UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de São José do Rio Preto

Ricardo Quiterio Sartori

Análises de diversidade e estrutura genética para a conservação de tamanduás-bandeiras (*Myrmecophaga tridactyla*) no estado de São Paulo, Brasil

São José do Rio Preto
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Comissão Examinadora

Profª. Drª Lilian Castiglioni
UNESP / IBILCE – Universidade Estadual Paulista
Orientadora

Profª. Drª. Lilian Madi Ravazzi
UNESP / IBILCE – Universidade Estadual Paulista

Prof. Dr. Kaio César Chaboli Alevi
UNESP / IBB – Universidade Estadual Paulista

Prof. Dr. Luís Gustavo da Conceição Galego
UFTM - Universidade Federal do Triângulo Mineiro

Prof. Dr. Tiago Lucena da Silva
UFAC - Universidade Federal do Acre

São José do Rio Preto
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“Tudo o que você toca. Você muda. Tudo o que você muda. Muda você. A única verdade perene. É a mudança”

Octavia E. Butler (2018, p. 6)

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RESUMO

O tamanduá-bandeira (*Myrmecophaga tridactyla*) tem ampla distribuição na região Neotropical e é encontrado em todos os biomas brasileiros, no entanto, é classificado como vulnerável pela IUCN em nível global, nacional e estadual. As ameaças à espécie são relacionadas à forte pressão antrópica e os efeitos da fragmentação ou perda de habitat podem ser avaliados a partir da diversidade genética e características da paisagem. O objetivo do trabalho foi avaliar a diversidade genética e a estrutura populacional da espécie no estado de São Paulo, utilizando os marcadores moleculares SRY, 16S, RAG2 e DRB, em conjunto com microssatélites (SSR). As amostras foram obtidas a partir de resgate e atendimentos em Hospital Veterinário, além de amostras cedidas de campanhas de capturas em Estação Ecológica, totalizando 41 indivíduos amostrados, por meio do Esquema de Amostragem Individual (ISS). Após a amplificação, genotipagem e sequenciamento de DNA, diferentes análises estatísticas foram realizadas em programas específicos. A análise do gene SRY permitiu a identificação precisa do sexo dos indivíduos, o 16S não apresentou polimorfismos, sendo altamente conservado na espécie. O RAG2 foi utilizado para inferir eventos históricos, apresentou quatro haplótipos e revelou baixa diversidade genética (diversidade nucleotídica=0,0039 e diversidade haplotípica=0,273). Por outro lado, o gene DRB foi utilizado para inferir eventos recentes devido à alta taxa de mutação, assim como os SSR. Os resultados do gene DRB indicaram alta diversidade genética, mesmo para um gene adaptativo, revelando a presença de 21 alelos SSCP distintos em 41 amostras. Os índices de diferenciação genética indicaram de baixa a moderada diferenciação genética entre todos os indivíduos do estado de São Paulo (Fst=0,0322) e alta diferenciação ao comparar apenas as duas populações geográficas em Estações Ecológicas (áreas protegidas), além de correlação entre a as distâncias genéticas e geográficas. Não há evidências de Isolamento por Distância e existem evidências de Isolamento por Barreira, tanto em relação ao Índice de Agregação Alélica (Rjave = 0,467216994; p= 0,026000000), e aos métodos de detecção de barreiras, que identificaram duas barreiras ao fluxo gênico na paisagem. Outros resultados de análise Bayesiana indicaram isolamento populacional e a presença de dois agrupamentos (*clusters*), corroborando os resultados dos SSR. Portanto, a abordagem genética combinada, utilizando marcadores de genes com diferentes taxas de mutação permitiu inferir a diversidade, dinâmica e as características históricas e recentes da população. A análise integrada permitiu inferir que as barreiras detectadas são recentes, causadas provavelmente pelas ações antrópicas. As populações são prioritárias para a conservação e as áreas protegidas servem de reduto para a espécie, mesmo em ambientes altamente antropizados. A proteção dessas populações pode ajudar na conservação de outras espécies e de seu habitat. Além disso, o modelo de estudo apresentado pode servir para ecologistas e conservacionistas como uma abordagem genética viável para auxiliar na conservação e pode ser aplicada a outras espécies e populações, especialmente em áreas fragmentadas. Estratégias de conservação para o tamanduá-bandeira que beneficiem outras espécies devem ser adotadas e os dados científicos, incluindo as informações genéticas, devem ser implementados em políticas públicas, em planos de manejo e para ações de conservação.

Palavras-chave: Genética da Conservação. Xenarthra. Ecótonos. São Paulo.

ABSTRACT

The giant anteater (Myrmecophaga tridactyla) has a wide distribution in the Neotropical region and is found in all Brazilian biomes, however, it is classified as vulnerable by the IUCN at a global, national, and state level. The threats to the species are related to strong anthropic pressure and the effects of fragmentation or habitat loss can be evaluated from the genetic diversity and landscape characteristics. The objective of this work was to evaluate the genetic diversity and population structure of the species in the state of São Paulo, using the molecular markers SRY, 16S, RAG2, and DRB, in conjunction with microsatellites (SSR). The samples were obtained from rescue and consultations at a Veterinary Hospital, in addition to samples provided from capture campaigns at Ecological Station, totaling 41 individuals sampled, through the Individual Sampling Scheme (ISS). After amplification, genotyping, and DNA sequencing, different statistical analyzes were performed in specific programs. The analysis of the SRY gene allowed the precise identification of the sex of the individuals, the 16S did not present polymorphisms, being highly conserved in the species. The RAG2 was used to infer historical events, presented four haplotypes and revealed low genetic diversity (nucleotide diversity=0.0039 and haplotypic diversity=0.273). In contrast, the DRB gene was used to infer recent events due to the high mutation rate, as well as the SSR. The DRB gene results indicated high genetic diversity, even for an adaptive gene, revealing the presence of 21 distinct SSCP alleles in 41 samples. The genetic differentiation indices indicated low to moderate genetic differentiation among all individuals in the state of São Paulo (Fst=0.0322) and high differentiation when comparing only the two geographic populations in Ecological Stations (protected areas), in addition to the correlation between genetic and geographic distances. There is no evidence of Isolation by Distance and there is evidence of Isolation by Barrier, both in relation to the Allelic Aggregation Index (Rjave = 0.467216994; p= 0.026000000) and to the barrier detection methods, which identified two barriers to the gene flow in the landscape. Other Bayesian analysis results indicated population isolation and the presence of two clusters, corroborating the SSR results. Therefore, the combined genetic approach, using markers of genes with different mutation rates, allowed us to infer the diversity, dynamics and the characteristics of historical and recent patterns of the population. The integrated analysis allowed us to infer that the detected barriers are recent, probably caused by anthropic actions. The populations are a priority for conservation and the protected areas serve as a refuge for the species, even in highly anthropic environments. The protection of these populations may help the conservation of other species and their habitat. In addition, the study model presented can serve ecologists and conservationists as a viable genetic approach to aid conservation and can be applied to other species and populations, especially in fragmented areas. Strategies for conservation of the giant anteater that benefit other species must be adopted and scientific data, including genetic information, must be implemented in public policies, management plans, and for conservation actions.

Keywords: Conservation Genetics. Xenarthra. Ecotones. São Paulo.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

AFLP: Polimosfismo de Comprimento de Fragmento Amplificado
CAPES: Coordenação de Aprimoramento de Pessoal de Nível Superior
cpDNA: DNA cloroplastidial
DNA: Ácido desoxirribonucleico
EENP: Estação Ecológica do Noroeste Paulista
EESB: Estação Ecológica de Santa Bárbara
FAMERP: Faculdade de Medicina de São José do Rio Preto
FAPESP: Fundação de Amparao à Pesquisa do estado de São Paulo
FCAV: Faculdade de Ciências Agrárias e Veterinárias
FENP: Floresta Estadual Noroeste Paulista
Fst: Índice de fixação
GABI: Grande Intercâmbio Biótico das Américas
IBGE: Instituto Brasileiro de Geografia e Estatística
IBILCE: Instituto de Biociências, Letras e Ciências Exatas
IF: Instituto Florestal
ISS: Esquema de Amostragem Individual
IUCN: União Internacional para a Conservação da Natureza
Km: Kilômetros
MHC: Complexo de Histocompatibilidade Principal
mtDNA: DNA mitocondrial
NGS: Sequenciamento de Próxima Geração
NP: Noroeste Paulista
Rjave: Distribuição espacial geral de todos alelos e loci
SACCAS: Setor de Atendimento Clínico Cirúrgico de Animais Selvagens
scnDNA: DNA nuclear de cópia única
SMAURB: Secretaria do Meio Ambiente e Urbanismo de São José do Rio Preto
SNPs: Polimosfirmos de Nucleotídeo Único
SP: São Paulo
SSR: Microssatélites / Repetições de Sequência Simples
UNESP: Universidade Estadual Paulista
UNIRP: Centro Universitário de Rio Preto

SUMÁRIO

1. INTRODUÇÃO

1.1 Biologia da Conservação: biodiversidade e ameaças à vida terrestre

A destruição e a fragmentação do habitat são os principais fatores de declínio e extinção de espécies, uma vez que podem aumentar a vulnerabilidade das populações de vida selvagem, reduzindo o espaço e os recursos disponíveis para elas e aumentando a probabilidade de conflito com os seres humanos. Além disso, criam habitats menores que suportam populações menores, que são mais propensas a serem extintas (FRANKHAM; 2005; FRANKHAM *et al.,* 2019).

Desde a metade do último século, foi amplamente reconhecido que a taxa de extinção de espécies estava aumentando e que muitas espécies estavam em perigo iminente de extinção. Os principais fatores relacionados a essas extinções e declínios foram o desmatamento, caça, pesca e outras matanças; perda, degradação e fragmentação do habitat; e introdução de espécies não nativas, patógenos, predadores e competidores (DIAMOND, 1989; HEDRICK; HURT, 2012).

Um número crescente de ecossistemas contendo espécies ameaçadas está desaparecendo e estima-se que, devido às atividades humanas, as taxas atuais de extinção de espécies são cerca de 1.000 vezes maiores do que sem influência antrópica (PIMM *et al.*, 2014). Mais de 27.000 espécies estão em risco de extinção, que incluem 25% são mamíferos, 14% são pássaros e 40% são anfíbios e estima-se que 1 milhão de espécies em todo o mundo podem enfrentar a extinção (DIAZ *et al*., 2019; IUCN, 2021).

Para medir as taxas e riscos de extinção em espécies, diversos fatores são avaliados. Os biólogos da conservação medem e aplicam medidas estatísticas de registros, taxas de perda de habitat e várias outras variáveis como perda de biodiversidade, características genéticas, entre outros aspectos para tentar compreender os padrões de extinções em diferentes escalas (REGAN *et al.,* 2001; MACKENZIE *et al.,* 2003). A criação de ferramentas como a Lista Vermelha de Espécies Ameaçadas da União Internacional para a Conservação da Natureza (IUCN), é essencial para a conservação, pois é a fonte de informação mais abrangente do mundo sobre o status de risco de extinção global de espécies de animais, fungos e plantas. Além disso, é importante criar métodos para otimizar possíveis problemas

relacionados às ferramentas de categorização de espécies ameaçadas (BALMFORD; GREEN; JENKINS, 2003; GARNER; HOBAN; LUIKART, 2020).

A Teoria da Biogeografia de Ilhas é possivelmente uma das contribuições mais significativas para a compreensão científica do processo e das taxas de extinção em espécies, envolvendo fatores que afetam a riqueza de espécies e comunidades, como o equilíbrio dinâmico entre as taxas de imigração e extinção, competição, colonização e equilíbrio, a partir da qual se pode determinar o número de espécies em equilíbrio em uma ilha (MACARTHUR; WILSON, 2001). As estimativas das taxas de extinção reais podem ser calculadas em ordens de magnitude mais altas e por isso, algumas estimativas de taxas de extinção foram criticadas por usar suposições que podem superestimar a gravidade da crise de extinção. Para isso diversas metodologias foram desenvolvidas a fim de evitar a superestimação das taxas de extinção que utilizam suposições extremamente conservadoras (HOFFMANN *et al*., 2010; CEBALLOS *et al.,* 2015).

Vale ressaltar que mesmo em análises extremamente conservadoras, os atuais esforços de conservação permanecem insuficientes para compensar os principais fatores de perda de biodiversidade. Assim, questionamentos surgiram a cerca de uma provável extinção do Holoceno, também conhecida como a sexta extinção em massa ou extinção do Antropoceno, um evento de extinção de espécies em curso que está intimamente ligado ao resultado da atividade humana (DIRZO *et al*., 2014). Com a degradação generalizada de habitats altamente biodiversos, como recifes de corais e florestas tropicais, bem como outras áreas, acredita-se que a maioria dessas extinções não seja documentada, além disso a taxa atual de extinção de espécies é estimada como sendo 100 a 1.000 vezes maior do que as taxas de extinção naturais de fundo, sem os efeitos antrópicos (PIMM *et al.,* 1995; DE VOS *et al.*, 2015)

A atividade humana tornou o período a partir de meados do século 20 diferente o suficiente do resto do Holoceno para considerar o Antropoceno uma nova época geológica (SYVITSKY *et al.,* 2020). Portanto, o Antropoceno fornece uma medida independente da escala e do ritmo das mudanças causadas pelo homem e o Antropoceno é um lembrete de que o Holoceno, durante o qual sociedades humanas complexas se desenvolveram, foi um ambiente estável (STEFFEN *et al*., 2011).

Além disso, a baixa diversidade atual, principalmente de grandes mamíferos em áreas continentais é um fenômeno antropogênico, não natural, com importantes implicações para o manejo da natureza (SANDOM *et al*., 2014)

Desta maneira, à medida que avançamos no Antropoceno, corremos o risco de conduzir o Sistema Terrestre em uma trajetória rumo à extirpação de várias espécies em todo o globo (STEFFEN *et al*., 2011).

Muitas publicações baseadas em métodos independentes demonstram a realidade da crise na biodiversidade. A sexta extinção em massa pode já estar acontecendo ou pode não ter ocorrido ainda, mas taxas elevadas de extinção e diversos declínios populacionais já ocorreram a uma taxa maior do que na ausência de influências antropogênicas (Figura 1). Isto é um fato e a hipótese de que a sexta extinção em massa tenha começado em terra e em água doce é cada vez mais provável (COWIE; BOUCHET; FONTAINE, 2022).

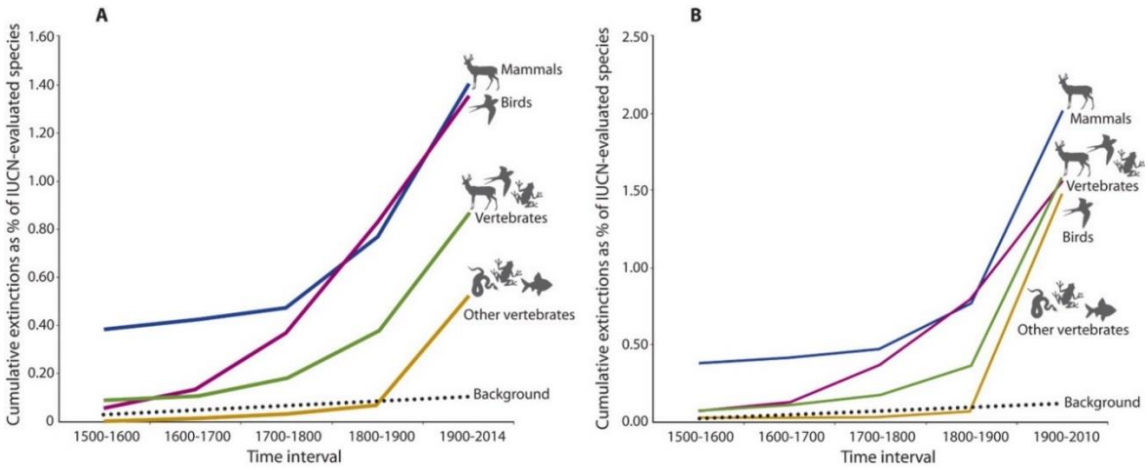

Figura 1. Espécies de vertebrados, registradas como extintas ou extintas na natureza pela IUCN, 2012. Os gráficos mostram a porcentagem do número de espécies avaliadas. A curva preta tracejada representa o número de extinções esperadas. (A) Estimativa altamente conservadora. (B) Estimativa conservadora. Fonte: CEBALLOS *et al*., 2015.

Independente das teorias sobre a sexta extinção em massa, o fato é que a extinção de espécies ocorre em níveis alarmantes e não só estamos perdendo espécies em uma taxa maior do que o normal, como também é evidente que os processos de evolução e especiação não conseguem acompanhar essa perda (CEBALLOS; EHRLICH, 2018).

Devido à essa constante perda da biodiversidade e altas taxas de extinção de espécies, tentativas de estabelecer a conservação da Biodiversidade foram e são cada vez mais necessárias. Assim, a partir de 1970, a Biologia da Conservação surgiu como um novo campo do conhecimento. Uma área interdisciplinar baseada na gestão

de recursos naturais a partir da combinação entre a teoria em ecologia e a genética evolutiva, por um lado, e da política e prática de conservação, por outro (DASHMAN, 1968; EHRENFELD, 1970; SOULÉ, 1985; GERBER, 2010).

A base multidisciplinar inerente para a biologia da conservação levou a novas subdisciplinas, incluindo a ciência social da conservação, o comportamento da conservação, a fisiologia da conservação e a genética da conservação (FRANKHAM, 2005; COOKE *et al*., 2013; BENNETT *et al*., 2017).

Além disso, a disciplina de Biologia da Conservação está intimamente ligada à ecologia populacional, envolvendo a dispersão, migração, demografia, tamanho efetivo da população, depressão endogâmica e viabilidade populacional mínima, principalmente de espécies raras ou ameaçadas de extinção. Os estudos na área permitem a compreensão dos fenômenos que afetam a manutenção, perda e restauração da biodiversidade e a entender processos evolutivos que geram diversidade genética, populacional, de espécies e de ecossistemas (SAHNEY, BENTON, FERRY, 2010).

Portanto, a Biologia da Conservação é o estudo da conservação da natureza e da biodiversidade da Terra com o objetivo de proteger as espécies, seus habitats e ecossistemas de taxas excessivas de extinção entre outras preocupações (MEINE; SOULÉ; NOSS, 2006).

Apesar da perda da biodiversidade mundial e dos esforços realizados, os conservacionistas estão longe de ajudar todas as espécies ameaçadas e estimativas sugerem que até 50% de todas as espécies do planeta desaparecerão nos próximos 50 anos, levando à crise na biodiversidade global e demonstrando a importância de esforços para a conservação de espécies e dos ecossistemas (KOH *et al*., 2004).

Assim, estratégias que visam a conservação de espécies são de extrema importância e uma das estratégias desenvolvidas baseia-se na identificação de "*hotspot*s de biodiversidade" (Figura 2), na qual concentrações de espécies endêmicas sofrem grande perda de habitat. Uma vez que até 44% de todas as espécies de plantas vasculares e 35% de todas as espécies em quatro grupos de vertebrados estão confinados aos *hotspots* que compreendem em torno de apenas 2% da superfície terrestre da Terra, esta pode ser uma estratégia viável para conservação de diversas espécies (MYERS *et al.*, 2000).

Vale ressaltar que há uma controvérsia considerável sobre quais métricas usar para delinear os *hotspots* de biodiversidade e as consequências da aplicação de

diferentes métricas para evitar incongruência entre os locais definidos usando diferentes métricas e diminuir a preocupação com a noção de que os locais de riqueza de espécies devem se sobrepor àqueles identificados usando outras métricas de diversidade (POSSINGHAM; WILSON, 2005). Porém, embora existam controvérsias, os *hotspots* de biodiversidade são alvos promissores de conservação para mitigar as perdas e continuam sendo prioridades como estratégia para a conservação da biodiversidade em todas as regiões biogeográficas (NOSS *et al*., 2015; CARTWRIGHT, 2019).

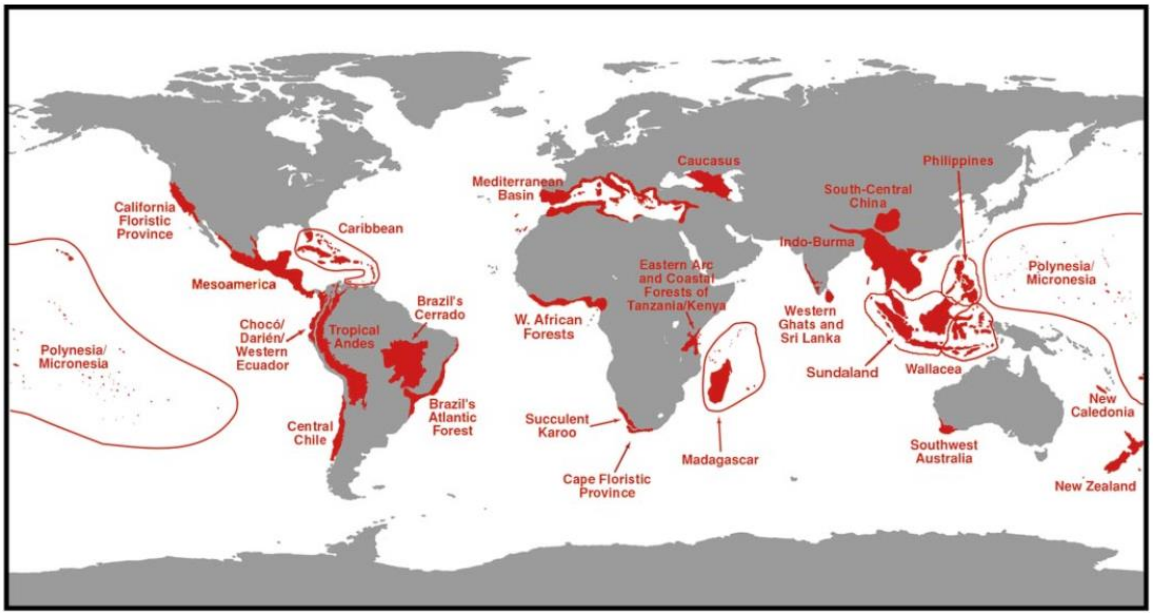

Figura 2. Mapa indicando os principais *hotspots* de biodiversidade. Fonte: MYERS *et al.*, 2000

Em relação às regiões biogeográficas, uma das regiões de maior biodiversidade e que apresenta ecossistemas amplamente diversos é a região Neotropical, o qual compreende a América Central, incluindo a parte sul do México e da península da Baixa California, o sul da Flórida, as ilhas do Caribe e a América do Sul (MORRONE, 2014; ANTONELLI, 2021).

As hipóteses para explicar a alta diversidade e endemismo na região Neotropical estão frequentemente relacionadas à heterogeneidade climática, geralmente associada à expectativa de que espécies tropicais tenham tolerâncias fisiológicas restritas. Essa heterogeneidade climática, tanto histórica quanto contemporânea afetaria diretamente a distribuição das linhagens por meio de

restrições fisiológicas espécie-específicas e indiretamente por meio da aptidão a parasitas, patógenos, predadores e competidores (CARNAVAL *et al*., 2014).

A região Neotropical é uma das mais importantes reservas de biodiversidade da Terra e isso demonstra a importância de estudos para preservação de recursos naturais tanto em áreas protegidas e não protegidas, além da conservação de espécies e seus habitats (MAGIOLI *et al.*, 2021; BURBANO-GIRÒN *et al*., 2022).

Dentre os *hotspots* de biodiversidade na região Neotropical, mais especificamente na América do Sul podemos destacar a biodiversidade encontrada no território brasileiro, uma região mega diversa devido ao seu grande número de espécies e endemismos. O Brasil é considerado o país com a maior biodiversidade de qualquer país do planeta e é o segundo país do mundo com as espécies mais endêmicas (COSTA *et al.*, 2005)

Essa alta diversidade de fauna pode ser explicada em parte pelo tamanho do Brasil e pela grande variação em ecossistemas como Floresta Amazônica, Mata Atlântica e Cerrado, os quais encontram-se altamente ameaçados por ações antrópicas. Devido à ascensão econômica e demográfica do país no último século, a capacidade do Brasil de proteger seus habitats ambientais está cada vez mais ameaçada (BOLZANI, 2016).

Portanto, proporcionalmente à enorme biodiversidade são os impactos ambientais causados pelo desenvolvimento econômico, visíveis principalmente no Sudeste Brasileiro (Figura 3), onde inúmeras espécies estão sob ameaça de extinção (CUBAS, 2006).

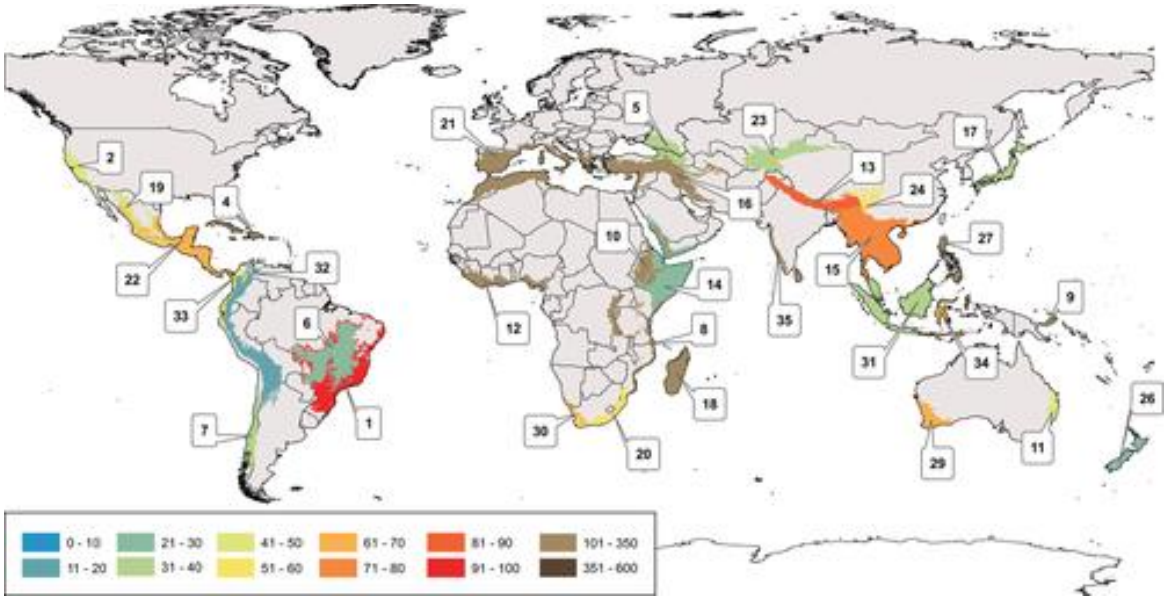

Figura 3. Mapa de projeção dos níveis de ameaça nos principais *hotsposts* de biodiversidade, ilustrando como a mudança climática e a pressão agro econômica afetam a região do Estado de São Paulo (áreas vermelhas indicam maior pressão antrópica). Fonte: HABEL *et al*., 2019.

Diante do panorama e devido à importância dos fragmentos remanescentes para a manutenção da biodiversidade e de espécies ameaçadas, é necessária a investigação da situação das populações silvestres remanescentes.

No presente trabalho, a amostragem experimental permitiu a avaliação em áreas protegidas e não protegidas de ecótonos entre Cerrado e Mata Atlântica no estado de São Paulo. Assim, duas Estações Ecológicas inseridas na zona de contato Cerrado/Mata Atlântica compõem a área de amostragem (Figura 4). É importante esclarecer que essas Estações Ecológicas sofreram diferentes impactos antrópicos com a fragmentação de habitat ao longo dos anos (IF, 2011; IF, 2014; IF, 2019).

Além da avaliação de populações em áreas protegidas e entorno, a avaliação das populações em áreas não protegidas e da paisagem também é fundamental e devem ter a mesma importância de conservação que áreas protegidas (LI *et al*., 2021).

Particularmente no Brasil, as áreas não protegidas compõem um importante cenário, principalmente em ambientes altamente fragmentados. Assim, conservar a vegetação nativa em terras privadas e não protegidas pode ser um dos mecanismos mais importantes para proteger a biodiversidade em biomas brasileiros, em especial na conservação de mamíferos terrestres (BERTASSONI; BIANCHI; DESBIEZ, 2021). Além disso, estudos indicam que diversos mamíferos, incluindo os tamanduás-

bandeiras usam as áreas protegidas e plantações em zonas de amortecimento, além de estradas não pavimentadas (PAOLINO *et al*., 2016; VERSIANI *et al.*, 2021).

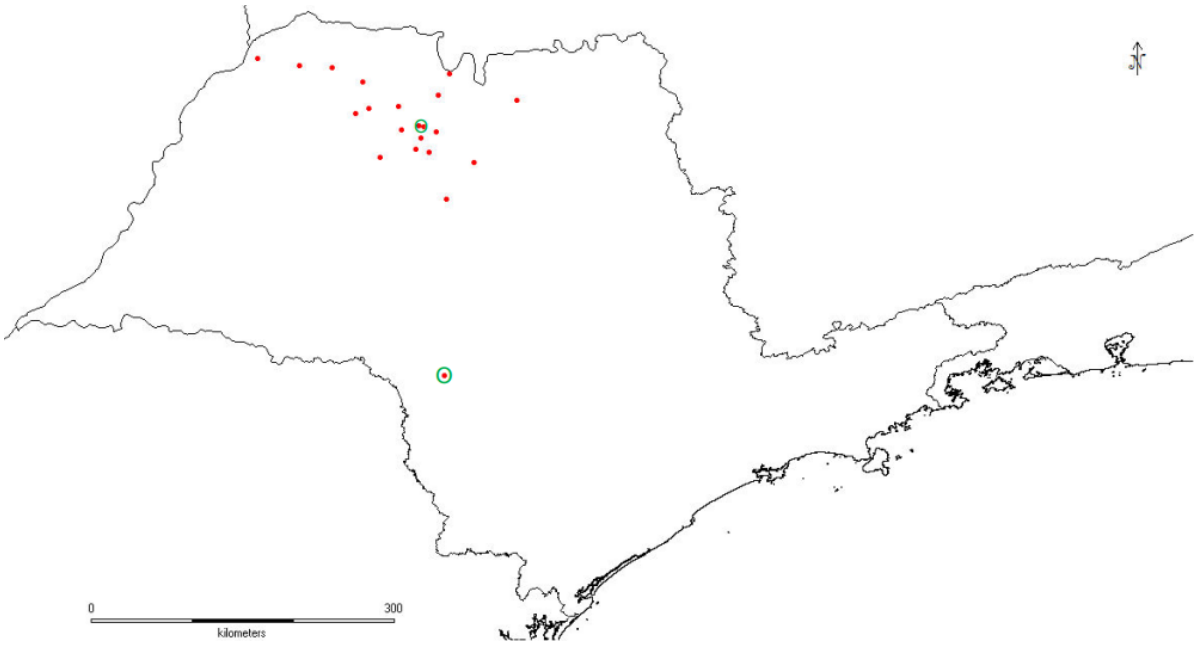

Figura 4. Mapa do estado de São Paulo com locais de amostragem (pontos vermelhos) e indicação das Estações Ecológicas (círculos verdes). Estação Ecológica do Noroeste Paulista (região noroeste) e Estação Ecológica de Santa Bárbara (região centro-sul). Fonte: SARTORI *et al*., 2021.

