steps (e.g. evaluated scat age and exposure to field conditions or storage methods - PALOMARES et al., 2002), five addressed DNA extraction protocols, three assessed processes for DNA quantification, 19 validated or tested markers for specific applications (e.g., identification of hybrids, ADAMS et al., 2007, genotyping efficiency and individualization, ZHANG et al., 2018), eight developed or adapted capture-and-recapture statistics for fecal sampling and three studies addressed other methods. The only study on the family Phocidae included in our data set evaluated the applicability of various sample types (blood, skin, hair, urine, buccal swabs and scats), storage and extraction protocols for the obtention of genetic material from two seal species (*Phoca vitulina* and *Halichoerus grypus*, STEINMETZ et al., 2021). They recommended sampling scats as a reliable source of DNA from wild individuals of these species. The development and application of qPCR essays for quantification of template DNA is a promising tool that can decrease costs associated with the number of replicates needed and the genotyping errors to produce more reliable results (CULLINGHAM et al., 2010; HAUSKNECHT et al., 2010; REDDY et al., 2012). qPCR essays can be employed by amplifying one species-specific microsatellite locus (CULLINGHAM et al., 2010; HAUSKNECHT et al., 2010), or other nuclear genes (REDDY et al., 2012). This is an important step towards more cost-effective analyses, but the initial costs for primer design and testing, when necessary, should be considered.

Other technologies such as the use of robotic liquid handling platforms that can automatically and simultaneously extract DNA from dozens of samples are promising but yet little explored, probably due to the initial costs involved in purchasing such equipment. Nonetheless, it was advocated to reduce the time and cost required for sample processing, excluding the cost of the equipment (QUASIM; MACDONALD; SARRE, 2018; VELLI et al., 2019).

4.3.6. Conservation Implications

Fecal DNA has become a well-established method for continuous monitoring of several aspects of rare carnivores, including species that are invasive (*V. vulpes* in Australia, MARLOW et al., 2015), conflict-prone (*C. lupus* in Finland, GRANROTH-WILDING et al., 2017), reintroduced (*L. lutra* in Netherlands, KOELEWIJN et al., 2010), under recovering (*C. lupus* in Italy, DUCHAMP et al., 2012), threatened (*Vulpes macrotis mutica* in California/USA, WILBERT et al., 2015), elusive (*Gulo gulo* in Norway and Sweden, BISCHOF et al., 2020), among others (Table S5). It is also under evaluation as a continuous monitoring tool for the conservation of other species (*P. pardus orientalis* in Russia, CHO et al., 2022). However, fecal DNA is still in its early stages for some species, for example those belonging to the family Phocidae (STEINMETZ et al., 2021). Although many of the studied species included in this review are globally classified as Least Concern by the IUCN (Table S5), they may face local threats. This is the case of the San Joaquin Kit Fox, which is federally endangered and threatened in the state of California (USA). Other example is the Wolverine (*G. gulo*) that is classified as Vulnerable in Europe, but it is classified as Least Concern globally (ABRAMOV, 2016). Nonetheless, the species included in this review were classified by authors in their respective study areas as either rare, elusive, endangered, threatened, cryptic, vulnerable, or endemic (words included as strings in our database search), reflecting the unique conditions of the different populations studied.

Given the high discriminatory power of molecular markers in distinguishing between species and individuals, these are essential tools for estimating demographic parameters, being more efficient than alternative methods in identifying individuals present in a study area. The findings presented in this review can help guide researchers and governmental agencies to effectively implement and improve genetic analyses from fecal samples, with a focus on aiding species conservation, being especially valuable for Canidae, Felidae, Ursidae and Mustelidae species.

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5 CAPÍTULO III - ASSESSMENT OF NONINVASIVE METHODS FOR DETECTING MEDIUM-SIZED MAMMALS IN AN ATLANTIC FOREST BIODIVERSITY HOTSPOT

Foto: Marina Elisa de Oliveira

Assessment of noninvasive methods for detecting medium-sized mammals in an Atlantic Forest biodiversity hotspot

Abstract

The Pernambuco Endemism Center (PEC) is the most threatened region of the Brazilian Atlantic Forest. A few mammal and bird species have gone locally extinct in this region mainly due to anthropogenic pressure. Given the pressing need to enhance our understanding of the PEC biodiversity and the limited availability of resources, we aimed at surveying the entire medium mammal community in one representative forest fragment immersed in a matrix of sugar cane monoculture. This forest fragment represents the reality of most forest remnants in the region and was evaluated as suitable for the reintroduction of species. We also compared the efficiency of camera trapping, hair trapping, line transect survey, molecular identification of feces (scat survey) and opportunistic encounter with carcasses and bones, while controlling for sampling effort, to assess the community of medium-sized mammals. We calculated species accumulation curves, the latency to first detection and the proportional detection success (PDS) for each method. We identified thirteen species, six of which were recorded only by one method. Camera trap detected the most species, but had the lowest PDS, whereas scat survey detected less species but had higher PDS. We discussed the implications of survey methods and sampling design for mammal community assessment in the threatened PEC scenario.

Keywords: Alagoas curassow; conservation; method comparison; non-invasive survey; pilot study

5.1. INTRODUCTION

The Pernambuco Endemism Center (PEC) is the Atlantic Forest region north of the São Francisco River in northeastern Brazil. This region spans four Brazilian states (Alagoas, Pernambuco, Paraíba and Rio Grande do Norte, from south to north) and is bordered by the São Francisco River to the south, the Atlantic Ocean to the east and north, and the Caatinga biome do the west. Originally covering approximately 4 million hectares, the PEC comprises mostly coastline forest vegetation (FUNDAÇÃO SOS MATA ATLÂNTICA; INPE, 2021; RIBEIRO et al., 2009). The biogeographical history of connection and isolation with other forest formations have led to a unique biota composition and the existence of many endemic species (FARIA; MELO, 2011; NEMÉSIO; SANTOS JUNIOR, 2014; PONTES et al., 2013; SILVA; SOUSA; CASTELLETTI, 2004), resulting in its recognition as an endemic center. However, the PEC is also the most threatened region of the Atlantic Forest, mainly due to past and contemporary habitat loss and fragmentation caused by anthropogenic activities (BARRETO, 2013; DA SILVA; MENDES PONTES, 2008; DA SILVA; TABARELLI, 2000; DE SOUZA; LANGGUTH; GIMENZES, 2004; GARBINO et al., 2018). Only approximately 11% of its original cover remains and there are no forest fragments larger than 10 000 hectares (FUNDAÇÃO SOS MATA ATLÂNTICA; INPE, 2021; RIBEIRO et al., 2009). PEC forest fragments are severely reduced and isolated by a matrix composed mostly of sugar cane plantation, but also by pasture and silviculture (BERNARD; MELO; PINTO, 2011; LINS-E-SILVA; FERREIRA; RODAL, 2021; RIBEIRO et al., 2009; TRINDADE et al., 2008).

Although there are limited studies conducted in the PEC region, the existing research indicates a reduction in species abundance and diversity, particularly among mammal species (ASFORA; PONTES, 2009; BELTRÃO et al., 2019; DA SILVA; MENDES PONTES, 2008). The composition of medium-sized mammals in this region has been investigated through museum records and bibliographic material (FEIJÓ; LANGGUTH, 2013; FEIJÓ; NUNES; LANGGUTH, 2016; MENDES PONTES et al., 2016), as well as through camera trapping and line transect surveys (BELTRÃO, 2019; DA SILVA; MENDES PONTES, 2008; MENDES PONTES et al., 2016; RAMOS, 2019). Nonetheless, uncertainties persist regarding the composition of mammal assemblages in the PEC, but all authors point to the extinction of all large mammals, except for the Capybara, *Hydrochoerus hydrochaeris* (GARBINO et al., 2018; MENDES PONTES et al., 2016; PONTES; BELTRÃO; SANTOS, 2019). Regarding bird species, at least three species have been confirmed as extinct in the PEC: the Alagoas curassow (*Pauxi mitu*), Alagoas Foliage-gleaner (*Philydor novaesi*) and Cryptic Treehunter (*Cichlocolaptes mazarbarnetti*) (BUTCHART et al., 2018).

In an effort to restore biodiversity in the PEC, the Chico Mendes Institute for Biodiversity Conservation (Ministry of Environment, Brazil) launched the National Action Plan for the Conservation of the Alagoas curassow in 2008 (SILVEIRA et al., 2008). Possible areas for the reintroduction of this species in the state of Alagoas include patches of Atlantic Forest on private properties owned by alcohol and sugar companies. In communities, mammals play primary roles as top-down regulators, seed dispersers, and other important ecological functions (ALMEIDA-NETO et al., 2008; ROEMER; GOMPPER; VAN VALKENBURGH, 2009; TURKINGTON, 2009). However, the presence of carnivores can significantly impact reintroduction plans, especially for birds (DESTRO; DE MARCO; TERRIBILE, 2018). Thus, in this work we aimed at characterizing the community of medium-sized mammals in one of the potential areas for reintroduction of the Alagoas curassow, with especial interest in evaluating the presence of carnivores (native and domestic) that could impact the reintroduction of this and other bird species.

Furthermore, in tropical environments, it is crucial to understand the effectiveness of survey methods due to the high species richness, limited resources available and the need to generate data rapidly aiming at species conservation (ARDENTE et al., 2017; ESPARTOSA; PINOTTI; PARDINI, 2011; SRBEK-ARAUJO; CHIARELLO, 2005). To our knowledge no work has attempted to combine survey methods to evaluate the medium-sized mammal community under the unique conditions of the PEC. Moreover, the use of noninvasive sampling, i.e., sampling approaches that are not based on the physical capture of individuals, may contribute to characterizing mammal species that occur in low abundances (LONG et al., 2012), as is the case of PEC. Thus, we also evaluated the effectiveness of several noninvasive sampling methods (camera trapping, hair trapping, line transect survey, scat surveys associated with molecular identification and opportunistic encounter with carcasses and bones) in detecting medium-sized mammals considering the sampling effort employed.

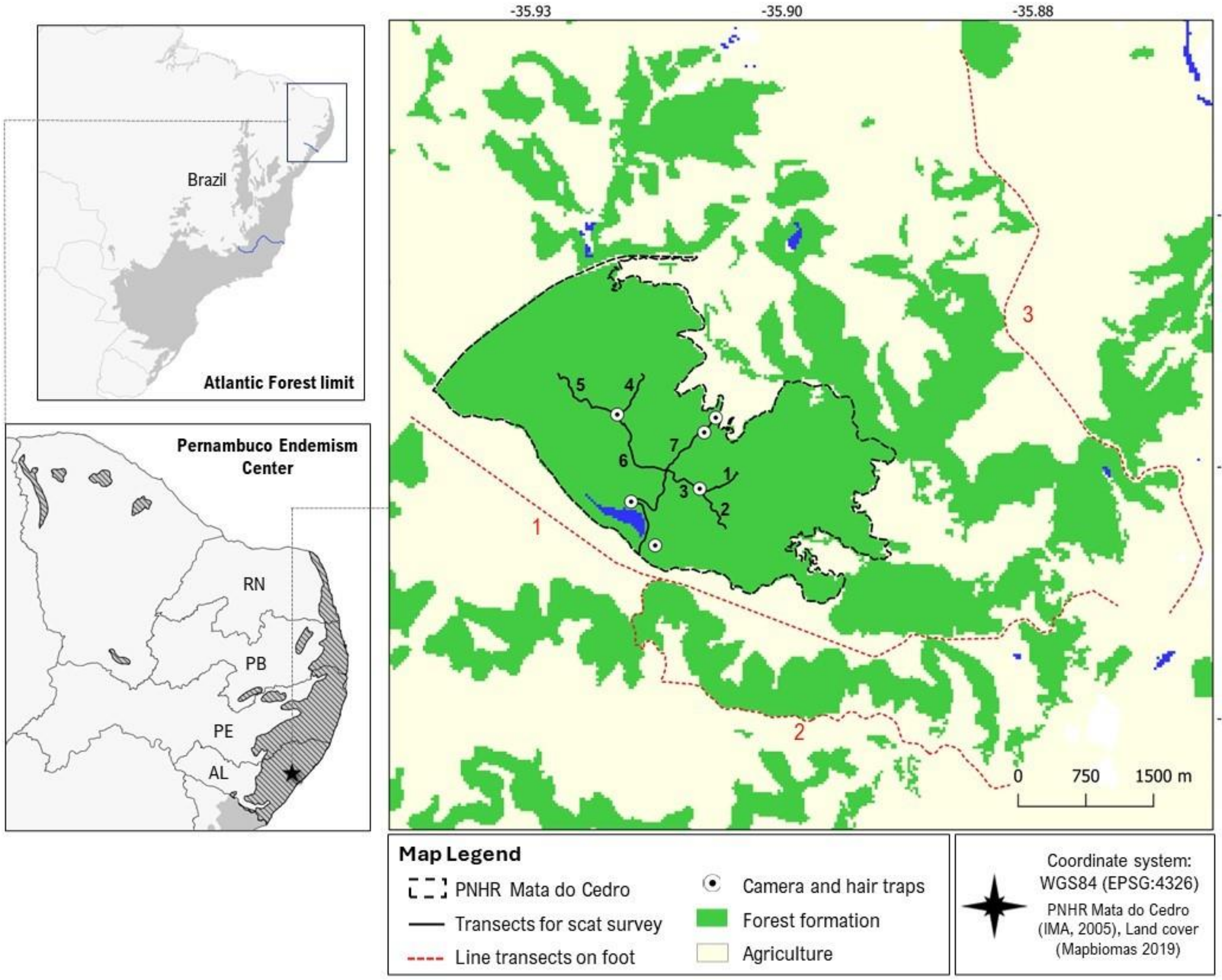
5.2. MATERIAL AND METHODS

5.2.1. Study area

The Private Natural Heritage Reserve (PNHR) Mata do Cedro (9° 31’ 23.82” S, 35° 55’ 6.53” W) covers 978.8 hectares and is located in the municipality of Rio Largo, Alagoas state, Brazil. This forest patch is composed mostly of secondary ombrophilous forest and surrounded by sugarcane monoculture (Figure 3.1) (RODA; SANTOS, 2005; SILVEIRA; OLMOS; LONG, 2003). During the 1970s and 1980s this forest patch experienced intense selective logging, but it is currently under recovery (SILVEIRA et al., 2008). As a result of this process, a trail that was previously used by trucks for timber extraction now traverses the forest, connecting the northeast and southwest edges. Although most of this trail is no longer accessible to large vehicles due to dense vegetation regrowth, it remains navigable by motorcycles and serves as the primary pedestrian access route to the interior of the forest. Access to the forest is restricted, however, instances of illegal hunting and fishing persist, especially near a water reservoir located in the southwestern portion of the forest patch. The sugarcane plantation is subjected to burning to facilitate manual harvesting, which also poses a threat to the fauna that uses the matrix. In northeastern Brazil, the sugarcane harvest usually takes place from November to April, exceptionally starting in September in 2019. Despite this scenario, this area is one of the top priorities for conservation in the Pernambuco Endemism Center (DIAS et al., 2022).

We surveyed the area during October and November of 2019. Besides the main trail (length = 2.17 km), we used six additional pre-opened line transects in the forest interior, with lengths varying from 0.5 to 1.02 km and that were at least 100 meters apart. The main trail plus the six line transects totaled 6.36 km (PRADO et al., 2022). These seven transects were used for line transect surveys on foot and as reference for camera and hair trap placement (Figure 3.1).