Estação Ecológica do Noroeste Paulista e entorno

A Estação Ecológica do Noroeste Paulista (EENP), situada entre as coordenadas 220º49´ S a 47º23´ W, possui uma área de 500 hectares em conjunto com a Floresta Estadual Noroeste Paulista (FENP) e seu entorno é composto pelas áreas com alta influência antrópica pertencentes aos municípios de São José do Rio Preto e Mirassol. Devido ao histórico de degradação do local, não restam fragmentos de floresta madura, especialmente da Estacional e do Cerradão e hoje se encontra altamente fragmentada e em contato com áreas muito urbanizadas (IF, 2014). Vale ressaltar que a urbanização e a expansão imobiliária ameaçam seriamente a área e apesar de a região de estudo ser muito pobre de fragmentos florestais preservados e não haver muitos estudos sobre a fauna que restou, os dados mostram que a região ainda pode abrigar uma rica diversidade, mesmo sendo uma área cercada por pastagens, plantações e bairros urbanos. Além disso, é um dos poucos fragmentos

protegidos que representa este bioma na região noroeste paulista (PEREIRA; BRASILEIRO, 2015).

Estação Ecológica de Santa Bárbara e entorno

A Estação Ecológica de Santa Bárbara (EESB) conta com 2.712 hectares (ha) entre as coordenadas geográficas 22°46’ a 22°41’ S e 49°16’ a 49°10’ W e a maior parte da área da Estação Ecológica de Santa Bárbara é ocupada por vegetação nativa, com poucas evidências de perturbação antrópica e faz contato, na maior parte de suas divisas, com pastagens e reflorestamentos (IF, 2011)

A EESB está entre as maiores áreas protegidas em São Paulo, e é uma das poucas a contemplar um mosaico dos diferentes tipos de vegetação de Cerrado. Diversas espécies registradas na Estação Ecológica encontram-se regionalmente ameaçadas de extinção, sendo que cinco são ameaçadas globalmente e as formações campestres destacam-se pelo número de espécies ameaçadas (LUCINDO *et al.*, 2015). Portanto a proteção e preservação da área é de extrema importância, uma vez que esta área é um dos últimos remanescentes de Cerrado aberto no estado de São Paulo.

Áreas não protegidas no Noroeste Paulista

Além das áreas protegidas, foram avaliadas as populações e indivíduos em áreas não protegidas ao longo do Noroeste Paulista (NP), por uma amostragem de conveniência, a partir de atendimentos realizados pelo Hospital Veterinário “Dr. Halim Atique” e pelo Zoológico Municipal de São José do Rio Preto para tentativa de reabilitação de indivíduos atropelados, queimados, atacados por cães ou perdidos em áreas urbanas. Dessa maneira, avaliar animais em áreas não protegidas pode ser de grande relevância, pois os processos populacionais da vida selvagem que ocorrem fora das áreas protegidas podem afetar a conservação das espécies dentro das áreas protegidas. Além das áreas protegidas serem eficazes para a manutenção de populações reprodutoras, áreas não protegidas também apresentam resultados positivos para algumas populações de espécies silvestres (LEÓN-ORTEGA, 2017).

Estudos sugerem que áreas não protegidas devem ter a mesma importância de conservação que as áreas protegidas, principalmente em relação aos mamíferos, no que diz respeito ao planejamento de conservação baseado na paisagem. Para alcançar os objetivos de conservação em longo prazo é cada vez mais reconhecido que as áreas não protegidas devem ser incorporadas às estratégias de conservação (LI *et al*., 2021; THAPA *et al*., 2021).

O Brasil, em sua legislação, obriga os proprietários de terras a separarem áreas de preservação. Assim, uma parte dos remanescentes de vegetação nativa devem ser protegidos em vez de convertidos para outros usos da terra em áreas de antigas fronteiras econômicas e esses remanescentes podem ser os pilares de sistemas de conservação regionais eficazes principalmente em áreas altamente antropizadas (DA SILVA; PINTO; SCARANO, 2021).

Portanto, o estudo de espécies ameaçadas em áreas protegidas e não protegidas é essencial para entender as respostas de organismos e populações frente às alterações antrópicas (VERSIANI *et al*., 2021). Ainda, a utilização de abordagens interdisciplinares incluindo abordagens genéticas pode contribuir para avaliar como a destruição e a fragmentação de habitat recente atua estruturando as populações de uma determinada espécie.

1.2 Genética da Paisagem e Filogeografia: escalas temporais, espaciais e diferentes marcadores moleculares

Para uma melhor compreensão dos efeitos da fragmentação e degradação de habitats sobre as espécies ameaçadas, é importante relacionar os dados genéticos com o contexto ecológico, a fim de compreender padrões e mecanismos evolutivos que conectam a genética do organismo e os fatores ambientais (MANEL *et al*., 2003).

Estudos conservacionistas geralmente envolvem os vórtices de extinção, no qual incluem a variação ambiental, a variação demográfica e a perda da variabilidade genética. Esses três fatores agem em conjunto e o declínio no tamanho da população causado por um destes fatores aumentará a vulnerabilidade da população aos outros fatores (GILPIN; SOULÉ, 1986; PRIMACK, 2004).

Assim, os vórtices de extinção são uma classe de modelos através dos quais biólogos conservacionistas, geneticistas e ecologistas podem entender a dinâmica e categorizar as extinções no contexto de suas causas. Este modelo mostra os eventos que levam as pequenas populações a se tornarem cada vez mais vulneráveis à medida que se aproximam da extinção (GILPIN; SOULÉ, 1986).

Muitas populações que sucumbem a um vórtice de extinção experimentam fortes fatores genéticos que fazem com que populações já pequenas diminuam de tamanho ao longo do tempo. Assim, as populações pequenas são particularmente vulneráveis a mudanças rápidas na estrutura genética populacional devido à

amostragem aleatória de gametas, podendo ser extintas principalmente localmente e regionalmente (FRANKHAM *et al*., 2019).

Portanto estudos genéticos por meio de abordagens interdisciplinares são essenciais para a conservação de espécies e auxiliar em planos para conservação e a Genética da Conservação é uma área de estudo interdisciplinar (AVISE, 2008), que abrange as áreas da ecologia, biologia molecular, genética de populações, modelagem matemática, sistemática e visa compreender a dinâmica dos genes em populações, principalmente para evitar a extinção de espécies (FRANKHAM; BRISCOE; BALLOU, 2002).

Ao contrário da Genética de Populações, a Genética da Conservação (apesar de intrinsicamente relacionadas) lida com os efeitos da perda de diversidade genética e mudanças recentes na estruturação genética visando a sobrevivência a longo prazo de espécies ameaçadas. Por isso aplica métodos genéticos, muitas vezes específicos para investigar os efeitos da estruturação genética contemporânea, principalmente em espécies e populações pequenas sob algum risco de extinção (WAN *et al*., 2004).

No geral, a estrutura da teoria genética evolutiva fornece uma abordagem para interpretar as medidas de variação genética e prever os efeitos futuros de fatores evolutivos para estratégias de gerenciamento na conservação de espécies ameaçadas (HEDRICK; HURT, 2012).

Quando uma população é pequena, qualquer mudança nos alelos pode impactar desproporcionalmente a população. Assim, a deriva genética leva pequenas populações a perder a diversidade genética (WRIGHT, 1932; STAR; SPENCER, 2013).

Além disso, quando as populações se tornam pequenas, a endogamia aumenta porque os indivíduos são mais propensos a acasalar com outros com um genoma que contém muitos dos mesmos alelos, o que pode levar à depressão endogâmica na população, podendo causar menos descendentes, mais defeitos congênitos, mais indivíduos propensos a doenças, diminuição da sobrevivência, da reprodução e da aptidão, assim como a diminuição da diversidade genética na população. Além disso, o fluxo gênico limitado devido à fragmentação de habitat pode levar pequenas populações à espiral da extinção (FRANKHAM *et al*., 2019).

A variabilidade genética influencia tanto na saúde quanto na sobrevivência a longo prazo das populações, uma vez que a diminuição da diversidade genética tem sido associada à redução da aptidão como alta mortalidade juvenil, diminuição do

crescimento populacional, redução da imunidade e maior risco de extinção (FERGUSON; DRAHUSHCHAK, 1990; LEBERG, 1990; SACCHERI *et al.*, 1998; FRANKHAM, 2005; KING; LIVELY, 2012).

De maneira geral, a Genética da Conservação objetiva compreender a dinâmica dos genes em populações para evitar a extinção, por diferentes métodos e em uma abordagem interdisciplinar. Assim, entre as áreas interdisciplinares relacionadas à Genética da Conservação destacam-se a Filogeografia e a Genética da Paisagem.

A Filogeografia e a Genética da Paisagem surgiram respectivamente, nos últimos 30 e 20 anos aproximadamente, no qual o termo Filogeografia foi utilizado pela primeira vez em 1987 e a Genética da Paisagem surgiu como disciplina própria em 2003, apesar da ideia de que os padrões da paisagem afetam a forma como os organismos são distribuídos remonta aos séculos XVIII e XIV (AVISE *et al*., 1987; MANEL *et al*., 2003).

A Filogeografia é considerada a ligação entre a genética de populações e a sistemática, e a Genética da Paisagem considerada a ligação entre a ecologia da paisagem e a genética de populações. Ambos os campos podem ser considerados simplesmente como a combinação da biogeografia clássica com a genética e a genômica. No entanto, as duas áreas diferem nas escalas temporais, espaciais e de organismo, além de geralmente serem distintas em relação à metodologia e aos marcadores moleculares utilizados nas análises (RISSLER, 2016).

Dessa maneira, a Filogeografia é importante para entendermos se diferentes populações isoladas possuem conexão evolutiva e auxiliam na definição de unidades evolutivas significativas (ESU), unidades de manejo (MU), além de identificar dispersões históricas intra ou interespecíficas, o que pode servir de subsídio para o planejamento de conservação, manejo, proteção e em translocações de indivíduos ou populações (NEAVES *et al*., 2016). Ou seja, a abordagem holística da filogeografia é fundamental para garantir medidas de conservação eficazes e permite que estudos populacionais locais em menor escala, possam ser integrados a um contexto mais amplo, auxiliando a maximizar a conservação da diversidade genética. Assim estudos filogeográficos em escalas maiores podem fornecer uma estrutura sólida para encaixar informações de estudos locais, como no caso do presente estudo, em um contexto geográfico maior (MORITZ, 1994; NEAVES *et al*., 2016).

Na Filogeografia, os eventos passados que podem ser inferidos incluem expansão populacional, gargalos populacionais, vicariância, dispersão e migração; que influenciaram na distribuição passada e/ou atual das espécies. Essas análises permitem entender os papéis dessas diferentes forças históricas na formação de padrões atuais nas populações (AVISE, 2000; CRUZAN; TEMPLETON, 2000; ROSENBERG; NORDBORG, 2002).

Estudos nesse campo podem focar no estudo de como os ciclos de glaciação dos últimos dois milhões de anos restringiram periodicamente algumas espécies a refúgios disjuntos e prováveis gargalos populacionais em faixas restritas que reduziram a variação genética histórica (CRUZAN; TEMPLETON, 2000). Como pode também, por outro lado, ser útil para abordar questões relacionadas a conservação como auxiliar na priorização de áreas de alto valor para conservação a partir da distribuição geográfica única e padrões genéticos nucleares e principalmente mitocondriais (MORITZ, 1994).

Sob outra perspectiva, a abordagem baseada na Genética da Paisagem é especificamente útil na conservação por utilizar genes com altas taxas de mutação, com a finalidade de esclarecer questões evolutivas e ecológicas relacionadas a eventos recentes como a fragmentação antrópica de habitat principalmente em escalas locais e regionais (MANEL *et al*., 2003; WANG, 2010).

A Genética da Paisagem permite a análise dos processos micro evolutivos que afetam as espécies à luz dos padrões espaciais da paisagem, proporcionando uma visão de como as populações interagem com seus ambientes (HOLDEREGGER; WAGNER, 2006).

Ainda, as análises possibilitam determinar quais características da paisagem são barreiras à dispersão e ao fluxo gênico, entender como as mudanças na paisagem induzidas pelo homem afetam a evolução das populações, suas dinâmicas e como doenças ou espécies invasoras se espalham pelas paisagens. Auxiliam também do manejo e escolha de unidades de conservação (DIFFENDORFER, 1998; (GALEGO; CARARETO, 2010; STORFER *et al*., 2010).

A genética da paisagem fornece, portanto, informações em escalas temporais e espaciais mais finas, ou seja, no nível de variação genética individual dentro de uma população. Por se concentrar na amostragem de indivíduos, a genética da paisagem tem a vantagem de não ter que definir subjetivamente populações discretas antes da análise e ferramentas genéticas são usadas para detectar diferenças genéticas

abruptas entre indivíduos dentro de uma população a fim de correlacionar descontinuidades genéticas com características da paisagem e do ambiente (MANEL *et al.*, 2003).

Nas análises, a amostragem em nível individual ou de populações pequenas se torna possível e integra a metodologia aplicada à Genética da Paisagem. Assim, o ISS – *Individual Sampling Scheme* ou Esquema de Amostragem Individual é uma alternativa metodológica à amostragem populacional convencional utilizada principalmente em estudos locais e regionais. Essa alternativa oferece maior flexibilidade no esquema de amostragem espacial e é uma abordagem eficiente, mesmo em amostragens reduzidas (PRUNIER *et al.*, 2013). Portanto é uma estratégia de amostragem importante na avaliação de espécies em extinção ou em áreas altamente fragmentadas, onde há poucos indivíduos remanescentes ou é difícil delimitar populações.

É importante a compreensão de que análises estatísticas específicas devem ser implementadas nesse tipo de estudo e em amostragem baseada em indivíduos. Por isso são utilizados métodos que agrupam indivíduos em subpopulações com base na diferenciação genética ou distância, por atribuição bayesiana além de outros métodos alternativos (MILLER, 2005; KOPELMAN *et al*., 2015; MUÑOZ-VALENCIA *et al*., 2021).

Portanto, métodos em genética da paisagem permitem avaliações a nível individual a partir da informação genética por métodos específicos de identificação de populações e atribuição individual com base em agrupamento estatístico de genótipos de espécimes, inclusive em análises de dados genéticos que utilizam um ou vários indivíduos de diferentes locais de amostragem (MANEL; BERTHIER; LUIKART, 2002; MANK; AVISE, 2004; GUILLOT *et al.*, 2009).

Em suma, a Genética da Paisagem facilita a compreensão de como o fluxo gênico e a adaptação ocorrem em paisagens heterogêneas reais. Também permite a estimativa da conectividade funcional entre as paisagens e ajuda a elucidar características da paisagem que atuam como barreiras ou facilitadores de dispersão, indicando possíveis locais para a criação ou preservação de corredores ecológicos para vida selvagem. Assim, pode ajudar a prever quão bem as populações se adaptam ou se adaptarão às mudanças contínuas (WAGNER; HOLDEREGGER, 2008; MANEL; HOLDEREGGER; 2013).

Essas duas abordagens podem contribuir para a compreensão de aspectos ecológicos e evolutivos em relação às populações de espécies ameaçadas e a combinação dessas áreas em estudos conservacionistas pode ser essencial para melhor atingir os objetivos na conservação. Para isso, a partir do ponto de vista de que as áreas possuem suas especificidades, é importante desenvolver conhecimentos interdisciplinares, além da comunicação e colaborações entre geneticistas, ecologistas, biogeógrafos e estatísticos espaciais (DINIZ-FILHO *et al*., 2008).

As duas disciplinas reúnem ferramentas moleculares e geoespaciais avançadas, fornecendo noções críticas sobre questões vitais para o campo da biogeografia e conservação, incluindo como e onde a biodiversidade surge, como as espécies respondem às mudanças climáticas e ambientais em diferentes escalas espaciais e temporais e auxiliam a entender como e onde os esforços de conservação devem ser focados (ALI, 2020).

Em conclusão, a diferença entre as duas áreas não é meramente semântica e entender as diferenças entre esses campos é importante para integrar as duas metodologias a fim de obter resultados robustos e que auxiliem em ações de conservação.

A compreensão das diferenças e similaridades entre as duas áreas (Figura 5) é um passo fundamental para interpretar apropriadamente quais informações os dados realmente suportam, garantindo que a interpretação dos resultados seja adequada. Além disso é de extrema importância a escolha de ferramentas apropriadas para as diferentes escalas de análise, em relação às metodologias e aos tipos de marcadores moleculares utilizados (WANG, 2010).

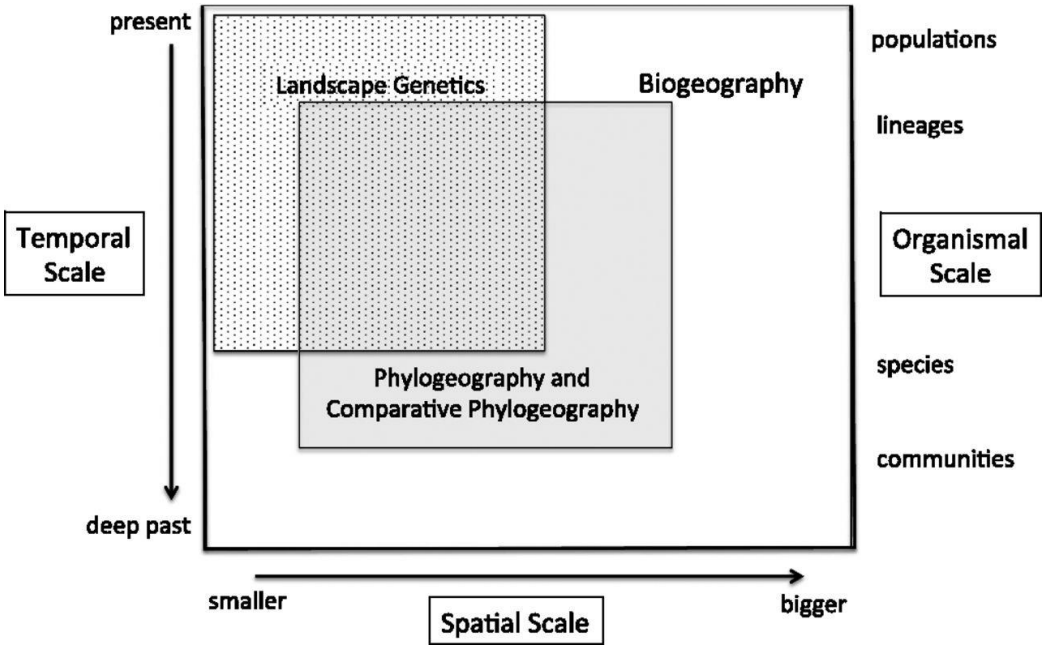

Figura 5. Ilustração comparativa entre a Genética da Paisagem (pontilhada) e a Filogeografia (cinza), mostrando a relação e as diferenças das duas áreas em relação à extensão da escala temporal, espacial e da escala do organismo. Fonte: RISSLER, 2016.

Marcadores moleculares e diferentes taxas de mutação

De maneira geral, a Filogeografia e a Genética da Paisagem possuem semelhanças, mas apresentam diferenças quanto à classe de marcadores moleculares mais apropriados para cada estudo (STORFER *et al*., 2007). A maioria dos estudos em Genética da Paisagem utiliza genotipagens por meio de marcadores microssatélites (SSR), enquanto a maior parte dos estudos em Filogeografia utiliza sequências de DNA organelar e em alguns casos sequências de DNA nuclear de cópia única (scnDNA), os quais possuem menores taxas de mutação (AVISE *et al.*, 1987; WAN *et al*., 2004; STORFER *et al*., 2007).

Com uma alta taxa de mutação média estimada, os SSR fornecem excelente resolução dos processos micro evolutivos recentes e o DNA mitocondrial (mtDNA), mesmo com taxas de mutação relativamente altas, geralmente apresenta uma taxa de mutação bem menor que a dos microssatélites (WAN *et al*., 2004; WANG, 2010). Notavelmente, a diferença de taxas de mutação entre mtDNA e SSR é grande e essa diferença estabelece as utilidades distintas desses marcadores para inferências em escalas temporais diferentes (VANDERGAST *et al*., 2007).

Os marcadores neutros são úteis, porém a utilização de genes não neutros pode expandir o grau de conhecimento na conservação de espécies. Ou seja, além

dos marcadores neutros como os SSR, mtDNA e scnDNA, os genes adaptativos (não neutros) também podem ser incorporados às metodologias de análise em Genética da Conservação (SATKOSKI TRASK *et al.*, 2013).

Evidências crescentes sugerem que a diversidade genética é particularmente importante em genes adaptativos, incluindo os genes do Complexo Principal de Histocompatibilidade (MHC), pois as variantes do MHC influenciam muitas características biológicas importantes relacionadas à conservação de espécies (como aspectos imunológicos e relacionados às preferências de acasalamento), especialmente em vertebrados (SOMMER, 2005).

A maioria dos estudos em Genética da Conservação infere os efeitos antrópicos utilizando marcadores de genes neutros para documentar padrões genéticos nas populações. Ou seja, a variação adaptativa ainda tem um papel limitado na genética da conservação, pois estudos com genes adaptativos são geralmente realizados em humanos e organismos modelo, mas são menos empregados para avaliação de outras espécies (GEBREMEDHIN *et al.*, 2009).

Entretanto, cada vez mais trabalhos utilizam genes adaptativos na genética da paisagem, incluindo análises robustas obtidas principalmente por Sequenciamento de Nova Geração (NGS) e em conjunto com genes mtDNA ou SSR (SATKOSKI TRASK *et al.*, 2013; CLOZATO *et al.*, 2015; MONTEIRO *et al.*, 2019).

De maneira geral, a distribuição da variação genética dentro de uma espécie resulta de uma combinação de processos e para isso diferentes marcadores são utilizados na compreensão dos efeitos da paisagem sobre as espécies em diferentes escalas (Figura 6). Assim, marcadores moleculares com diferentes taxas de substituição irão capturar assinaturas de processos populacionais em diferentes épocas na história evolutiva das espécies (AVISE *et al*., 1987; THOMSON; WANG; JOHNSON, 2010; WANG, 2010).

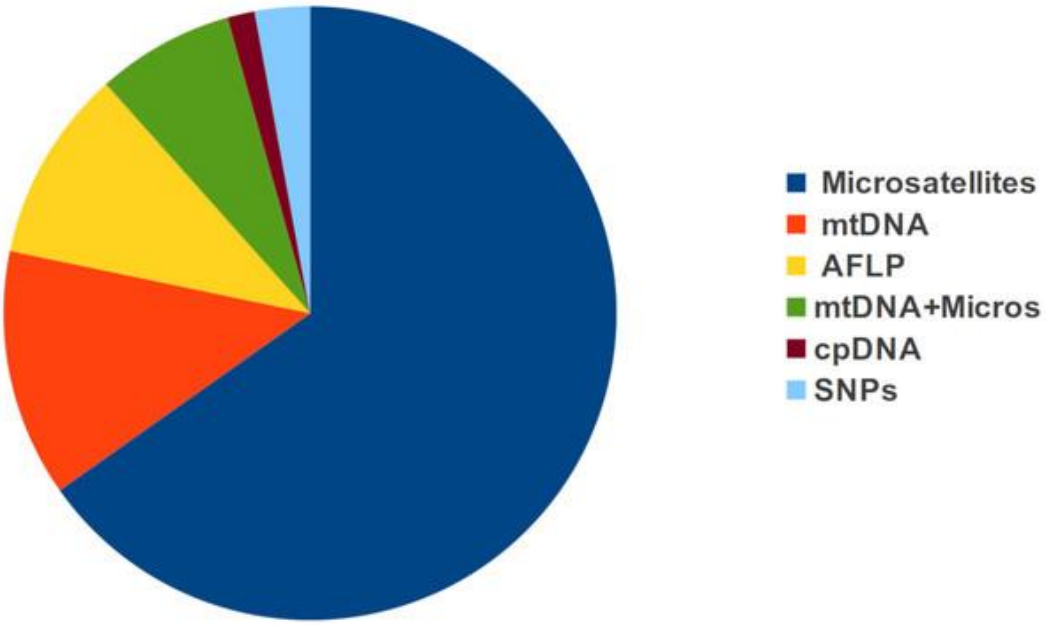

Figura 6. Proporção de estudos que utilizam diferentes tipos de marcadores moleculares para avaliar os efeitos ambientais e da paisagem no fluxo gênico em organismos de regiões tropicais. Fonte: MONTEIRO *et al*., 2019.

Portanto, a compreensão de como a paisagem atua estruturando as populações pode ser ampliada, a partir da combinação de diferentes marcadores moleculares e uma abordagem conjunta entre Genética da Paisagem e Filogeografia (RISSLER, 2016).

Para a análise de dados utilizando ambas abordagens é preciso ter cautela na utilização cruzada de materiais e métodos entre os dois campos, para a interpretação dos dados de acordo com as escalas temporais utilizadas (WANG, 2010).

A utilização adequada de genes com diferentes taxas de mutação em uma abordagem genética integrada pode fornecer excelentes complementos para a compreensão dos processos micro evolutivos, dos fatores que afetam a variação genética e das respostas das espécies frente às mudanças ambientais em diferentes escalas espaciais e temporais, possibilitando inferências sobre como e onde devem ser realizadas ações conservacionistas (PEASE *et al*., 2009; ALI, 2020).

Consequentemente, os resultados obtidos a partir desse tipo de abordagem genética podem ser bastante úteis e devem, quando possível, ser integrados ao desenvolvimento de estratégias de conservação, manejo e políticas formais. Principalmente em países menos desenvolvidos com alta biodiversidade como no caso dos países da América Latina e do Brasil, onde diversas espécies estão sob

ameaça de extinção incluindo várias espécies de mamíferos aquáticos e terrestres (COSTA *et al*., 2005; TORRES-FLOREZ *et al.*, 2014; TORRES-FLOREZ *et al.*, 2018).

1.3 *Myrmecophaga tridactyla*, uma espécie ameaçada: extinções locais e estratégias de conservação

Mamíferos terrestres de médio e grande porte podem ser particularmente ameaçados e vulneráveis à extinção por causa da fragmentação e perda de habitat, especialmente espécies com grandes áreas de vida ou longos ciclos de vida (HAILER *et al*., 2006; MORRIS *et al.*, 2008; KEINATH *et al*., 2017).

Este é o caso do tamanduá-bandeira (*Myrmecophaga tridactyla*; Linnaeus, 1758) na América do Sul e no Brasil. A espécie é um mamífero de grande porte eu pesa em média 31 quilos e mede em média 1,20 metros de comprimento quando adulto. O tamanduá-bandeira é considerado uma espécie vulnerável no Brasil, principalmente devido à perda de habitat no Cerrado, Mata Atlântica e em outros biomas brasileiros (MIRANDA *et al*., 2015).

A espécie também apresenta características que evidenciam sua alta vulnerabilidade frente aos impactos ambientais. Devido aos seus longos pêlos facilmente inflamáveis e à baixa mobilidade, os tamanduás-bandeira são atingidos com freqüência por grandes incêndios florestais, que podem causar a morte desses indivíduos. Por ser um animal de baixa mobilidade e possuir visão fraca, além das mortes ocasionadas pelos incêndios e pela queima da cana-de-açúcar, a espécie é uma das principais vítimas de atropelamentos em rodovias. A mortalidade causada por atropelamentos pode ameaçar profundamente a persistência da população de tamanduá-bandeira em permanecer nas áreas extremamente fragmentadas (DINIZ; BRITO, 2013).

Devido às ameaças e características da espécie, a tendência é que as populações restantes de tamanduá-bandeira progressivamente tornem-se mais isoladas. Sabe-se que as reduções de tamanho e alcance das populações aumentam sua vulnerabilidade à extinção estocástica, levando em vários casos à extinção local (CLOZATO *et al.*, 2017).

Esta espécie é principalmente terrestre, em contraste com outros tamanduás vivos e preguiças, que são arborícolas ou semiarborícolas, é reconhecível por seu focinho alongado, cauda espessa, garras dianteiras longas e pelagem distintamente

colorida. Algumas características biológicas do animal podem ser indicativas de baixas taxas de crescimento populacional. Entre elas, podemos destacar: baixa taxa metabólica, baixa capacidade termorregulatória, pequeno potencial reprodutivo, cuidado parental prolongado, longo período de gestação e hábitos solitários (DINIZ; BRITO, 2013).

O tamanduá-bandeira tem uma temperatura corporal baixa para um mamífero e os xenartros em geral tendem a ter taxas metabólicas mais baixas do que a maioria dos outros mamíferos, uma tendência que se acredita estar correlacionada com suas especializações alimentares e baixa mobilidade (FRANCISCO; TEIXEIRA, 2018).

Os tamanduás-bandeira podem acasalar durante todo o ano e atingem a maturidade sexual entre 2,5 e 4 anos de idade. A fêmea tem um filhote por gestação (embora, em raríssimos casos, houve relatos de gêmeos) e cada gestação dura cerca de 190 dias. Após nascer, o filhote fica grudado nas costas da mãe por 6 a 9 meses, camuflando em meio a sua pelagem. À medida que cresce, ele começa a também se alimentar de insetos, reduzindo o consumo do leite materno, até que se torne independente (MIRANDA *et al.*, 2015).

Com relação à sua ocupação, as áreas de vida do tamanduá-bandeira variam em tamanho dependendo da localização. Os animais geralmente forrageiam em áreas abertas e descansam em áreas florestais, possivelmente porque as florestas são mais quentes do que as pastagens em dias frios e mais frias em dias quentes (MOURÃO; MEDRI, 2006).

A espécie pertence à superordem Xenarthra juntamente com outras espécies de tamanduá, as preguiças e os tatus. Assim, tamanduás e preguiças pertencem à ordem Pilosa e os tatus pertencem a ordem Cingulata (MCKENNA; BELL, 1997). Em relação às outras espécies de tamanduá, ainda surgem novidades evolutivas como a descoberta realizada em 2018 de pelo menos sete espécies distintas de tamanduaí (*Cyclopes*), sendo que quatro espécies previamente foram validadas e três novas espécies foram descritas pelos autores, sendo *Cyclopes xinguensis, Cyclopes rufus* e *Cyclopes thomasis* (MIRANDA *et al*., 2018).

Do ponto de vista evolutivo, a família Dasypodidae (Cingulata) é o táxon mais basal, tendo se separado da ordem Pilosa entre o período Cretáceo e o Paleógeno, há 70,5 milhões de anos atrás. Já os tamanduás divergiram das preguiças no meio do Paleoceno, há aproximadamente 60 milhões de anos. Por sua vez, as duas famílias de tamanduás (Vermilingua: família Myrmecophagidae e família Cyclopedidae), teriam

se separado no meio do Eoceno em torno de 40 milhões de anos atrás (DELSUC; DOUZERY, 2009).

Durante a maior parte da era cenozóica, os tamanduás foram confinados à América do Sul. Após a formação do istmo do Panamá há cerca de 3 milhões de anos, os tamanduás dos três gêneros existentes invadiram a América Central no Grande Intercâmbio Biótico das Américas - GABI (SHAW; MCDONALD, 1987).

O surgimento do Istmo do Panamá possibilitou que a placa continental sul-americana se conectasse com a porção norte do continente e com isso, este evento possibilitou que muitas famílias de xenartras, atualmente extintas, ocupassem a região norte, onde hoje seria os Estados Unidos. No entanto, apenas oito espécies viventes do grupo são encontradas fora da América do Sul. Outros eventos geológicos influenciaram na evolução do grupo, como o soerguimento das Cordilheiras dos Andes que promoveu alterações no clima atmosférico e serviu como barreira de isolamento geográfico (MORAES-BARROS; ARTEAGA, 2015).

Este evento teve influência direta na diversificação de tamanduás e tatus no Eoceno e a diversificação no gênero Dasypus no Mioceno superior, há 7 milhões de anos, quando houve aumento significativo na altura dos Andes. Ainda, a separação entre Floresta Amazônica e Mata Atlântica, juntamente com a distribuição longitudinal da Mata Atlântica também são eventos geológicos, que possuem forte influência na evolução dos xenartras na América do Sul (DELSUC; VIZCAÍNO; DOUZERY, 2004; MORAES-BARROS; ARTEGA, 2015).

O registro fóssil de tamanduás é geralmente escasso e o tamanduá-bandeira é o mais terrestre das espécies vivas de tamanduá. Seus ancestrais podem ter sido originalmente adaptados à vida arbórea e a transição para a vida no solo pode ter sido auxiliada pela expansão de habitats abertos (como a savana na América do Sul ou o Cerrado no Brasil) e pela disponibilidade de insetos, como formigas e cupins, os quais forneceram uma fonte importante de alimento para a espécie neste cenário (GAUDIN; BRANHAM, 1998).

Em relação à distribuição geográfica, o tamanduá-bandeira é nativo da América Central e do Sul, desde Honduras até o norte da Argentina. Além disso, restos fósseis foram encontrados na região noroeste de Sonora, no México (SHAW; MCDONALD, 1987).

A espécie apresenta ampla distribuição geográfica, mas sofre extinções locais regionais e nacionais. Provavelmente está extinta na Guatemala, em Belize, no

Uruguai e em Santa Catarina (Figura 7). Também é considerada extinta localmente nos estados do Rio de Janeiro, do Espírito Santo e criticamente ameaçada no Paraná e no Rio Grande do Sul (MIRANDA *et al.*, 2015).

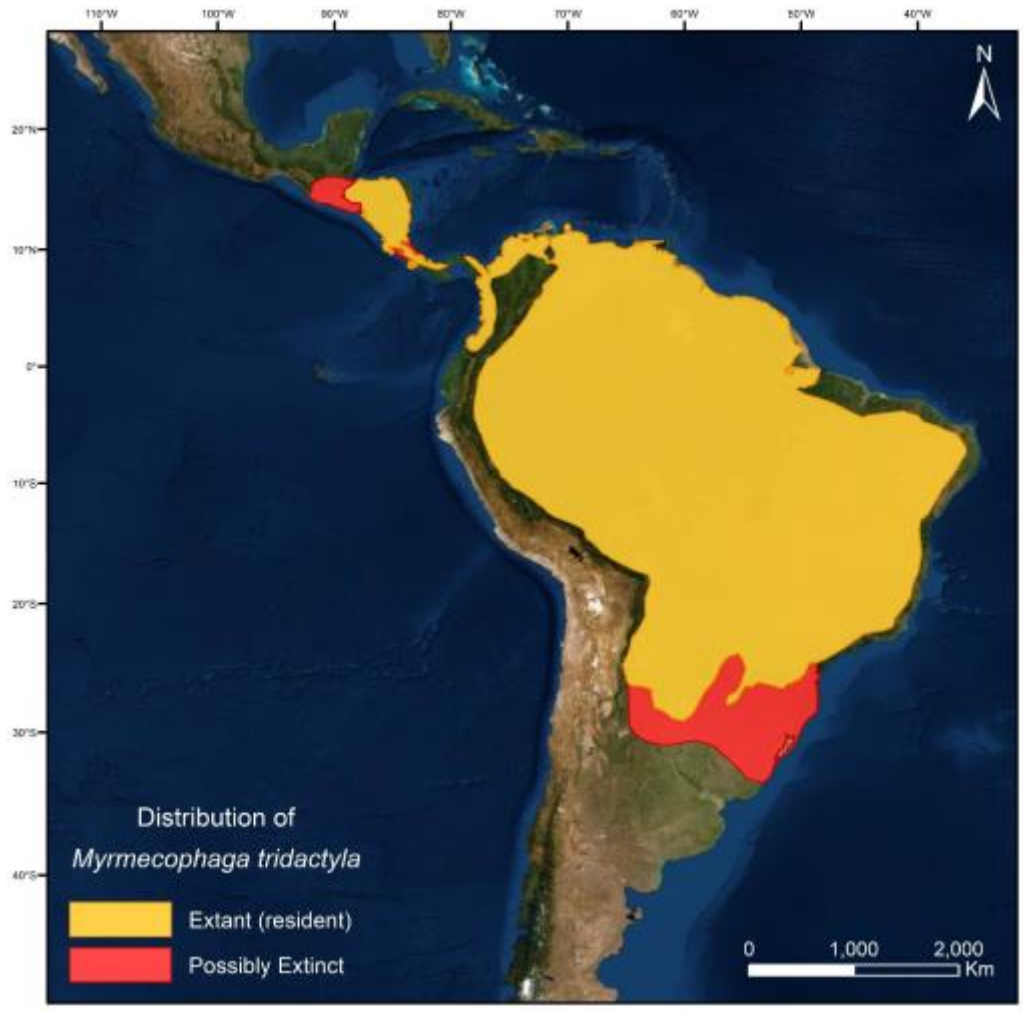

Figura 7. Mapa da distribuição geográfica da espécie *Myrmecophaga tridactyla* (em amarelo) e locais de possíveis extinções (em vermelho). Fonte: ALBERICI *et al*., 2020, MIRANDA *et al.*, 2014.

Devido às características ambientais, ameaças antrópicas e características biológicas da espécie, análises para conservação do tamanduá-bandeira são necessárias. A espécie está listada como “Vulnerável” (VU) pela IUCN e ações de conservação são urgentes para tentar aumentar a chance de persistência da espécie em áreas antropizadas e diminuir o número de extirpações regionais (MIRANDA; BERTASSONI, ABBA, 2014).

Estimativas mostram que entre 2000 e 2010, a população total diminuiu cerca de 30%, com diversos indivíduos mortos devido a incêndios florestais no Brasil mesmo em unidades de conservação (SILVEIRA *et al*., 1999.) O animal é particularmente

vulnerável a incêndios devido ao seu movimento lento e pelagem inflamável. Além de incêndios florestais e em áreas agrícolas, as ameaças induzidas pelo homem incluem colisão com veículos, ataques de cães, destruição de habitat e caça. Particularmente no estado de São Paulo, a espécie enfrenta diversas ameaças devido as características da região (BERTASSONI *et al.*, 2019). Dessa maneira, é necessário estabelecer estratégias de conservação para a espécie e seu habitat, levando em consideração fatores genéticos do táxon e questões ambientais como as características dos biomas e da paisagem (MIRANDA *et al.*, 2022).

As ameaças sofridas pela espécie podem ser observadas na área abrangida pelo presente trabalho, composta pelo bioma Cerrado e ecótonos compostos por Cerrado e Mata Atlântica. Apenas na região Noroeste Paulista foram atendidos 161 tamanduás-bandeiras em um período de 10 anos pelo Hospital Veterinário “Dr. Halim Atique”, no Setor de Atendimento Clínico Cirúrgico de Animais Selvagens (SACCAS). Dos animais atendidos, 42% foram vítimas de atropelamento, 13% de queimaduras e 45% de outros traumas, como por exemplo ataques por cães ou inconclusivos. Ou seja, foram atendidos em média 16 animais por ano, dos quais 57% vieram a óbito durante o tratamento, foram eutanasiados ou já chegaram mortos. Em relação aos animais reabilitados, 25% foram reintroduzidos e 18% destinados para zoológicos (ANDRADE FILHO *et al.*, 2017).

Em relação à situação das populações de outras espécies na mesma região, ao realizar um levantamento de todos os animais atendidos pelo Hospital de 2003 a 2017, constatou-se que foram realizados 2.928 atendimentos, no qual as aves corresponderam aos atendimentos mais frequentes (59%), seguido pelos mamíferos (34%) e répteis (8%). Deste total, 56% dos animais vieram a óbito e 44% foram reabilitados (PALA *et al.*, 2017).

Ou seja, os índices referentes à porcentagem de sobrevivência das diferentes espécies atendidas foram muito similares às taxas de sobrevivência dos tamanduás-bandeira. A alta porcentagem de atendimentos e óbitos de tamanduás-bandeira e de outras espécies indicam que a situação atual das populações remanescentes na região citada é extremamente preocupante em relação aos níveis de risco de extirpação local (CRUVINEL *et al.*, 2010).

Além disso, análises filogeográficas e de modelagem ecológica para avaliação da espécie reforçam a importância do Cerrado como um bioma prioritário para a conservação do tamanduá-bandeira (COIMBRA *et al.*, 2022).

Dessa maneira, urge a criação de planos e estratégias para conservação das populações de espécies silvestres, que devem ser baseadas em dados científicos. Todavia, para integrar dados científicos em ações de conservação é necessário interligar diferentes setores da sociedade e envolver comunidade científica, sociedade civil e o setor público; para a efetiva utilização da ciência em políticas de conservação (LAURANCE *et al.*, 2012).

Para a implementação de políticas púbicas baseadas em dados científicos, é necessário a integração entre cientistas e tomadores de decisão, porém as prioridades para cientistas e políticos tomadores de decisão na agenda da conservação normalmente não coincidem, resultando no fato de que a ciência não está sendo suficientemente aplicada para apoiar as políticas de conservação no Brasil (KARAM-GEMAEL *et al*., 2018).

Assim, os formuladores de políticas ambientais normalmente não utilizam o conhecimento produzido por instituições acadêmicas e geralmente não se baseiam em pesquisas científicas, fazendo com que as medidas para a conservação não sejam apoiadas por evidências baseadas em conhecimento, mas motivadas meramente por interesses políticos ou econômicos (CARNEIRO; DA-SILVA-ROSA, 2011).

Portanto, se a atual lacuna entre a ciência e as políticas de conservação não for preenchida, o país ameaçará a manutenção de seu capital natural a longo prazo, uma vez que pesquisas acadêmicas poderiam fazer maiores contribuições diretas para a conservação ambiental (MATIOLI; FERNANDES, 2012; AZEVEDO-SANTOS *et al.*, 2017).

Isso demonstra a necessidade de um diálogo mais proativo entre cientistas e profissionais da conservação ao definir prioridades de pesquisa e de ações para a conservação, com abordagens multidisciplinares que possam integrar as ciências biológicas à outras áreas como economia, sociologia e política. Além disso, é fundamental a comunicação mais direta e o estabelecimento de parcerias com o setor público para avançar na conservação ambiental (LAURANCE *et al*., 2012).

Para a integração entre cientistas, profissionais da conservação e políticos, estudos locais interdisciplinares podem ser uma ferramenta importante no envolvimento e longevidade das iniciativas de conservação (BENNETT *et al*., 2019).

Estudos locais são de grande importância para essa integração, uma vez que existem diversos benefícios em adaptar projetos de conservação regionais para circunstâncias locais, o que pode aumentar sua influência e custo benefício, antecipar

ações de conservação, auxiliar na longevidade de projetos e nas ações diretas em locais críticos para ações de conservação (PRESSEY *et al.*, 2016).

A partir dos pontos de vista apresentados, as estratégias para implementar ações de conservação efetivas devem envolver: estudos locais e regionais, análises interdisciplinares incluindo abordagens genéticas integradas e ecológicas, avaliação em áreas protegidas e não protegidas, fomento do uso de resultados acadêmicos em ações, comunicação com tomadores de decisão, entre outras abordagens que integrem o mundo acadêmico e o manejo prático da vida silvestre, com a finalidade de alcançar os objetivos de conservação em longo prazo (MURILLO *et al.*, 2011; LI *et al.*, 2021; THAPA *et al.*, 2021).

Estratégias baseadas em espécie guarda-chuva e bandeira

Dentre as estratégias para conservação de espécies e ecossistemas, estão a utilização de espécies bandeiras e de espécies guarda-chuvas.

Por definição, as espécies bandeiras destinam-se principalmente à sensibilização do público e à angariação de fundos para a conservação. Já as espécies guarda-chuvas são aquelas em que a proteção beneficia uma ampla gama de espécies coocorrentes (CARO, 2010; VERÍSSIMO *et al.*, 2011).

Espécies que integram explicitamente ambas as funções são chamadas de guarda-chuvas emblemáticas ou *flagship umbrellas* (CARO, 2010; LI; PIMM, 2016; KALINKAT *et al.*, 2017).

Assim, o tamanduá-bandeira pode ser considerado uma espécie “guarda-chuva emblemática” e como consequência, a sua conservação pode auxiliar na conservação de outras espécies e seus habitats (ROBERGE; ANGELSTAM, 2004; MIRANDA *et al.*, 2015; PINTO *et al.*, 2018).

Em outras palavras, a espécie pode atuar simultaneamente como uma espécie bandeira e guarda-chuva, pois é uma espécie carismática que está presente em praticamente todos os biomas brasileiros e sua proteção pode beneficiar muitos mamíferos, aves e outras espécies ocorrentes na mesma área e seus habitats (DINIZ, BRITO; 2013).

A identificação e utilização de espécies bandeiras e guarda-chuvas, como no caso do carismático tamanduá-bandeira (*Myrmecophaga tridactyla*), pode ter grande impacto na conservação de outras espécies e seus habitats, a partir de contextos locais. Assim, a estratégia baseada em espécies emblemáticas serve justamente

como iniciativa de projetos locais para a conservação da diversidade biológica (BOWEN-JONES; ENTWISTLE, 2002; DINIZ; BRITO, 2012; QIAN *et al.*, 2020).

Porém, existe um debate de longa data sobre a utilidade das abordagens de conservação centradas em espécies específicas, uma vez que diferentes espécies necessitam de diferentes estratégias de conservação. No entanto, a avaliação de espécies emblemáticas continua popular porque fornece “atalhos” úteis e necessários para programas de conservação bem-sucedidos (ROBERGE; ANGELSTAM, 2004).

As estratégias baseadas em espécies emblemáticas são amplamente utilizadas e podem ser efetivas na conservação de outras espécies, apesar das críticas. Outro fato é que como nem todas as espécies se beneficiam igualmente, é possível integrar abordagens alternativas ou complementares à essa estratégia, visando espécies que requerem ações de conservação especializadas (RUNGE *et al.*, 2019).

Abordagens baseadas em espécies emblemáticas podem ser utilizadas em conjunto com outras estratégias locais e regionais para fomentar a conservação da vida silvestre junto às comunidades locais e aos tomadores de decisão (NEW, 2011).

Devido às características da espécie, da paisagem e do panorama apresentado, utilizar a abordagem de espécie guarda-chuva emblemática e análises integradas, em um contexto interdisciplinar são fundamentais para a conservação da espécie alvo e podem auxiliar na conservação de outras espécies e seus habitats (CARO, 2010).

Dessa maneira, o tamanduá-bandeira encontra-se ameaçado, com populações em áreas altamente fragmentadas, assim como outras espécies com populações na área de estudo.

A utilização da abordagem genética em escala local e regional, a partir da estratégia baseada em espécie emblemática pode ser fundamental, uma vez que a identificação de populações prioritárias do ponto de vista genético, indica que as populações da espécie devem ser protegidas. Consequentemente, a proteção dessas populações em áreas protegidas e não protegidas poderá auxiliar na conservação de outras espécies (FRANKHAM; BALLOU; BRISCOE, 2002; FRANKHAM *et al.*, 2019).

Assim, avaliamos a diversidade genética e a existência de estruturação genética populacional na espécie *M. tridactyla*, além da relação entre a estrutura genética e a distribuição geográfica das populações em relação às características da paisagem.

A problemática do presente trabalho é evidente e avaliações por modelagens ecológicas predizem que as populações da espécie no estado de São Paulo estão fadadas à não persistir em longo prazo nos habitats altamente fragmentados, mesmo em cenários otimistas (BERTASSONI; BIANCHI; DESBIEZ, 2021).

Portanto, o presente trabalho visa avaliar, por meio de análises genéticas moleculares, os efeitos relacionados a eventos recentes como a fragmentação antrópica do habitat e eventos históricos relacionados à dispersão histórica das populações. Elucidar estes fatores pode contribuir inicialmente para a conservação da espécie, para identificar populações prioritárias ou em risco, e para a implementação de estratégias e ações que auxiliem na conservação de outras espécies e seus habitats.

2. OBJETIVOS

2.1. Objetivo geral

- O trabalho teve como objetivo principal a caracterização genética em nível molecular da espécie *Myrmecophaga tridactyla* no estado de São Paulo, a partir da análise de gene não neutro e genes neutros.

2.2. Objetivos específicos

- Analisar as sequências dos genes 16S e RAG2 para avaliar padrões históricos dos indivíduos remanescentes em áreas protegidas e não protegidas em São Paulo.

- Analisar o exon 2 do gene DRB para avaliar as variantes genéticas relacionadas a eventos recentes, em comparação com SSR.

- Avaliar a diversidade genética e estrutura populacional em diferentes escalas temporais por meio de marcadores moleculares neutros e não neutros.

- Verificar a contribuição de eventos demográficos e ambientais na riqueza e distribuição da diversidade genética adaptativa na espécie.

- Inferir a diferença entre barreiras históricas e barreiras recentes ao fluxo gênico em uma região altamente antropizada.

- Iniciar a criação de uma base de dados para outras pesquisas que visam compreender as dinâmicas das populações de tamanduás-bandeiras, os riscos de extinções locais da espécie e a estabelecer estratégias viáveis de conservação.

3. MATERIAIS E MÉTODOS

As metodologias utilizadas para a obtenção e análise dos dados estão apresentadas nos Capítulos a seguir, em formato de manuscrito (artigo científico). Os métodos apresentam variações, portanto os diferentes parâmetros estatísticos implementados nas análises e a amostragem utilizada estão descritos separadamente em cada Capítulo.

A lista dos animais amostrados e informações como a localidade, ocorrência, sexo e entre outras, estão arquivadas no repositório de dados Dryad (https://doi.org/10.5061/dryad.cfxpnvx31).

4. RESULTADOS

Os resultados, discussões e conclusões estão organizados em dois Capítulos, apresentados a seguir, na forma de artigos científicos submetidos a revistas especializadas e indexadas. Resultados já publicados estão em anexo (ANEXO A): “Identifying priority giant anteater (Myrmecophaga tridactyla) populations for conservation in São Paulo State, Brazil”

Capítulo I: “Genetic diversity and molecular sexing of giant anteater: efforts for conservation and effects on other species”

Capítulo II: “Adaptive molecular polymorphism in giant anteater using a viable low-cost method”

4.1 Capítulo I

Manuscrito submetido para a revista **Animal Conservation**
(Qualis A1 – Biodiversidade); (Fator de Impacto 3.650)

Original Article

Genetic diversity and molecular sexing of giant anteater: efforts for conservation and effects on other species

Ricardo Sartori[1], Cinara Brandão[2], Lilian Castiglioni[1,2]

[1] São Paulo State University, UNESP IBILCE, São José do Rio Preto - SP, Brazil
[2] São José do Rio Preto Medical School, FAMERP, São José do Rio Preto - SP, Brazil
Correspondence: Ricardo Sartori - Av. Brigadeiro Faria Lima, Vila São José, 5416, 15090-000, São José do Rio Preto, São Paulo – Brazil. ricardoquiterio@gmail.com

Conservation genetics of giant anteaters and its effects

Abstract

Environmental impacts caused by economic development include the potential loss of habitats, leading countless species under threat of extinction, and the process of deforestation leads to the formation of isolated habitat fragments. The effects of fragmentation and habitat loss over a wildlife species population can be assessed when genetic diversity and landscape characteristics are considered for the investigation of anthropogenic impacts. The aims are to analyze the genetic structure of the giant anteater (*Myrmecophaga tridactyla*) in a region of the state of São Paulo, Brazil. This species has been one of the main victims of habitat fragmentation, and it is included in the “Vulnerable” category by the IUCN. The SRY marker was used for molecular sexing and proved to be an efficient, reliable and fast method for identifying the sex of the animals. The 16S gene is highly conserved in the species, and results corroborate the slow mutation rates in mitochondrial DNA for Xenarthra. The RAG2 gene results indicated low genetic diversity in the population, revealing four polymorphic sites and four haplotypes with significant p-value (p=0.030). The nucleotide diversity was 0.00039, and haplotypic diversity was 0.273, indicating low diversity in the nuclear gene. The results corroborate other studies in these populations including the CytB gene. The low levels of diversity found in genes usually employed in phylogeographic analysis indicate that the species must continue in the red list. Also, previous microsatellite analysis showed the recent fragmentation of the population in the state of São Paulo. The combined genetic approach by comparing different mutation ratio genes allowed us to infer the population's historical and recent diversity, dynamics and structure. Actions may be taken based on the “flagship umbrella” strategy once the conservation of these populations can help to preserve other species and to protect their habitat.

Keywords: conservation genetics, mitochondrial, nuclear, Brazil, São Paulo.

Introduction

Many natural populations are threatened by the dramatic reduction in the total area of available habitat, by increasing habitat fragmentation, and even by species characteristics, leading to population decline (Kinnaird et al. 2003).

Thus, there is a great effort regarding the conservation of fauna worldwide, especially concerning threatened species in Neotropical regions (Moraes-Barros & Arteaga 2015).

Brazil is home to the greatest biological diversity among the world's megadiverse countries, but great environmental impacts caused by economic development are visible mainly in Southeast Brazil, where numerous species are at risk of extinction and there are countless threatened populations (Bolzani 2016).

Due to the intense impact caused by human actions related to the deforestation of large areas intended for planting crops and building roads, the fragmentation of habitat leads to the emergence of barriers to gene flow because the exchange of individuals between subpopulations is restricted (Frankham et al. 2002). In this scenario, one of the most threatened species in Brazil is the giant anteater, therefore it is essential to carry out studies that address the genetic profile of the species (Clozato et al. 2017; Diniz & Brito 2013).

Genetic studies on the remaining populations of a species can be fundamental for understanding population dynamics, migration rates, genetic structure, similarities between population isolates, and the effects of habitat fragmentation, also providing a tool for the development of conservation programs (Collevatti et al. 2007). This way, conservation genetic studies are highly relevant and can help create action plans for the conservation of remaining giant anteater populations (Costa et al. 2016).

The dispersions and evolutionary history of a population can be measured combining conserved genes and genes with a high mutation rate, since the comparison between the patterns revealed by different mutation rate genes can provide valuable clues about the dynamics and behavioral changes of the species, helping to differentiate recent and historical aspects in populations, and to understand adaptation through time and space (Rissler 2016). This ensemble approach of genes frequently used in landscape genetic analysis with genes used in phylogeography is mainly useful in anthropized areas to help understand the current impacts of habitat fragmentation on the species. Thus, in the present study, we evaluated genes with different mutation

rates, mitochondrial (mtDNA) and autosomal nuclear (nDNA), to understand evolutionary aspects of the species compared to previous results using microsatellite (SSR) markers (Garcia et al. 2005; Sartori et al. 2021). Mitochondrial genes have maternal inheritance and relatively high mutation rates, between five to ten times that of single-copy nuclear DNA, and the period of evolution is dissimilar for different regions of mtDNA (Palumbi et al. 1991; Barros et al. 2003; Wan et al. 2014).

Additionally, the investigation of a nuclear marker may be important for the evaluation of isolated populations and for comparison of the levels of intraspecific genetic diversity and population structure, mainly related to historical events (Teeling et al. 2001; Clozato et al. 2017).

In a previous study, we performed analyses on the same giant anteater populations using specific microsatellite molecular markers (Garcia et al. 2005; Sartori et al. 2021). Thus, the comparison of genes with different mutation rates can help to understand differences between historical and recent evolutionary events, such as anthropic habitat fragmentation (Muniz et al. 2019).

It may be difficult to separate the effects of recent anthropogenic fragmentation from the genetic signature of prehistoric fragmentation due to previous natural geological and climatic changes. So, to address these challenges, the examination of the phylogenetic and population genetic structure of a population can be performed to confirm contemporary patterns of genetic structure and the possibility to be produced by biologically reasonable assumptions about model parameters (Vandergast et al. 2007).

The aim of this work is to evaluate, through different approaches, the genetic and dynamic characteristics of the population of giant anteaters in the most urbanized and populous state of Brazil.

Due to the great threat of extinction suffered by the species *M. tridactyla* and the economic characteristics of the studied region, which has been an intense target of anthropic actions mainly related to the deforestation of large areas for planting crops, it is essential to carry out studies that address the genetic structure of this terrestrial species (Costa et al. 2016).

Ecological studies carried out in the central-south region of the state of São Paulo indicate that giant anteaters are possibly unable to persist in habitats composed only of altered environments. The low rate of population persistence in the study area

in the long term draws special attention and measures to mitigate human impacts on the species should be established (Bertassoni et al. 2021).

Thus, conservationist genetic studies are highly relevant and can help to create action plans for the conservation of the remaining giant anteater populations and other species populations in the studied region.

Methods

Sampling and molecular markers

A total of 41 individuals were analyzed: 30 samples obtained in partnership with the Veterinary Hospital “Dr. Halim Atique”, including eight samples from the surroundings of the Noroeste Paulista Ecological Station (protected area), five samples collected from animals from the São José do Rio Preto Municipal Zoo, and six samples provided from capture campaigns at the Santa Bárbara Ecological Station (protected area). The genomic DNA was extracted using the QIAamp DNA kit according to the manufacturer's instructions. The marker pair for the SRY gene was used (Takami et al. 1998), a pair for the amplification of the 16S mitochondrial gene fragment (Palumbi et al. 1991), and a pair for the RAG2 nuclear gene fragment (Teeling et al. 2001; Clozato et al. 2017).

Amplification conditions and statistical analysis

The amplification was performed in a Veriti 9700 thermocycler with a standardized total volume of 25μL, containing 3μL DNA (approximate concentration 100 ηg/μL), 1μL forward primer, 1μl of reverse primer, 12.5μL of Gotaq® Master Green Promega, and 7.5μL of ultra-pure water.

For molecular sexing, a fragment of the SRY gene was used, from the pair of primers F 5' AAGCGACCCATGAATGCATTCATGGTGTGGT 3' and R 5' GAGGTCGATACTTATAGTTCGGGTATTTCTCT 3', designed by Takami et al. (1998). The amplification conditions were: denaturation at 95ºC for three minutes, 35 cycles of 93ºC for one minute, 55ºC for two minutes, 72ºC for three minutes for the annealing, and an additional extension step at 72ºC for seven minutes. After electrophoresis in 2% agarose gel, it was possible to identify the sex of the animals for carrying out the aforementioned complementary analyses.

For the 16S mitochondrial gene fragment, the amplification conditions were adapted from Barros et al. (2003): denaturation at 95ºC for three minutes, 35 cycles of 94°C for 30 seconds, 50°C for one minute, 72°C for two minutes for annealing, and an additional extension step at 72ºC for 10 minutes. For the analysis of the 16S, three sequences of theGenbankAY245753.1 (SP), AY245754.1 (PA), and AY245755.1 (PI) were used, totaling 44 sequences.

For the analysis of the RAG2 nuclear gene, the amplification was performed by the method touchdown PCR: denaturation at 95ºC for three minutes, 15 cycles of 94ºC for 30 seconds, (68 – 54ºC) for one and a half minute, 72ºC for two minutes and 20 cycles of 94ºC for 30 seconds, 54ºC for a minute and a half, 72ºC for two minutes for the annealing and an additional extension step at 72ºC for 10 minutes. Forty sequences were used for this fragment, as we were not successful in sequencing one of the samples (Korbie & Mattick 2008).

Amplifications were performed in a Veriti 9700 thermocycler and in a volume 25μL total, containing 50ηg/μL of DNA, 1μL of primer forward, 1μl of primer reverse, 12.5μL of Gotaq® Master Green Promega, and 8.5μL of ultra-pure water.

PCR products were purified with the commercial GenElute PCR Clean-Up (Sigma-Aldrich) following the manufacturer's instructions, and the samples were sequenced with the set BigDye Terminator v3.1 Cycle Sequencing Kitin automatic sequencer ABI 3500 (Applied Biosystems) by the Sanger method (Sanger et al. 1977). Confirmation of the homology of the fragments of all sequences was obtained by the BLAST tool (www.ncbi.nlm.nih.gov/Blast.cgi).

The quality of the electropherograms was verified using the PHPH online tool (Towaga & Brigido 2003). To edit and align sequences by method ClustalW, we used the software BioEdit v. 7.2.5 (Hall 1999).

After alignment, the mitochondrial and nuclear gene fragments showed 330 and 725 base pairs (bp). The haplotypic network was built with the aid of the computational resource TCS 1.21 (Clement et al. 2000). For the RAG2 gene, genetic diversity, distance, pairwise distribution, and gene flow analyses using the δst and λst indices (Nei 1982), the MEGA 7 program (Kumar et al. 2016), DnaSP 4.0 program (Librado & Rozas 2009) and GENEPOP 1.2 (Raymond & Rousset 1995) were used to calculate. The gametic phase was verified, with the PHASE function in DnaSP 4.0 (Librado & Rozas 2009; Stephens et al. 2001) and the AMOVA performed with the ARLEQUIN

3.5 program (Excoffier & Lischer 2010). The maps were generated in DIVA-GIS version 7.5 (Hijmans et al. 2012).

DNA sequence data will be deposited in the GenBank nucleotide sequence database (https://www.ncbi.nlm.nih.gov/genbank/). All biological material collection was authorized for scientific purposes by the Brazilian Federal Environmental Agency (IBAMA) and approved by Research Ethics Committees (CEUA) under the numbers: 43854-4; 04/2014PP; 2014/05302-8; COTEC 429/2014; D23/201; 38326-5 and 003414/13.

Results

The molecular sexing allowed us to identify males and females for further statistical analysis. This method proved to be efficient, reliable, and fast in identifying the sex of giant anteaters. Each individual was tested three times in different amplification reactions to avoid possible errors in molecular sexing. PCR products were visualized on 2% agarose gel, and male individuals showed a band of approximately 210 pairs of bases (Figure 1).

After performing electrophoresis and visualizing the gels, 24 males and 17 females were identified. Thus, the reliable identification of the sex of the individuals made it possible to carry out complementary analyses to investigate genetic patterns related to sex.

Groups of males and females were analyzed in order to elucidate possible differences in genetic patterns of evolutionary and ecological scale. This investigation is relevant due to the characteristics of the species, such as the long gestation period, long caution parental behavior, and solitary habits; and these analyses make it possible to evaluate differences in rates of migration between males and females.