Figure 3.1 – Location of the study area (PNHR Mata do Cedro) in relation to the Pernambuco Endemism Center (black star) and the Atlantic Forest. Transects for line transect survey on foot are numbered from shortest to longest. Transects used for line transect by car and scat survey are in red. Brazilian states: AL – Alagoas, PE – Pernambuco, PB – Paraíba, RN – Rio Grande do Norte. The Brazilian rivers (blue lines in the top left insert) refer to the São Francisco River in the north and the Doce River in central Atlantic Forest



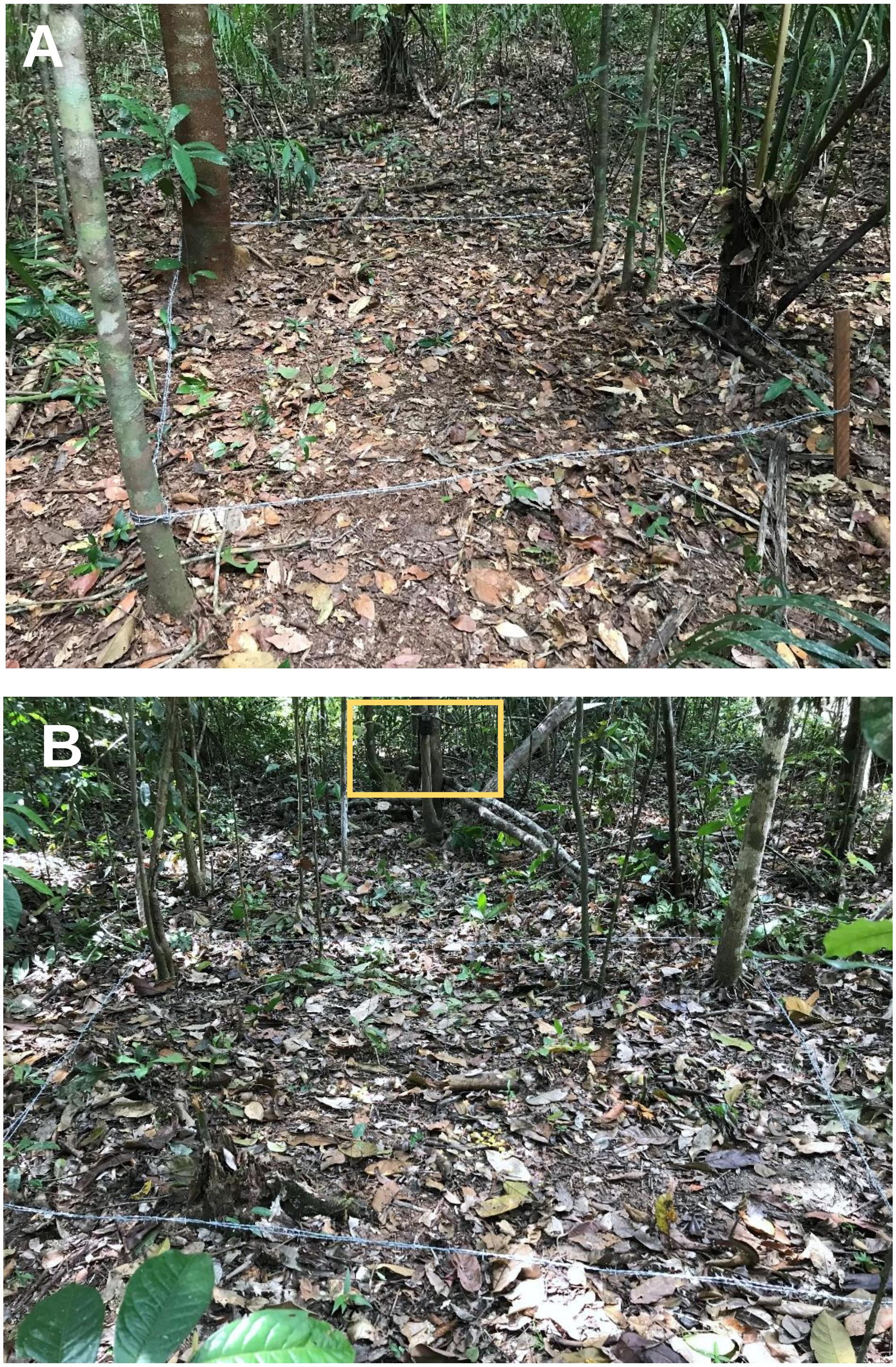
5.2.2. Camera trapping

We strategically deployed six camera traps (Bushnell Trophy Cam) across the study area to maximize sampling coverage, considering terrain access constraints. The camera traps were installed close to the main trail, water sources, and at the intersections of the open transects, avoiding placement in the matrix due to high theft risk. The camera traps were installed 50 cm above the ground with the beam directed slightly downwards and operated continuously day and night (Figure 3.2A). Cameras were set to capture 1-minute videos at 1 minute interval, automatically recording date and time. Five cameras were active for 28 days and one camera was active for 20 days, resulting in 160 trap-days. To increase species detection, we baited camera stations (details provided below). We revised all cameras every three days to replace baits, memory cards, and batteries. A record was defined as one detection per species per day per camera trap.

5.2.3. Hair trapping

We assembled six corral-type hair traps (KENDALL; MCKELVEY, 2008) in front of each camera trap, aligned with the camera's beam (Figure 3.2A). Each hair trap consisted of an area of approximately 4 m^2 enclosed with barbed wire supported by four trees or wooden sticks at a height of 20 cm from the ground. To increase barb density, the barbed wire was wrapped twice around supporting trees or sticks, positioning the barbs of the second round between the barbs of the first round (Figure 3.2B). We placed baits aimed at attracting carnivores, herbivores and omnivores (bacon or sardines and corn or fruits; MAGIOLI et al., 2019) and an attractant (cinnamon; PORTELLA et al., 2013) in the center of the corral. We checked traps for hairs and replaced bait and attractant every three days. Hair tufts were individually placed in paper bags and stored at room temperature. Hair traps were active during the same days as camera traps (sampling effort = 160 trap-days).

Figure 3.2 - Hair and camera traps installed. (A) Configuration of the hair trap. (B) Positioning of the camera trap indicated by a yellow rectangle, affixed to a tree and facing the corral



5.2.4. Line transect survey

We systematically walked each of the seven transects (the main trail plus the six opened transects) seven times in different orders from 4:30 to 9:00 am. A single observer walked at a constant speed of 1.5 km/h and recorded the geographic position of all sighted medium-sized mammals, as well as the number of individuals observed. Field work was not carried out on rainy or windy days. Each day the observer covered on average 2.02 km (SD ± 0.76). In the study area, the sun rose between 4:50 and 5:10 am. We also considered sightings made on the motorized transect that linked the headquarters of the nongovernmental organization Instituto de Preservação da Mata Atlântica (IPMA), where the researchers were based, to the reservoir at the entrance PNHR Mata do Cedro (Figure 3.1, distance = 8.19 km). This route was traveled between 4:00 - 5:00 am at a constant speed of 20 km/h with two or three watchers in the vehicle on the same days as the line transect survey on foot. In both cases, we conducted 22 days of field work, covering 44.52 km on foot and 180.12 km by car in total.

5.2.5. Scat survey

We delimited three transects on dirt roads of the matrix that are used for sugarcane transportation based on the availability and accessibility of roads (Figure 3.1). From a motorized vehicle moving at a constant speed of 10 km/h, two or three experienced spotters actively searched for feces. Given the sandy composition of the matrix soil, feces were readily visible from the vehicle during surveys. However, within the forest, the litter layer was sufficiently dense to impede the detection of feces, hence we concentrated our efforts on the dirt roads surrounding the forest fragment. We collected a portion of 3 to 4 cm of feces with disposable sticks, placed it in sterile plastic falcon tubes containing 95% ethanol and stored at -20ºC until DNA extraction. The collected feces were identified as belonging to mammals based on size and morphology (CHAME, 2003). Total survey effort was 10 days of field work, with each fieldwork day conducted from one to seven days apart (3 days on average), covering approximately 70 km in total. This strategy allowed time for species defecation, while avoiding DNA degradation due to excessive exposure to environmental conditions (REDDY et al., 2012).

We extracted DNA from fecal samples using the QIAamp® DNA Stool Mini Kit (Qiagen), according to the manufacturer's recommendations with minor modifications. For species identification, we used a primer set developed for canid and felid identification

(RODRÍGUEZ-CASTRO et al., 2018) to amplify a fragment of the 12S rRNA region of mitochondrial DNA (~190 bp). This primer was chosen because all feces collected matched canid or felid morphology. PCR protocol followed Rodríguez-Castro et al. (2018). PCR products were visualized on a 1.5% agarose gel. Successful PCR products were purified using Exo+SAP enzymes (Cellco) and sequenced using a 3730xl DNA Analyzer automated sequencer (Applied Biosystems), by the Sanger method. The quality of the sequences obtained was analyzed in the Geneious 8.0.2 software (KEARSE et al., 2012) and only high-quality sequences were used for molecular identification of the species. These sequences were compared with reference sequences available in the GenBank database (BENSON et al., 2012) of the National Center of Biotechnology Information (NCBI) and the database of the Laboratório de Biodiversidade Molecular e Conservação (UFSCar, Brazil), where molecular analyses were conducted.

5.2.6. Encounter of carcasses and bones

We opportunistically collected all tissue and bone samples from dead animals found during line transect and scat surveys, as well as during the route to revise camera and hair traps. All collection of biological material (feces, tissue, and bones) were performed under appropriate authorizations (SISBIO 71604, CEUA UFSCar 3472121119).

5.2.7. Data analysis

To assess sampling effort sufficiency of each method separately, we constructed species accumulation curves using the ‘specaccum’ function of the package *vegan* applying the “random” method and 1000 permutations in the R environment (R CORE TEAM, 2022).

Sampling effort was standardized across survey methods as the number of fieldwork days. Thus, the sampling effort was 28 days of field work for camera and hair trapping (considering five cameras and hair traps active for 8 days and six traps active for the remaining 20 days), 22 days for line transect survey on foot and by car, and 10 days for scat survey. We calculated the proportional detection success (PDS) per species per method as: number of sampling days a species was detected by a focal method/sampling effort x 100. We evaluate if the PDS differed significantly among survey methods through an analysis of variance (ANOVA) with Tukey’s pairwise comparison, conducted in the R environment (R CORE TEAM, 2022). We also calculated the latency to initial detection (LDT), as the time in days until the first detection of a species (FORESMAN; PEARSON, 1998). We did not include encounters with carcasses and bones in PDS and LDT calculations because sampling effort was not standardized with other methods.

We analyzed the potential risk of predation of the Alagoas curassow considering the behavior and size of this species (diurnal, approximately 3 kg for adult individuals) and the feeding habits of the carnivore species detected in the area.

5.3. RESULTS

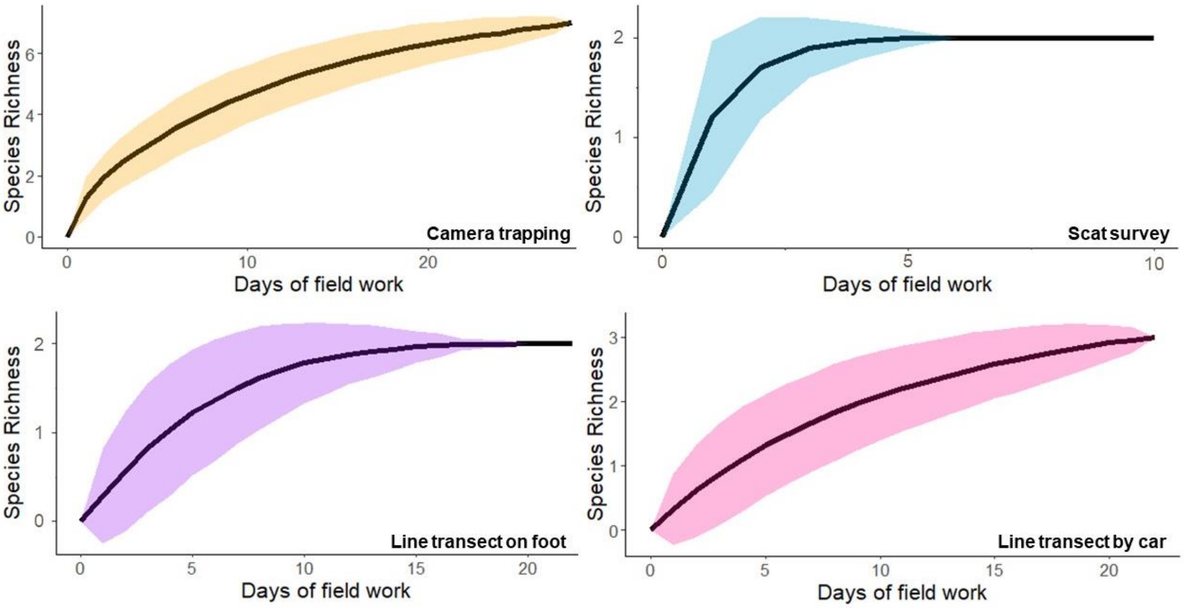
We detected a total of 13 medium sized mammal species in the PNHR Mata do Cedro (Table 3.1). Hair traps did not detect any species and were excluded from further analyses. The species accumulation curves for scat survey and line transect survey on foot reached a plateau with a narrow confidence interval, whereas for camera trapping and line transect survey by car, it increases with increasing sampling days but did not reach a plateau (Figure 3.3).

Camera traps detected the highest number of species (seven species, Table 3.1). For this method, species of the genus Didelphis were the most detected, totaling 38 video records, followed by a non-focal species (the lizard *Tupinambis merianae*, 14 detections), and the species *Tamandua tetradactyla* and *Dasypus novemcinctus* with three detections each. Four species were detected exclusively by this method: *D. novemcinctus*, *T. tetradactyla*, *Didelphis aurita* and *Didelphis albiventris*. Line transect survey recorded five species (Table 3.1). We observed contrastingly different species depending on if the survey was carried out on foot (inside the forest fragment) or by car (on the sugar cane matrix, Table 3.1). We collected 26 feces morphologically identified as belonging to mammals. We successfully extracted DNA and molecularly identified 25 feces, yielding an identification success of 96.15%. We identified 14 feces as belonging to *Cerdocyon thous* and 11 to domestic dogs (*Canis familiaris*). Finally, we encountered carcasses and bones from six species, two of which were exclusively recorded by this method (*Bradypus variegatus* and *Eira barbara*, Table 3.1).

Table 3.1 – Species identified by survey method employed and proportional detection success (PDS) in parenthesis. For line transect survey, ^{W} and ^{M} indicate species that were detected exclusively on walking and motorized transects, respectively.