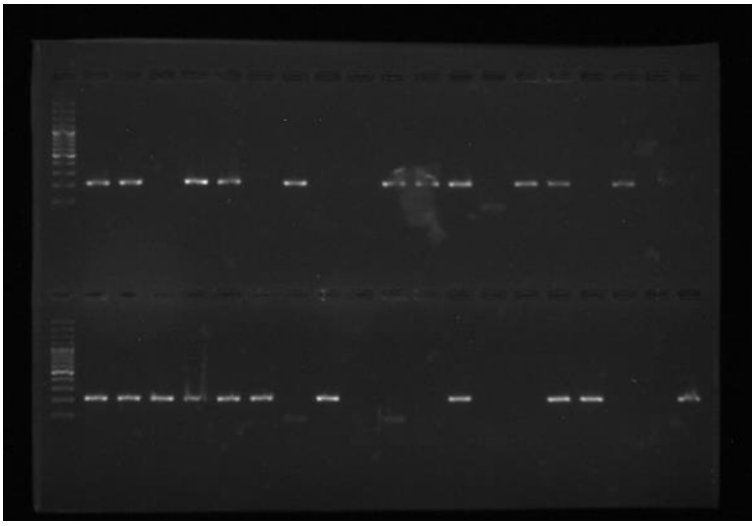

Figure 1. 2% agarose gel, illustrating the presence of bands in males and absence in females. A simple and efficient way to identify the sex of animals.

The analysis of the 16S fragment indicated that there is no genetic diversity or distance, as it revealed an average value of 0.000 between individuals, and only one haplotype was identified, indicating that the 16S gene is extremely conserved. The evaluation of conserved genes is fundamental for the approach proposed in this work, but the nullity of polymorphisms did not allow further exploration of the data in relation to this marker.

Thus, despite the 16S intraspecific polymorphisms found in other mammalian species, this marker is not efficient for intraspecific analyses in relation to the species *M. tridactyla*, instead, it is a highly recommended tool for identification of the species. Nonetheless, analysis of the RAG2 fragment presented polymorphisms, and the marker was useful for the intraspecific approach and identification of the species.

RAG2 Results

Haplotypic distribution

The generated network (Figure 2 a.b) identified four haplotypes, being: (I) with the highest frequency and found in 25 individuals (individuals from the EENP, NP, and EESB); (II) shared by 13 individuals (EENP, NP, and ESSB); (III) and (IV) two unique haplotypes (NP and EESB). The haplotypes I and II are shared by all population groupings and there is no historical subdivision evidence for the population of the state of São Paulo (Figure 3).

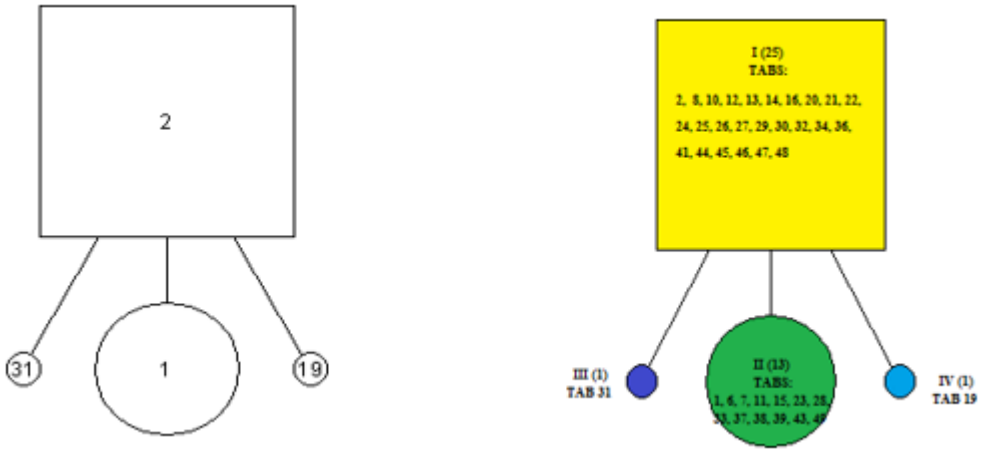

Figure 2. Haplotypic network illustrating the distribution of shared haplotypes among individuals sampled for the RAG2 gene. a. Original network generated by the program. b. The edited network contains the identification of individuals by haplotype.

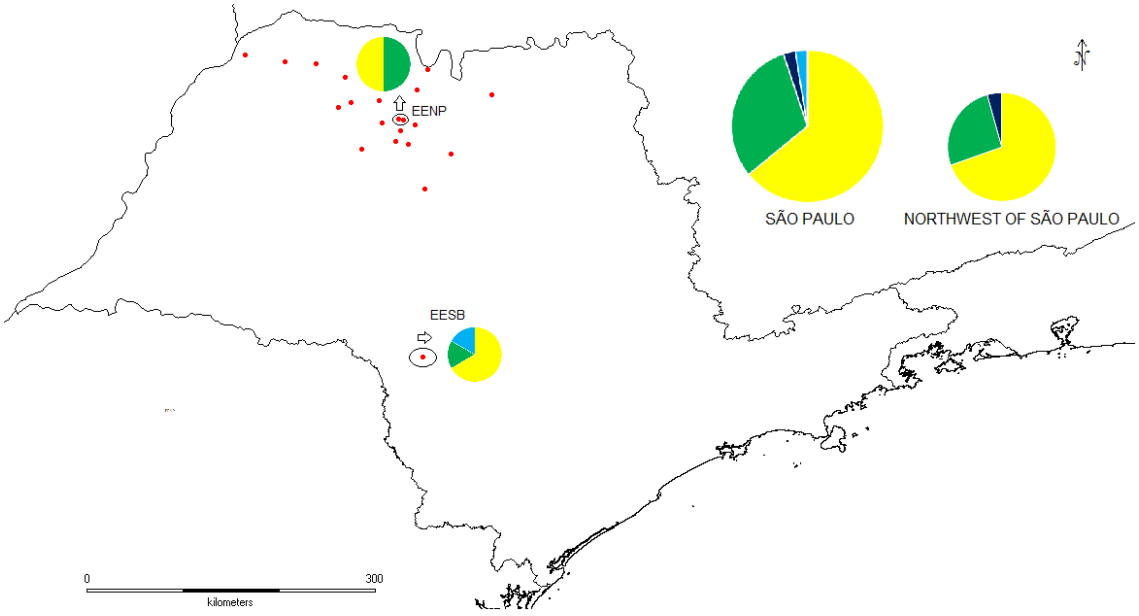

Figure 3. Spatial haplotypic distribution among population groupings for the RAG2 gene. The haplotypes I and II are shared by all population groupings revealing a lack of evidence of historical subdivision for the population in São Paulo in this gene history. EESB (Santa Bárbara Ecological Station) and EENP (Noroeste Paulista Ecological Station). Unique haplotypes were identified in protected and non-protected areas.

Genetic diversity indices

The results indicated low genetic diversity. Four polymorphic sites, four haplotypes with significant p-value (p=0.030), and four mutations were identified. Nucleotide diversity was 0.00039 and haplotypic diversity was 0.273, indicating low genetic diversity in populations for this gene.

Gene flow analysis

The values of gene frequency between populations of Ecological Stations and the interpopulation proportion of genetic variability were δst = 0.00005 and λst = 0.08246, and the number of migrant sites (Nm) was 2.78, indicating high historical gene flow.

Population genetic structure

Molecular analysis of variance indicated that the greatest genetic variance is contained within the populations (Tables 1 and 2) and p values were significant, except for θCT. In summary, low diversity and genetic distance were found, high gene flow and greater diversity within population groups.

Table 1. Mean F(θ) Statistics and Significance Tests

Fixation index		Significance Tests
θST:	0.08083	p = 0.00489
θSC:	0.14842	p = 0.00587
θCT:	-0.07937	p = 1.00000

Table 2. Analysis of Molecular Variance (AMOVA)

Source of variation	Sum of squares	Variance components	Percentage of variation
Among groups	0.500	-0.03294	-7.94
Among populations	3.210	0.06648	16.02
Within populations	28.990	0.38145	91.92
TOTAL	32.700	0.41500	

Nucleotide and haplotype diversity by male and female groups

The analyses from the RAG2 gene sequences indicated no correlation between the haplotypic distribution and the geographic region of the individuals. Even in a more conserved gene, females showed slightly greater genetic diversity than males in the analyzed area. Diversity indexes for females: two haplotypes, Haplotype diversity (Hd) = 0.121 and Standard Deviation of Haplotype diversity = 0.075, Nucleotide diversity (Pi) = 0.00017 and Average number of nucleotide differences (k) = 0.12097. For males: two haplotypes, Haplotype diversity (Hd) = 0.082 and Standard Deviation of Haplotype diversity = 0.053, Nucleotide diversity (Pi) = 0.00011 and Average number of nucleotide differences (k) = 0.08156.

These results allow us to infer the importance for the species of the dispersion of females, and highlight that they have greater dispersal capacity and greater genetic diversity than males in historical patterns point of view.

Discussion

The investigation of the effects of habitat fragmentation through the analysis of mitochondrial and nuclear genes with different mutation levels was fundamental to investigate evolutionary and ecological aspects, allowing to make inferences about historical dispersal and recent events of giant anteater populations in the state of São Paulo.

Molecular evolution rates in the genes of different species can vary substantially due to biological aspects of each species, evolutionary history, and effective population size (Gissi et al. 2000; Huang et al. 2008; Welch et al. 2008; Lanfear et al. 2014; Allio et al. 2017).

In mammals, the 16S gene generally has highly conserved regions across species (Yang et al. 2014), but presents intraspecific polymorphisms in populations of bats, mice, and humans (Lloyd 2003; Galtier et al. 2006; Abhyankar et al. 2009). The only haplotype found in the 16S analysis indicates that this may be the oldest haplotype for this population of the species, having a higher probability fixation within populations, leading to a lack of genetic differentiation of the population.

It is worth mentioning that another study using the less conservated mitochondrial gene CytB also showed null diversity for some giant anteater populations in the states of Mato Grosso and Paraná, in which only one haplotype was found and made it impossible to calculate the diversity indices. For other populations of the

species, this same CytB fragment was polymorphic and revealed different levels of genetic diversity and haplotypes, including in the state of São Paulo (Clozato et al. 2017).

We thus assume that genetic diversity can vary considerably in different mitochondrial genomic regions, and different populations of *M. tridactyla* showed different levels of genetic diversity in the same mitochondrial region. This fact elucidates the importance of multilocus genetic studies for a better understanding of population dynamics.

The high gene flow and low genetic diversity found with the RAG2 nuclear gene in populations from the state of São Paulo demonstrate that populations from different locations exchanged genetic material, and that the population expansion and gene flow found may reflect the ancestral history of dispersal in the species (Frankham 2005).

Ribeiro and Morales (2016) analyzed the mitochondrial gene CytB for evaluation of giant anteater populations in the same areas of the state of São Paulo and identified three haplotypes, genetic uniformity, evidence of gene flow, and low haplotypic diversity, without population structure. Thus, our results of a nuclear gene corroborate previous findings using mtDNA. Both studies also revealed low genetic diversity, and seem to indicate that female migration was fundamental in the process of species dispersion in the state of São Paulo, as the high gene flow found in a mitochondrial gene suggests that, historically, giant anteater populations were interconnected, and female dispersal may have been a likely mechanism that helped to connect populations and fix genes of matrilineal origin in these populations (Frankham et al. 2002).

Therefore, the slightly higher migration rate and diversity found in females with the nuclear gene RAG2 may indicate the importance of females in the dispersion of the species through the Cerrado of São Paulo. In addition, the low genetic diversity found associated with characteristics of the species and the strong anthropic actions in the areas make the species remain on the endangered animal lists.

This way, the results of another study using the mitochondrial gene CytB in populations from the state of São Paulo presented similar results when compared with our findings for the RAG2 nuclear gene that indicated the presence of more haplotypes than mitochondrial gene fragments. This fact can also be observed in the study performed by Clozato et al. (2017), in which nuclear markers (RAG2 and iAMELY) were also more polymorphic than mitochondrial genes (HVI and CytB) in populations of the state of São Paulo, with a greater number of haplotypes and diversity.

Indeed, comparative studies of mitochondrial gene mutation rates between different species and orders of mammals demonstrated that the superorder Xenarthra has the lowest mutation rates in mitochondrial genes when compared to other orders or clades. Bininda-Emonds (2007) investigated several genes, including 15 mitochondrial gene loci for several mammalian clades, and observed that the Xenarthra clade presented the lower rates of mutation in mitochondrial genes when compared to other mammalian clades. This feature extends to the entire Xenarthra clade, including the *M. Tridactyla* species.

Therefore, the use of a marker in a more conserved gene region of mitochondria and an autosomal nuclear marker, combined with the comparison of previous results using high mutation rate genes, provided us with a deeper knowledge of the historical time of this species, making it possible to understand it in ecological and evolutionary scales.

Also concerning high mutation rate genes, our previous studies evolving the analysis of microsatellite markers indicated low levels of gene flow, population structure, and high genetic diversity in populations, in contrast to the results of the other more conserved genes (Sartori et al. 2021). These differences found between the microsatellites and the other analyzed fragments can be explained by the characteristics of each molecular marker. Despite mitochondrial DNA (such as the CytB) and microsatellites have high mutation rates, the rates of mutation can vary considerably depending on the species. In addition, the microsatellites have faster mutation rates than mitochondrial and autosomal nuclear genes in mammals, and can detect more clearly recent events in populations (Wan 2004).

In this way, the comparison of results in different mutation ratio genes made it possible to understand recent events, such as the impacts of habitat fragmentation on populations in the state of São Paulo, which are of great importance to understand ecological issues about these populations. The comparison of microsatellites and more conserved genes results provided valuable clues for us to infer the behavioral changes in the species' populations. Thus, the results indicate that recent habitat fragmentation directly influences the genetic structure of giant anteater populations in the state of São Paulo. Also, the populations showed historical connection, which can be important for conservation actions in possible future translocations and breeding programs.

The comparison of these different targeted genes can be addressed as a combination of landscape genetics and phylogeographic approach, mainly because

genes used for historical investigation were already evaluated in other studies with a longer geographical range in a phylogeographic approach (Clozato et al. 2017). Our results corroborate the studies made with fewer samples in this region of São Paulo, so the current results contribute to implementing fine-scale data to the understanding of the historical distribution of the species in the state of São Paulo.

In summary, our previous results of the microsatellites indicated a low rate of migration, population structure, and high genetic variability, which shows the isolation of these population groupings, and some hypotheses indicate that evolutionary, ecological and biological factors may have led to this population pattern (Sartori et al. 2021). Genetic drift seems to be the most reasonable factor to explain the high diversity genetics found, since the low migration rate leads to regional differences in the gene frequency of the subpopulations. In a metapopulation with populations isolated, different alleles are fixed, increasing the variance between subpopulations due to the involuntary mixing of separate populations throughout the process of habitat fragmentation (Ridley 2003).

According to Frankham et al. (2002), in a short period, without the occurrence of extinction, the various small populations are expected to show fixation faster, but retain greater total genetic diversity than large and unique populations. That is, the genetic consequences of the fragmentation of habitat in a large and unique population are different from the genetic consequences in several fragments of small, completely isolated populations, which have initially the same population size on different time scales. The probability that an allele is lost in a large population is greater than for all small populations combined. However, in the long term, when extinctions of small populations occur, the total of small surviving populations will retain less genetic diversity than the large and unique ones (Frankham et al. 2002; Frankham 2005).

This theory supports the high genetic diversity obtained with SSR markers in populations of highly fragmented areas, and the gene flow levels do not just depend on the distance between populations, but also on the nature of the surrounding landscape between the populations (Templeton et al. 2001).

In addition to the evolutionary and environmental factors raised as the characteristics' genetics, demographic history of each population, and ecological aspects of the landscape; profile analyses of wild animal populations should also take into account the biological questions of the species (Lacy 1997). The biological characteristics of some species can influence the levels of loss of genetic diversity in

populations, and a factor that seems to contribute to the maintenance of genetic variability in threatened populations is a long time of life span and generational period of some species, as long-lived animals can take longer to present a clear genetic response to the impacts' environmental factors (Hailer et al. 2006).

Thus, we argue that the long-life span of giant anteaters, between 20 and 30 years (Nowak 1999), together with the demographic and evolutionary history of analyzed populations, buffered the loss of genetic diversity. That is, species with greater longevity may have virtually allele maintenance ancestors and a lower decline in genetic variability after population bottlenecks.

The role of evolutionary factors, such as genetic drift and natural selection, can fix different alleles in different subpopulations, reflecting the genetic diversity found in these populations for the microsatellite loci. So, our results indicate that some populations of vulnerable and threatened species of extinction can maintain high levels of genetic diversity even in areas intensely fragmented, and that different populations of the same species can respond in different ways to environmental impacts.

In general, it was possible to characterize the genetic structure of the populations of giant anteater in the western region of the state of São Paulo through the following analyses: sequencing of a non-coding 16S mitochondrial gene fragment, sequencing of the autosomal nuclear gene RAG2, and analysis by sex groups through the analysis of the SRY marker. The comparison with SSR markers and the analysis of different molecular markers with different mutation rates, made it possible to assess evolutionary and ecological issues, allowing us to understand recent fragmentation and produce knowledge for the conservation of the species.

Thus, we suggest that populations of the species in the state of São Paulo demonstrate a recent genetic response to habitat fragmentation, and results indicate the need to establish management plans for the conservation of these populations, as the impacts of habitat fragmentation increase the risk of local extinctions and the loss of genetic diversity, which is essential for the recovery of the species, whether in breeding programs in captivity, reintroductions, translocations or genetic rescue.

The comparison of different molecular markers and the high rates of mutation of the microsatellite markers allowed us to identify the changes of the population dynamics in the face of the effects of habitat fragmentation in these populations and differentiate recent and historical patterns.

In line with our findings, ecological studies carried out in the state of São Paulo, more specifically at the Santa Bárbara Ecological Station, indicate that giant anteaters are possibly unable to persist in composite habitats only by altered environments, and the low rate of population persistence in the study area in the long term (less than 8 individuals remaining in 100 years) demonstrates that measures to mitigate the anthropic impacts on the species must be established (Bertassoni et al. 2021).

Finally, we suggest that strategies for the conservation of a “flagship umbrella” species, such as the giant anteater, can be a valid strategy for the protection of other species, including populations of the protected areas and surroundings. For example, some of the mammal species that could benefit from the protection of the giant anteater, and that share the same areas at Noroeste Paulista Ecological Station, are the puma (*Puma concolor*), the black howler (*Alouatta caraya*), the maned wolf (*Chrysocyon brachyurus*), the little wildcat (*Leopardus guttulus*), the ocelot (*Leopardus pardalis*) and the jaguarundi (*Puma yagouaroundi*) (Instituto Florestal 2019). At Santa Bárbara Ecological Station, seven mammal species are in some category of threat, from of which one is a small rodent (*Cerradomys scotii*), the soft-tailed armadillo (*Cabassous unicinctus*), the maned wolf (*Chrysocyon brachyurus*), the ocelot (*Leopardus Pardalis*), the puma (*Puma concolor*) and the pampas deer (*Ozotoceros bezoarticus*) (Instituto Florestal 2011). Thus, different threatened species may take benefit of conservation when strategies like that are implemented in protected and non-protected areas.

The present work contributed to the production of knowledge concerning the conservation of the species and strengthened the database to establish the need for the creation of local management plans. The results can be used to support and guide the creation of conservation programs for the species.

Conclusions

Populations of the northwest and central-south regions of the state of São Paulo are important for the conservation and recovery of the genetic diversity of the species, and both regions presented unique haplotypes for the RAG2 gene.

The combination of the results of high mutation rates and more conserved genes revealed how recent habitat fragmentation directly influences the genetic structure of giant anteater populations in the state of São Paulo.

The effective protection of Ecological Stations and private lands (including legally protected areas and Permanent Protection Areas) are extremely important for the remaining populations of giant anteaters in the state of São Paulo.

The protection of these populations based on genetic priority may help to conserve other species in highly anthropized and protected areas.

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Conflict of interest

None declared

Author contribution statement

R.Q.S: Data curation; formal analysis; investigation; methodology; project administration; software; visualization; writing-original draft; writing-review and editing. C.C.B.: Supervision; visualization; writing-review and editing. L.C.: Conceptualization; formal analysis; funding acquisition; project administration; resources; supervision; validation; visualization; writing-review and editing. Reviewed by all authors.

References

Abhyankar, A., Park, H-B., Tonolo, G. & Luthman, H. (2019). Comparative Sequence Analysis of the Non-Protein-Coding Mitochondrial DNA of Inbred Rat Strains. *PLoS ONE*. 12, 4, 8148. https://doi.org/10.1371/journal.pone.0008148

Allio, R., Donega, S., Galtier, N. & Nabholz, B. (2017). Large Variation in the Ratio of Mitochondrial to Nuclear Mutation Rate across Animals: Implications for Genetic Diversity and the Use of Mitochondrial DNA as a Molecular Marker, *Mol. Biol. Evol*. 34, 11, 2762–2772. doi: 10.1093/molbev/msx197. PMID: 28981721.

Barros, M.C., Sampaio, I. & Schneider, H. (2003). Phylogenetic analysis of 16S mitochondrial DNA data in sloths and anteaters. *Genet. Mol. Biol.* [online]. 26, 1, 5-11. doi:10.1590/S1415-47572003000100002

Bertassoni, A., Bianchi, R.D.C., & Desbiez, A.L.J. (2021). Giant Anteater Population Density Estimation and Viability Analysis Through Motion-Sensitive Camera Records. *J. Wildl. Manag*. 85, 1554-1562. doi:10.1002/jwmg.22123

Bininda-Emonds, O.D.A. (2007). Fast Genes and Slow Clades: Comparative Rates of Molecular Evolution in Mammals. *Evol. Bioinform. Online*. 3, 59–85. PMID: 19461986. PMCID: PMC2684144.

Bolzani, V.S. (2016). Biodiversidade, bioprospecção e inovação no Brasil. *Cien. Cult.* [online]. 68, 1, 04-05. http://dx.doi.org/10.21800/2317-66602016000100002

Clement, M., Posada, D. & Crandall, K.A. (2000).TCS: a computer program to estimate gene genealogies. *Mol. Ecol*. 9, 10, 1657-1660. doi: 10.1046/j.1365-294x.2000.01020.x.

Clozato, C.L., Miranda, F.R., Lara-Ruiz, P., Collevatti, R.G. & Santos, F.R. (2017). Population structure and genetic diversity of the giant anteater (*Myrmecophaga tridactyla*: Myrmecophagidae, Pilosa) in Brazil. *Genet. Mol. Biol.* 40, 1. https://doi.org/10.1590/1678-4685-GMB-2016-0104

Collevatti, R.G., Leite, K.C.E., Miranda, G.H.B. & Rodrigues, F.H.G. (2007). Evidence of high inbreeding in a population of the endangered giant anteater, *Myrmecophaga tridactyla* (Myrmecophagidae), from Emas National Park, Brazil. *Genet. Mol. Biol.* 30, 1, 112-120. https://doi.org/10.1590/S1415-47572007000100020

Costa, M.S., Araujo, A.M., Campelo, J.E.G., Machado, T.M.M., Pires, L.C., Egito, A.A. & Mariante, A.S. (2016). Survey and morphological characterization of Brazilian local goat breed Gurgueia in its origin. *Rev. Bras. de Saude e Prod. Anim*. 17, 2, 127-138. https://doi.org/10.1590/S1519-99402016000200001

Diniz, M.F. & Brito, D. (2013). Threats to giant anteater, *Myrmecophaga tridactyla* (Pilosa: Myrmecophagidae), viability in a protected Cerrado remnant encroached by urban expansion in central Brazil. *Zoologia*. 30, 151–156. https://doi.org/10.1590/S1984-46702013000200005

Excoffier, L. & Lischer, H.E. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour*. 10, 3, 564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Frankham, R., Ballou, J.D. & Briscoe, D.A. (2002). Introduction to Conservation Genetics. Cambridge University Press, Cambridge.

Frankham, R. (2005). "Genetics and extinction". *Biol. Conserv*. 126, 2, 131 140. doi:10.1016/j.biocon.2005.05.002.

Galtier, N., Enard, D, Radondy, Y., Bazin, E. & Belkhir, K. (2006). Mutation hot spots in mammalian mitochondrial DNA, *Genome Res*. 16, 215–222. doi: 10.1101/gr.4305906.

Garcia, J.E., Vilas Boas, L.A., Lemos, M.V.F., Macedo Lemos, E.G. & Contel, E.P.B. (2005). Identification of microsatellite DNA markers for the giant anteater *Myrmecophaga tridactyla*. *J. Hered.* 96, 600-602. https://doi.org/10.1093/jhered/esi089

Gissi, C., Reyes, A., Pesole, G. & Saccone, C. (2000). Lineage-specific evolutionary rate in mammalian mtDNA. *Mol. Biol. Evol*. 17, 7, 1022-31. doi: 10.1093/oxfordjournals.molbev.a026383.

Hailer, F., Helander, B., Folkestad, A.O., Ganusevich, S.A., Garstad, S., Hauff, P., Koren, C., Nygård, T., Volke, V., Vilà, C. & Ellegren, H. (2006). Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from population decline, *Biol. Lett*. 22, 2, 316-319. doi: 10.1098/rsbl.2006.0453

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis programfor Windows. *Nucleic Acids Symp. Ser.* 41, 95-98. doi: 10.14601/PHYTOPATHOL_MEDITERR-14998U1.29

Hijmans, R.J., Guarino, L., Bussink, C., Mathur, P., Cruz, M., Barrentes, I. & Rojas, E. (2012). DIVA-GIS 7.5. A geographic information system for the analysis of species distribution data. In: http://www.diva-gis.or

Huang, D., Meier, R., Todd, P.A. & Chou, L.M. (2008). Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding, *J. Mol. Evol.* 66, 167. doi: 10.1007/s00239-008-9069-5.

Instituto Florestal, I.F. (2011). Plano de Manejo da Estação Ecológica de Santa Bárbara. Governo do Estado de São Paulo.

Instituto Florestal, I.F. (2019). Plano de Manejo da Floresta Estadual Noroeste Paulista. Governo do Estado de São Paulo.

Kinnaird, M.F., Sanderson, E.W., O'Brien, T.G., Wibisono, H.T. & Woolmer, G. (2003). Deforestation Trends in a Tropical Landscape and Implications for Endangered Large Mammals, *Conserv. Biol.* 17, 1, 245–257. doi:10.1046/j.1523-1739.2003.02040.x

Korbie, D.J. & Mattick, J.S. (2008). TouchDown PCR for increased specifity and

sensitivity in PCR amplification, *Nat. Protoc*. 3, 1452-1456. doi: 10.1038/nprot.2008.133.

Kumar, S., Stecher, G. & Tamura, K. (2016). MEGA7:Molecular Evolutionary Genetics Analysis. V. 7.0 for Bigger Datasets, *Mol. Biol. Evol*. 33, 07, 1870-1874. doi: 10.1093/molbev/msw054.

Lacy, R.C. (1997) Importance of Genetic Variation to the Viability of Mammalian Populations, *J. Mammal*. 78, 2, 320-335. https://doi.org/10.2307/1382885

Lanfear, R., Kokko, H. & Eye-Walker, A. (2014). Population size and the rate of evolution, *Trends Ecol Evol*. 29, 1, 33-41. doi: 10.1016/j.tree.2013.09.009.

Librado, P. & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, Oxford. 25, 1451-1452. https://doi.org/10.1093/bioinformatics/btp187

Lloyd, B.D. (2003). Intraspecific Phylogeny of the New Zealand Short-Tailed Bat Mystacina tuberculata Inferred from Multiple Mitochondrial Gene Sequences, *Syst. Biol*. 52, 4, 460-476. doi: 10.1080/10635150390218187. PMID: 12857638.

Moraes-Barros, N. & Arteaga, M.C. (2015). Genetic diversity in Xenarthra and its relevance to patterns of neotropical biodiversity, *J. Mammal.* 96, 4, 3, 690–702. https://doi.org/10.1093/jmammal/gyv077

Muniz, A.C., Lemos-Filho, J.P., Buzatti, R.S.D.O., Ribeiro, P.C.C., Fernandes, F.M., Lovato, M.B. (2019). Genetic data improve the assessment of the conservation status based only on herbarium records of a Neotropical tree. *Sci. Rep.* 9, 5693. https://doi.org/10.1038/s41598-019-41454-0

Nei, M. (1982). Evolution of human races at the gene level. *Prog. Clin. Biol. Res.* 103, Pt A, 167-181. PMID: 7163193.

Nowak, R.M. (1999). Walker’s Mammals of the World. 1, 6 ed. Baltimore and London: The Johns Hopkins University Press.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. (1991). The Simple Fool's Guide to PCR. University of Hawaii Press, Honolulu.

Raymond, M. & Rousset, F. (1995). GENEPOP (Version 1.2): Population-Genetics Software for Exact Tests and Ecumenicism. *J. Hered*. 86, 248-249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Ribeiro, R.C & Morales, A.C. (2016). Genética populacional de *Myrmecophaga tridactyla* (Pilosa, Myrmecophagidae) no estado de São Paulo utilizando o gene mitocondrial CytB, 50 (Master´s dissertation), São Paulo State University. In: http://hdl.handle.net/11449/138129

Ridley, M. (2003). Evolution, 3rd ed. Wiley - Blackwell Science.

Rissler, L.J. (2016). Union of phylogeography and landscape genetics. *PNAS*. 113, 29, 8079-8086. doi:10.1073/pnas.1601073113

Sanger F., Nicklen S. & Coulson, A.R. (1997). DNA sequencing with chain-terminating inhibitors. *PNAS*. 74, 5463-7. doi: 10.1073/pnas.74.12.5463.

Sartori, R.Q., Lopes, A.G., Aires, L.P.N., Bianchi, R.C, Morales, A.C. & Castiglioni, L. (2021). Identifying Priority Giant Anteater (*Myrmecophaga tridactyla*) Populations for Conservation in São Paulo State, Brazil. *Ecol Evol*. 11, 700-713. https://doi.org/10.1002/ece3.6809.

Stephens, M., Smith, N. & Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet*. 68, 978-989. https://doi.org/10.1086/319501

Takami, K., Yoshida, M., Yoshida, Y. & Kojima, Y. (1998). Sex determination in Giant

Anteater (Myrmecophaga tridactyla) using hair roots by Polymerase Chain Reaction amplification. *J. Reprod. Dev*. 44, 1, 73-78. https://doi.org/10.1262/jrd.44.73

Teeling, E.C., Madsen, O., Van Den Busshe, R.A., De Jong W.W., Stanhope, M.J. & Springer, M.S. (2001). Microbat paraphyly and the convergent evolution of a key innovation in Old World rhinolophoid microbats*,* *PNAS*. 99, 1431-1436. https://doi.org/10.1073/pnas.022477199

Templeton, A.R., Robertson, R.J., Brisson, J. & Strasburg, J. (2001). Disrupting evolutionary processes: The effect of habitat fragmentation on collared lizards in the Missouri Ozarks, *PNAS.* 98, 10, 5426-5432. doi: 10.1073/pnas.091093098

Towaga, R.C. & Brigido, M.M. (2003). PHPH: Web based tool for simple electropherogram quality analysis. 1st International Conference on Bioinformatic and Computational Biology – *IcoBiCoBi*. Universidade de Brasília, Brasília, 73. In: http://asparagin.cenargen.embrapa.br/phph/reference.html

Vandergast A.G., Bohonak, A.J., Weissman, D.B. & Fisher, R.N. (2007). Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history: phylogeography and landscape genetics of a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: Stenopelmatus). *Mol. Ecol*. 16, 977–992. doi:10.1111/j.1365-294X.2006.03216.x

Wan, Q.H., Wu, H., Fujihara, T. & Fang, S.G. (2004). Which genetic marker for which conservation genetics issue? *Electrophoresis.* 25, 2165–2176. doi: 10.1002/elps.200305922. PMID: 15274000.