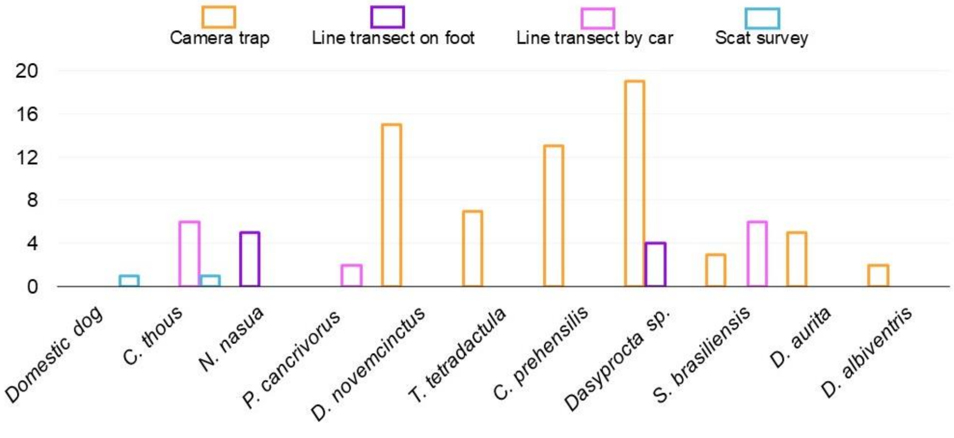
Species	**Conservation status**	**Camera trap**	**Line transect survey**	**Scat survey**	**Carcasses**
Carnivora					
Domestic dog (*Canis familiaris*)				X (50%)	
Crab-eating Fox (*Cerdocyon thous*)	LC		X (18.18%) ^{M}	X (70%)	X.
Tayra (*Eira Barbara*)	LC				X
South American Coati (*Nasua nasua*)	LC		X (18.18%) ^{W}		X
Crab-eating Raccoon (*Procyon cancrivorus*)	LC		X (4.54%) ^{M}		
Xenarthra					
Brown-throated Sloth (*Bradypus variegatus*)	LC				X
Nine-banded Armadillo (*Dasypus novemcinctus*)	LC	X (10.7%)			
Southern Tamandua (*Tamandua tetradactyla*)	LC	X (10.7%)			
Rodentia					
Brazilian Porcupine (*Coendou prehensilis*)	LC	X (7.1%)			X
Agouti (Dasyprocta sp.)		X (3.5%)	X (13.63%) ^{W}		X
Lagomorpha					
Tapeti (*Sylvilagus brasiliensis*)	EN	X (3.5%)	X (9.09%) ^{M}		
Didelphimorphia					
*White-eared Opossum (*Didelphis albiventris*)	LC	X (14.3%)			
*Brazilian Common Opossum (*Didelphis aurita*)	LC	X (75%)			

Figure 3.3 – Species accumulation curves with increasing sampling effort per survey method employed. Shaded colors indicate the confidence interval for the 1000 permutations



Proportional detection success varied among species and sampling methods, but the difference was not statistically significant ($p > 0.05$). For species detected with more than one survey method, the LDT for camera trapping was higher than for any other method, except for *Sylvilagus brasiliensis* (Figure 3.4). The agouti (*Dasyprocta sp.*) was more rapidly detected through line transect survey, whereas the *C. thous* and the domestic dog were more rapidly detected by scat survey.

Figure 3.4 - Latency to initial detection (LTD) per species and survey method (in days)



5.4. DISCUSSION

For the first time, a systematic survey using different sampling methods was conducted to assess medium-sized mammal diversity in the endangered Pernambuco Endemism Center. In total, 13 medium-sized mammal species were detected. The species accumulation curves displayed patterns of stabilization for scat survey and line transect within the forest, suggesting that we likely detected all species that could be detected by these methods. For line transect in the matrix and camera trapping, it is likely that we missed a few species. We did not intend to compare species accumulation curves between methods, as each method is biased towards distinct sets of species due to several reasons (GOMPPER et al., 2006; JOSHI et al., 2020; MAZZOLLI et al., 2017), which was also observed among the sampling methods employed here. For example, camera traps were not placed in the sugarcane matrix, reducing their ability to detect species that make more use of this habitat, such as *Cerdocyon thous*. On the other hand, the scats were collected exclusively on the sugarcane matrix, hindering the detection of forest-dwelling species.

Nonetheless, camera trapping was the method that detected most species, concentrated in the groups Xenarthra, Rodentia, Lagomorpha and Didelphimorphia. This method showed the highest LDT and lowest PDS. Most camera trap detections were of the only two marsupial species of the genus Didelphis that occur in the Brazilian Atlantic Forest. Previous assessments of medium mammal community in PEC did not include Didelphis species as a medium-sized mammal (BELTRÃO, 2019; DA SILVA; MENDES PONTES, 2008; GARBINO et al., 2018; MENDES PONTES et al., 2016), but rather included *D. aurita* and *D. albiventris* as part of the small size mammal assemblage (ASFORA; PONTES, 2009; BELTRÃO, 2019). We believe there are two main reasons for this. In Brazil, all marsupials are considered part of the small mammal community (ASFORA; PONTES, 2009; UMETSU; PARDINI, 2007). Although these two species are the larger marsupials in Brazil, they can be caught by methods designed to sample small mammals (e.g., live traps and pitfalls) (BARROS et al., 2015). However, considering that *D. aurita* and *D. albiventris* can be larger than the 1 kg threshold commonly used to define medium species (CÁCERES, 2005; CHIARELLO, 2000; DE ALMEIDA; TORQUETTI; TALAMONI, 2008; NOGUEIRA, 1988), their diet include a diversity of small mammals, reptiles, birds and their eggs (CÁCERES; MONTEIRO-FILHO, 2001; CARVALHO; FERNANDEZ; NESSIMIAN, 2005; COCKLE et al., 2016; DA SILVA et al., 2014; OLIVEIRA;

SANTORI, 1999), making them important predators of small vertebrates (MOURA; VIEIRA; CERQUEIRA, 2009), and the overly simplified composition of medium mammals in the PEC, we suggest that these two species should be included in the list of medium mammals for the PEC community.

Encounter of bones and carcasses yielded the second highest number of species, but it is important to note that the sampling effort for this method is not comparable to others as it encompassed the combined effort of line transect and scat surveys, plus the distance covered during camera trap revisions, and other additional field work conducted for independent projects. Line transect survey within the forest fragment (conducted on foot) detected three species, whereas line transect survey on the sugar cane matrix (conducted by car) and scat survey detected two species each. We observed contrastingly different species depending on if line transect survey was carried out within the forest fragment or on the sugar cane matrix. This could probably be related to the species preferential habitat and/or feeding habits, although all species detected are usually associated with areas of high human impact and are considered generalists (BOGONI et al., 2016; DESBIEZ; BORGES, 2010; DIAS et al., 2019; FERREGUETTI; TOMAS; BERGALLO, 2018; GOULART et al., 2009). We detected *P. cancrivorus* only once (a group of three individuals) at the edge of a sugar cane plot during a motorized transect at 4 am, near an area of human settlement. The low detection rate of the nocturnal *P. cancrivorus* (DUTRA et al., 2023) could be attributed to the fact that we only conducted line transect survey during the day.

Finally, our hair sampling was ineffective to detect any of the species present in the area. As the camera trap beam was directed towards the baits placed in the center of the hair trap, we recorded through video most animals passing over or under the hair trap to access the baits, but not touching the barbed wire (Supplementary material S2). Thus, the corral-type design of the trap, even mounted close to the ground, was not ideal for the detection of medium-sized mammals present in PEC. As hair traps with barbered wire have been reported to successfully record mammals elsewhere (MAGIOLI et al., 2019; ZIELINKI et al., 2006), we advocate that a more detailed evaluation of the most efficient design of hair trap to survey the PEC mammal community is needed.

We sampled only two species through fecal survey, but given the rapid plateau reached by the species accumulation curve, the lowest LDT and highest PDS, we believe that we detected all species that could be detected by this method in the study area, despite the lower number of sampling days. This method is particularly effective for detecting Canidae and Felidae (Chapter II), which explains why we only detected canid species. We observed the presence of domestic dogs next to human settlements scattered throughout the extensive sugarcane plantation; however, we have never observed this species in the vicinity or inside the PNHR Mata do Cedro during systematic surveys. During each field day of scat survey, once a portion of the feces was collected, we removed it from the transect to prevent sampling duplication, but dog scats were detected on the first three, 7th and 10th days of field work. Thus, the vicinity of the forest fragment was frequently visited by domestic dogs.

The total observed richness was comparable to other forest fragments of similar size in Paraíba (BELTRÃO, 2019; DA SILVA; MENDES PONTES, 2008), Pernambuco (RAMOS, 2019) and Alagoas (MENDES PONTES et al., 2016), but lower than similar-sized Atlantic Forest remnants outside PEC (BOGONI et al., 2016; CHIARELLO, 1999; PENIDO; CARLOS, 2012; REALE; FONSECA; UIEDA, 2014; SRBEK-ARAUJO; CHIARELLO, 2005). In contrast to findings in Paraíba's forest fragments, where the South American coati was less frequently recorded than other carnivores, as the ocelot (*Leopardus pardalis*, BELTRÃO et al., 2019), we made four independent observations of coati groups (comprising four to eight individuals) during line transect surveys on foot and no felid species were observed. Given the reduced community of medium-sized mammals in PEC and the need for rapid data on species occurrence, we sought to provide recommendations for surveying the PEC medium mammal community. Based on the species accumulation curves, we suggest that the duration of camera trap studies in this region to longer than 160 trap-days.

To enhance scat survey results, molecular identification should be employed in combination. Some feces were initially identified as belonging to felid species based on morphology and on the presence of hair, typical of felid feces (CHAME, 2003), but were later molecularly identified as belonging to *C. thous*. Despite its elevated cost, scat survey associated with genetic analyses can also be a valuable source of information on gene flow and connectivity, demographic parameters, among other important aspects for conservation of *C. thous* as feces of this species were frequently detected and showed high amplification success. Whenever possible we propose conducting diurnal and nocturnal line transect surveys as employed in Mendes Pontes et al. (2016), focusing survey effort on the forest interior as well as the matrix and edge areas.

Lastly, none of the individual approaches succeeded in detecting all the species present in the region. Therefore, given the unique landscape characteristics of PEC and the urge to produce fact and efficient data, we strongly recommend employing an integrated approach that combines multiple survey methods to assess the mammal community. Given that most forest remnants in the PEC are smaller than the one studied here, a relatively modest sampling effort (one sampling season of approximately 30 days) is sufficient to survey almost completely the community of mammals. We also highlight the importance of opportunistic records, such as the encounter of carcasses and bones, as these records can represent an important contribution to complement the species list (DIAS et al., 2019).

All native species of the Carnivora order detected in the Mata do Cedro, namely, *C. thous*, *N. nasua*, *P. cancrivorus* and *E. barbara* are not strict carnivores. These species are reported to have an omnivorous diet, including only small birds as a minor part of their diet (ALVES-COSTA; DA FONSECA; CHRISTÓFARO, 2004; BISBAL, 1986; GATTI et al., 2006; GROTTA-NETO et al., 2020; PEDÓ et al., 2006; QUINTELA; IOB; ARTIOLI, 2014; ROCHA-MENDES et al., 2010; TIRELLI et al., 2019). Given that, it is less expected that these species would prey on adult Alagoas curassow individuals. However, eggs and young individuals could be vulnerable. Other species known to predate bird eggs that were also detected in the study area are the Didelphis species, *Dasyprocta sp.* and the lizard *T. merianae* (COCKLE et al., 2016; GALETTI et al., 2009). The only domestic species detected in the area, the domestic dog is also omnivore and often rely on human-provided food as part of their diet (VANAK; GOMPPER, 2009). This dietary flexibility allows domestic dogs to thrive in natural environments at higher densities compared to native carnivores, as their population dynamics are not solely regulated by the resources available in natural areas (PASCHOAL et al., 2016). Thus, dogs can pose a significant threat to wildlife as predators of young and adult individuals of various taxonomic groups as well as vectors for diseases (GALETTI; SAZIMA, 2006; HUGHES; MACDONALD, 2013; MARTINEZ et al., 2013; YOUNG et al., 2011), particularly in South America (DOHERTY et al., 2017), but the extent of their impact on the PEC remains unknown. Thus, our findings suggest that the domestic dog might be the main mammal predator affecting the Alagoas curassow reintroduction program. Reintroduced individuals are particularly endangered if they move to the surroundings of the PHNR, where dogs were detected. Eggs and young individuals are also susceptible to predation by other omnivorous species detected. We highly recommend the prompt start of a control program aiming at reducing domestic dog usage of the PNHR vicinities and exploring the feasibility of incorporating an antipredator training in the reintroduction program.

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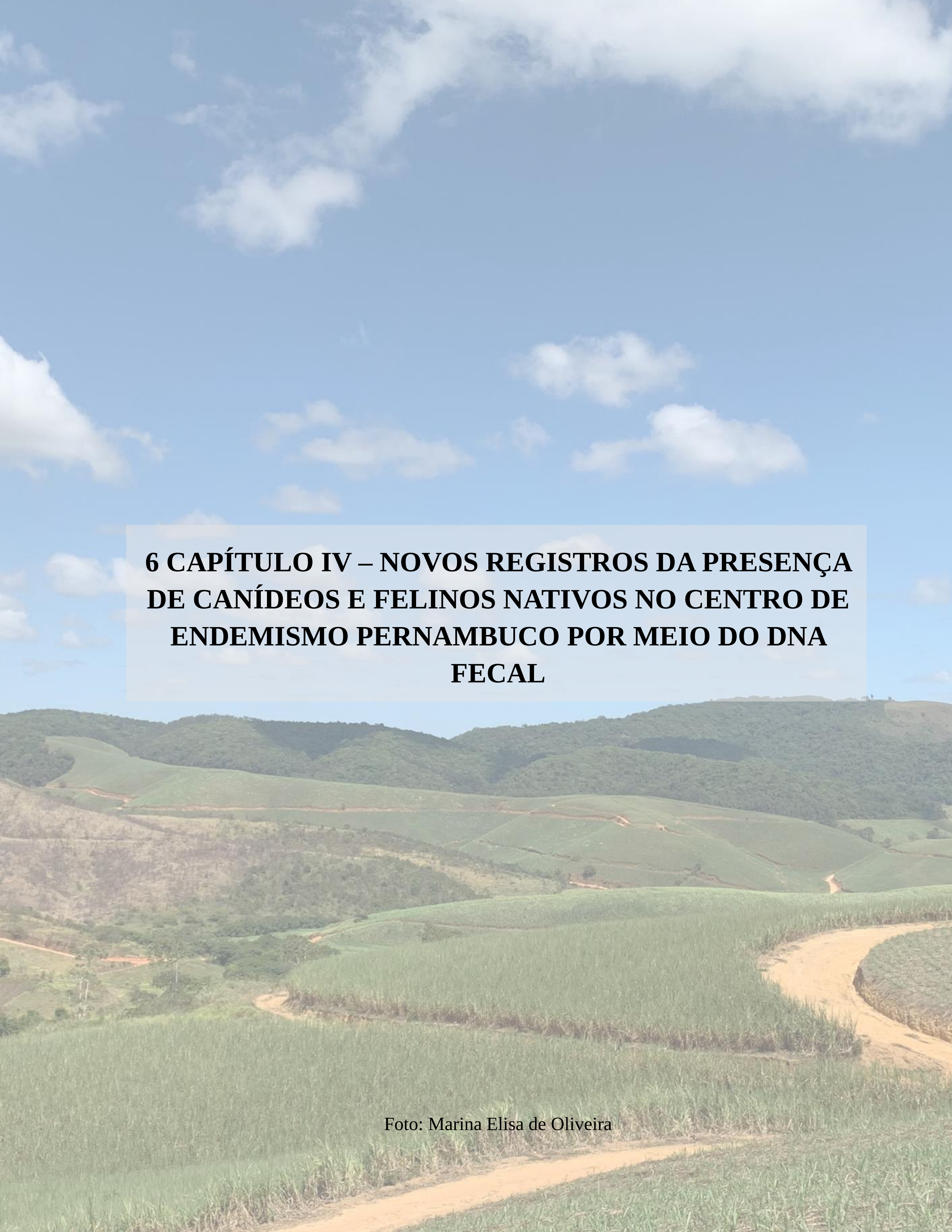