Welch, J., Bininda-Emonds, O. & Bromham, L. (2008). Correlates of substitution rate variation in mammalian protein-coding sequences, *BMC Evol. Biol.* 8, 53. https://doi.org/10.1186/1471-2148-8-53

Yang, L., Tan, Z., Wang, D., Xue, L., Guan, M., Huang, T. & Li, R. (2014). Species identification through mitochondrial rRNA genetic analysis, *Sci. Rep.* 4, 4089. https://doi.org/10.1038/srep04089.

4.2 Capítulo II

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Original Paper

Adaptive molecular polymorphism in giant anteater using a viable low-cost method

Ricardo Q. Sartori[1], Aparecida P. Silveira-Carvalho[2], Cinara C. Brandão[2], Luiz C. de Mattos[2], Lilian Castiglioni[1,2]

1 Universidade Estadual Paulista, UNESP/IBILCE. Sao Jose do Rio Preto - SP, Brazil
2 Faculdade de Medicina de São José do Rio Preto, FAMERP. Sao Jose do Rio Preto - SP, Brazil

Corresponding e-mail: ricardoquiterio@gmail.com

Abstract

Most of the biodiversity is located in countries of the Neotropical region. However, there are several limitations for conservation analysis due to socioeconomic factors, such as lack of financial resources, technology, and genomic/new generation sequence technical training. Therefore, viable low-cost alternatives to genetically assess populations of endangered species are critical to aid in conservation strategies. These limitations also occur in Brazil, and the use of economically viable methods in conservation genetics can be an important tool. One of the most threatened species in Brazil is the giant anteater (*Myrmecophaga tridactyla*), so we evaluated populations of the species in a highly anthropized area as a study model. The objective was to genetically characterize the species in the state of São Paulo through the analysis of the non-neutral gene DRB (exon 2), using a cost-effective approach from inter-individual analyzes based on Landscape Genetics. The results indicated high genetic diversity and correlation between genetic and geographic distance, evidencing the genetic distance between populations from different areas. We identified two clusters and also recent barriers between more distant populations. The approach allowed us to investigate the effects of recent habitat fragmentation on wild populations and to analyze aspects related to selection driven by environmental factors. In addition, the study model presented can serve ecologists and conservationists as a viable genetic tool for conservation issues evaluation and can be applied to other species and populations, mainly in anthropized areas.

Keywords: conservation, DRB, MHC, *Myrmecophaga tridactyla*, Brazil.

Introduction

The Neotropical region includes more rainforests than any other kingdom, as well as large savannah areas, stretching from southern Mexico to Central America, and northern South America to southern Brazil. These tropical forest and savannah regions are among the most important biodiversity reserves on Earth, and demonstrate the importance of studies for the preservation of natural resources in protected and unprotected areas for the conservation of species and their habitats (Magioli et al., 2021; Burbano-Girón et al., 2022).

Among the biodiversity hotspots in the Neotropical region, we can highlight the biodiversity found in the Brazilian territory, a megadiverse region due to its remarkable number of species and endemism. Brazil is considered the country with the greatest biodiversity on the planet, and it is the second country in the world with the most endemic species (Costa et al., 2005).

This high diversity of fauna can be partly explained by the size of Brazilian territory and its great variation in ecosystems, such as the Amazon and the Atlantic Forests and the Cerrado, which are highly threatened by human activities. Due to the country's relatively explosive economic and demographic rise in the last century, Brazil's ability to protect its environmental habitats is increasingly menaced (Bolzani, 2016).

In this way, proportionally to the enormous biodiversity, the environmental impacts caused by economic development are visible in Brazil, where countless species are in danger of extinction (Vieira et al., 2008).

To infer human impact effects, genetic analyses can be performed to address patterns of differentiation and levels of genetic diversity among potentially isolated populations using neutral or adaptive markers. Growing evidence suggests that genetic diversity is particularly important in adaptive genes and especially in genes in the major histocompatibility complex (MHC) level, since MHC variants influence many important biological characteristics related to species conservation (including immunological aspects and related to mating preferences), especially in the vertebrates (Sommer, 2005).

Thus, analyses of non-neutral loci can provide important additional information and other works use analyses of adaptive genes in landscape genetics combined with neutral genes, such as microsatellites (Manel et al., 2003; Satkoski Trask et al., 2013;

Clozato et al., 2015). We suggest that low-cost methods for genetic evaluation of adaptive genes can be an important tool for conservationists and ecologists, especially in developing countries, since genetic diversity is an essential part of biodiversity continually shaped by microevolutionary processes (Frankham et al., 2002).

Despite being widely recognized for the conservation and adaptation of species in rapidly changing environments, genetic diversity is largely ignored in practice management and policy implementation, mainly in developing countries. The lack of genetic data implemented in practice management could be attributed to factors such as lack of financial resources, capacity building, knowledge transfer, policy clarity regarding genetic diversity preservation goals, and lack of integration of genetics into guidelines for policy implementation at different levels that could be decision support tools for policymakers and practitioners (Klütsch and Laikre, 2021).

In this way, the aim of the present study is to evaluate the genetic diversity in an adaptive gene through a viable cost-effective approach, and to compare the results of the adaptive gene with other results obtained from microsatellite analyses (Garcia et al., 2005; Sartori et al., 2021).

Material and methods

Sampling and Study Area

Sampling was possible due to partnerships with SACCAS/Hospital Veterinário Dr. Halim Atique (Centro Universitário de Rio Preto/UNIRP, São José do Rio Preto, SP), FAMERP (Faculdade de Medicina de São José do Rio Preto, SP), UNESP/IBILCE (Universidade Estadual Paulista – Instituto de Biociências Letras e Ciências Exatas de São José do Rio Preto, SP), UNESP/FCAV (Universidade Estadual Paulista – Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, SP), the direct collaboration of the 4° Batalhão de Polícia Ambiental do estado de São Paulo and IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis).

The blood samples obtained from the treatment of run-over or burned animals and also from capture campaigns totalize 41 individuals of *Myrmecophaga tridactyla* in the state of São Paulo, Brazil.

DNA Extraction and Amplification

Genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instructions. After extraction, the genomic DNA was quantified in the EPOCH spectrophotometer (BioTek) for determination of DNA concentration. Then, 2 µl of each DNA sample was applied to a 2% agarose gel to verify the integrity of the material for molecular analysis. The methodology involved optimizing the SSCP technique, performing manual allelic genotyping of exon 2 of the DRB gene and specific statistical analysis. Genotyping was performed successfully, obtaining the first results regarding this gene fragment for the species.

After amplification of exon 2 of the DRB gene in 2% agarose gel, the SSCP technique was performed in 15% polyacrylamide gel, which allowed the detection of variants separated by a single base difference. For denaturation, 5 µl of the PCR product of each sample were diluted in 5 µl of the solution containing blue in bromophenol (0.025%), xylene cyanol (0.025%), formamide (98%), and 10 mM EDTA with pH 8.0 in a 1:1 ratio (Orita et al. 1989).

Thus, the DNA was denatured by incubation at 94ºC for 10 minutes and placed on ice for 5 minutes. The electrophoretic run was performed in vertical well with the following conditions: 20 minutes at 200V, followed by 3 or 4 hours at 300V at a constant temperature, using 0.5x TBE buffer. The gels were stained with silver nitrate (AgNO3) and stored (Bassam et al. 1993; Bassam et al. 2007). Subsequently, the genotyping of the SSCP alleles was performed to create the matrix and analyses in specific programs.

The biological material collection was authorized for scientific purposes by the Brazilian Federal Environmental Agency – IBAMA and approved by Research Ethics Committees – CEUA (numbers: 43854-4; 04/2014PP; 2014/05302-8; COTEC 429/2014; D23/201; 38326-5 and 003414/13).

Statistical analysis

The software used for statistical analysis were the Alleles in Space - AIS, the GENELAND 4.0.5 in the R 3.4.2 version, and the software Tools for Population Genetic Analysis – TFPGA (Miller, 1997; Miller, 2005; Guillot et al., 2005; R Core Team, 2021).

For the analyses, the binary matrix was generated from the SSCP patterns through the polyacrylamide gels. This method is informative, cost-effective, and reliably reproducible. The SSCP has advantages, but it is important to note that it also has

some limitations. Overall, despite its limitations, this molecular approach may be very useful and can be implemented in the analysis of different organisms (Boguhn et al., 2008; Kalwade, Devarumath, 2014).

TFPGA is a program for the analysis of population genetic data that calculates descriptive statistics, genetic distances, F statistics, and Hardy-Weinberg equilibrium tests; it performs exact genetic differentiation tests and UPGMA cluster/cluster analyses (Sokal and Michener, 1958; Miller, 1997).

The estimates of genetic diversity and population differentiation were calculated using the computer program TFPGA 1.3 (Miller, 1997), to calculate the Hardy-Weinberg equilibrium deviations with exact and Chi-square tests (Haldane, 1954), genetic distance (Nei, 1972; Nei, 1978), allelic frequencies, mean heterozygosity (observed–Ho and expected–He), percentage of polymorphic loci and, finally, the genetic divergence between populations was estimated by calculating the unbiased estimate of FST (Wright, 1965).

Parameters for the evaluation of the DRB exon2 were studied both in the population of the State of São Paulo (all individuals sampled) and within the subpopulations of the conservation areas.

In turn, AIS performs a series of analyzes based on individual information that can be used to detect or characterize patterns of spatial genetic structure. This program was essential for evaluation in our individual sampling method (Mank and Avise, 2004). The program makes it possible to perform several spatial analyses based on individuals to evaluate the correlations between the genetic and geographic distances of the individuals and populations sampled (Miller, 2005).

Thus, we use the AIS for individual-based assessments to detect the presence of barriers to gene flow through the Monmonier algorithm (Monmonier, 1973). Monmonier algorithm is supervised and the number of biogeographical barriers to be calculated must be specified in advance. Therefore, numerous simulations were performed and barriers were inferred using a connectivity network based on Delaunay triangulation and residual genetic distances (Monmonier, 1973; Brouns et al., 2003; Manni et al., 2004).

The Mantel Test (Mantel, 1967) based on inter-individual patterns was used to assess spatial structure at smaller local spatial scales using statistical procedures that depend on inter-individual patterns of genetic and geographic variation. Therefore, the Test was used to analyze the verification of Isolation by Distance through the

determination of the significance between genetic and geographic distances of observations in the landscape.

Aggregation indices were also calculated. They are generally used in ecological studies to quantify spatial patterns and can also be useful for testing patterns of genetic diversity in a given landscape (Clark and Evans, 1954; Miller, 2005). Three-dimensional graphics for genetic interpolation of landscape shape were produced to elucidate the spatial patterns of genetic diversity across the landscape (Miller et al., 2006). All AIS analyses were calculated with 100,000 permutations/iterations.

It is worth mentioning that, for the barrier detection analyses, we calculated all barriers that were contiguous to each other, since it is statistically recommended to analyze the number of barriers until the moment when the detected borders form a “loop” around a barrier population (Manni et al., 2004).

Also, GENELAND software was used for evaluation of population structure using the GENELAND version 4.0.5 (Guillot et al., 2005) and R version 3.4.2 (R Core Team, 2021). Spatial structure and posterior probability of belonging to a given cluster were possible using the matrix of SSCP and adapting the matrix multiple models specified in the software manual, and the following were determined using the correlated and null alleles models. Each run consists of 100,000 MCMC iterations with a thinning of 100 and a burn-in of 200. The correlated alleles model selected may be more powerful at detecting subtle differentiation. Thus, the correlated alleles model could be more informative than the default model of uncorrelated frequencies, and hence get more accurate results, in particular in the presence of low differentiation and when K is unknown and the uncertainty of coordinates was set to 10 (Guillot, 2008; Guillot et al., 2011).

Results

The results of the DRB gene fragment indicated high genetic diversity in the population (allelic richness), with the presence of 21 different SSCP alleles in 41 genotyped animals. The high allelic diversity is evident in our analyses for the species under study (*Myrmecophaga tridactyla*) since we included a sample of the lesser anteater (*Tamandua tetradactyla*) for comparative purposes, which presented a reduced number of alleles compared to the giant anteater samples. It is worth mentioning that the statistical analyses were performed using two different approaches: classical population analysis and Bayesian analysis based on individuals.

Population-based analyses

Genetic Diversity and Differentiation

To calculate the genetic diversity indices, analyses were performed according to the geographic locality of the populations to identify the diversity and the genetic differentiation of the population groups in the Ecological Stations in comparison with the other remaining individuals in non-protected areas along the northwest of the state of São Paulo. It was also possible to compare the indices of genetic diversity and genetic differentiation of the giant anteater subpopulations in the protected areas.

The results of the exact and chi-square tests indicated deviation in the Hardy-Weinberg equilibrium in all analyzed populations. The high allelic richness was evident in our analyses. In contrast, the mean values of observed heterozygosity (Ho) were drastically lower than expected (He) for all populations, and the DRB results indicate a high degree of inbreeding and heterozygote deficiency within the subpopulations in the state of São Paulo, despite the high genetic diversity (Fis=1 and Expected Mean Heterozygosity=0.3566).

About the population differentiation index (based on individual sampling), for the exon 2 of the DRB gene, the value (Fst=0.03220) indicates low to moderate genetic differentiation between the populations of the state of São Paulo (in all individuals sampled), presenting a very similar value when compared with the SSR result (Fst=0.03407). Also, high genetic diversity was revealed when comparing only the individuals from the two populations in the Ecological Stations (Fst=0.2420). The results of the adaptive gene (DRB) were compared to the previous neutral SSR results and indicated a higher differentiation in the adaptive gene (non-neutral exon).

In general, the results of the DRB marker corroborate the results of the SSR, indicating low to moderate population differentiation (when all individuals sampled were analyzed) and high genetic differentiation between the geographically distant populations of the Ecological Stations and surroundings (although with different levels of differentiation by Wright's F values), high allelic richness, and expected heterozygosity lower than that observed, indicating a deficit of heterozygotes.

Genetic Distances

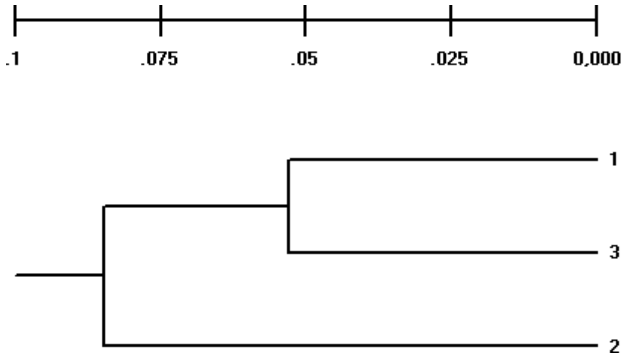
Genetic Distance analyses based on the Nei distance revealed that the greatest genetic distance was identified in the most geographically distant populations, evidencing the genetic distance between populations from different areas (Table 1; Figure 1). This result presented by the DRB gene also corroborates our previous SSR distance results.

Table 1. Genetic Distances show the greater genetic distance between populations more distant geographically, despite the low values presented.

Populations	Distance	Identity
1 (EENP) x 2 (EESB)	0.0634	0.9386
1 (EENP) x 3 (NP)	0.0530	0.9484
2 (EESB) x 3 (NP)	0.1058	0.8996

EENP: Ecological Station of the Northwest of the State of São Paulo. EESB: Santa Bárbara Ecological Station. NP: Northwest of São Paulo State.

Figure 1 – Dendrogram (UPGMA method) for the DRB gene. 1) EENP population. 2) EESB population. 3) Sampled individuals in the NP.



The UPGMA dendrogram demonstrated that there is a greater distance between individuals from the Northwest region of the state of São Paulo (clusters 1 and 3 in the UPGMA dendrogram) and individuals from the Central-South region (population 2 in the UPGMA dendrogram). The results also allowed us to infer that there is a greater differentiation between subpopulations than between individuals within subpopulations. These findings indicate that individuals from more

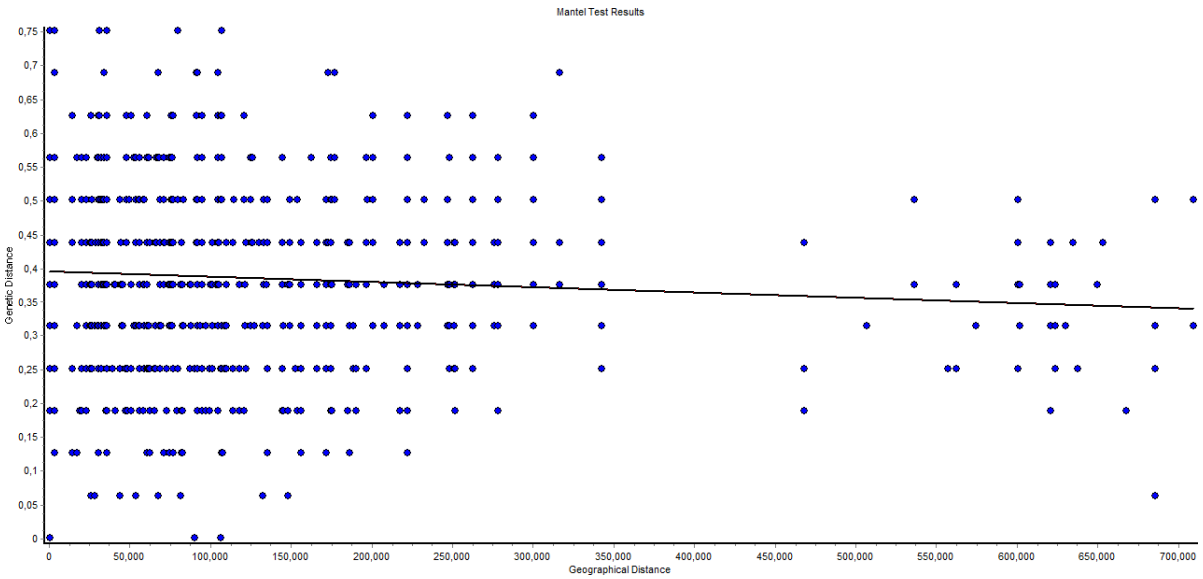
geographically distant subpopulations have greater differentiation than individuals within subpopulations. In addition, individuals more geographically distant have greater genetic distance.

Individual-based Bayesian analyses

Mantel Test

In addition to the classical analyses, Bayesian analyses were performed using individuals as the unit of analysis. The analyses were performed without “a priori” information from populations. The result of the Mantel Test revealed that there is no correlation between interindividual and geographic genetic distances (r=-0.0819231806, p=0.806), indicating that there is no evidence of Isolation by Distance-IBD (Figure 2).

Figure 2 – Mantel Test indicating that there is no Isolation by Distance between individuals.



Allelic Aggregation Index and Spatial Autocorrelation

In the analysis of the Allelic Aggregation Index, the global spatial distribution of all alleles and loci calculated was Rjave=0.467216994 and p=0.026000000, indicating that the samples present an aggregated spatial distribution (Rjave<1). This result can be corroborated by the classical analyses of distance and genetic differentiation mentioned above, allowing us to infer that there is an aggregation of genotypes in

space despite the high allelic diversity. Also, this Rjave value is smaller than the SSR result, showing the higher aggregated spatial distribution for the adaptive gene DRB.

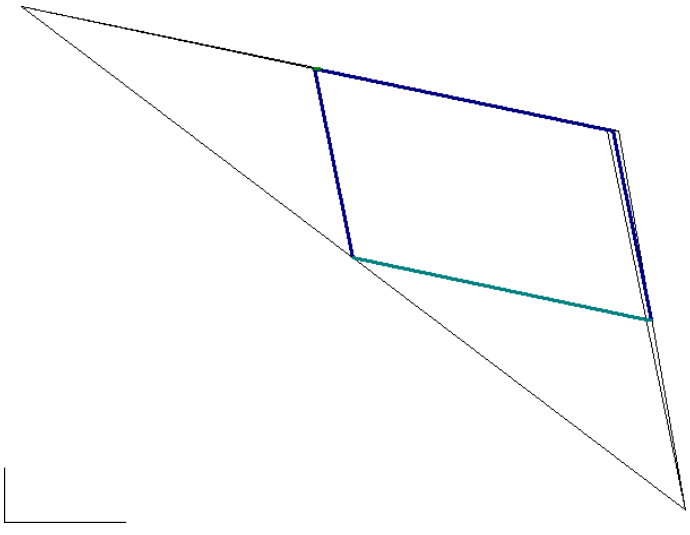
The Spatial Autocorrelation Analysis between genetic and geographic distances was not statistically significant (Ay=0.383993902, p=0.96299).

Barrier detection

The detection of barriers to gene flow in the landscape was performed using spatial analysis and was inferred through the connectivity network by the Monmonier algorithm, using Residual Genetic Distances and Delaunay Triangulations. At least two barriers were detected in the studied area. However, the interpretation must be carried out with caution so as not to overestimate the number of existing barriers. We calculated the barriers that were contiguous with each other. According to the software manual, the number of barriers must be analyzed until the border pattern closes in on itself, and the formation of a “loop” around a population indicates the maximum number of identifiable barriers.

The graph demonstrates the detected barriers (green and blue lines inside the triangle that separate the populations/individuals) and the triangulation (black lines) that represents the connection of the populations/individuals, in which the triangle ends define the area and position of the connected/separate individuals by the Delaunay triangulation (Figure 3). These results also corroborate previous SSR results and show stronger barriers for the DRB analysis.

Figure 3 – Analysis of barrier detection by the Monmonier Algorithm using the connectivity network based on the Delaunay triangulation.



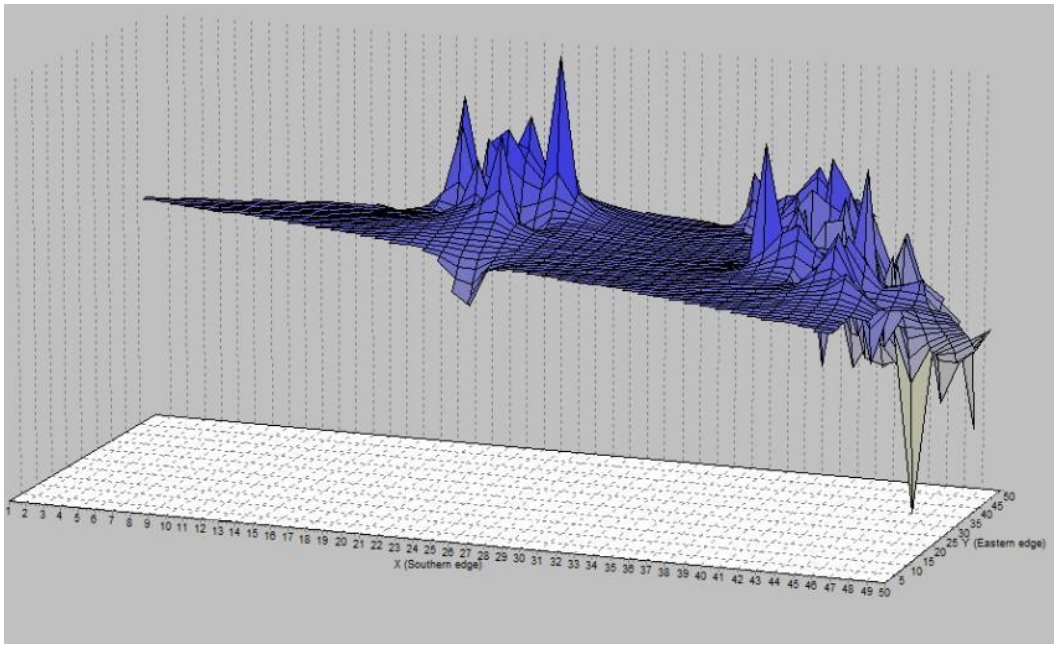
Genetic diversity and variability - Landscape Shape Interpolation

The analysis of the genetic surface of the landscape made it possible to elucidate patterns of genetic distance for the DRB gene. The Y and X axes correspond to the north and east UTM geographic coordinates, and the Z (height) axis corresponds to the genetic distance. Positive peaks represent areas with high genetic discontinuities (high paired genetic distance) and negative peaks/valleys are indicative of areas with high genetic similarities (low pair-to-pair genetic distance).

The three-dimensional graphic output facilitates the visualization of spatial patterns of genetic diversity across the landscape. The peaks indicate that the greatest distances between individuals correspond to regions that have historically been the target of greater disturbances (peaks correspond to the Northwest region of São Paulo. The valleys indicate the shortest distances and are grouped in the region corresponding to the EESB (area less impacted by human action in the studied perimeter).

Thus, the analysis of the graphic result indicates the genetic variability for this gene and illustrates the difference in the distribution pattern in relation to individuals from different areas (Figure 4).

Figure 4 – Three-dimensional graphic of the landscape genetic shape, elucidating the genetic diversity among individuals through peaks and valleys.



In conclusion, this result indicates that the characteristics of the landscape and the anthropic actions can be a fundamental factor in the genetic conformation and in the allelic distribution in these individuals and populations, which corroborates the results of classic analyses and other Bayesian analyses for this selective gene.

Individual spatial analysis - Population structure

The analyses in GENELAND were possible by using the generated matrix following the parameters indicated in the software manual. Results indicated the presence of two clusters (Fst=0.03898), showing the differences between the populations and individuals of the northwest and the central-south regions of the state of São Paulo (Figures 5, 6, and 7). The Fst result for all individuals was slightly higher in the DRB gene than in SSR genes (Fst=0.03407).

In general, the results corroborate other results obtained in different programs and statistical approaches.

Figure 5 – Graph generated in GENELAND showing the two clusters identified.

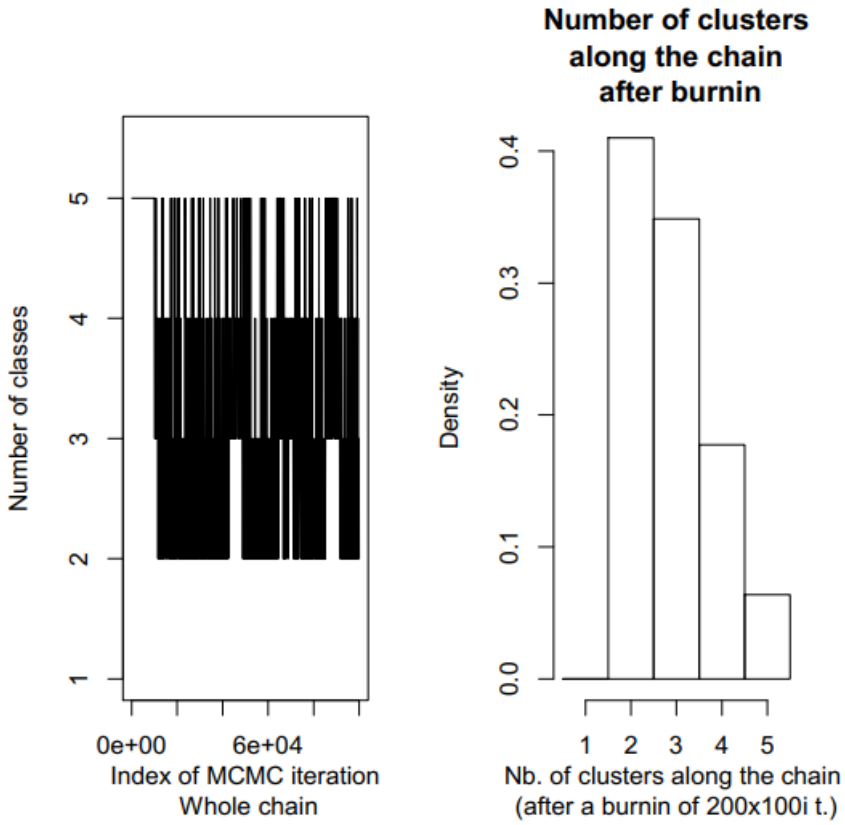


Figure 6 – Graph of the spatial analysis showing two well-defined clusters.

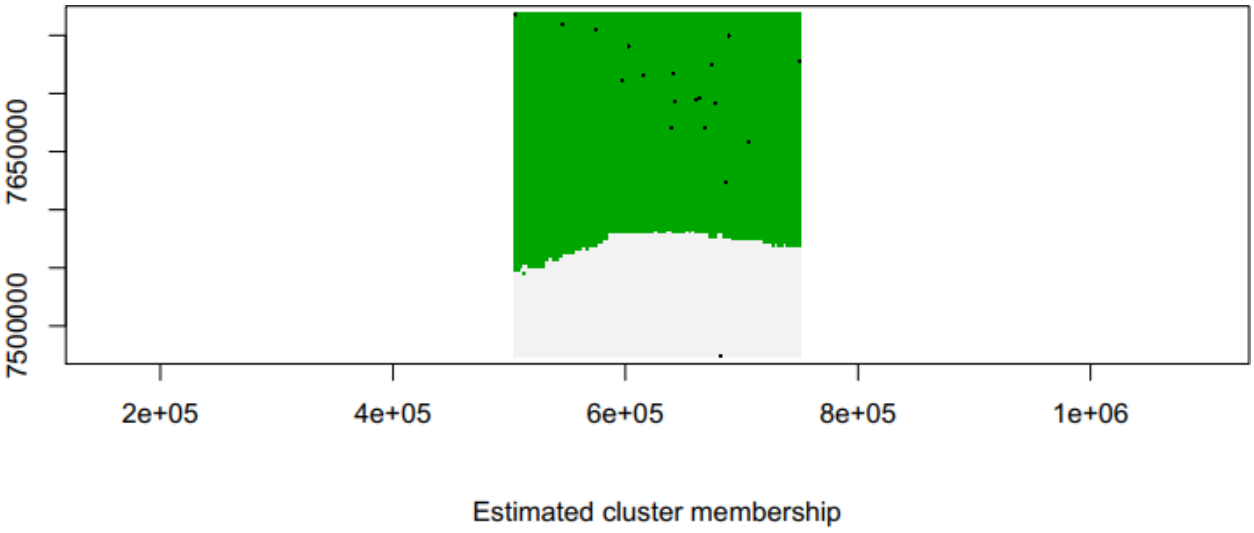
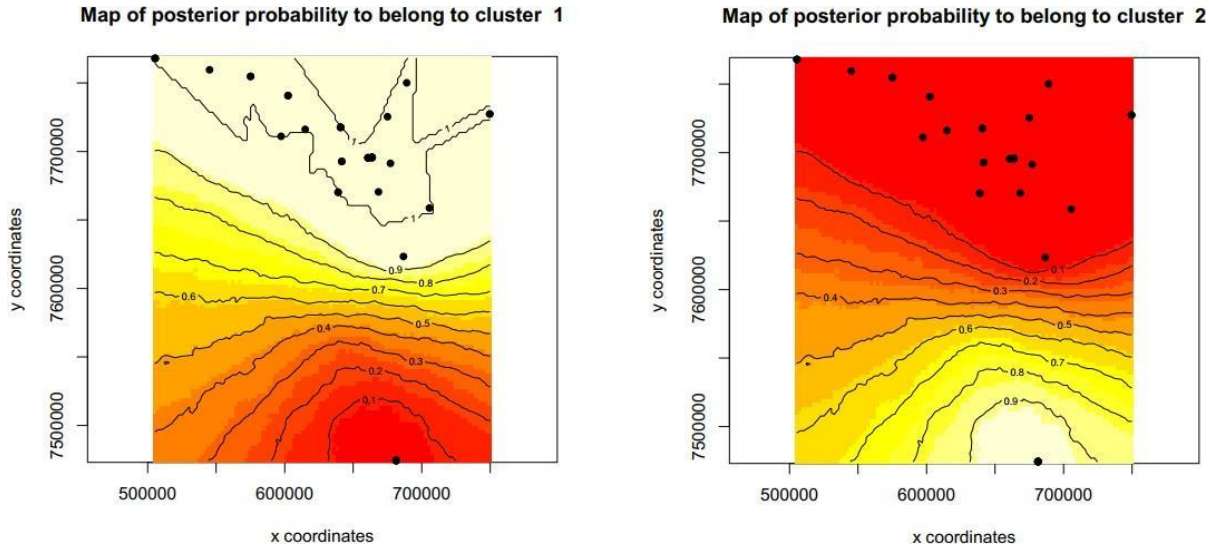


Figure 7 – Posterior probability of belonging between areas and individuals related to cluster 1 and cluster 2.