6 CAPÍTULO IV – NOVOS REGISTROS DA PRESENÇA DE CANÍDEOS E FELINOS NATIVOS NO CENTRO DE ENDEMISMO PERNAMBUCO POR MEIO DO DNA FECAL

Foto: Marina Elisa de Oliveira

Novos registros da presença de canídeos e felinos nativos no Centro de Endemismo Pernambuco por meio do DNA fecal

Resumo

O Centro de Endemismo Pernambuco (CEP), região da Mata Atlântica ao norte do Rio São Francisco, encontra-se intensamente ameaçada por atividades antrópicas que levam a um severo processo de defaunação na região. A maior parte dos fragmentos florestais remanescentes estão em propriedades privadas de usinas de cana-de-açúcar a álcool, sendo assim importantes focos de esforços para estudos visando a conservação das espécies do CEP. Os carnívoros, como os canídeos e felinos, geralmente ocorrem em baixas densidades e apresentam comportamento elusivo, o que dificulta sua detecção. Dessa forma, objetivamos avaliar a presença de canídeos e felinos nativos do CEP no estado de Alagoas por meio da identificação molecular do DNA presente nas fezes deixadas no ambiente. Amostramos 44 transectos em seis remanescentes representativos de Mata Atlântica: cinco dentro de propriedades privadas e uma unidade de conservação, sendo a maior do estado. Das 50 fezes coletadas, identificamos duas espécies nativas (*Cerdocyon thous* e *Leopardus tigrinus*) e o cão doméstico (*Canis familiaris*). *C. thous* foi detectado em maior frequência do que *L. tigrinus*, e provavelmente é a espécie dos grupos estudados que ocorre em maior abundância no CEP por estar mais associada com áreas abertas e antropizadas, como a matriz de cana-de-açúcar. Este trabalho apresenta o primeiro registro de *L. tigrinus* para o estado de Alagoas nos últimos 20 anos e a primeira tentativa bem-sucedida de detecção de mamíferos através do DNA fecal no CEP. A falta de detecção de outros felinos, como *Leopardus pardalis*, pode ser mais um indício do processo de defaunação do CEP.

Palavras-chave: amostragem não invasiva; cachorro-do-mato; Estação Ecológica Murici; gato-do-mato

6.1. INTRODUÇÃO

O Centro de Endemismo Pernambuco (CEP) é a região da Mata Atlântica brasileira que se estende ao norte do Rio São Francisco pelos estados de Alagoas, Pernambuco, Paraíba e Rio Grande do Norte. Essa região abriga grande riqueza e endemicidade de espécies, principalmente para as aves, grupo que já foi mais estudado em comparação aos outros vertebrados da região (SILVA; SOUSA; CASTELLETTI, 2004; SILVEIRA; OLMOS; LONG, 2003). Com relação aos mamíferos, cinco espécies de mamíferos de médio porte são consideradas endêmicas ao CEP: *Coendou prehensilis* (MENEZES et al., 2021), *Coendou speratus* (PONTES et al., 2013), *Sylvilagus brasiliensis* (RUEDAS et al., 2017), *Dasyprocta iacki* (FEIJÓ; LANGGUTH, 2013) e *Sapajus flavius* (VALENÇA-MONTENEGRO et al., 2021). Contudo, ainda existe uma grande lacuna de conhecimento com relação a ocorrência e distribuição dos mamíferos no CEP (FEIJÓ et al., 2021).

A comunidade de carnívoros que ocorrem no CEP compreende 11 espécies, distribuídas nas famílias Felidae, Canidae, Mustelidae e Procyonidae (FEIJÓ et al., 2021; GARBINO et al., 2018). Contudo, o processo de defaunação é mais intenso para o CEP do que para as outras regiões da Mata Atlântica (BOGONI et al., 2018). Ou seja, essa é a região que mais tem perdido indivíduos e espécies de médio e grande porte em todo o bioma devido a caça e a perda e fragmentação do habitat, principalmente com a crescente expansão da monocultura de cana-de-açúcar (BARBOSA; AGUIAR; ALVES, 2020; BELTRÃO, 2019; BOGONI et al., 2018; DE SOUZA; ALVES, 2014; FEIJÓ et al., 2021; RIBEIRO et al., 2009). Dentre os carnívoros presentes no CEP, quatro pertencem à família Felidae (*Leopardus pardalis*, *Leopardus wiedii* e *Leopardus tigrinus* e *Puma yagouaroundi*) e um à família Canidae, *Cerdocyon thous* que também é a única espécie do seu gênero. As duas grandes espécies da família Felidae que habitam a Mata Atlântica e ocorriam nessa região, a onça-pintada (*Panthera onca*) e a onça-parda (*Puma concolor*), encontram-se localmente extintas (GARBINO et al., 2018; MENDES PONTES et al., 2016).

O uso sistemático de armadilhas fotográficas para o estudo e monitoramento de mastofauna tem acrescentado à compreensão sobre a ocorrência de espécies raras no CEP, como os canídeos e felinos (BELTRÃO et al., 2019; FEIJÓ et al., 2021; RAMOS, 2019), contudo não proporcionam acesso ao material genético dessas espécies. Feijó et al. (2021) destacam que o uso de métodos complementares pode auxiliar na identificação de espécies nessa região e que, até o momento, a amostragem de mamíferos tem sido principalmente direcionada para algumas áreas protegidas nas capitais dos estados que compõe o CEP. Dentre eles, o estado de Alagoas é particularmente promissor para novos estudos por ter sido proporcionalmente menos estudado até o momento (CARVALHO et al. 2021; FEIJÓ et al., 2021).

Considerando a defasagem do conhecimento científico para a região do CEP e seu elevado status de ameaça, a utilidade da amostragem não invasiva associada as ferramentas moleculares para identificação de espécies de carnívoros raros (Capítulo II), objetivamos neste trabalho avaliar a presença de canídeos e felinos nativos na Mata Atlântica do estado de Alagoas por meio do DNA presente nas fezes depositadas no ambiente. Focamos nossa amostragem em remanescentes de Mata Atlântica de propriedades privadas e na maior unidade de conservação do estado afim de fornecer um panorama mais completo sobre a ocorrência dessas espécies.

6.2. MATERIAL E MÉTODOS

6.2.1. Escolha dos transectos amostrais

Foram escolhidas seis áreas de remanescentes florestais de Mata Atlântica no estado de Alagoas para serem amostradas por serem áreas indicadas como mais viáveis para manter a biodiversidade de vertebrados a longo prazo (BELTRÃO et al., em prep.), sendo elas: Mata da Cachoeira, Mata do Coimbra, Reserva Particular do Patrimônio Natural Mata do Cedro (RPPN Mata do Cedro), Complexo Maceió-Paripueira e Estação Ecológica Murici (ESEC Murici, Figura 4.1). Dessas seis áreas, cinco são fragmentos em propriedades particulares e uma é uma unidade de conservação de proteção integral (ESEC Murici) (Figura 4.1, Tabela 4.1). Cada área apresenta uma composição variável da matriz, sendo principalmente composta por cana-de-açúcar, mas também por pasto e eucalipto, com a eventual presença de assentamentos humanos de baixa densidade.

Usamos as áreas de remanescentes florestais como referência para a escolha de oito transectos amostrais no interior e ao redor de cada uma para serem percorridos em busca de fezes depositadas por canídeos e felinos. Na RPPN Mata do Cedro apenas 4 transectos foram escolhidos devido ao reduzido acesso ao remanescente no momento de coleta, totalizando 44 transectos amostrais de 2 km de extensão cada (Figura 4.1). Para a delimitação dos transectos, geramos um buffer de 15 km a partir da borda de cada área de referência (Figura 4.2A). O *shapefile* dos remanescentes de referência foi obtido a partir das análises de Beltrão et al. (in prep.) baseado no uso e cobertura do solo da plataforma MapBiomas (Coleção 5, www.mapbiomas.org). Ao buffer, foi sobreposto uma grade de células de 4 km^2 (Figura 4.2B). O tamanho do buffer e das células da grade foram escolhidos baseado na distância de movimentação e extensão da área de vida das espécies de canídeos e felinos que podem ocorrer no CEP (DI BITETTI; PAVIOLO; DE ANGELO, 2006; GOULART et al., 2009a; MACDONALD; COURTENAY, 1996; MAGALHÃES; SRBEK-ARAUJO, 2022). As células de cada grade foram categorizadas dependendo da cobertura florestal (0-15%, 15-30%, 30-60% e maior que 60% de cobertura), de acordo com o mapa de cobertura do solo também da plataforma MapBiomas (Coleção 6, Figura 4.2C e D). Todas as análises foram realizadas no *software* QGIS v. 3.18.

A alocação dos transectos amostrais foi feita por meio da inspeção visual das células categorizadas buscando por transectos visíveis de extensão mínima de 2 km no *software* Google Earth Pro 7.3.6, que apresenta composição de imagens do satélite LandSat 8 de resolução de até 0,5 m (Figura 4.2E e F). Os transectos foram distribuídos o mais igualitariamente o possível nas quatro categorias de cobertura florestal para contemplar espécies que tenham maior ou menor sensibilidade à cobertura florestal (REGOLIN et al., 2017), tentando manter uma distância mínima de uma célula entre transectos.

Figura 4.1 – Localização das áreas de referência e dos transectos amostrais. Estado brasileiros: RN – Rio Grande do Norte, PB – Paraíba, PE – Pernambuco, AL – Alagoas. Áreas de referência: A – Mata da Cachoeira, B – Mata do Coimbra, C – Estação Ecológica Murici, D – Complexo Maceió-Parirupeira, E – Reserva Particular do Patrimônio Natural Mata do Cedro, F – Mata de Coruripe. Os rios brasileiros referem-se ao Rio São Francisco ao norte e Rio Doce na região central da Mata Atlântica no mapa superior a esquerda

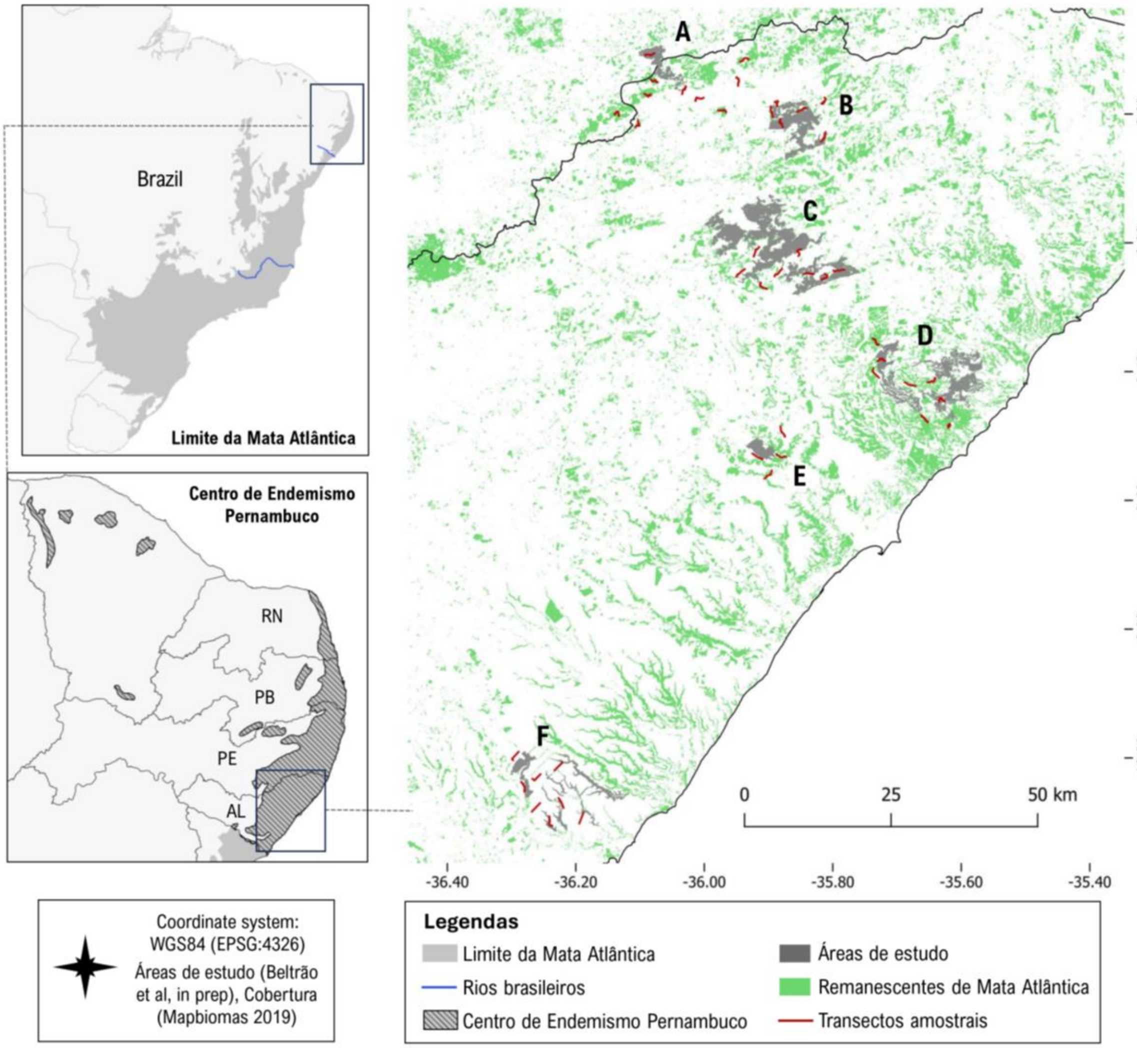
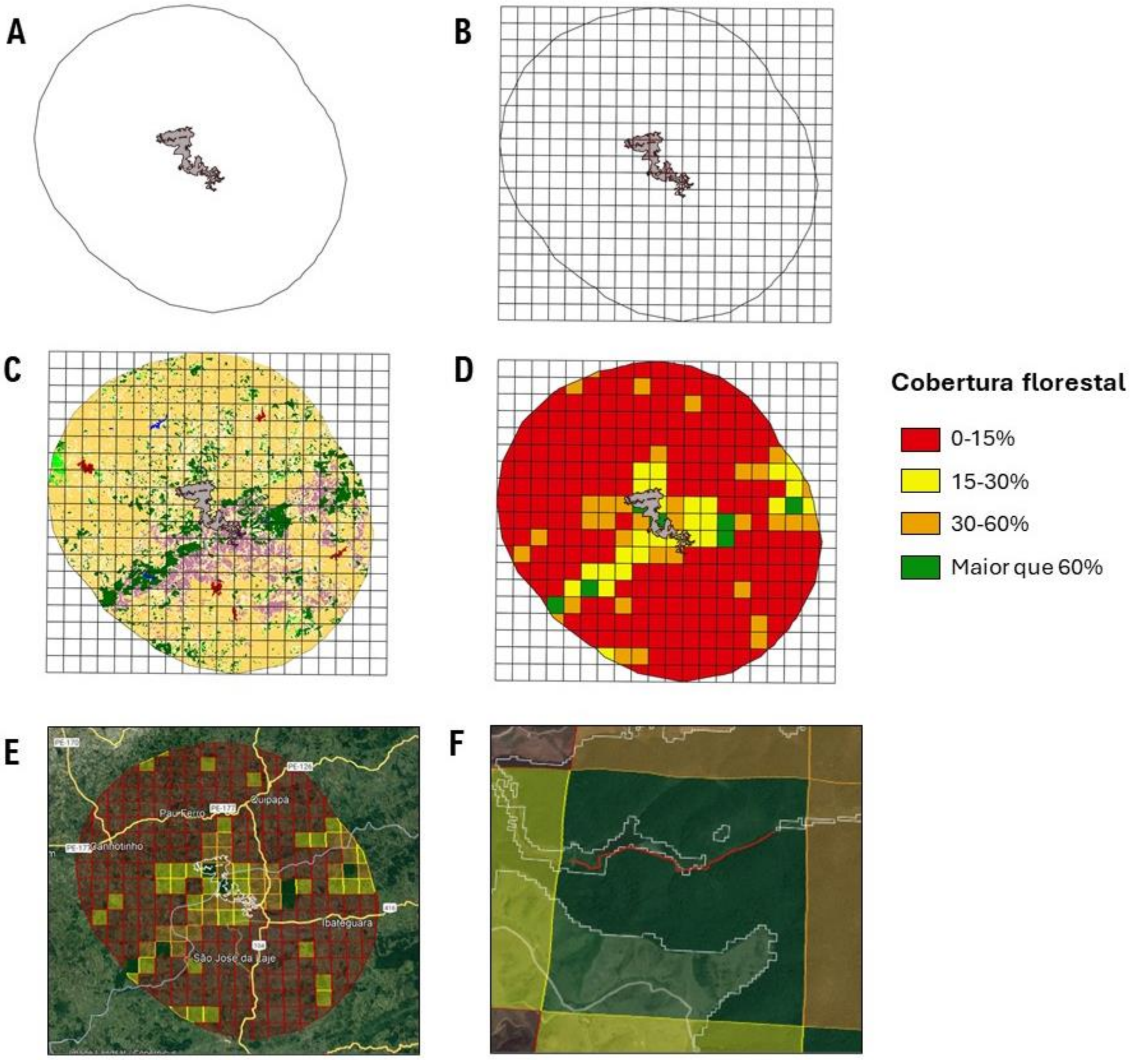