In summary, the results allowed us to infer that selective pressures act structuring the genetic patterns of the populations in the analyses of an adaptive gene. These findings corroborate previous neutral SSR results and reinforce the effects of recent fragmentation over the populations in these areas.

Discussion

The analysis of highly polymorphic genes can be fundamental in understanding populations in highly anthropized environments (Wan et al., 2004).

The average number of alleles found can be considered high when compared with the results described in the literature for this same gene in other mammalian species, indicating high genetic diversity through the allelic richness (Schad et al., 2011; Lau et al., 201; Sommer et al., 2013). In addition, the average number of alleles is higher than that described in a study carried out with the species *Tamandua tetradactyla* for this same gene region (Clozato et al., 2015).

The presence of barriers to gene flow seems to be an important factor in the segregation of populations, and the segregation of population groups or individuals from different geographic areas can be better explained by Isolation by Barriers-IBB and not by Isolation by Distance-IBD.

Also, the discrepancy found between allelic richness and heterozygote deficit can be explained according to the characteristics of the diversity parameters themselves, as these measures reflect different types of genetic diversity in natural populations. First, the FIS inbreeding coefficient, also known as the heterozygote deficit, is a measure of the reduction in an individual's heterozygosity as a result of non-random mating within its subpopulation, and is better suited to reflect recent processes because it has faster dynamics than the genetic diversity measured by the number of alleles. In turn, allelic wealth is more useful for identifying historical processes (Widmer and Lexer, 2001). An example of another study that identified this difference is the study performed by Comps et al. (2001), who found a negative correlation between allelic richness and genetic diversity (He) in recolonized areas.

Therefore, our results demonstrate that the maintained alleles may reflect the historical characteristics of these populations, whereas the heterozygote deficiency may be the result of recent events, such as the intense fragmentation of the habitat that affects these populations.

Thus, our results indicate that the cost-effective approach may be very informative for conservation of species in highly anthropized regions. Concerning the methodology, the assessment of adaptive genes such as MHC genes can be performed by different methods, and the SSCP analysis through specific statistical software can be an important tool in the toolbox of ecologists and conservationists (Piertney and Oliver, 2006).

Although analyses using NGS are more accurate (Stipoljev et al., 2020), analyses using more accessible methods make it possible to obtain important results (Osborne et al., 2013), mainly in places with fewer resources available for this type of analysis. Therefore, the proposed experimental method is a viable way that guarantees initial results and cost-effectiveness for the analysis of threatened species in less developed countries and with major problems in the conservation of species and their habitats (Myers et al., 2000).

Thus, we suggest that the use of heterologous primers with SSCP polyacrylamide gel genotyping may be an alternative analysis, especially in developing regions that have limitations in terms of funding for genomic analysis or next-generation sequencing (NGS). It is worth noting that it is important to perform the analysis of adaptive genes together with neutral genes of high mutation rate like the microsatellites, as the combined approach generates more robust results (Schenekar and Weiss, 2017; Clozato et al., 2015). We also suggest that other option for microsatellites (if there are no markers available for the species and no conditions for the development of markers) would be the use and analysis of available heterologous inter simple sequence repeat (ISSR) based on adequate screening and statistical evaluations, which also allow for the analysis of diversity and genetic differentiation at a low-cost and can be highly informative (Felix et al., 2020; Sarwat, 2012; Aytekin et al., 2011; Barbosa et al., 2014).

Therefore, in the present study, previous results obtained through microsatellites in the same population of giant anteaters were incorporated for comparison with the results of the DRB gene (Sartori et al., 2021).

From this point of view, comparing the results of the DRB and the SSR, some conclusions were possible regarding the characteristics and the status of giant anteaters in the state of São Paulo, even with a reduced sample size. The analysis of these genes allowed us to infer the presence of recent inbreeding within the populations due to the results of heterozygote deficiency in different genes.

Furthermore, it is known that recent events such as habitat fragmentation may not clearly reflect the loss of MHC alleles and SSR alleles (even though they are highly polymorphic genes). Thus, recent ecological events may take time to present a more evident genetic response regarding the loss of alleles, especially in species with a long-life span, as in the case of the giant anteater (Hailer et al., 2006).

In general, the results of the DRB gene corroborated the results of the SSR about the high allelic richness and the presence of barriers to gene flow, distance, and differentiation of populations from Ecological Stations, in addition to the identification of structure in the subpopulations of the state of São Paulo. The DRB gene showed a higher degree of deficiency in heterozygotes when compared with the SSR, and this result may be an alert for the situation of these populations, as our findings indicate a decrease in heterozygosity in a gene related to the immune system and sexual selection (DRB – MHC II), which may influence the susceptibility of these individuals to pathogens (Sommer, 2005). Thus, it is urgent to create management plans and reproduction programs for these populations of the species. On the other hand, the maintenance of allelic diversity of the DRB gene demonstrates the importance of these individuals, and since the maintenance of allelic diversity in the MHC should be an additional goal of genetic management, this result confirms the importance of these populations in the conservation of the species' genetic resources (Hughes, 1991; Aguilar et al., 2004).

Our results for the SSR and DRB genes demonstrate that the remaining individuals in the state of São Paulo are a priority in conservation management plans and in preservation of a diverse set of fitness-related genes, along with neutral variation, may be the key to long term survival of threatened populations (Funk et al., 2012; Matala et al., 2014).

It is noteworthy that MHC variants influence many important biological traits, including immune recognition, susceptibility to infectious and autoimmune diseases, individual odors, mating preferences, and even morphological issues. These diverse functions and characteristics place MHC genes among the best candidates for studying the mechanisms and significance of molecular adaptation in vertebrates (Sommer, 2005; Ujvari and Belov, 2011; Ivy-Israel et al., 2021).

Thus, the levels of MHC diversity presented in other studies also demonstrate high MHC diversity in mammals, even in isolated populations that have low diversity of mtDNA haplotypes (Wilbert et al., 2020).

Therefore, the use of highly polymorphic genes is of great importance to understand recent events and investigate conservation issues (Wan et al., 2004; Bos et al., 2008). The study allowed the identification of molecular markers of extreme importance for conservation studies in this species and allowed us to identify important patterns for the conservation of populations. Some hypotheses to explain the genetic

patterns found are typically non-random mating, the presence of population structure, genetic drift, selection, maintenance of ancestral alleles, among other biological and evolutionary factors such as long-life span of the species (Neff et al., 2008; Widmer and Lexer, 2001; Sharma et al., 2016; Hailer et al., 2006; Barrandeguy and García, 2021).

The results of the analysis of the DRB gene indicate that selection may be a fundamental factor in recent genetic differentiation that influences the non-random sexual selection of individuals and the genetic patterns found. In other words, the maintenance of high diversity of alleles, together with the high deficiency of heterozygotes, can be directly related to the recent fragmentation of the habitat, which can be justified by the presence of barriers detected in the area, in the analysis of the neutral SSR genes and the non-neutral DRB genes (Sartori et al., 2021).

Although widely used, the sequencing of SSCP alleles after excision of polyacrylamide gel proved to be a challenge in the analysis and proved to be a method with a high rate of contamination in our experiments, even with all the necessary laboratory procedures. Therefore, NGS analyses are the most suitable for nucleotide analysis and especially for allele identification (Okano et al., 2020).

In addition to the DNA mixing that occurred, another limitation may be that the final excised products, especially the larger ones, do not present ideal concentrations or adequate quality for sequencing, even with the re-amplification of the excised bands and different extraction methods (Tautz and Renz, 1983; Santos et al., 2017).

Therefore, although sequencing of SSCP alleles excised from polyacrylamide gel is a widely used technique (Sunnucks et al., 2000), in our analysis, the degree of contamination was extremely high and we cannot suggest this technique as a highly viable approach. The analysis of the allelic pattern by SSCP was informative, although simple, and together with the results of previously analyzed microsatellites, this approach is a highly informative alternative tool.

In addition to the joint allelic approach of neutral and non-neutral genes, the use of direct sequencing in the analysis of MHC genes may be an alternative strategy to cloning and sequencing or NGS, even if it results in smaller analyzable fragments (Osborne et al., 2013). Using Sanger sequencing together with computational allele inference can be a very useful method, particularly in situations where the variation is very high, since it eliminates the time-consuming and expensive validation of results from mutation detection methods (Sanger et al., 1977; Stephens et al., 2001; Stephens

and Donnely, 2003). Also, the non-neutrality of highly polymorphic MHC sequences violates the assumptions of the coalescent model, but the method performs well in the analysis of real MHC data and can be a valid tool in the analysis of adaptive genes (Babik, 2010).

It is clear that the analysis by NGS or cloning followed by Sanger sequencing should be performed if there is this possibility and funding, but more economically viable methods must be described for initial analysis, especially in populations that are in the process of local extinction in regions with fewer resources. We emphasize that NGS analysis would be the ideal method, but its cost, especially in less developed countries, is usually high for conservation research. The NGS analysis has advantages over cloning and sequencing, as cloning can generate additional artifacts introduced by bacterial incompatibility repair systems and a large number of clones need to be sequenced for accurate genotyping, making cloning to be a laborious and expensive work (Babik, 2010).

Thus, as an alternative to NGS and other sequencing analyses, the combined approach proposed, by using SSCP-PCR for allelic genotyping of the DRB gene (or other adaptive MHC genes) and the comparison with SSR (or ISSR in some cases), can serve as an extremely viable alternative to evaluate evolutionary and ecological issues in the conservation of endangered species.

The proposed strategies are viable alternatives to assess wild populations and to help in conservation plans and actions for threatened small populations. In summary, this approach can be applied as an assessment tool also for other species, mainly in populations of highly anthropized areas.

In addition to genetic analyses, a comprehensive and interdisciplinary approach is necessary, since it will also be important to evaluate other aspects, such as environmental and immunological factors, analysis of conservation policies, and other issues, to verify the feasibility of creating and defining management plans, to the possibility of implementing strategies for the conservation of the species, which are the main purposes of any conservationist study (Matioli and Fernandes, 2012; Frankham et al., 2002; Frankham et al., 2019).

Our results indicate the importance of these populations for the conservation of the species and the importance of the use of genetic data in conservation plans. However, there is an incompatibility between the availability of genetic data and its use in conservation policies. Thus, it is necessary to increase the number of collaborative

partnerships between genetic researchers and conservation managers to support strategies for managing wild populations based on scientific results, which demonstrates the need for multidisciplinary approaches in conservation biology (Taft et al., 2020; Lacy, 1997).

In this way, multidisciplinary studies are needed and the use of the genetic results obtained can be an important step to effectively contribute to the processes of decision-making and adequate management plans for the threatened species (Desalle and Amato, 2004).

We emphasize that the giant anteaters of the state of São Paulo should be prioritized for conservation measures due to their high allelic diversity and genetic variability. It is necessary to go further and integrate all the genetic and ecological knowledge obtained to assist in the creation of adequate management plans with local actions through decision-makers, requiring involvement in the areas of conservation policy (Mello-Théry, 2011; Bertassoni et al., 2019; Bertassoni et al., 2021)

In summary, our analysis results made it possible to obtain unprecedented results for this species with the DRB gene, allowing the description of these findings for the first time using a non-neutral marker until now. These results can help in the creation of management plans for the species and should be used to effectively contribute to the conservation of populations of the species.

Conclusions

The low-cost approach presented can be used for genetic evaluation of other species, mainly in highly anthropized environments.

The populations of giant anteaters in the state of São Paulo have priority for the conservation and recovery of the species' genetic diversity.

Selection and genetic drift are key evolutionary factors in the differentiation of the population in the state of São Paulo.

Recent habitat fragmentation directly influences patterns genetics of populations in areas.

Interdisciplinary studies and the integration with decision-makers in conservation policies are necessary for the effective contribution of the results in conservation actions of the species' populations.

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Conflict of interest

None declared.

Author contribution statement

R.Q.S: Data curation; formal analysis; investigation; methodology; project administration; software; visualization; writing-original draft; writing-review and editing. A.P.S-C: methodology; technical support; visualization; writing-review and editing. L.C.M: Methodology; visualization; validation; resources; supervision; writing-review and editing. C.C.B.: Supervision; validation; visualization; writing-review and editing. L.C.: Conceptualization; data curation; formal analysis; funding acquisition; project administration; resources; supervision; validation; visualization; writing-original draft; writing-review and editing. All authors reviewed the manuscript.

References

Aguilar A, Roemer G, Debenham, S, Binns M, Garcelon D, Wayne R K (2004) High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. PNAS 101, 10:3490 3494. https://doi.org/10.1073/pnas.0306582101

Aytekin I, Ozdil F, Zulkadir U, Boztepe S, Sariyel V (2011) Evaluation of ISSR markers for genetic diversity analysis in Anatolian water buffaloes. J Sci Food Agric 91, 11:1957-62. doi: 10.1002/jsfa.4397. PMID: 21480268.

Babik W (2010) Methods for MHC genotyping in non-model vertebrates. Mol Ecol Resour 10, 2:237–251. doi:10.1111/j.1755-0998.2009.02788.x

Barbosa NCCP, Freitas S, Morales AC (2014) Distinct genetic structure in populations of Chrysoperla externa (Hagen) (Neuroptera, Chrysopidae) shown by genetic markers ISSR and COI gene. Rev Bras Entomol 58, 2:203–211. doi:10.1590/S0085-56262014000200012.

Barrandeguy ME, GARCÍA MV (2021) The Sensitiveness of Expected Heterozygosity and Allelic Richness Estimates for Analyzing Population Genetic Diversity. In: Genetic Diversity [Online First] IntechOpen. doi: 10.5772/intechopen.95585

Bassam BJ, Caetano-Anollés G (1993) Silver staining of DNA in polyacrylamide gels. Appl Biochem Biotechnol 42:181-188. doi: 10.1016/0003-2697(91)90120-i.

Bassam BJ, Gresshoff PM (2007) Silver staining of DNA in polyacrylamide gels. Nat Protoc 2:2649-2654. https://doi.org/10.1038/nprot.2007.330

Bertassoni A, Bianchi RDC, Desbiez ALJ (2021) Giant Anteater Population Density Estimation and Viability Analysis Through Motion-Sensitive Camera Records. J Wildl Manage. doi:10.1002/jwmg.22123

Bertassoni A, Costa RT, Gouvea JA, Bianchi RC, Ribeiro JW, Vancine MH, Ribeiro MC (2019) Land-use changes and the expansion of biofuel crops threaten the giant anteater in southeastern Brazil. J Mammal 100, 2:435-444. http://dx.doi.org/10.1093/jmammal/gyz042

Boguhn J, Strobel E, Witzig M, Tebbe CC, Rodehutscord M (2008) Description of the structural diversity of rumen microbial communities in vitro using single-strand conformation polymorphism profiles. Arch Anim Nutr 62, 6:454-67. doi: 10.1080/17450390802453443. PMID: 19143229.

Bolzani VS (2016) Biodiversidade, bioprospecção e inovação no Brasil. Cien Cult [online] 68, 1:04-05. http://dx.doi.org/10.21800/2317-66602016000100002

Bos DH, Gopurenko D, Williams RN, Dewoody JA (2008) Inferring population history and demography using microsatellites, mitochondrial DNA, and major histocompatibility complex (MHC) genes. Evolution 62, 6:1458-68. doi: 10.1111/j.1558-5646.2008.00364.x.

Brouns G, De Wulf A, Constales D (2003) Delaunay triangulation algorithm useful for multibeam echosounding. J Surv Eng 129:79-84. doi:10.1061/(ASCE)0733-9453(2003)129:2(79)

Burbano-Girón J, Jantke K, Molina-Berbeo MA, Buriticá-Mejía N, Urbina-Cardona N, Sánchez-Clavijo LM, Etter A (2022) An assessment of spatial conservation priorities for biodiversity attributes: Composition, structure, and function of Neotropical biodiversity. Biol Conserv 265:109421. https://doi.org/10.1016/j.biocon.2021.109421

Clark PJ, Evans FC (1954) Distance to nearest neighbor as a measure of spatial relationships in populations. Ecology 35:445-453. https://doi.org/10.2307/1931034

Clozato CL, Mazzoni CJ, Moraes-Barros N, Morgante JS, Sommer S (2015) Spatial pattern of adaptive and neutral genetic diversity across different biomes in the lesser anteater (*Tamandua tetradactyla*). Ecol Evol 5, 21:4932-48. doi: 10.1002/ece3.1656. PMID: 26640672. PMCID: PMC4662318.

Comps B, Gömöry D, Letouzey J, Thiébaut B, Petit RJ (2001) Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European beech. Genetics 157, 1:389-97. doi: 10.1093/genetics/157.1.389.

Costa LP, Leite YLR, Mendes SL, Ditchfield AD (2005) Mammal Conservation in Brazil, Conserv Biol 19, 3:672–679. doi:10.1111/j.1523-1739.2005.00666.x

Desalle R, Amato G (2004) The expansion of conservation genetics. Nat Rev Genet 5:702-712. doi: 10.1038/nrg1425.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol Ecol 14:2611-2620. doi: 10.1111/j.1365-294X.2005.02553.x.

Felix FC, Das Chagas KPT, Ferrari CS, Vieira FA, Pacheco MV (2020) Applications of ISSR markers in studies of genetic diversity of Pityrocarpa moniliformis. Forest Science, Rev Caatinga 33, 4. https://doi.org/10.1590/1983-21252020v33n417rc

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to Conservation Genetics. Cambridge University Press, Cambridge.

Frankham R, Ballou JD, Ralls K, Eldridge M, Dudash MR, Fenster CB, Lacy RC, Sunnucks P (2019) A Practical Guide for Genetic Management of Fragmented Animal and Plant Populations, Oxford Scholarship Online. doi: 10.1093/oso/9780198783411.001.0001

Funk WC, Mckay JK, Hohenlohe PA, Allendorf FW (2012) Harnessing genomics for delineating conservation units. Trends Ecol Evol 27:489-496. doi: 10.1016/j.tree.2012.05.012.

Garcia JE, Vilas Boas LA, Lemos MVF, Macedo Lemos EG, Contel EPB (2005) Identification of microsatellite DNA markers for the giant anteater *Myrmecophaga tridactyla*. J Hered 96:600-602. https://doi.org/10.1093/jhered/esi089

Guillot G, Mortier F, Estoup A (2005) GENELAND: A computer package for landscape genetics, Mol Ecol Notes 5:712-715. doi:10.1111/j.1471-8286.2005.01031.x

Guillot, G (2008) Inference of structure in subdivided populations at low levels of genetic differentiation - the correlated allele frequencies model revisited. Bioinformatics 24, 19:2222-2228. https://doi.org/10.1093/bioinformatics/btn419

Guillot G, Santos F, Estoup A (2011) Population genetics analysis using R and the Geneland program. In: http://www2.imm.dtu.dk/~gigu/Geneland/

Hailer F, Helander B, Folkestad AO, Ganusevich AS, Garstad S, Hauff P, Koren C, Nygård T, Volke V, Vilà, C, Ellegren H (2006). Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from population decline. Biol Lett 22, 2:316-319. doi: 10.1098/rsbl.2006.0453

Haldane JBS (1954) An exact test for randomness of mating. J Genet 52, 1:631-635. https://doi.org/10.1007/BF02985085

Hughes AL (1991) Circumsporozoite protein genes of malaria parasites (Plasmodium spp.): evidence for positive selection on immunogenic regions. Genetics 127, 2:345-353. doi: 10.1093/genetics/127.2.345.

Ivy-Israel NMD, Moore CE, Schwartz TS et al (2021) Association between sexually selected traits and allelic distance in two unlinked MHC II loci in white-tailed deer (*Odocoileus virginianus*). Evol Ecol 35:513-535. https://doi.org/10.1007/s10682-021-10108-x

Kalwade SB, Devarumath RM (2014) Single strand conformation polymorphism of genomic and EST-SSRs marker and its utility in genetic evaluation of sugarcane. Physiol Mol Biol Plants 20, 3:313-321. doi:10.1007/s12298-014-0231-9

Klütsch CFC, Laikre L (2021) Closing the Conservation Genetics Gap: Integrating Genetic Knowledge in Conservation Management to Ensure Evolutionary Potential. In: Ferreira C.C., Klütsch C.F.C. (eds) Closing the Knowledge-Implementation Gap in Conservation Science. Wildlife Research Monographs, vol 4. Springer, Cham. https://doi.org/10.1007/978-3-030-81085-6_3

Lacy, RC (1997) Importance of Genetic Variation to the Viability of Mammalian Populations. J Mammal 78, 2:320-335. https://doi.org/10.2307/1382885

Lau Q, Chow N, Gray R et al (2015) Diversity of MHC DQB and DRB Genes in the Endangered Australian Sea Lion (Neophoca cinerea). J Hered 106, 4:395-402. doi: 10.1093/jhered/esv022.

Magioli M, Rios E, Benchimol M et al (2021) The role of protected and unprotected forest remnants for mammal conservation in a megadiverse Neotropical hotspot. Biol Conserv. 259:109-173. https://doi.org/10.1016/j.biocon.2021.109173

Manel S, Schwartz MK, LUIKART G. et al (2003) Landscape genetics: Combining landscape ecology and population genetics. Trends Ecol Evol 18, 4:189-197. https://doi.org/10.1016/S0169-5347(03)00008-9

Mank J, Avise J (2004) Individual organisms as units of analysis: Bayesian-clustering alternatives in population genetics. Genet Res 84, 43:135-143. doi: 10.1017/s0016672304007190

Manni F, Guerard E, Heyer E (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. Hum Biol 76, 2:173-190. doi: 10.1353/hub.2004.0034.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res, Birmingham, 27, 2:209-220. PMID: 6018555.

Matala AP, Ackerman MW, Campbell, MR et al (2014) Relative contributions of neutral and non-neutral genetic differentiation to inform conservation of steelhead trout across highly variable landscapes. Evol Appl 7:682-701. doi: 10.1111/eva.12174

Matioli SR, Fernandes FMC (2012) Biologia molecular e evolução, Ribeirão Preto, Holos. ISBN: 85-86699-75-7.

Miller MP (1997) Tools for population genetic analyses –TFPGA 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author.

Miller MP (2005) Alleles in space (AIS): Computer software for the joint analysis of interindividual spatial and genetic information. J Hered 96, 6:722-724. https://doi.org/10.1093/jhered/esi119

Miller MP, Bellinger MR, Forsman ED et al (2006) Effects of historical climate change, habitat connectivity, and vicariance on genetic structure and diversity across the range of the red tree vole (Phenacomys longicaudus) in the Pacific Northwestern United States. Mol Ecol 15:145-159. doi: 10.1111/j.1365-294X.2005.02765.x.

Monmonier MS (1973) Maximum-difference barriers: An alternative numerical regionalization method. Geogr Anal 5, 3:245-261. https://doi.org/10.1111/j.1538-4632.1973.tb01011.x

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca G.A.B, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858. https://doi.org/10.1038/35002501

Neff B, Garner S, Heath J, Heath D (2008) The MHC and non-random mating in a captive population of Chinook salmon. Heredity 101:175-185. https://doi.org/10.1038/hdy.2008.43

Nei M (1972) Genetic Distance between populations. Am Nat 106, 949:283-292. http://dx.doi.org/10.1086/282771

Nei M (1978) Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals. Genetics 89, 3:583-90. doi: 10.1093/genetics/89.3.583.

Okano M, Miyamae J, Suzuki S et al (2020) Identification of Novel Alleles and Structural Haplotypes of Major Histocompatibility Complex Class I and DRB Genes in Domestic Cat (*Felis catus*) by a Newly Developed NGS-Based Genotyping Method. Front Genet 11. doi:10.3389/fgene.2020.00750

Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T (1989) Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. PNAS 86, 8:2766-2770. doi: 10.1073/pnas.86.8.2766. PMID: 2565038. PMCID: PMC286999.

Osborne AJ, Zavodna M, Chilvers BL et al (2013) Extensive variation at MHC DRB in the New Zealand sea lion (*Phocarctos hookeri*) provides evidence for balancing selection. Heredity 111, 1:44–56. doi:10.1038/hdy.2013.18

Piertney S, Oliver M (2006) The evolutionary ecology of the major histocompatibility complex. Heredity 96, 7–21. https://doi.org/10.1038/sj.hdy.6800724

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. neff 74:5463-7. doi: 10.1073/pnas.74.12.5463

Santos F, Gómez-Manzo S, Sierra-Palacios E et al (2017) Purification, concentration and recovery of small fragments of DNA from Giardia lamblia and their use for other molecular techniques. MethodsX, Elsevier 4:289-296. doi: 10.1016/j.mex.2017.08.005.

Sartori RQ, Lopes AG, Aires LPN, Bianchi RC, Morales AC, Castiglioni L (2021) Identifying Priority Giant Anteater (*Myrmecophaga tridactyla*) Populations for

Conservation in São Paulo State, Brazil. Ecol Evol, Wiley 11:700-713. https://doi.org/10.1002/ece3.6809.

Sarwat M (2012) ISSR: A Reliable and Cost-Effective Technique for Detection of DNA Polymorphism. In: Sucher N., Hennell J., Carles M. (eds) Plant DNA Fingerprinting and Barcoding. Methods Mol Biol (Methods and Protocols), vol 862. Humana Press. https://doi.org/10.1007/978-1-61779-609-8_9

Satkoski Trask J, George D, Houghton P, Kanthaswamy S, Smith DG (2013) Population and Landscape Genetics of an Introduced Species (*M. fascicularis*) on the Island of Mauritius. PLoS ONE 8, 1:e53001. https://doi.org/10.1371/journal.pone.0053001

Schad J, Dechmann D, Voigt C et al (2011) MHC class II DRB diversity, selection pattern and population structure in a neotropical bat species, *Noctilio albiventris*. Heredity 107:115-126. https://doi.org/10.1038/hdy.2010.173

Schenekar T, Weiss S (2017) Selection and genetic drift in captive versus wild populations: an assessment of neutral and adaptive (MHC-linked) genetic variation in wild and hatchery brown trout (Salmo trutta) populations. Conserv Genet 18:1011-1022. doi:10.1007/s10592-017-0949-3

Sharma R, Kumar B, Arora R, Ahlawat S, Mishra AK, Tantia, MS (2016) Genetic diversity estimates point to immediate efforts for conserving the endangered Tibetan sheep of India. Meta Gene, Elsevier 8:14-20. doi: 10.1016/j.mgene.2016.01.002

Sokal RR, Michener CD (1958) A statistical method for evaluating systematic relationships. Univ Kans Sci Bull 38:1409-1438.

Sommer S (2005) The importance of immune gene variability (MHC) in evolutionary ecology and conservation. Front Zool 2, 16. https://doi.org/10.1186/1742-9994-2-16

Sommer S, Courtiol A, Mazzoni CJ (2013) MHC genotyping of non-model organisms using next-generation sequencing: a new methodology to deal with artefacts and allelic dropout. BMC Genom 14, 542. https://doi.org/10.1186/1471-2164-14-542

Stephens M, Donnelly P (2003) A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 73, 5:1162-9. doi: 10.1086/379378.

Stephens M, Smith N, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68:978-989. https://doi.org/10.1086/319501

Stipoljev S, Bužan E, Rolečková B, Iacolina L, Šprem N (2020) MHC Genotyping by SSCP and Amplicon-Based NGS Approach in Chamois. Animals 10, 9:1694. doi:10.3390/ani10091694

Sunnucks P, Wilson AC, Beheregaray LB, Zenger K, French J, Taylor AC (2000) SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. Mol Ecol 11:1699-710. doi: 10.1046/j.1365-294x.2000.01084.x.

Taft HR, McCoskey DN, Miller JM, Pearson SK, Coleman MA, Fletcher NK, Mittan CS, Meek MH, Barbosa S (2020) Research-management partnerships: an opportunity to integrate genetics in conservation actions. Conserv Sci Pract 2, e218. doi: doi.org/10.1111/csp2.218

Tautz D, Renz M (1983) An optimized freeze-squeeze method for the recovery of DNA fragments from agarose gels. Anal Biochem 132, 1:14-19. doi: 10.1016/0003-2697(83)90419-0.

Mello-Théry NA (2011) Conservation of natural areas in São Paulo. Estud Av 25, 71:175-188. doi:10.1590/s0103-40142011000100012.

Ujvari B, Belov K (2011) Major Histocompatibility Complex (MHC) Markers in Conservation Biology. Int J Mol Sci 12, 8:5168-5186. https://doi.org/10.3390/ijms12085168

Vieira ICG, Toledo PM, Silva JMC, Higuchi H (2008) Deforestation and threats to the biodiversity of Amazonia. Braz J Biol 68, 4:949–956. doi:10.1590/S1519-69842008000500004

Wan QH, Wu H, Fujihara T, Fang SG (2004) Which genetic marker for which conservation genetics issue? Electrophoresis 25:2165–2176. doi: 10.1002/elps.200305922. PMID: 15274000.

Widmer A, Lexer C (2001) Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. Trends Ecol Evol 16, 6:267-269. doi:10.1016/S0169-5347(01)02163-2

Wilbert TR, Maldonado JE, Tsuchiya MTN et al (2020) Patterns of MHC Polymorphism in Endangered San Joaquin Kit Foxes Living in Urban and Non-urban Environments. In: Conservation Genetics in Mammals, Springer. https://doi.org/10.1007/978-3-030-33334-8_13

Wright S (1965) The interpretation of population structure by F-statistics with special regard to system of mating. Evolution 19:395-420. https://doi.org/10.2307/2406450

5. CONSIDERAÇÕES FINAIS

-A combinação dos resultados de altas taxas de mutação e genes mais conservados revelou que a fragmentação recente do habitat influencia diretamente na estrutura genética das populações da espécie no estado de São Paulo.