Tabela 4.1 – Nome e tamanho das áreas de referência amostradas em Alagoas. O código refere-se à codificação da Figura 4.1.

Código	**Nome da área**	**Tamanho (ha)**
A	Mata da Cachoeira (Usina Serra Grande)	1544
B	Mata do Coimbra (Usina Serra Grande)	3785
C	Estação Ecológica de Murici	11691
D	Complexo Maceió-Paripueira	6599
E	RPPN Mata do Cedro	1943
F	Mata de Coruripe (Usina Coruripe)	3064

Figura 4.2 – Esquema das etapas de seleção dos transectos amostrais, utilizando o fragmento Mata da Cachoeira como exemplo (em cinza). A) Buffer de 15 km ao redor do fragmento de referência. B) Grade sobreposta ao buffer com células de 4 km^2. C) Grade sobreposta ao mapa de cobertura do solo obtido na plataforma MapBiomas (coleção 6). D) Classificação das células baseado na porcentagem de cobertura florestal presente. E) Inspeção visual das células. F) Escolha e marcação (em vermelho) de um transecto amostral



6.2.2. Coleta de fezes

Sempre que possível respeitamos a seleção inicial dos transectos, porém houve o remanejamento de alguns transectos devido as condições de acesso em campo. Cada transecto foi percorrido a pé uma única vez em velocidade constante de 1km/h por dois observadores experientes em busca de fezes de canídeos e felinos, identificadas pela morfologia (CHAME, 2003) durante os meses de novembro e dezembro de 2021. Coletamos todas as fezes encontradas, desde que não apresentassem sinais de degradação pela ação do tempo e condições ambientais que pudessem impedir a identificação molecular. Fotografamos e coletamos uma porção de pelo menos 4 cm de uma das extremidades das fezes, e acondicionamos individualmente em tubos do tipo Falcon (15 ou 50 mL) em álcool 95%. As amostras foram mantidas em temperatura ambiente até o momento da extração de DNA que se deu no Laboratório de Biodiversidade Molecular e Conservação (LabBMC, UFSCAR – Campus São Carlos/SP, Brasil). A extensão das trilhas, assim como os pontos de coleta das amostras foram marcados com o uso de um equipamento GPS (GPSMAP 62, Garmin). A coleta e transporte de amostras biológicas foram realizados sob a autorização do Sistema de Autorização e Informação em Biodiversidade (Licença SISBIO – MMA/ICMBIO número 71604), aprovação da Comissão de Ética no Uso de Animais da Universidade Federal de São Carlos (CEUA/UFSCAR – protocolo 3472121119) e acesso ao patrimônio genético pelo Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen - A9F8717).

6.2.3. Identificação molecular

A extração do DNA das fezes foi feita utilizando o kit QIAamp® Fast DNA Stool Mini Kit (Qiagen). As células epiteliais do intestino da espécie depositora estão presentes na parte externa das fezes e podem se desprender da amostra fecal durante a movimentação e transporte, passando para o álcool utilizado na preservação. Dessa forma, um fragmento de 1 cm da porção externa das fezes mais 1 mL do álcool onde a amostra foi preservada foram transferidos para um tubo tipo Eppendorf de 2 mL e centrifugados por 10 min em centrífuga refrigerada a –4ºC. Após centrifugação, o álcool sobrenadante foi vertido e apenas o material precipitado foi utilizado para a extração, seguindo as recomendações do fabricante do kit. O volume final de eluição foi de 100 µL, divido em duas eluições de 50 µL cada. A identificação molecular foi feita por meio da amplificação e sequenciamento de um fragmento do DNA mitocondrial das regiões 12S ou 16S. Primeiramente tentamos a amplificação de uma região do gene 12S (~190 pb), utilizando o par de primers Car12Ss1-F e Car12Ss1-R desenvolvidos para a identificação de canídeos e felinos sul-americanos (RODRÍGUEZ-CASTRO et al., 2018). As reações de PCR foram realizadas em um volume final de 12 µL, contendo: 0,25 µM de dNTPs; 1X do buffer tris-KCl (Tris–HCl 20 mM pH 8,4 e KCl 50 mM); 2,5 mM de MgCl2 (50 mM, Invitrogen); 0,25 mg/mL de BSA (Bovine Serum Albumin); 0,6 µM de cada primer; 1 unidade de *Taq* DNA Polymerase Platinum (Invitrogen); 3 µL do DNA extraído e água destilada ultrapura (q.s.p 12 µL, Invitrogen). O ciclo da PCR compreendeu uma etapa inicial de desnaturação a 95ºC durante 4 min; 40 ciclos de amplificação a 95ºC por 30 s, 56ºC por 45 s e 70ºC por 45 s; e um ciclo final de extensão a 70ºC por 10 min. Os produtos de PCR foram visualizados em gel de agarose 1,5%. Quando a amplificação foi malsucedida, tentamos a amplificação da região 16S (130-138 pb), menor e menos específica, utilizando o par de primers 16Smam1 e 16Smam2 desenvolvidos para a identificação de mamíferos (TAYLOR, 1996). As reações de PCR foram realizadas em um volume final de 10 µL, contendo: 0,2 µM de dNTPs; 1X do buffer tris-KCl (Tris–HCl 20 mM pH 8,4 e KCl 50 mM, Invitrogen); 4 mM de MgCl2 (50 mM, Invitrogen); 0,4 µM de cada primer; 1 unidade de *Taq* DNA Polymerase Platinum (Invitrogen); 3 µL do DNA extraído e água destilada ultrapura (q.s.p 10 µL, Invitrogen). O ciclo da PCR compreendeu uma etapa inicial de desnaturação a 95ºC durante 10 min; 42 ciclos de amplificação a 95ºC por 30 s, 64ºC por 30 s e 72ºC por 1 min; e um ciclo final de extensão a 72ºC por 10 min. Os produtos de PCR também foram visualizados em gel de agarose 1,5%.

Todas as reações de PCR incluíram controles negativos (sem DNA molde) e positivos (DNA molde de boa qualidade) e foram preparadas em uma capela de fluxo laminar esterilizada por UV e hipoclorito de sódio 2% para evitar contaminações. Todas as amplificações foram realizadas em termociclador Veriti 96 Well Thermal Cycler (Applied Biosystems). As amplificações bem-sucedidas foram purificadas pelo método enzimático (ExoSAP-IT, Thermofisher) e sequenciadas em sequenciador automático 3730xl DNA Analyzer (Applied Biosystems).

A qualidade das sequências obtidas foi analisada no *software* Genious 6.0 (KEARSE et al., 2012) e apenas sequências de alta qualidade foram mantidas. Para a identificação das espécies, as sequências geradas foram comparadas com sequências obtidas na base de dados *GenBank* do *National Center of Biotechnology Information* (NCBI) por meio da ferramenta BLAST (*Basic Local Alignment Search Tool*) (BENSON et al., 2012) e alinhadas com sequências de referências obtidas a partir de amostras de tecido usadas como controle e disponíveis por Rodriguez-Castro et al. (2018).

6.3. RESULTADOS

Nós coletamos um total de 50 amostras fecais potencialmente pertencentes a canídeos e felinos (0,56 fezes por km percorrido, Figura 4.3). A identificação molecular foi alcançada para 17 amostras fecais (34% de sucesso de identificação ou 0,19 fezes identificadas por km percorrido): 10 pela amplificação e sequenciamento da região do gene 12S e sete pela região do gene 16S. Identificamos três espécies nativas, sendo duas dos grupos focais deste trabalho, e uma espécie doméstica. Nove fezes foram identificadas como pertencentes a cachorro-do-mato (*C. thous*) distribuídas em oito transectos amostrais diferentes, uma fezes de gato-do-mato (*L. tigrinus*) localizada em um transecto adjacente a ESEC Murici, uma fezes identificada apenas a nível do gênero Leopardus pela amplificação do gene 16S, cinco fezes de cachorro doméstico (*Canis familiaris*) em quatro transectos e apenas uma amostra fecal pertencente a espécie não focal, o sagui (*Callithrix jacchus*) (Figura 4.3).

Considerando o status de ameaça de *L. tigrinus* (Em Perigo de acordo com Livro Vermelho da Fauna Brasileira Ameaçada de Extinção, TRIGO et al., 2018) e a sugestão de que os indivíduos do CEP, da Caatinga e parte da Amazônia brasileira podem fazer parte de uma nova espécie do complexo tigrina (*Leopardus emiliae*, NASCIMENTO; FEIJÓ, 2017; TRIGO et al., 2013a), levantamos os registros de ocorrência dessas espécies para melhor compreender como os dados produzidos neste trabalho podem ajudar a compreender sua distribuição na região do CEP em Alagoas. Realizamos uma busca na base Google Scholar (www.scholar.google.com) utilizando as palavras-chave: ("*Leopardus tigrinus*" OR "*Leopardus emiliae*") AND ("Centro de Endemismo Pernambuco" OR "Pernambuco Endemism Center"). Nós compilamos os pontos de ocorrência disponíveis em artigos científicos e na literatura cinza (trabalhos de conclusão de curso, dissertações e teses, Tabela 4.2). Também entramos em contato com coleções biológicas para obter os dados de localidade e ano de coleta dos materiais depositados e classificados como *L. tigrinus* e *L. emiliae* por Feijó e Langguth (2013) e Nascimento e Feijó (2017) (Tabela 4.2). Como a filogenia dos chamados tigrina é complexa (TRINDADE et al., 2021) e apenas recentemente explorada (NASCIMENTO; FEIJÓ, 2017), consideramos *L. tigrinus* e *L. emiliae* como sinônimos para o levantamento de registros.

Figura 3.3 – Registros obtidos neste trabalho nas seis áreas de referência amostradas (quadros de contorno tracejado) e registros históricos de *Leopardus tigrinus* obtidos de coleções biológicas e da literatura (quadro de contorno sólido)

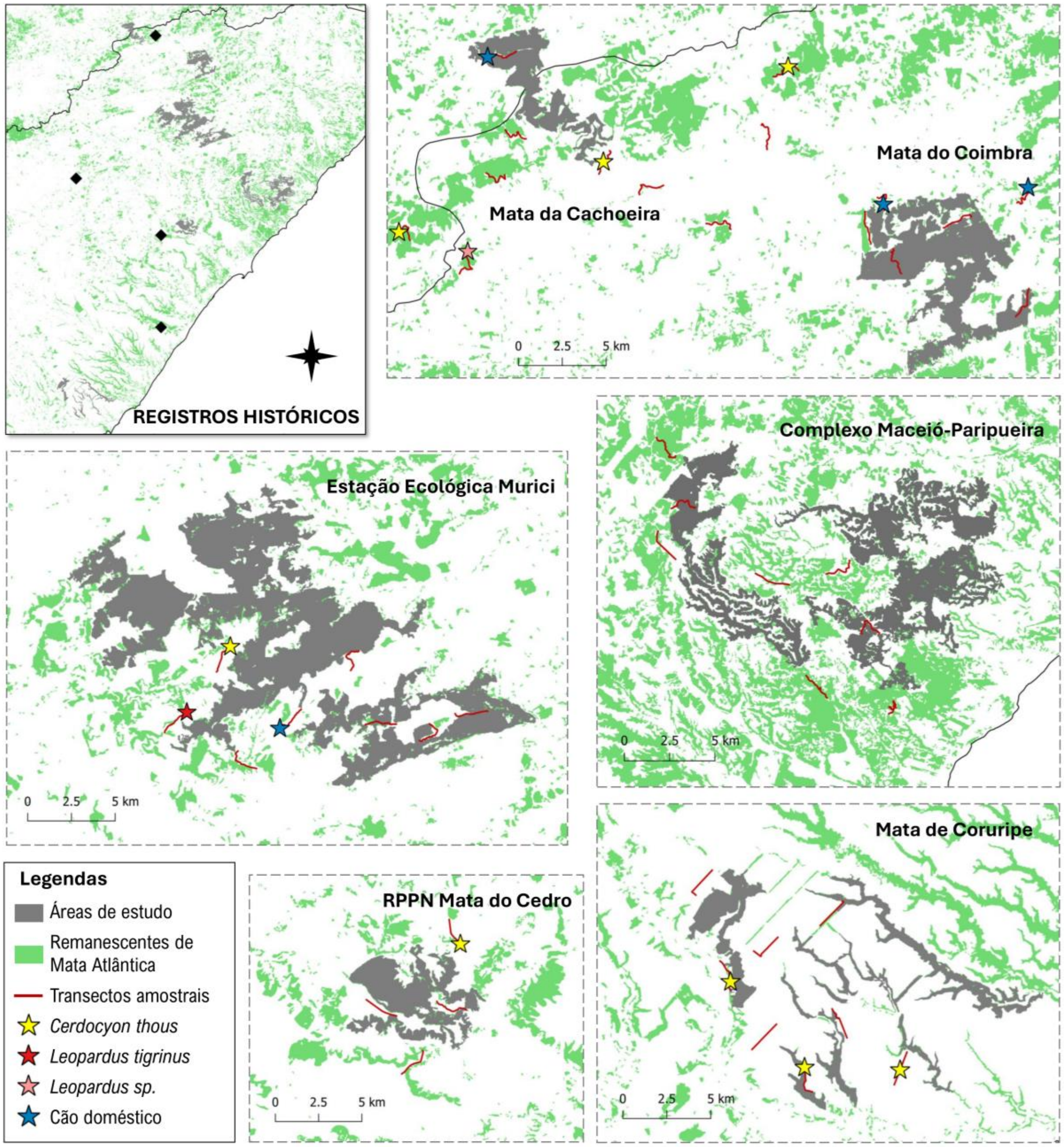


Tabela 4.2 – Ocorrências de *Leopardus tigrinus* e *Leopardus emiliae* descritas na literatura e em coleções biológicas. Localizações são aproximadas baseadas nas informações de depósito ou descrição de localidade da literatura. NI – Não informado. MPEG – Museu Paraense Emílio Goeldi, UFPB – Coleção de Mamíferos do Departamento de Sistemática e Ecologia da Universidade Federal da Paraíba, MZUSP – Museu de Zoologia da Universidade de São Paulo, MN – Museu Nacional da Universidade Federal do Rio de Janeiro, UFPE – Coleção de Mamíferos do Departamento de Zoologia da Universidade Federal de Pernambuco. Estados brasileiros: PE – Pernambuco, PB – Paraíba.

Registros para o estado de Alagoas

Local	**Localização**		**Ano**	**Método**	**Referência**
Viçosa	-9.376	-36.242	NI	Coleção biológica	MPEG 24895
Entre Pilar e Atalaia	-9.551	-35.979	1999	Coleção biológica	UFPB 3567
Quebrângulo	-9.318	-36.473	1957	Coleção biológica	MZUSP 8299
Viçosa	-9.376	-36.242	1952	Coleção biológica	MN 24895
Roteiro	-9.835	-35.979	1984	Coleção biológica	UFPE 978
Ibateguara (Mata do Coimbra)	-8.935	-35.996	2002	Encontro visual	FERNANDES, 2003
Registros recentes para os outros estados do CEP					
Lagoa dos Gatos/PE (Mata Bernardina)	-8.707	-35.859	2005-2006	Encontro visual/Vocalização	DA SILVA; MENDES PONTES, 2008
São Lourenço da Mata/PE (Estação Ecológica de Tapacurá)	-8.041	-35.196	2019	Armadilhamento fotográfico	RAMOS, 2019
Mamanguape/PB (Rebio Guaribas II)	-6.679	-35.213	2016-2018	Armadilhamento fotográfico	BELTRÃO, 2019

6.3. DISCUSSÃO

Até o momento, a principal metodologia para obtenção de dados de ocorrência das duas espécies de canídeos e felinos nativos detectadas aqui, e para mamíferos de médio porte em geral, no CEP tem sido o avistamento em transectos lineares (DA SILVA; MENDES PONTES, 2008; FERNANDES, 2003; SÁ-NETO et al., 2013), o levantamento em coleções biológicas (FEIJÓ; LANGGUTH, 2013; MENDES PONTES et al., 2016) e, mais recentemente, o armadilhamento fotográfico (BELTRÃO, 2019; RAMOS, 2019). Contudo, os estudos conduzidos na Mata Atlântica do estado de Alagoas estão temporalmente defasados. Até onde sabemos, o último levantamento sistemático de mamíferos de médio porte publicado para o estado foi realizado em

2002 (FERNANDES, 2003), além do levantamento apresentado no Capítulo III. Dessa forma, este trabalho apresenta o primeiro registro publicado na literatura de *L. tigrinus* para o estado de Alagoas em 20 anos. Este trabalho também representa o primeiro estudo conduzido no CEP que realizou, com sucesso, a identificação molecular de fezes de mamíferos que ocorrem em baixas densidades. Em nossas buscas, encontramos apenas um trabalho focado na coleta e identificação molecular de fezes de mamíferos conduzido na Mata Atlântica do nordeste (DE OLIVEIRA et al., 2022a). O trabalho destes autores focou na detecção de cervídeos do gênero Mazama, contudo, apesar do auxílio de cães farejadores, os autores não detectaram fezes das espécies focais em Alagoas, tendo sido localizadas apenas na Mata Atlântica abaixo do Rio São Francisco.

As duas espécies de canídeos e felinos nativos identificadas nesse trabalho estão avaliadas com diferentes riscos de extinção. O gato-do-mato é classificado como Em Perigo e Vulnerável de acordo com o Livro Vermelho da Fauna Brasileira Ameaçada de Extinção e a IUCN, respectivamente (PAYAN; DE OLIVEIRA, 2016; TRIGO et al., 2018). Também está incluído no Plano de Ação Nacional para a Conservação dos Pequenos Felinos instaurado em 2014. Por outro lado, o cachorro-do-mato é classificado como Menos Preocupante tanto na avaliação brasileira (BEISIEGEL et al., 2013) como na avaliação global da IUCN (LUCHERINI, 2015). As duas espécies também apresentam diferentes habitats preferenciais, *L. tigrinus* é geralmente associado com habitats florestais (GOULART et al., 2009b; TRIGO et al., 2013b), enquanto *C. thous* é frequentemente associado a áreas abertas (FERRAZ et al., 2010; GOULART et al., 2009b).

Apesar de contrastantes em termos de habitat e status de ameaça, essas duas espécies sofrem de forma similar com a caça, sendo caçadas principalmente porque as pessoas acreditam que assim estariam protegendo seus animais domésticos, principalmente galinhas (DE SOUZA; ALVES, 2014). Também são consideradas espécies com maior adaptabilidade a ambientes com intensa pressão antrópica, ocorrendo em áreas onde outros carnívoros mais sensíveis já podem ter sido extintos (OLIVEIRA-SANTOS et al., 2012; REGOLIN et al., 2017). Em análise de materiais coletados no nordeste brasileiro e depositados em coleções científicas Feijó e Langguth (2013) descreveram *L. tigrinus* como a espécie que apresentou a maior abundância entre os felinos nas coleções visitadas, sendo amplamente distribuída na Caatinga e Mata Atlântica. Por outro lado, *C. thous* está entre os carnívoros com maior número de registros em coleções científicas (FEIJÓ; LANGGUTH, 2013). Apesar de *L. tigrinus* ter sido a única espécie de felino detectada no nosso estudo, não se trata de uma espécie abundante. Em levantamentos de fauna na região da Mata Atlântica, *L. tigrinus* frequentemente ocorre em menos áreas e é menos abundante em comparação com *C. thous* (BELTRÃO, 2019; CHIARELLO, 1999; RIOS et al., 2022; ROSSANEIS, 2014), como observado neste trabalho. *L. tigrinus* tem hábitos noturnos, podendo apresentar flexibilidade na presença de outras espécies de felinos, e ocorre em baixas densidades (7 a 13 indivíduos/100 km^2), o que dificulta sua deteção (DE OLIVEIRA et al., 2020; OLIVEIRA-SANTOS et al., 2012). Por outro lado, *C. thous* também tem padrão de atividade noturno, mas é frequentemente considerado uma espécie abundante entre os canídeos e felinos (70 a 94 indivíduos/100 km^2) (FARIA-CORRÊA et al., 2009; MONTEIRO-ALVES et al., 2019). Contudo, essa espécie tem sido registrada em baixa abundância em levantamentos mais recentes conduzidos no CEP, o que pode estar relacionado a uma real baixa abundância dessa espécie no CEP ou ao desenho amostral empregado pelos autores que focou na amostragem no interior dos remanescentes de Mata Atlântica (ALBUQUERQUE, 2017; BELTRÃO, 2019). Com a distribuição dos transectos amostrais em áreas com diferentes porcentagens de cobertura florestal empregada aqui, pudemos detectar espécies associadas a habitats florestais e áreas abertas. Considerando o número de deteções, a maior utilização de áreas abertas por *C. thous* e a maior extensão das plantações de cana-de-açúcar e pastagens em relação aos remanescentes florestais, *C. thous* provavelmente é a espécie dentre os canídeos e felinos mais abundante do CEP.

Dentre as espécies que ocorrem no CEP, nós esperávamos registrar também a presença da jaguatirica (*Leopardus pardalis*) nas áreas amostradas, em especial na área mais preservada (ESEC Murici). *L. pardalis* já foi registrada em fragmentos florestais do CEP na Paraíba (ALBUQUERQUE, 2017; BELTRÃO et al., 2019) e em Pernambuco (RAMOS, 2022) com o uso de armadilhamento fotográfico. Na Reserva Biológica Guaribas na Paraíba, *L. pardalis* teve maior número de registros do que *L. tigrinus* (BELTRÃO, 2019). Em Alagoas, foi registrada na Mata de Coimbra (área também amostrada neste trabalho), juntamente com *L. tigrinus*, por avistamento em transecto linear conduzido em 2002 (FERNANDES, 2003), tendo sido feito apenas um registro de cada espécie. Contudo, nós não detectamos *L. pardalis* em nenhuma das áreas, apesar da metodologia de identificação molecular das fezes ser eficiente para a detecção e estudo dessa espécie (SARANHOLI, 2018). Nossos resultados podem indicar uma ausência ou reduzida abundância de *L. pardalis* nas áreas estudadas. A redução de espécies especializadas em florestas, como a jaguatirica, em paisagens fragmentadas e cercadas por plantações de cana-de-açúcar já foi documentada para a mais estudada região de Mata Atlântica do estado de São Paulo, onde foi observada a substituição das espécies especialistas por espécies exóticas e/ou espécies com maior preferência por habitats não florestais, como o cachorro doméstico e o cachorro-do-mato (BECA et al., 2017). É possível que a não detecção de *L. pardalis* no estado de Alagoas esteja associada a presença de cães domésticos e/ou ao reduzido tamanho dos fragmentos florestais de Mata Atlântica (MASSARA et al., 2015). Outra possibilidade é que, apesar da metodologia de coleta de fezes em transectos já ter sido empregada com sucesso para amostragem de *L. pardalis* em outras regiões (DEMATTEO et al., 2014; FARRELL; ROMAN; SUNQUIST, 2000; SARANHOLI, 2018), é possível que para *L. pardalis* no CEP a coleta de fezes tenha maior sucesso quando focada na amostragem de latrinas (RODGERS et al., 2014, 2015). Ainda, apesar de uma das amostras ter sido identificada apenas a nível do gênero Leopardus, que poderia indicar a presença de *L. pardalis*, não podemos afirmar a presença dessa espécie baseado apenas na informação da sequência obtida, pois apenas uma amplificação bem-sucedida foi alcançada para essa amostra.

Apesar de termos identificado fezes de cães domésticos em apenas três dos 44 transectos, nós observamos sua presença ou evidências nos arredores de todas as áreas de referência, sozinhos ou acompanhados de humanos, indicando que estão amplamente distribuídos em todas as áreas amostradas. Os cães domésticos também podem apresentar impactos negativos para as espécies detectadas, pela transmissão de doenças, interações agonísticas ou competição por recursos (CARNIELI et al., 2008; DE OLIVEIRA et al., 2020; LEMOS et al., 2011; VANAK; GOMPPER, 2010), potencialmente estando presentes em maior abundância do que as espécies nativas (PASCHOAL et al., 2016). Dessa forma, os efeitos que os cães domésticos exercem na distribuição e padrão de atividades dos carnívoros nativos do CEP merecem maiores estudos.

Por fim, *C. thous* e *L. tigrinus* apresentam questões de isolamento genético de indivíduos do CEP com relação a outras ecorregiões em que ocorrem, ressaltando a importância das populações do CEP (TCHAICKA et al., 2007; TRIGO et al., 2013a; TRINDADE et al., 2021). Baseado em dados de cinco regiões do DNA nuclear e mitocondrial, Tchaicka et al. (2007) apontaram alta diversidade genética e a existência de dois clados filogenéticos para *C. thous*, um compreendendo a Mata Atlântica nordestina e Caatinga, e outro compreendendo o Cerrado, Pantanal e a Mata Atlântica ao sul, com a presença de porções da Amazônia em ambos. Para *L. tigrinus*, Nascimento e Feijó (2017) sugerem, baseado em dados morfológicos, que os indivíduos da Mata Atlântica do nordeste, Caatinga, Cerrado e regiões da Amazônia compreendam a espécie

L. emiliae, enquanto *L. tigrinus* estaria restrito à Amazônia. Dados genéticos provenientes de SNPs, microssatélites e regiões do DNA nuclear e mitocondrial confirmam a existência de um clado nordestino de *L. tigrinus* (TRIGO et al., 2013a; TRINDADE et al., 2021). Contudo, a filogenia dos chamados tigrina é complexa, podendo haver plesiomorfismo dentro do grupo, o que dificulta a identificação das espécies (TRINDADE et al., 2021).

6.4 IMPLICAÇÕES PARA A CONSERVAÇÃO

A baixa riqueza de espécies detectadas aqui, em comparação com remanescentes de Mata Atlântica fora do CEP, é mais um indicativo do processo de defaunação no CEP. Contudo, também indicam como as unidades de conservação, como a ESEC Murici, são fundamentais para a preservação da biodiversidade, considerando que o ameaçado *L. tigrinus* foi registrada apenas nesta área. Os resultados deste trabalho reforçam o potencial do DNA fecal em registrar espécies ameaçadas, crípticas e que ocorrem em baixa densidade, ou seja, são consideradas raras, como o caso de *L. tigrinus*. Mesmo com o relativo baixo sucesso de amplificação das amostras coletadas, provavelmente pela falta de um controle rígido para a idade das fezes, observamos que as fezes podem ser utilizadas como fonte de material genético do cachorro-do-mato e do gato-do-mato para estudos no CEP. A coleta sistemática de fezes repetida em diversos períodos amostrais, controlando para a idade das fezes, e associada a análises genéticas, em especial aquelas baseadas em marcadores de herança biparental (microssatélites e SNPs) podem ser empregadas para melhor entender os parâmetros demográficos dessas espécies (número mínimo de indivíduos, sexo, parentesco, etc.), para estudar o fluxo gênico, diversidade genética, entre outras aplicações. Dessa forma, estudos futuros conduzidos empregando essa metodologia podem nos ajudar a melhor compreender os efeitos da paisagem fragmentada do CEP nas populações de cachorro-do-mato e gato-do-mato, auxiliando planejamento de ações de conservação.

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CONSIDERAÇÕES FINAIS

Ao longo deste trabalho, pudemos compreender melhor a distribuição dos mamíferos de médio e grande porte em toda a extensão da Mata Atlântica brasileira. Encontramos claros indícios da vulnerabilidade da comunidade de mamíferos desse bioma as mudanças climáticas preditas para o futuro, bem como da reduzida comunidade de mamíferos presentes no Centro de Endemismo Pernambuco. Também avaliamos a amostragem genética de fezes como uma alternativa viável para a detecção de espécies consideradas raras, como é o caso de muitas espécies de carnívoros que apresentam comportamento elusivo, são ameaçadas e/ou ocorrem em baixas densidades. Concluímos que essa abordagem, que tem sido amplamente aplicada, mas ainda não explorada no CEP, é capaz de produzir dados tão eficazes quanto outras metodologias de amostragem. Procuramos otimizar os esforços de amostragem no ameaçado CEP, provendo métricas importantes para auxiliar na aplicação de metodologias de amostragem de mamíferos não invasivas, incluindo a amostragem genética de fezes, e demonstrando a eficácia dessas metodologias em amostrar espécies raras. Por fim, aplicamos com sucesso a metodologia da identificação molecular das fezes de canídeos e felídeos no estado que abriga a maior área de Mata Atlântica no CEP. De forma geral, os resultados apontam para a importância e ameaça do Cendro de Endemismo Pernambuco e guiam novos esforços de pesquisa para a região.