-As populações de tamanduás-bandeira no estado de São Paulo são prioritárias para a conservação e recuperação da diversidade genética da espécie, principalmente devido aos resultados a partir de genes com altas taxas de mutação.

-A baixa diversidade e haplótipos únicos identificados a partir do gene RAG2 (e de mtDNA) em conjunto com dados ecológicos, indicam que a espécie deve continuar na lista de espécies ameaçadas.

-A seleção, além da deriva genética, é um dos fatores evolutivos principais que atua na diferenciação genética das populações, inferida com base nas análises realizadas a partir do gene DRB.

-Migrações em curtas distâncias ocorrem e mantém o fluxo gênico local e alta diversidade genética intrapopulacional. Entretanto, não há evidências de migração em longas distâncias entre as populações de tamanduá-bandeira, o que em conjunto com a intensa ação antrópica leva à diminuição de populações.

-A conservação e proteção das populações de tamanduá-bandeira, baseada na prioridade genética, pode contribuir para a realização de ações que contribuam para a conservação de outras espécies e seus habitats.

-Estudos interdisciplinares e a integração com tomadores de decisão em políticas de conservação são necessários para a efetiva contribuição de dados científicos e genéticos visando a implementação em ações de conservação.

REFERÊNCIAS

ALBERICI, V. *et al*. Survival Blueprint for the conservation of the giant anteater, Myrmecophaga tridactyla, in the Brazilian Cerrado. An output from the Anteaters & Highways Project, Brazil, and EDGE of Existence fellowship. **Zoological Society of London,** London, UK, 2020.

ALI, T. A Conceptual Framework for Designing Phylogeography and Landscape Genetic Studies. **Critical Reviews in Plant Sciences**, v. 39, n. 6, p. 457-478, 2020.

ANDRADE FILHO, E. A*. et al*. Casuística de atendimentos de Tamanduás Bandeira (Myrmecophaga tridactyla Linnaeus, 1745) de vida livre de 2008 a setembro de 2017 no Hospital Veterinário "Dr. Halim Atique". In: XIV Congresso Científico UNIRP, XIV, 2017, São José do Rio Preto, **Anais.** São José do Rio Preto: CEPECC / UNIRP, 2017.

ANTONELLI, A. The rise and fall of Neotropical biodiversity. **Botanical Journal of the Linnean Society**, v. 20, p. 1-17, 2021.

AVISE, J. C. *et al.* Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. **Annual Ver. of Ecology and Systematics,** v. 18, p. 489-522, 1987.

AVISE, J. C. **Phylogeography: The History and Formation of Species.** President and Fellows of Harvard College, 2000.

AVISE, J. C. **The history, purview, and future of conservation genetics**. In: CAROLL, S. P.; FOX C. W., eds., Conservation Biology: Evolution in Action, Oxford University Press, USA, 2008.

AZEVEDO-SANTOS, V. M. *et al.* Removing the abyss between conservation science and policy decisions in Brazil. **Biodivers. Conserv.,** v. 26, p. 1745–1752, 2017.

BALMFORD, A.; GREEN, R. H. Y. S. E.; JENKINS, M. Measuring the changing state of nature. **Trends in Ecology & Evolution**, v. 18 n. 7, p. 326–30, 2003.

BENNETT, N. J. *et al*. Conservation social science: Understanding and integrating human dimensions to improve conservation. **Biological Conservation**, v. 205, p. 93-108, 2017.

BENNETT, N. J. *et al.* Local support for conservation is associated with perceptions of good governance, social impacts, and ecological effectiveness. **Conservation Letters**, v. 12, e12640, 2019.

BERTASSONI, A.; BIANCHI, R. D. C.; DESBIEZ, A. L. J. Giant Anteater Population Density Estimation and Viability Analysis Through Motion-Sensitive Camera Records. **J. Wildl. Manage.,** v. 85, p. 1554-1562, 2021.

BERTASSONI, A. *et al*. Land-use changes and the expansion of biofuel crops threaten the giant anteater in southeastern Brazil. **J. of Mammalogy**, v. 100, n. 2, p. 435-444, 2019.

BOLZANI, V. S. Biodiversidade, bioprospecção e inovação no Brasil. **Ciência e Cultura,** vol.68, n.1, p. 04-05, 2016.

BOWEN-JONES, E.; ENTWISTLE, A. Identifying appropriate flagship species: The importance of culture and local contexts. **Oryx,** Cambridge University Press, v. 36, n.2, 189-195, 2002.

BROOKS, T. M. *et al*. "Habitat Loss and Extinction in the Hotspots of Biodiversity. **Conservation Biology**, v. 16, n.4, p. 909-923, 2002.

BURBANO-GIRÓN, J.; JANTKE, K.; MOLINA-BERBEO, M. A.; BURITICÁ-MEJÍA, N.; URBINA-CARDONA, N.; SÁNCHEZ-CLAVIJO, L. M.; ETTER, A. An assessment of spatial conservation priorities for biodiversity attributes: Composition, structure, and function of Neotropical biodiversity. **Biol. Conserv.,** v. 265,109421, 2022.

BUTLER, O. E. **A Parábola do Semeador**. São Paulo: Editora Morro Branco, 2018.

CARNAVAL, A. C. *et al*. Prediction of phylogeographic endemism in an environmentally complex biome**. Proc. R. Soc. B.,** v. 281, p. 1-8, 2014.

CARNEIRO, M. J.; DA-SILVA-ROSA, T. The use of scientific knowledge in the decision making process of environmental public policies in Brazil. **J. com.,** v. 10, n.1, A03, 2011.

CARO, T. **Conservation by proxy: indicator, umbrella, keystone, flagship, and other surrogate species**. Island Press, Washington, D.C., 2010.

CARTWRIGHT, J. Ecological islands: conserving biodiversity hotspots in a changing climate. Frontiers in Ecology and the Environment. **Front. Ecol. Environ.,** v. 17, n. 6, p. 331–340, 2019.

CEBALLOS, G.; EHRLICH, P. R.; BARNOSKY, A. D.; GARCÍA, A.; PRINGLE, R. M.; PALMER, T. M. Accelerated modern human–induced species losses: Entering the sixth mass extinction. **Science Advances**, v. 1, n. 5, e1400253, 2015.

CEBALLOS, G.; EHRLICH, P. R. The misunderstood sixth mass extinction. **Science**, v. 360, n. 6393, p. 1080-1081, 2018.

CLOZATO, C. L.; MAZZONI, C. J.; MORAES-BARROS, N.; MORGANTE, J. S.; SOMMER, S. Spatial pattern of adaptive and neutral genetic diversity across different biomes in the lesser anteater (Tamandua tetradactyla). **Ecol. Evol.,** v. 5, n. 21, p. 4932–4948, 2015.

COIMBRA, R. T. F; MAGALHÃES, R. F.; LEMES, P.; MIRANDA, F. R.; SANTOS, F. R. Integrative Phylogeography Reveals Conservation Priorities for the Giant Anteater *Myrmecophaga tridactyla* in Brazil. **Diversity**, v. 14, n. 7, p. 542, 2022.

COOKE, S. J.; SACK, L.; FRANKLIN, C. E.; FARRELL, A. P.; BEARDALL, J.; WIKELSKI, M.; CHOWN, S. L. What is conservation physiology? Perspectives on an increasingly integrated and essential science. **Conservation Physiology**, v. 1, n. 1, cot001, 2013.

COSTA, L. P.; LEITE, Y. L. R.; MENDES, S. L.; DITCHFIELD, A. D. Mammal Conservation in Brazil, **Conserv. Biol.,** v. 19, n. 3, p. 672–679, 2005.

COWIE, R. H.; BOUCHET, P.; FONTAINE, B. The Sixth Mass Extinction: fact, fiction or speculation? **Biological Reviews**, 1464-7931, 2022.

CRUVINEL, C. A. T. *et al*. Atendimento de tamanduás-bandeira realizado pelo SACCAS – Setor de Atendimento Clínico Cirúrgico de Animais Selvagens do Hospital Veterinário "Dr. Halim Atique". Casouística entre 2007 e 2010. In: VII Congresso Científico UNIRP, VII, 2010, São José do Rio Preto, **Anais**. São José do Rio Preto: UNIRP, 2010.

CRUZAN, M. B.; TEMPLETON, A. R. Paleoecology and coalescence: phylogeographic analysis of hypotheses from the fossil record. **Trends in Ecology and Evolution**, v. 15, n. 12, p. 491-496, 2000.

CUBAS, Z. S.; SILVA, J. C. R.; CATÃO-DIAS, J. L. **Tratado de animais selvagens – Medicina Veterinária**. São Paulo, Roca, p. 1354, 2006.

DA SILVA, J. M. C.; PINTO, L. P.; SCARANO, F. R. Toward integrating private conservation lands into national protected area systems: Lessons from a megadiversity country. **Conservation Science and Practice**, v. 3, e433, 2021.

DASHMAN, R. F. **Environmental Conservation**. New York, NY: John Wiley and Sons, 1968.

DE VOS, J. M.; JOPPA, L. N.; GITTLEMAN, J. L.; STEPHENS, P. R.; PIMM, S. L. Estimating the normal background rate of species extinction. **Conservation Biology**, v. 29, p. 452-462, 2015.

DELSUC, F.; DOUZERY, E. J. P. Armadillos , anteaters , and sloths (Xenarthra). **The timetree of life,** Oxford University Press, Oxford Biology, p. 475–478, 2009

DELSUC, F.; VIZCAÍNO, S. F.; DOUZERY, E. J. P. Influence of Tertiary paleoenvironmental changes on the diversification of South American mammals: a relaxed molecular clock study within xenarthrans. **BMC Evol. Biol.,** v. 28, n. 4, p.11, 2004.

DIAMOND, J. Overview of recent extinctions. In: WESTERN, D.; PEARL, M.C. editors. **Conservation for the Twenty-first Century**. New York, NY: Oxford Univ. Press, p. 37–41, 1989.

DIAZ, S.; SETTELE, J.; BRONDIZIO, E. Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental

Science-Policy Platform on Biodiversity and Ecosystem Services. **IPBES**, p. 1-39, 2019.

DIFFENDORFER, J. E. Testing Models of Source-Sink Dynamics and Balanced Dispersal. **Oikos,** v. 81, n. 3, p. 417–433, 1998.

DINIZ, M. F.; BRITO, D. The Charismatic Giant Anteater (Myrmecophaga tridactyla): A Famous John Doe? **Edentata, v.** 13, n. 1, p. 76-83, 2012.

DINIZ, M. F.; BRITO, D. Threats to giant anteater, Myrmecophaga tridactyla (Pilosa: Myrmecophagidae), viability in a protected Cerrado remnant encroached by urban expansion in central Brazil. **Zoologia**, v. 30, p. 151–156, 2013.

DINIZ-FILHO, J. A. F. et al. Mapping the evolutionary twilight zone: molecular markers, populations and geography. **Journal of Biogeography**, v. 35, p. 753-763, 2008.

DIRZO, R.; YOUNG, H. S.; GALETTI, M.; CEBALLOS, G.; ISAAC, N. J. B.; COLLEN, B. Defaunation in the Anthropocene. **Science**, v. 345, n. 6195, p. 401-406, 2014.

EHRENFELD, D. W. **Biological Conservation**. New York, NY: Holt, Rinehart and Winston, 1970.

FERGUSON, M. M.; DRAHUSHCHAK, L. R. Disease resistance and enzyme heterozygosity in rainbow trout. **Heredity,** v. 64, n. 3, p. 413-417, 1990.

FERRARI, V. M; CASTIGLIONI, L. Isolamento e caracterização genética de Toxoplasma gondii em Myrmecophaga tridactyla (Linnaeus, 1758). Dissertação (Mestrado) – Universidade Estadual Paulista. São José do Rio Preto, p. 47, 2016.

FRANCISCO, A. R.; TEIXEIRA, P. S. dos S. Biologia e manejo nutricional de tamanduás das espécies *Myrmecophaga tridactyla* e *Tamandua tetradactyla* mantidos em cativeiro: revisão. **Revista De Ciência Veterinária e Saúde Pública**, v. 5, n. 1, p. 085-096, 2018

FRANKHAM, R.; BALLOU, J. D.; RALLS, K.; ELDRIDGE, M.; DUDASH, M. R.; FENSTER, C. B.; LACY, R. C.; SUNNUCKS, P. **A Practical Guide for Genetic Management of Fragmented Animal and Plant Populations**. Oxford Scholarship Online, 2019.

FRANKHAM, R. Genetics and extinction. **Biological Conservation,** v. 126, n. 2, p. 131-140, 2005

FRANKHAM, R.; BALLOU, J. D.; BRISCOE, D. A. **Introduction to Conservation Genetics**. Cambridge University Press, Cambridge, 2002.

GALELO, L. G. C.; CARARETO, C. M. A. Scenario of the spread of the invasive species Zaprionus indianus Gupta, 1970 (Diptera, Drosophilidae) in Brazil. **Genetics and Molecular Research**, v. 33, n. 5, p. 767-773, 2010.

GARNER, B. A.; HOBAN, S.; LUIKART, G. IUCN Red List and the value of integrating genetics. **Conserv. Genet.,** v. 21, p. 795–801, 2020.

GAUDIN, T. J.; BRANHAM, D. G. The phylogeny of the Myrmecophagidae (Mammalia, Xenarthra, Vermilingua) and the relationship of Eurotamandua to the Vermilingua. **Journal of Mammalian Evolution**, v. 5, n.3, p. 237–65, 1998.

GEBREMEDHIN, B. *et al.* Frontiers in identifying conservation units: from neutral markers to adaptive genetic variation. **Animal Conservation**, v. 12, p. 107-109, 2009.

GERBER, L. Conservation Biology. **Nature Education Knowledge,** 3(10):14, 2010.

GILPIN, M. E.; SOULÉ, M. E. **Minimum Viable Populations: Processes of Species Extinction.** In: SOULÉ, M. E. (ed.), Conservation Biology: The Science of Scarcity and Diversity. Sunderland, Mass: Sinauer, p. 19–34, 1986.

GUILLOT, G.; LEBLOIS, R.; COULON, A.; FRANTZ, A. C. Statistical methods in spatial genetics. **Molecular Ecology**, v. 18, p. 4734–4756, 2009

HABEL, J. C. *et al*. Final countdown for biodiversity hotspots. **Conservation Letters**, v. 12, e12668, 2019.

HAILER, F.; HELANDER, B.; FOLKESTAD A. O.; GANUSEVICH, S. A.; GARSTAD, S.; HAUFF, P.; KOREN, C.; NYGÅRD, T.; VOLKE, V.; VILÀ, C.; ELLEGREN, H. Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from population decline. **Biology Letters**, v. 22, n. 2, p. 316-319, 2006.

HEDRICK, P. W.; HURT, C. R. Conservation genetics and evolution in an endangered species: research in Sonoran topminnows. **Evol. Appl.,** v. 5, n.8, p. 806–819, 2012.

HOFFMANN, M. *et al*. The Impact of Conservation on the Status of the World's Vertebrates. **Science,** p. 330, n. 6010, p. 1503–1509, 2010.

HOLDEREGGER, R.; WAGNER, H. H. A brief guide to Landscape Genetics. **Landscape Ecology,** v. 21, n. 6, p. 793–796, 2006.

IBGE. INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Mapa dos Biomas Brasileiros**. Rio de Janeiro: IBGE, 2019.

IF. INSTITUTO FLORESTAL. **Plano de Manejo da Estação Ecológica de Santa Bárbara**. São Paulo: IF, 2011.

IF. INSTITUTO FLORESTAL. **Plano e Manejo da Floresta Estadual Noroeste Paulista.** São Paulo: IF, 2019.

IF. INSTITUTO FLORESTAL. **Proposta de criação da floresta estadual do Noroeste Paulista.** São Paulo: IF, 2014.

IUCN. INTERNATIONAL UNION FOR CONSERVATION OF NATURE. **The IUCN Red List of Threatened Species.** Gland: IUCN, 2021

KALINKAT, G. *et al*. Flagship umbrella species needed for the conservation of overlooked aquatic biodiversity. **Conservation Biology,** v. 31, n. 2, p. 481-485, 2017.

KARAM-GEMAEL, M.; LOYOLA, R.; PENHA, J.; IZZO, T. Poor alignment of priorities between scientists and policymakers highlights the need for evidence-informed conservation in Brazil. **Perspectives in Ecology and Conservation,** v. 16, n. 3, p. 125-132, 2018.

KEINATH, D. A.; DOAK, D. F.; HODGES, K. E.; PRUGH, L. R.; FAGAN, W.; SEKERCIOGLU, C. H.; BUCHART, S. H. M.; KAUFFMAN, M. A global analysis of traits predicting species sensitivity to habitat fragmentation. **Global Ecology and Biogeography**, v. 26, p. 115–127, 2017.

KING, K. C.; LIVELY, C. M. Does genetic diversity limit disease spread in natural host populations?. **Heredity,** v. 109, n. 4, p. 199–203, 2012.

KOH, L. P. *et al.* Species coextinctions and the biodiversity crisis. **Science,** v. 305, n. 5690, p. 1632-1634, 2004.

KOPELMAN, N. M.; MAYZEL, J.; JAKOBSSON, M.; ROSENBERG, N. A; MAYROSE, I. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. **Molecular Ecology Resources**, v. 15, n. 5, p. 1179-1191, 2015.

LAURANCE, W. F.; *et al*. Making conservation research more relevant for conservation practitioners. **Biological Conservation,** v. 153, p. 164-168, 2012.

LEBERG, P. L. Influence of genetic variability on population growth: implications for conservation. **Journal of Fish Biology,** v. 37, p. 193–195, 1990.

LEÓN-ORTEGA, M.; MARTÍNEZ, J. E.; PÉREZ, E.; LACALLE, J. A.; CALVO, J. F. The contribution of non-protected areas to the conservation of Eurasian Eagle-owls in Mediterranean ecosystems. **Ecosphere,** v. 8, n.9, e01952, 2017.

LI, B. V.; PIMM, S. L. China's endemic vertebrates sheltering under the protective umbrella of the giant panda. **Conservation Biology**, v. 30 p. 329–339, 2016.

LI, W.; YANG, P.; LI, B.; LIU, C.; SUN, L.; LI, J. Habitat characteristics or protected area size: What is more important for the composition and diversity of mammals in nonprotected areas? **Ecology and Evolution,** v. 11, n. 12, p. 7250-7263, 2021.

LUCINDO, A. S.; ANTUNES, A. Z; KANASHIRO, M. M.; DIAS, M. M. Birds at Santa Bárbara Ecological Station, one of the last Cerrado remnants in the state of São Paulo, Brazil. **Biota neotrop.,** v. 15, n. 4, e0155, 2015.

MACARTHUR, R. H.; WILSON, E. O. **The Theory of Island Biogeography.** Princeton, N.J: Princeton University Press, 2001.

MACKENZIE, D. *et al.* Estimating Site Occupancy, Colonization, and Local Extinction When a Species Is Detected Imperfectly. **Ecology,** v. 84 n. 8, p. 2200–07, 2003.

MAGIOLI, M. *et al.* The role of protected and unprotected forest remnants for mammal conservation in a megadiverse neotropical hotspot. **Biol. Conserv.,** v. 259, p. 109-173, 2021.

MANEL, S.; BERTHIER, M. P.; LUIKART, G. Detecting Wildlife Poaching: Identifying the Origin of Individuals with Bayesian Assignment Tests and Multilocus Genotypes. **Conservation Biology,** v. 16, n. 3, p. 650-659, 2002

MANEL, S. *et al*. Landscape genetics: Combining landscape ecology and population genetics. **Trends in Ecology and Evolution,** v. 18, n. 4, p. 189-197, 2003.

MANEL, S.; HOLDEREGGER, R. Ten years of landscape genetics. **Trends in Ecology & Evolution,** v. 28, n. 10, p. 614–621, 2013.

MANK, J.; AVISE, J. Individual organisms as units of analysis: Bayesian-clustering alternatives in population genetics. **Genet. Res.,** v. 84, n. 43, p. 135-143, 2004.

MATIOLI, S. R.; FERNANDES, F. M. C. **Biologia molecular e evolução**. Ribeirão Preto, Holos, 2012.

MCKENNA, M. C.; BELL, S. K. **Classification of mammals above the species level.** Columbia University Press, New York, p. 631, 1997.

MEINE, C.; SOULÉ, M.; NOSS, R. F. A Mission-Driven Discipline: the Growth of Conservation Biology. **Conservation Biology,** v. 20, p. 631-651, 2006.

MILLER, M. P. Alleles in space (AIS): Computer software for the joint analysis of interindividual spatial and genetic information. **J. Hered.,** v. 96, n. 6, p. 722-724, 2005.

MIRANDA, F. R.; BERTASSONI, A.; ABBA, A. M. Myrmecophaga tridactyla. **The IUCN Red List of Threatened Species,** v. 8235, p. 1-14, 2014.

MIRANDA, F. R.; CASALI, D. M.; PERINI, F. A.; MACHADO, F. A.; SANTOS, F. R. Taxonomic review of the genus Cyclopes Gray, 1821 (Xenarthra: Pilosa), with the revalidation and description of new species. **Zoological Journal of the Linnean Society,** v. 183, n. 3, p. 687 721, 2018.

MIRANDA, F. R.; CHIARELLO, A. G.; RÖHE, F.; BRAGA, F. G.; MOURÃO, G. M.; MIRANDA, G. H. B.; SILVA, K. F. M.; FARIA-CORRÊA, M. A.; VAZ, S. M.; BELENTANI, S. C. S. Avaliação do Risco de Extinção de Myrmecophaga tridactyla Linnaeus, 1758 no Brasil. Processo de avaliação do risco de extinção da fauna brasileira. **ICMBio,** 2015.

MIRANDA, F. R.; MACHADO, A.; CLOZATO, C.; SILVA; S. Nine biomes and nine challenges for the conservation genetics of Neotropical species, the case of the

vulnerable giant anteater (*Myrmecophaga tridactyla*). **Biodiversity and Conservation**, v. 31, p. 1-27, 2022.

MONTEIRO W. P. *et al.* Everything you always wanted to know about gene flow in tropical landscapes (but were afraid to ask). **Peer J.**, v. 7, e6446, 2019.

MORAES-BARROS, N.; ARTEAGA, M. C. Genetic diversity in Xenarthra and its relevance to patterns of neotropical biodiversity. **Journal of Mammalogy,** v. 96, n. 4, p. 690-702, 2015.

MORITZ, C. Defining "evolutionary significant units" for conservation. **Trends in Ecology and Evolution,** v. 9, n. 10, p. 373–375, 1994.

MORRIS, W. F. *et al.* Longevity can buffer plant and animal populations against changing climatic variability. **Ecology,** v. 89, p. 19–25, 2008.

MORRONE, J. J. Biogeographical regionalisation of the Neotropical region. **Zootaxa,** v. 3782, p. 110, 2014

MOURÃO, G.; MEDRI, I. M. Activity of a specialized insectivorous mammal (*Myrmecophaga tridactyla*) in the Pantanal of Brazil. **Journal of Zoology**, v. 271, n. 2, p. 187–192, 2006

MUÑOZ-VALENCIA, V.; MONTOYA-LERMA, J.; SEPPÄ, P.; DIAZ, F. Landscape genetics across the Andes Mountains: Environmental variation drives genetic divergence in the leaf-cutting ant Atta cephalotes. **Cold Spring Harbor Laboratory,** p. 1-24, 2021.

MURILLO, L. P. P.; AGUILAR, R. S.; NARANJO, E. J.; VAZQUEZ, L. B. Conservation effectiveness of protected areas in Mexico: Effects on medium and large mammals at local and regional scales. **International Journal of Biodiversity and Conservation**, v. 3, n. 10, p. 487-496, 2011.

MYERS, N.; MITTERMEIER, R. A.; MITTERMEIER, C. G.; DA FONSECA, G. A. B.; KENT, J. Biodiversity hotspots for conservation priorities. **Nature,** v. 403 p. 853–858, 2000.

NEAVES, L. E. *et al*. Phylogeography of the Koala, (Phascolarctos cinereus), and Harmonising Data to Inform Conservation. **PLoS ONE,** v. 11, n. 9, e0162207, 2016.

NEW, T. 2011 Launching and steering flagship Lepidoptera for conservation benefit. **Journal of Threatened Taxa**, v. 3, n. 6, p. 1805-1817, 2011.

NOSS, R. F. *et al*. How global biodiversity hotspots may go unrecognized: lessons from the North American Coastal Plain. **Divers. Distrib.,** v. 21, p. 236– 44, 2015.

PALA, E. K. *et al*. Casuística do setor de atendimento clínico cirúrgico de animais selvagens (SACCAS) do Hospital Veterinário "Dr. Halim Atique" de janeiro de 2003 a agosto de 2017. In: XIV Congresso Científico UNIRP, XIV, 2017, São José do Rio Preto, **Anais.** São José do Rio Preto: CEPECC / UNIRP, 2017.

PAOLINO, R. M.; VERSIANI, N. F.; PASQUALOTTO, N.; RODRIGUES, T. F.; KREPSCHI, V. G.; CHIARELLO, A. G. Buffer zone use by mammals in a Cerrado protected area. **Biota Neotropica**, v. 16, n. 2, 2016

PEASE K. M. *et al*. Landscape genetics of California mule deer (Odocoileus hemionus): the roles of ecological and historical factors in generating differentiation. **Molecular Ecology,** v. 18, p. 1848–1862, (2009).

PENA, H. F. J.; FERRARI, V. M.; AIRES, L. P. N.; SOARES, H. S.; OLIVEIRA, S.; ALVES, B. F.; GENNARI, S. M., DUBEY, J. P.; DE MATTOS, L. C.; DE MATTOS, C. C. B., CASTIGLIONI L. First isolation and genotyping of Toxoplasma gondii in a free-living giant anteater (Myrmecophaga tridactyla) revealed a unique non-archetypal genotype. **Acta Trop.,** v. 204, 105426, 2020.

PEREIRA, J. H.; BRASILEIRO, L. A. **Análise de impactos ambientais gerados pela implantação de loteamentos fechados de alto padrão**. Dissertação (Mestrado) – Universidade Estadual Paulista. Ilha Solteira, p. 261, 2015.

PIMM, S. L.; JENKINS, C. N.; ABELL, R.; BROOKS, T. M.; GITTLEMAN, J. L.; JOPPA, L. N.; RAVEN, P. H.; ROBERTS, C. M.; SEXTON, J. O. The biodiversity of species and their rates of extinction, distribution, and protection. **Science,** v. 344, n. 6187, 1246752, 2014.

PIMM, S. L.; RUSSELL, G. J.; GITTLEMAN, J. L.; BROOKS, T. M. The Future of Biodiversity. **Science,** v. 269, n. 5222, p. 347-350, 1995.

PINTO, F. A. S.; BAGER, A.; CLEVENGER, A. P.; GRILO, C. Giant anteater (Myrmecophaga tridactyla) conservation in Brazil: Analysing the relative effects of fragmentation and mortality due to roads. **Biological Conservation,** v. 228, p. 148-157, 2018.

POSSINGHAM, H. E.; WILSON, K. Turning up the heat on hotspots. **Nature,** v. 436, p. 919-920, 2005.

PRESSEY, R. L.; MILLS, M.; WEEKS, R.; DAY, J. C. The plan of the day: Managing the dynamic transition from regional conservation designs to local conservation actions. **Biological Conservation,** v. 166, p. 155-169, 2013.

PRIMACK, R. B. **A Primer of Conservation Biology.** 3rd ed. Sinauer Associates, p. 320, 2004.

PRUNIER, J. G.; KAUFMANN, B.; FENET, S.; PICARD, D.; POMPANON, F.; JOLY, P.; LENA, J. P. Optimizing the trade-off between spatial and genetic sampling efforts in patchy populations: Towards a better assessment of functional connectivity using an individual-based sampling scheme. **Molecular Ecology,** v. 22, n. 22, p. 5516–5530, 2013.

QIAN, J. *et al.* Selecting flagship species to solve a biodiversity conservation conundrum. **Plant Diversity,** v. 42, n. 6, p. 488 491, 2020.

REGAN, H. M.; LUPIA, R.; DRINNAN, A. N.; BURGMAN, M. A. The Currency and Tempo of Extinction. **The American Naturalist,** v. 157, n. 1, p. 1-10, 2001.

RISSLER, L. J. Union of phylogeography and landscape genetics. **PNAS,** v. 113, n. 29, p. 8079–8086, 2016.

ROBERGE, J-M.; ANGELSTAM, P. Usefulness of the umbrella species concept as a conservation tool. **Conservation Biology,** v. 18, p. 76– 85, 2004.

ROSENBERG, N. A.; NORDBORG, M. Genealogical Trees, Coalescent Theory and the Analysis of Genetic Polymorphisms. **Nature Reviews Genetics,** v. 3, p. 380–390, 2002.

RUNGE, C. A. *et al.* Single species conservation as an umbrella for management of landscape threats. **PLoS ONE,** v. 14, n. 1, e0209619, 2019.

SACCHERI, I.; KUUSSAARI, M.; KANKARE, M.; VIKMAN, P.; FORTELIUS, W.; HANSKI, I. Inbreeding and extinction in a butterfly metapopulation. **Nature,** v. 392 n. 6675, p. 491-494. 1998.

SAHNEY, S.; BENTON, M. J.; FERRY, P. A. Links between global taxonomic diversity, ecological diversity and the expansion of vertebrates on land. **Biology Letters,** v. 6 n. 4, p. 544-547, 2010.

SANDOM, C.; FAURBY, S.; SANDEL, B.; SVENNING, J. C. Global late Quaternary megafauna extinctions linked to humans, not climate change. **Proceedings of the Royal Society B.,** v. 281, n. 1787, 20133254, p. 1-9, 2014.

SARTORI; R. Q.; LOPES, A. G.; AIRES, L. P. N.; BIANCHI, R. C.; DE MATTOS, C. C. B.; MORALES, A. C.; CASTIGLIONI, L. Identifying Priority Giant Anteater (Myrmecophaga tridactyla) Populations for Conservation in São Paulo State, Brazil. **Ecology and Evolution,** Wiley, v. 11, p. 700-713, 2021.

SATKOSKI TRASK, J.; GEORGE, D.; HOUGHTON, P.; KANTHASWAMY, S.; SMITH, D. G. Population and Landscape Genetics of an Introduced Species (M. fascicularis) on the Island of Mauritius. **PLoS ONE,** v. 8, 1:e53001, 2013.

SHAW C. A.; MCDONALD, H. G. First Record of Giant Anteater (Xenarthra, Myrmecophagidae) in North America. **Science,** v. 236, n. 4798, p. 186-188, 1987.

SILVEIRA, L. *et al*. Impact of wildfires on the megafauna of Emas National Park, central Brazil. **Oryx,** v. 33, n. 2, p. 108–14, 1999.

SOMMER, S. The importance of immune gene variability (MHC) in evolutionary ecology and conservation. **Front. Zool.,** v. 2, n. 16, 2005.