No Capítulo I, foi demonstrado que as mudanças climáticas preditas para o futuro, em especial aquelas que levam a um maior aumento da temperatura máxima são uma séria ameaça as espécies de mamíferos da Mata Atlântica brasileira. A maioria das espécies respondeu negativamente frente as mudanças climáticas preditas para os próximos 60 a 80 anos, reduzindo sua distribuição. A intensidade e velocidade dos efeitos negativos das mudanças climáticas dependerá dos efeitos sinergísticos e aditivos de outras ameaças antrópicas, como as mudanças do uso do solo. Medidas para a mitigação dos efeitos das mudanças climáticas são imperativas para todo o território da Mata Atlântica, em especial para as áreas de contato com os biomas Caatinga e Cerrado, visando frear a perda de riqueza de espécies e, consequentemente, da história evolutiva dos refúgios. Ainda, os padrões de endemicidade e riqueza das espécies encontrados são congruentes com os refúgios propostos por Carnaval e Moriz (2008). Apesar de haver certa discussão sobre a influência dos refúgios paleoclimáticos na distribuição das espécies, nossos resultados apontam que o padrão de distribuição dos mamíferos de 21 000 anos atrás foi influenciada pela dinâmica de contração e expansão da floresta.

No Capítulo II, demonstramos as tendências, o esforço amostral necessário e a eficácia do uso do DNA fecal para a amostragem de carnívoros considerados caros a partir de 250 trabalhos científicos compilados por meio de uma revisão sistemática da literatura. O DNA presente nas fezes tem sido amplamente utilizado para a identificação molecular de espécie, individual e do sexo dos indivíduos presentes em uma determinada área. Para além dessas aplicações, o DNA fecal também é utilizado para fornecer importantes dados para a conservação das espécies, como parâmetros demográficos, parentesco, fluxo gênico e conectividade, uso preferencial de habitats, entre outros. Para otimizar as coletas de fezes visando análises genéticas, as amostras fecais devem ser coletadas o mais frescas o possível, considerando o tempo necessário para a deposição das fezes e o tempo de degradação do DNA. Apesar da grande variabilidade decorrente das diferentes espécies estudas, locais de estudo e suas condições ambientais específicas, calculamos um esforço amostral médio de aproximadamente 2,5 km de transectos percorridos para a coleta de uma amostra fecal molecularmente identificada da espécie focal. Para os trabalhos que compararam o DNA fecal e do armadilhamento fotográfico para detecção de uma espécie focal, o esforço amostral médio foi de 3,8 km de transectos percorridos ou 25 armadilhamento-dias, respectivamente. Os resultados desse capítulo visam guiar novos estudos focados em estudar carnívoros raros, otimizando esforços amostrais e reduzindo custos associados a análises moleculares.

No Capítulo III, tendo em vista a reduzida comunidade de mamíferos, fruto de um severo processo de defaunação e da necessidade de produzir mais informações para a região do CEP, focamos nossos esforços de amostragem em uma área representativa da região (RPPN Mata do Cedro). Somando todas as metodologias não invasivas aplicadas, identificamos 13 espécies de mamíferos de médio porte, valor de riqueza compatível com outros fragmentos de tamanho similar já avaliados no CEP, mas menor que em outros fragmentos de Mata Atlântica fora do CEP. Dentre as metodologias aplicadas, o armadilhamento fotográfico detectou mais espécies, mas apresentou menor sucesso de detecção proporcional, ou seja, menor número de detecções por esforço amostral. A amostragem de fezes seguida da identificação molecular detectou menos espécies, mas apresentou maior sucesso de detecção proporcional para as duas espécies registradas por essa metodologia (*Cerdocyon thous* e cachorro doméstico) e menor latência de detecção, ou seja, detectou essas espécies mais rápido. Sugerimos que a combinação de diferentes metodologias de amostragem reduz os vieses de amostragem que cada metodologia apresenta individualmente e otimiza a detecção de espécies. Também sugerimos que um curto período de coleta (30 dias) parece ser suficiente para realizar um levantamento compreensivo da comunidade de mamíferos de áreas de tamanho similar quando metodologias complementares são aplicadas. Ainda, este estudo serviu como um estudo piloto para a aplicação da metodologia do DNA fecal para detecção de mamíferos no Centro de Endemismo Pernambuco. A experiência e resultados obtidos nesse estudo foram utilizados para o delineamento experimental do estudo apresentado no Capítulo IV.

No Capítulo IV, demonstramos que a amostragem de fezes é uma fonte valiosa de material genético das espécies *Cerdocyon thous* e *Leopardus tigrinus* no CEP. Também demonstramos como a amostragem não invasiva pode evidenciar a presença de espécies elusivas e de baixa detecção, como o caso de *L. tigrinus.* Contudo, também observamos a reduzida comunidade principalmente de felinos da região, com apenas um registro de *L. tigrinus* nos últimos 20 anos, ressaltando novamente os efeitos da defaunação no CEP. Ainda, argumentamos que o DNA derivado das fezes apresenta alto potencial para ser utilizado em análises populacionais, visando melhor entender a conectividade entre os fragmentos florestais remanescentes do CEP, bem como a diversidade genética das populações, fornecendo subsídios para ações de conservação das espécies detectadas.

Nossos resultados destacam o status de ameaça da Mata Atlântica devido a atividades antrópicas contemporâneas. Focamos em fornecer dados que contribuam para pesquisas mais eficientes e com maior custo-benefício visando monitorar as espécies e entender melhor sua distribuição e grau de ameaça em pequenas e largas escalas. O processo de defaunação observado no Centro de Endemismo Pernambuco provavelmente será agravado pelas mudanças climáticas, sendo necessárias medidas imediatas de mitigação para a possível reversão desses efeitos. Muitas propriedades privadas localizadas no bioma da Mata Atlântica encontram-se em desacordo com a legislação vigente, sendo necessários o reflorestamento e restauração da vegetação remanescente (REZENDE et al., 2018). Se realizado, esse processo pode proteger a biodiversidade dos efeitos das mudanças climáticas, bem como prover condições para populações maiores e mais conectadas entre si, sendo fundamental sua implementação especialmente no CEP. A preservação da biodiversidade não somente protege espécies, indivíduos ou habitats, mas também preserva serviços ecossistêmicos insubstituíveis para a humanidade, promovendo maior qualidade de vida e o acesso das futuras gerações a condições dignas de sobrevivência.

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APÊNDICES

APÊNDICE A – MATERIAL SUPLEMENTAR REFERENTE AO CAPÍTULO I.

Table S1 – Medium and large mammal species occurring in the Brazilian Atlantic Forest per refugia based on the distribution maps available at the IUCN (International Union for Conservation of Nature and Natural Resources) assessments, the Red Book of Brazilian Fauna Threat of Extinction (INSTITUTO CHICO MENDES DE CONSERVAÇÃO DA BIODIVERSIDADE, 2018) and the Extinction Risk Assessments published by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, Ministério do Meio Ambiente), inclusion in the species distribution model (SDM) and phylogenetic diversity analysis (PD), and summary statistics for the best SDM model. 1 indicates occurrence in the refugium, and 0 indicates absence; X indicates the inclusion in the analysis and * indicates non-inclusion.

Species	**IUCN status**	**Pernambuco**	**Bahia**	**São Paulo**	**SDM**	**PD**	**N of unique occurrences used in SDM**	**Omission rate**	**AUC**
Alouatta belzebul	Vulnerable	1	0	0	X	X	16	0.066	0.767
Alouatta caraya	Near Threatened	0	0	1	X	X	19	0.315	0.798
Alouatta guariba	Vulnerable/Critically Endangered	0	1	1	X	X	405	0.004	0.751
Blastocerus dichotomus	Vulnerable	0	0	1	*	X	*	*	*
Brachyteles arachnoides	Critically Endangered	0	0	1	X	X	32	0.031	0.709
Brachyteles hypoxanthus	Critically Endangered	0	1	1	X	X	15	0	0.908
Bradypus torquatus	Vulnerable	0	1	1	X	X	110	0.058	0.667
Bradypus variegatus	Least Concern	1	1	1	X	X	168	0.006	0.837
Cabassous tatouay	Least Concern	1	1	1	X	X	128	0.047	0.774
Cabassous unicinctus	Least Concern	0	1	1	X	X	38	0	0.604
Callicebus barbarabrownae	Critically Endangered	0	1	0	X	X	17	0	0.772
Callicebus coimbrai	Endangered	0	1	0	*	X	*	*	*

Callicebus melanochir	Vulnerable	0	1	0	*	X	*	*	*
Callicebus nigrifrons	Near Threatened	0	1	1	X	X	36	0.027	0.737
Callicebus personatus	Vulnerable	0	1	1	*	X	*	*	*
Callithrix aurita	Endangered	0	0	1	X	X	78	0.012	0.645
Callithrix flaviceps	Critically Endangered	0	0	1	X	X	22	0.045	0.771
Callithrix geoffroyi	Least Concern	0	1	1	X	X	62	0.023	0.644
Callithrix jacchus	Least Concern	1	1	1	X	X	157	0	0.745
Callithrix kuhlii	Vulnerable	0	1	0	X	X	40	0.05	0.707
Callithrix penicillata	Least Concern	0	1	1	X	X	191	0	0.688
Cavia aperea	Least Concern	1	1	1	X	X	58	0.125	0.689
Cavia fulgida	Least Concern	0	1	1	*	X	*	*	*
Cavia magna	Least Concern	0	0	1	*	X	*	*	*
Cerdocyon thous	Least Concern	1	1	1	X	X	766	0.003	0.728
Chaetomys subspinosus	Vulnerable	0	1	1	X	X	38	0.027	0.763
Chrysocyon brachyurus	Near Threatened	0	1	1	X	X	271	0	0.663
Coendou insidiosus	Least Concern	0	1	1	X	X	26	0.041	0.599
Coendou prehensilis	Least Concern	1	1	1	X	X	16	0	0.630
Coendou speratus	Not assessed	1	0	0	*	X	*	*	*
Coendou spinosus	Least Concern	0	1	1	X	X	114	0.038	0.658
Conepatus chinga	Least Concern	0	0	1	X	X	49	0.020	0.650
Conepatus semistriatus	Least Concern	1	1	1	X	X	129	0.015	0.583
Cuniculus paca	Least Concern	1	1	1	X	X	101	0.025	0.811
Cyclopes didactylus	Data Deficient	1	0	0	*	X	*	*	*

Dasyprocta azarae	Data Deficient	0	1	1	X	X	76	0.027	0.593
Dasyprocta iacki	Data Deficient	1	1	0	*	*	*	*	*
Dasyprocta leporina	Least Concern	0	1	1	X	X	35	0.030	0.882
Dasyprocta prymnolopha	Least Concern	1	1	0	*	X	*	*	*
Dasypus hybridus	Near Threatened	0	0	1	*	X	*	*	*
Dasypus novemcinctus	Least Concern	1	1	1	X	X	1061	0	0.729
Dasypus septemcinctus	Least Concern	0	1	1	X	X	119	0.033	0.707
Didelphis albiventris	Least Concern	1	1	1	X	X	308	0	0.718
Didelphis aurita	Least Concern	1	1	1	X	X	341	0.009	0.772
Eira barbara	Least Concern	1	1	1	X	X	461	0	0.740
Euphractus sexcinctus	Least Concern	1	1	1	X	X	416	0.002	0.696
Galea spixii	Least Concern	1	0	0	*	X	*	*	*
Galictis cuja	Least Concern	1	1	1	X	X	184	0.048	0.678
Herpailurus yagouaroundi	Least Concern	1	1	1	X	X	322	0.003	0.743
Hydrochoerus hydrochaeris	Least Concern	1	1	1	X	X	286	0.021	0.664
Leontopithecus caissara	Endangered	0	0	1	*	X	*	*	*
Leontopithecus chrysomelas	Endangered	0	1	1	X	X	21	0	0.709
Leontopithecus chrysopygus	Endangered	0	0	1	X	X	26	0.192	0.570
Leontopithecus rosalia	Endangered	0	0	1	*	X	*	*	*
Leopardus colocolo	Near Threatened	0	1	1	*	X	*	*	*
Leopardus geoffroyi	Least Concern	0	0	1	*	X	*	*	*
Leopardus guttulus	Vulnerable	0	1	1	X	*	364	0.002	0.591

Leopardus pardalis	Least Concern	1	1	1	X	X	481	0	0.730
Leopardus tigrinus	Vulnerable	1	1	0	X	X	24	0	0.718
Leopardus wiedii	Near Threatened	1	1	1	X	X	264	0.060	0.700
Lontra longicaudis	Near Threatened	1	1	1	X	X	264	0.003	0.782
Lycalopex gymnocercus	Least Concern	0	0	1	X	*	60	0	0.597
Lycalopex vetulus	Near Threatened	0	1	1	X	*	94	0	0.646
Mazama americana	Data Deficient	0	1	1	X	X	92	0.010	0.798
Mazama gouazoubira	Least Concern	1	1	1	X	X	229	0.013	0.817
Mazama jacunda	Vulnerable	0	0	1	X	X	36	0.055	0.845
Mazama nana	Vulnerable	0	0	1	X	X	60	0.016	0.658
Myocastor coypus	Least Concern	0	0	1	X	X	90	0.224	0.564
Myrmecophaga tridactyla	Vulnerable	0	1	1	X	X	221	0	0.760
Nasua nasua	Least Concern	1	1	1	X	X	569	0	0.736
Ozotoceros bezoarticus	Vulnerable	0	0	1	*	X	*	*	*
Panthera onca	Near Threatened	0	1	1	X	X	146	0.164	0.572
Pecari tajacu	Least Concern	1	1	1	X	X	140	0.042	0.811
Potos flavus	Least Concern	1	1	1	X	X	36	0	0.857
Priodontes maximus	Vulnerable	0	1	1	X	X	16	0.125	0.582
Procyon cancrivorus	Least Concern	1	1	1	X	X	526	0.003	0.770
Puma concolor	Least Concern	1	1	1	X	X	492	0	0.763
Sapajus cay	Vulnerable	0	0	1	*	X	*	*	*
Sapajus flavius	Endangered	1	0	0	X	X	19	0.176	0.716
Sapajus libidinosus	Near Threatened	1	0	1	*	X	*	*	*
Sapajus nigritus	Near Threatened	0	1	1	X	X	159	0.006	0.710
Sapajus robustus	Endangered	0	1	0	*	X	*	*	*

Sapajus xanthosternos	Critically Endangered	0	1	0	X	X	21	0.045	0.759
Speothos venaticus	Near Threatened	0	1	1	X	X	16	0	0.815
Sylvilagus brasiliensis	Endangered	1	1	1	X	X	64	0.031	0.774
Tamandua tetradactyla	Least Concern	1	1	1	X	X	671	0	0.728
Tapirus terrestris	Vulnerable	0	1	1	X	X	114	0.007	0.811
Tayassu pecari	Vulnerable	0	1	1	X	X	65	0.015	0.880
Tolypeutes tricinctus	Vulnerable	0	1	0	*	X	*	*	*

Table S2 – Databases and data sets used to obtain occurrence records.

Source	Type	Description	Available at
SALVE	Database	Biodiversity Conservation Status Assessment System – ICMBio, 2-23: organize species occurrence records that are reviewed by specialists to be used in biodiversity status assessment by Brazilian governmental agencies.	salve.icmbio.gov.br
GBIF	Database	Imports curated records from other databases such as iNaturalist	www.gbif.org
SpeciesLink	Database	SpeciesLink is a distributed information system that integrates primary data from scientific collections	www.specieslink.cria.org.br
Souza et al. (2019)	Data set	ATLANTIC MAMMALS: a data set of assemblages of medium- and large-sized mammals of the Atlantic Forest of South America	
Culot et al. (2018)	Data set	ATLANTIC-PRIMATES: a dataset of communities and occurrences of primates in the Atlantic Forests of South America	
Santos et al. (2019)	Data set	NEOTROPICAL XENARTHRANS: a data set of occurrence of xenarthran species in the Neotropics	
Nagy-Reis et al. (2020)	Data set	NEOTROPICAL CARNIVORES: a data set on carnivore distribution in the Neotropics	
de Oliveira et al. (2022)	Data set	Elusive deer occurrences at the Atlantic Forest: 20 years of surveys	

Table S3 – Characteristics of mean annual precipitation and mean annual temperature increase compared to baseline years range (1981-2010) in each tested General Circulation Model (GCM) for the years of 2081 to 2100.

GCM	Precipitation	Temperature
MRI-ESM2-0	High increase	Moderate increase
CanESM5	High decrease	High increase
MIROC6	Fluctuations	Fluctuations
INM-CM4-8	Low decrease	Low increase

Table S4 – Metrics of phylogenetic diversity adjusted for species richness measured for the three Atlantic Forest refugia. Significant departures from null expectations are indicated with asterisk (*) representing phylogenetic overdispersion. No phylogenetic clustering pattern was detected. PD_{Faith}: Faith's phylogenetic diversity; MPD: mean pairwise distance; MNTD: mean nearest taxon distance, all by standardized effect size (ses).

	Observed metric in community	Mean metric in null communities	Standardized effect size *vs* null communities	p-value (KEMBEL et al., 2010)
		ses.PD_{Faith}		
Pernambuco	1340.91	1138.28	2.09	0.99*
Bahia	1548.37	1484.13	0.98	0.85
São Paulo	1594.42	1581.19	0.27	0.53
		ses.MPD		
Pernambuco	163.58	150.18	1.70	0.95*
Bahia	156.03	150.37	1.45	0.98*
São Paulo	153.84	150.27	1.25	0.95*
		ses.MNTD		
Pernambuco	40.81	32.29	1.32	0.91
Bahia	19.28	21.35	-0.63	0.29
São Paulo	16.50	19.16	-1.07	0.13

Figure S1 – Species richness lost (red) or gained (green) in each future (2080-2100) scenario tested in comparison to present species richness.

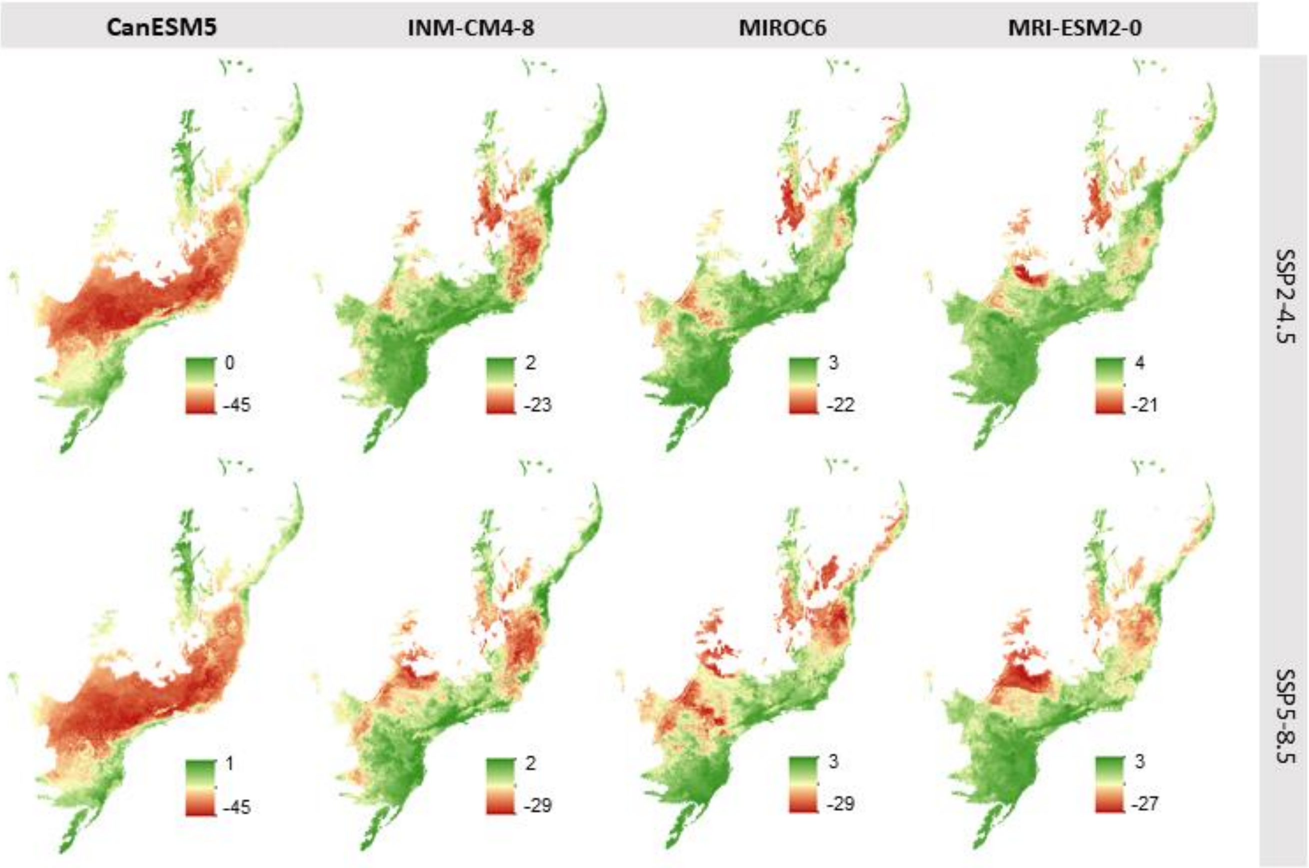


Figure S2 – Percentage of predicted changes in distribution of mammal species analyzed per refugia in the four GCM under the SSP5-8.5.

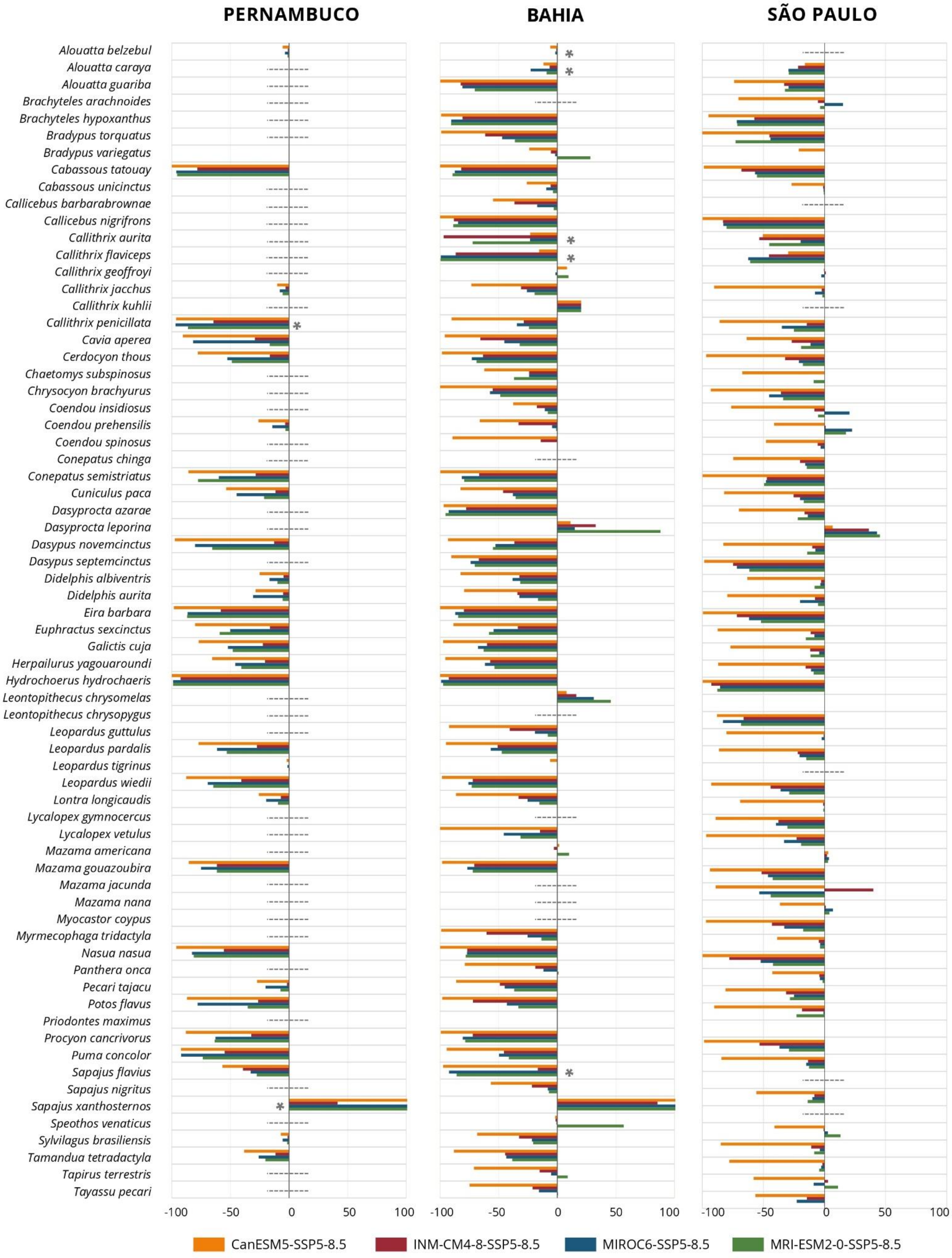
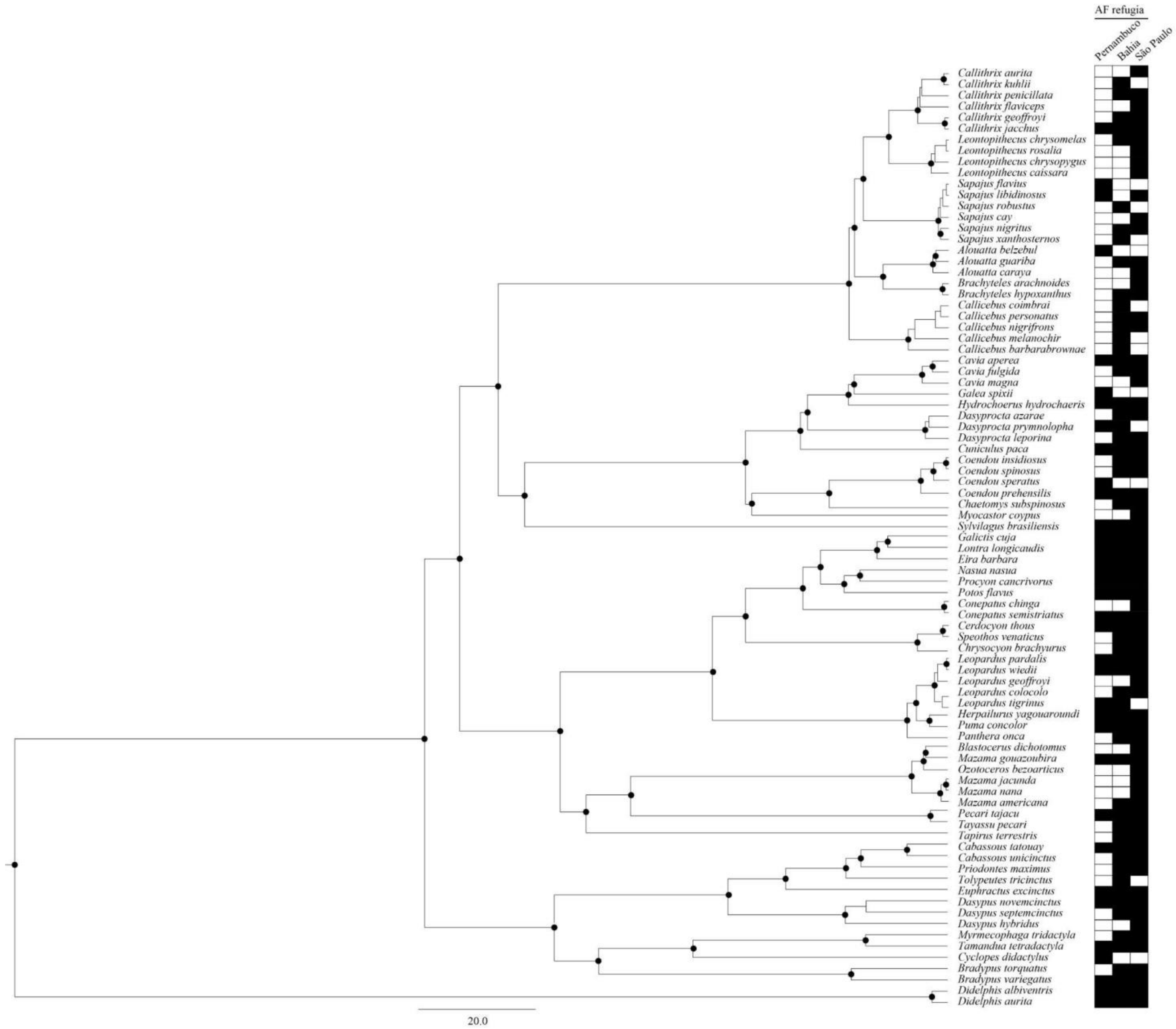


Figure S3 – Bayesian phylogenetic analysis based on 31 gene-matrix (UPHAM et al., 2019) and occurrence site (black square) for each mammal species. Nodes with black circles represent posterior probabilities above 0.9.



APÊNDICE B – MATERIAL SUPLEMENTAR REFERENTE AO CAPÍTULO II.

Table S5 – Information extracted from the 250 papers included in this review.

Table S6 – Information extracted from papers that disclaimed total sampling effort in the form of kilometers traveled and number of molecularly identified species and for papers that employed both fecal DNA survey and camera trapping.

Table S7 – Information extracted from papers that compared the fecal DNA approach with an alternative sampling method.

Supplementary Material S1 – R script used for the analysis of effect sizes, based on the script made available by Carvalho et al. (2022)

APÊNDICE C – MATERIAL SUPLEMENTAR REFERENTE AO CAPÍTULO III.

Supplementary material S2 – A video record of a *Sylvilagus brasiliensis* individual jumping over the hair trap.

O download direto de uma pasta comprimida (.zip) contendo todos os arquivos dos Apêndices B e C pode ser feito clicando no link abaixo ou copiando no navegador:
https://drive.google.com/uc?export=download&id=1OEamdHudSxJFcoVpUupcnaHvZExxMx1m