SOULÉ, M. E. What is Conservation Biology? A new synthetic discipline addresses the dynamics and problems of perturbed species, communities, and ecosystems. **BioScience,** v. 35, n. 11, p. 727–734, 1985.

STAR, B.; SPENCER, H. G. Effects of genetic drift and gene flow on the selective maintenance of genetic variation. **Genetics,** v. 194, n. 1, p. 235–44, 2013.

STEFFEN, W. *et al.* The Anthropocene: From Global Change to Planetary Stewardship. **Ambio,** v. 40, n. 7, p. 739-761, 2011.

STORFER, A. *et al*. Putting the 'landscape' in landscape genetics. **Heredity,** v. 98, p. 128–142, 2007.

STORFER, A.; MURPHY, M. A.; SPEAR, S. F.; HOLDEREGGER, R.; WAITS, L. P. Landscape genetics: where are we now? **Molecular Ecology,** v. 19, n. 17, p. 3496-3514, 2010.

SYVITSKI, J. *et al*. Extraordinary human energy consumption and resultant geological impacts beginning around 1950 CE initiated the proposed Anthropocene Epoch. **Commun. Earth Environ.,** v. 1, n. 32, 2020.

THAPA, A. *et al*. Non-protected áreas demanding equitable conservation strategies as of protected areas in the Central Himalayan region. **PLoS ONE,** v. 16, n. 8, e0255082, 2021.

THOMSON, R. C.; WANG, I. J.; JOHNSON, J. R. Genome-enabled development of molecular markers for ecology, evolution, and conservation. **Molecular Ecology,** v. 19, p. 2184–2195, 2010.

TORRES-FLOREZ, J. P. *et al.* The coming of age of conservation genetics in Latin America: what has been achieved and what needs to be done. **Conservation Genetics**, v. 19, p. 1–15, 2018

TORRES-FLOREZ, J. P.; HUCKE-GAETE, R.; ROSENBAUM, H.; FIGUEROA, C. C. High genetic diversity in a small population: the case of Chilean blue whales. **Ecology and Evolution,** v. 4, n. 8, p. 1398-1412, 2014.

VANDERGAST, A. G.; BOHONAK, A. J.; WEISSMAN, D. B.; FISHER, R. N. Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history: phylogeography and landscape genetics of a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: Stenopelmatus). **Molecular Ecology,** v. 16, p. 977–992, 2007.

VERÍSSIMO, D.; MACMILLAN, D. C.; SMITH, R. J. Toward a systematic approach for identifying conservation flagships. **Conservation Letters,** v. 4, p. 1–8, 2011.

VERSIANI, N. F., BAILEY, L. L.; PASQUALOTTO, N.; RODRIGUES, T. F.; PAOLINO, R. M.; ALBERICI, V.; CHIARELLO, A. G. Protected areas and unpaved roads mediate habitat use of the giant anteater in anthropogenic landscapes. **Journal of Mammalogy**, v. 102, n. 3, p. 802–813, 2021

WAGNER, H. H.; HOLDEREGGER, R. Landscape Genetics. **BioScience,** v. 58, n. 3, p. 199-207, 2008.

WAN, Q. H.; WU, H.; FUJIHARA, T.; FANG, S. G. Which genetic marker for which conservation genetics issue? **Electrophoresis,** v. 25, p. 2165-2176, 2004.

WANG, I, J. Recognizing the temporal distinctions between landscape genetics and phylogeography. **Molecular Ecology,** v. 19, p. 2605–2608, 2010.

WRIGHT, S. The Roles of Mutation, Inbreeding, Crossbreeding and Selection in Evolution. **Proceedings of the Sixth International Congress of Genetics,** v. 1, p. 356–366, 1932.

APÊNDICE A

ANEXO A

Capa da edição e artigo científico publicados em 2021 (na revista *Ecology and Evolution – Wiley;* QUALIS A2, Fator de Impacto: 3.002).

Artigo publicado no formato Open Access (Creative Commons 4.0). Permitida a cópia e redistribuição do material por qualquer meio ou formato, podendo ser anexado no presente documento e inserido em Repositório online. Importante registrar os novos resultados e otimizações nas análises estatísticas dos marcadores microssatélites, em comparação com os resultados prévios.

ANEXO B

Autorização do Comitê de Ética no Uso de Animais (Faculdade de Medicina de São José do Rio Preto - FAMERP).

Projeto de Pesquisa realizado no laboratório de Imunogenética, iniciado a partir de amostras destinadas ao projeto desenvolvido anteriormente (*Toxoplasma gondii* em *Myrmecophaga tridactyla*) por Ferrari e Castiglioni (2016) e Pena *et al.*, (2020).

ANEXO C

Parceria realizada com o Zoológico Municipal de São José do Rio Preto (Secretaria do Meio Ambiente e Urbanismo - SMAURB).

A colaboração visa a avaliação genética de tamanduás-bandeira no Zoológico, para iniciar a criação de uma base de dados e caracterizar os animais cativos da espécie, o que pode contribuir em longo prazo para planos de manejo e reprodução em cativeiro, a fim de auxiliar na conservação da espécie.

ANEXO A

Capa da edição e artigo científico publicados em 2021 (na revista *Ecology and Evolution* – *Wiley;* QUALIS A2, Fator de Impacto: 3.002).

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ORIGINAL RESEARCH

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Identifying Priority Giant Anteater (*Myrmecophaga tridactyla*) Populations for Conservation in São Paulo State, Brazil

Ricardo Quiterio Sartori[1] | **Alessandro Garcia Lopes**[1] | **Luiz Paulo Nogueira Aires**[4] | **Rita de Cassia Bianchi**[2] | **Cinara Cássia Brandão de Mattos**[3] | **Adriana Coletto Morales**[2] | **Lilian Castiglioni**[1,3,4]

[1]School of Biosciences, Humanities, and Exact Sciences, Graduate Program in Biosciences, São Paulo State University (UNESP), São José do Rio Preto, São Paulo State, Brazil

[2]School of Agricultural and Veterinary Science, São Paulo State University (UNESP), Jaboticabal, São Paulo State, Brazil

[3]São José do Rio Preto Medical School (FAMERP), São José do Rio Preto, São Paulo State, Brazil

[4]Rio Preto University Center (UNIRP), São José do Rio Preto, São Paulo State, Brazil

Correspondence
Lilian Castiglioni, São José do Rio Preto Medical School (FAMERP), Department of Epidemiology and Public Health. Address: A Brigadeiro Faria Lima, 5416, Vila São Pedro, 15090-000, São José do Rio Preto, São Paulo, Brazil.
Email: clilian@terra.com.br

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Abstract

Habitat loss is the main threat to biodiversity conservation worldwide. Some species may be particularly susceptible to the effects of fragmentation and the isolation of populations. The impacts of human activity on wild animal populations may be understood through relationships between individual genetic data and spatial landscape variables, particularly when considering local population dynamics influenced by fragmented habitats. Thus, the objective of this study was to analyze the population structure and genetic diversity of the giant anteater (*Myrmecophaga tridactyla*) using an individual sampling scheme (ISS) on a regional geographic scale. Data were collected from 41 specimens from twenty different locations in São Paulo State, Brazil, and six polymorphic microsatellite loci were genotyped. Our results indicate that barriers to gene flow exist and have segregated individuals of the farther away areas into two spatially structured clusters. The populations were also found to have high genetic diversity. The experimental sampling approach used herein enabled an analysis of the population dynamics of the giant anteater on a regional scale, as well as the identification of priority populations for genetic resource conservation for this species. The results reflect the need for adequate management plans. The efficacy of the sampling scheme may vary based on the study model used, but we argue that the use of an ISS combined with suitable molecular markers and statistical methods may serve as an important tool for initial analyses of threatened or vulnerable species, particularly in anthropized regions where populations are small or hard to characterize.

KEYWORDS

Brazil, conservation genetics, giant anteater, microsatellite markers, population structure, São Paulo

1 | INTRODUCTION

Decreases in and losses of habitat connectivity lead to increases in the existing decline in wild animal populations, thus raising the risk of their extinction (Butchart et al., 2010). The transformation of a continuous habitat into "small pieces of habitat" may affect biodiversity at the population level, with changes in species number, distribution, reproduction, and survival (Carvalho et al., 2009; Fahrig, 2003; Rocha et al., 2018; Wolff et al., 1997).

Medium- and large-sized land mammals may be particularly vulnerable to habitat fragmentation and loss, particularly for species with large home ranges or long life cycles (Keinath et al., 2017; Morris et al., 2008). A large-sized animal averaging 31 kg, the giant anteater (*Myrmecophaga tridactyla*; Linnaeus, 1758) (Figure 1), is considered a vulnerable species in Brazil largely because of its habitat loss in the Cerrado and other Brazilian biomes (Miranda et al., 2015).

There is a tendency for the remaining populations of *M. tridactyla* to become progressively more isolated, which, in turn, further increases their vulnerability and leads to various cases of local extinction. Some of the species' biological characteristics may lead to low rates of population growth: a low metabolic rate and low thermoregulatory capacity, limited reproductive potential, prolonged parental care, a long gestation period, and solitary habits (Clozato et al., 2017). The species is a frequent victim of roadkills, as well as of fires caused by sugar cane crop burning and prolonged periods of drought. These factors threaten the persistence of giant anteater populations in anthropized areas (Diniz & Brito, 2013, 2015).

The extinction of small populations is expected in many fragments over time; the study of local population dynamics influenced by the existence of highly fragmented habitat networks may provide clearer information that can be used to implement strategies to help protect and manage isolated populations (Canale et al., 2012; Weiss & Leese, 2016).

Conservation genetics focuses on the effects of contemporary genetic structuring on long-term survival of a species, and genetic analyses have been used to determine the prospective status of several endangered species (Wan et al., 2004). This study sought to characterize the giant anteater's population structure in fragments of Cerrado and Cerrado/Atlantic forest ecotones, to evaluate its genetic diversity, to detect any barriers to gene flow, and to determine the current degree of gene flow in anthropized areas in São Paulo State, Brazil.

FIGURE 1 The giant anteater, *Myrmecophaga tridactyla* (Pilosa: Myrmecophagidae). Photo credit: Jason Woolgar

2 | MATERIAL S AND METHODS

2.1 | Study area

The animals sampled herein were largely distributed in the northwestern and central-southern region of São Paulo State, which is the most industrialized state in Brazil. This region is currently experiencing intense expansion and urban development, with native vegetation being highly affected by human activity (Durigan et al., 2007). Before colonization, this region was covered by a mosaic of Cerrado and seasonal semi-deciduous forest; however, the forests have since been almost entirely depleted (Myers et al., 2000). The vegetation cover of São Paulo State has been drastically reduced, and the remnants correspond to small areas surrounded by pasture, sugarcane, soybean, reforestation, perennial crops, and urban zones (Kronka et al., 2005).

The study area included some animals that had been obtained, most frequently after car accidents, in cities that border the Noroeste Paulista Conservation Area (EENP) located in the cities of São José do Rio Preto and Mirassol. Other animals were obtained in search-and-capture campaigns in and around the Santa Bárbara Conservation Area (EESB) located in the city of Águas de Santa Bárbara. The use of animals from these locations permitted inferences about the status of individuals and populations in habitats in and around these conservation areas.

The EENP conservation area is 500 ha in size when combined with the Noroeste Paulista State Forest and is considered to be seriously threatened by urbanization and real estate speculation. It is surrounded by pastures, farms, and urban neighborhoods (IF – Instituto Florestal, 2014). The EESB conservation area is 2,712 ha in size, and most of the area is occupied by native vegetation. There is little evidence of human disturbance, and the area is surrounded by pastures and reforestation sites (IF – Instituto Florestal, 2011).

2.2 | Sampling

The current study included a local analysis involving an ISS—individual sampling scheme (Prunier et al., 2013). The ISS used allowed for giant anteaters in the northwestern and central-southern regions of São Paulo State to be analyzed.

The samples included in this study (n = 41) were obtained from different sources. Some were collected by veterinarians and park rangers after the collection of animals that had been run over or had been victims of crop burning (n = 35). In other cases, blood samples

were obtained from animals captured alive in field studies for other purposes and were provided to our laboratory (n = 6).

Samples were obtained from a total of twenty different sites. The geographic distances between the sampling points ranged from 10 km to 340 km (Figure 2). The maps were generated in DIVA-GIS, version 7.5 (Hijmans et al., 2012).

The analyses of interindividual information used in this study may be applied on practically any spatial scale, including in analyses of genetic data that use one or several individuals from different sampling sites (Guillot et al., 2009; Mank & Avise, 2004; Miller, 2005; Safner et al., 2011). The number of individuals required to evaluate genetic structure depends on the extent of population differentiation, as well as on the degree of polymorphism in the markers; these factors mean that a small number of sample animals may be appropriate in certain circumstances (Landguth et al., 2012).

All biological material collection herein was authorized for scientific purposes by the Biodiversity Information and Authorization System (SISBIO) from the Brazilian Federal Environmental Agency (IBAMA) under certificate number 43854-4 and was approved by the research ethics committees at the universities involved in this study (Rio Preto University Center [UNIRP] under authorization number 04/2014PP and São José do Rio Preto Medical School [FAMERP] under authorization number 2014/05302-8). For the captures in the EESB conservation area (the samples provided from previous studies), sampling licenses were obtained (COTEC No. 429/2014 D23/2013 PGH; SISBIO No. 38326-5), as was authorization from the local institutional animal care and use committees (CEUA No. 003414/13). Details on the origin of the samples, geographic coordinates, and genotypes are available in the Dryad Data Repository (https://doi.org/10.5061/dryad.cfxpnvx31).

2.3 | Amplification conditions

Six pairs of microsatellite-specific oligonucleotides were used in the molecular analyses, as detailed in Table 1 (Garcia et al., 2005). Genomic DNA was extracted using the QIAmp DNA kit (Qiagen) following the manufacturer's instructions. It was quantified using an Epoch™ Spectrophotometer System (BioTek) to measure DNA quality and concentration.

FIGURE 2 (a) Map of the study area (São Paulo State, Brazil). (b) Red dots represent the sampling points, and green circles represent the conservation areas

TABLE 1 Microsatellite-specific oligonucleotides (Garcia et al., 2005)

Locus	Repeat motif	Primer sequences	T (°C)	Size (bp)
MtriUSP 04	(gt)09	5′GGGTCAGATATCCTAATGGG3′ 5′TGTCTTCTTTACTCAGTGCTCC3′	61	158
MtriUSP 07	(gt)42	5′AGGAGGATAAGATTAGGCAG3′ 5′TGTGTCCTGTGAAGTAATGG3′	62	274
MtriUSP 11	(gt)15	5′TGTCTCTGTGTTAGGGTTCTTC3′ 5′TCACCTTCATTGGAGCTTC3′	59	174
MtriUSP 13	(gt)14	5′CTGCTCAGGTAACATTCC3′ 5′TGGTAAAGAATGAGGTC3′	57	223
MtriUSP 17	(gt)20	5′CCCAGAATGAACTTACTTG3′ 5′ACTGGCAACTTGTTGTTG3′	55	191
MtriUSP 20	(gt)14	5′CTTTCCTCATATCTCCCTG3′ 5′CTATATGCTTGCCTTTGG3′	55	157

Amplification via PCR was performed with the addition of the M13 tail, which allowed for the use of the tailed primer method (Schuelke, 2000). The amplifications were performed using touchdown PCR in a total volume of 17.2 μl, which contained 1 μl of DNA (~50 ηg), 0.2 μl of the forward primer (5 μM), 0.4 μl of the M13 primer dyed with fluorophore (5 μ), 0.6 μl of the reverse primer (5 μM), 5 μl of the Gotaq® Colorless Master Mix (Promega; 2X), and 10 μl of ultrapure water (Don et al., 1991; Korbie & Mattick, 2008).

The amplification conditions included denaturation at 95°C for 2 min, 12 cycles at 95°C for 1 min, between 64°C and 52°C for 40 s (−1°C per cycle), 72°C for 30 s, plus 25 additional cycles (1 min at 95°C, 40 s at 52°C, and 30 s at 72°C), and the final extension for 5 min at 72°C. After the amplifications were confirmed in 3% agarose gel, the fragments were identified in an ABI 3500 Genetic Analyzer (Applied Biosystems). The DS-33 GeneScan™ Installation Standard (Applied Biosystems) was used for the capillary electrophoresis run, and the reactions were organized into two groups based on the expected fragment sizes.

The results and peak sizes were analyzed using GeneMapper®, version 4.1 (Applied Biosystems, 2012). The correct identification of the alleles is crucial for a reliable interpretation of the microsatellite data (Arif et al., 2010). No artifacts or stutter peaks negatively affected the differentiation between homozygote and heterozygote, and the alleles exhibited well-resolved peaks. The fragments were larger in size relative to the size of the original markers due to the addition of the use of the M13-tailed primer, but the sizes corresponded to the expected sizes based on the information available on their development (Garcia et al., 2005).

2.4 | Statistical analyses

GENEPOP Software, version 4.2 (Raymond & Rousset, 1995), was used to determine the presence of null alleles based on the maximum likelihood method, (Dempster et al., 1977). No genotyping failure was detected and the original dataset was used for the remaining analyses.

To characterize the population structure, individual-based Bayesian analyses were employed using the programs STRUCTURE v.2.3.4 (Pritchard et al., 2000) and Geneland 4.0.5 (Guillot et al., 2005) to determine the most likely number of clusters (K).

In STRUCTURE, we ran 25 replicate runs for each potential number of genetic clusters with 100,000 burn-in steps, 20,000 MCMC repetitions, and 25 iterations per run. Values of $K = 1$ to $K = 5$ were tested using the model with admixture, correlated allele frequencies, and the ancestry prior option "separate α for each population" (Falush et al., 2003). The results were then used in STRUCTURE HARVESTER (Earl & Vonholdt, 2012) to select the K value associated with the highest mean posterior probability of the data (Evanno et al., 2005) with postprocessing in the CLUMPAK program (Kopelman et al., 2015).

Unbalanced sampling has a large impact on STRUCTURE analysis. It reduces the quality of individual assignments to populations and the accuracy of the estimated number of populations. However, these adverse effects of unbalanced sampling on Structure analysis can be largely overcome by simply switching to the alternative ancestry prior, at least when the number of populations is not large and STRUCTURE can yield highly accurate inferences of individual ancestries (Wang, 2017).

Population structure was also analyzed with the software GENELAND 4.0.5 (Guillot et al., 2005) and R version 3.4.2 (R Core Team, 2019). GENELAND differs from STRUCTURE in that geographical information can be incorporated to produce more accurate inferences of population structure based on the spatial distribution of individuals (Levy et al., 2012).

The spatial structure and posterior probability of belonging to a given cluster were determined using the correlated and null alleles models, for runs of $K = 1$ to $K = 5$, and 10 multiple runs were done to check convergence. Each run consisted of 100,000 MCMC iterations with a thinning of 100 and a burn-in of 200. The correlated alleles model may be more powerful at detecting subtle differentiation. Thus, the correlated alleles model could be more informative than the default model of uncorrelated frequencies and hence get

more accurate results, in particular in presence of low differentiation and when K is unknown (Guillot, 2008). The uncertainty of coordinates was set to 10, as this is a computational step that allows for the inclusion of information on the mobility of the species in question (Guillot et al., 2011), according to the characteristics of the species (Medri et al., 2006) and the landscape (Durigan et al., 2007).

Many types of statistical analyses have been applied to understand how the landscape structures genetic variation in natural populations (Guillot et al., 2005; Manel et al., 2003). Bayesian spatial cluster analysis and edge detection methods are frequently useful for describing patterns and revealing microevolutionary processes between individuals and within populations (Guillot et al., 2009; Safner et al., 2011). Thus, in addition to the Bayesian spatial cluster analysis implemented in GENELAND, we performed the edge detection method in the Alleles in Space (AIS) Software (Miller, 2005).

Monmonier's algorithm (Monmonier, 1973) was implemented in the individual-based program AIS to detect the presence of barriers to gene flow and evidence of isolation by barrier (IBB). Monmonier's algorithm is supervised, and the number of biogeographical barriers to be calculated must be specified beforehand (Miller, 2005).

We computed the two first barriers that were contiguous to each other, once the forming boundary has closed on itself by forming a loop around a population (Manni et al., 2004). The barriers were inferred using a Delaunay triangulation-based connectivity network and residual genetic distances (Manni et al., 2004; Monmonier, 1973). This combination of spatial statistical analyses involving Bayesian clustering and barrier detection methods involving Monmonier's algorithm may be a powerful tool for maximizing accuracy and minimizing type-1 errors in barrier detection (Blair et al., 2012).

Aggregation indices were also calculated in AIS. These indices are usually used in ecological studies to quantify spatial patterns and can also be useful for testing genetic diversity patterns over a given landscape. All of the analyses in AIS were calculated with 100,000 permutations/iterations (Miller, 2005).

To determine whether there was evidence of isolation by distance (IBD), the test provided by Mantel (Mantel, 1967; Sokal, 1979) was performed in the VEGAN package of the R software, version 3.4.2 (Oksanen et al., 2019; R Core Team, 2019) using Spearman's correlation, Euclidean dissimilarity indices for geographic distances, and the Bray–Curtis index for genetic distances. These values were used in the interindividual genetic analyses due to their consistent performance in this context; they effectively replace the genetic distances commonly used in population analyses (Bowlby et al., 2016; Shirk et al., 2017).

Subsequently, we performed the principal component analyses (PCA) of individual genetic variation using R version 4.0.2 (R Core Team, 2019) and ADEGENET 2.1.3, centered and scaled (Jombart, 2008).

The main asset of PCA is the absence of any assumption about the underlying population genetic model, and PCA has been suggested as an alternative to individual-based analysis such as Bayesian clustering algorithms and edge detection methods (Patterson et al., 2006; Jombart et al., 2009). However, PCA does not provide a group assessment and would require a priori definition of clusters to study population structures (Jombart et al., 2010).

Thus, the subdivision a priori were defined according to the geographic area of the sampled individuals: (a) the population of the conservation area—EENP, (b) the population of the conservation area—EESB, and (c) a population defined by the animals sampled in other areas across the northwest region of São Paulo State—NP. This subdivision allowed us to compare and evaluate the genetic patterns among the animals in conservation areas and animals farther away from the protected areas.

Cluster analysis was also performed in PAST (Hammer et al., 2001) using the same subdivision and Bray–Curtis index (Bowlby et al., 2016; Shirk et al., 2017), according to the Neighbor-Joining (NJ) method (Saitou and Nei, 1987). The nodes were supported by bootstrap analysis with 10,000 replicates.

Parameters for the evaluation of the microsatellite loci were studied. Estimates of genetic diversity and microsatellite variation in the population of São Paulo State (all sampled individuals) and within the subpopulations of the conservation areas, from which the average allelic richness and gene diversity (HE) were calculated. Finally, genetic divergence between populations was estimated by calculating the unbiased estimate of F_{ST}, and the gene flow analysis was computed using the number of migrants per generation based on the method involving private alleles (Barton & Slatkin, 1986; Weir & Cockerham, 1984) using the GENEPOP program, version 4.2 (Raymond & Rousset, 1995).

3 | RESULTS

3.1 | Population structure

3.1.1 | Bayesian clustering analyses

The results of all Bayesian clustering methods yielded the same optimal number of clusters. We identified $K = 2$ in STRUCTURE as the most probable number of clusters after calculating ΔK (Figures 3 and 4). These clusters corresponded to the individuals of the northwestern region of São Paulo State (blue) and central-southern region/EESB individuals (orange). In the spatially explicit analyses performed in GENELAND, the most probable number of clusters also was $K = 2$ (Figure 5) and the results showed that the individuals are spatially structured across the area ($F_{ST} = 0.03407$ in the dataset containing all individuals and $F_{ST} = 0.09$ in the dataset containing only the populations of conservation areas). The posterior probabil- ity maps indicated that the individuals belong to specific clusters, with a low probability of occupation in farther away areas (Figure 6). Despite the low Delta *K* value (9.118790) and the posterior probability for individuals belonging to cluster 1 and cluster 2 (<0.56), both results indicate the population subdivision and corroborate the hypothesis that there are two groups in the studied area. Besides, the relatively low values may be due to the experimental sampling method and the number of individuals used.

3.1.2 | Isolation by barrier and Isolation by distance

Monmonier's algorithm placed the main boundary separating the individuals/populations of the northwestern region and the central-southern region. The other contiguous barrier showed that the individuals sampled in the most urbanized area are isolated (including the EENP area population), and the result is suggestive of isolation by barrier—IBB (figure not shown).

The Mantel test was performed to test the isolation by distance (IBD) hypothesis, and no evidence of IBD was found (figure not shown) since there was no correlation between genetic and geographic distances ($r = 0.06982$, $p = .195$). The allelic aggregation index, which is calculated based on the overall spatial distribution of all of the alleles and loci (RjAVE), was 0.778477556 ($p = .00633$). This result indicates that the samples presented a clumped spatial distribution (RjAVE < 1) and supports the structure found in the Bayesian spatial analysis.

In other words, the analyses as a whole indicate that spatial structuring does exist, but that distance is not an isolated factor that led to this population structure pattern.

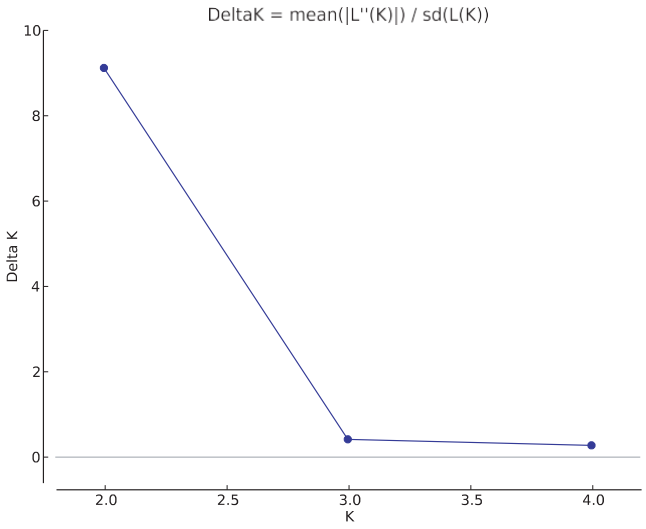

FIGURE 3 Results of the Structure Harvester showing delta K values from the Evanno method

3.2 | Multivariate analysis

3.2.1 | Principal component analysis

The principal component analysis (PCA) was carried out to define in few vectors all data collected from the genotypes studied (Figure 7). The PCA results indicated that only 5 components explain the variation of the data set. The numbers of principal components have been defined according to the relationship between the eigenvalues. The first component (PC1, eigenvalue = 5.283) contributed by 10.358% of the total variability, while PC2 (eigenvalue = 4.435) accounted for 8.696% of the total variability. The distribution of genotypes in the PCA analysis revealed the differences in the distribution of the allelic frequencies in the conservation area populations.

EENP population diverges from the others (NP and EESB). Also, NP and EESB have the same average, but NP has much greater variation than EESB. This variation was expected due to the number of individuals sampled per population. It is important to emphasize that the existing variability in the total population of the state of São Paulo shows the importance of the conservation of these populations for the maintenance of the genetic resources to the species *M. tridactyla*.

The EESB population showed less variation, but a similar average to the NP population (ellipses centers almost together), and the EENP population stands out mainly to PC2. Each ellipse comprises (in general) 95% of the population, and this explains the elements that are external to the ellipse (outliers).

3.2.2 | Clustering methods

The dendrogram based on the NJ algorithm revealed two discrete clusters.

Results presented high bootstrap support values (Figure 8), a finding that corroborates the results of the individual-based clustering analyses.

3.3 | Genetic diversity and population differentiation

The population of São Paulo State (all sampled individuals) presented high genetic diversity, both in relation to the expected

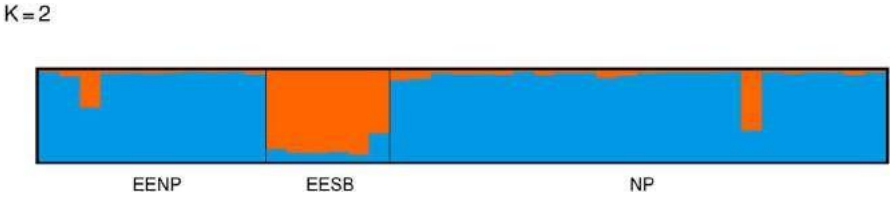

FIGURE 4 Result of the STRUCTURE Software analysis indicating the presence of two population clusters ($K = 2$) based on the higher Delta K value. Noroeste Paulista Conservation Area population (EENP), Santa Bárbara Conservation Area population (EESB), and isolated individuals sampled in other regions of the northwest area of São Paulo (NP)

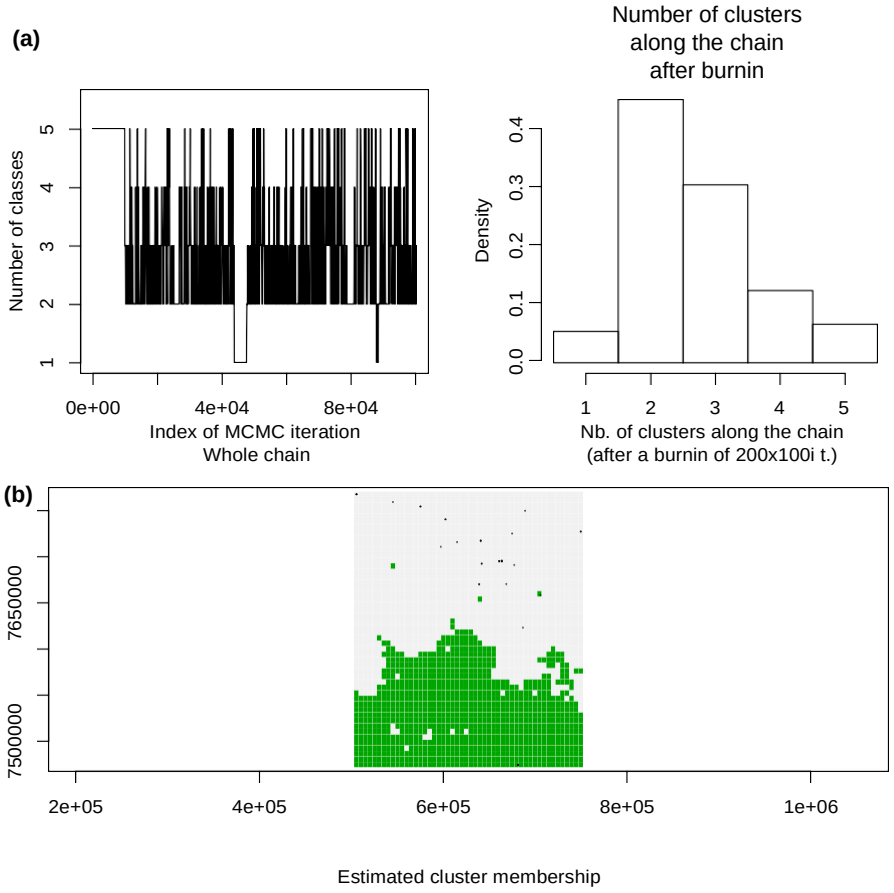

FIGURE 5 (a) Identification of two clusters using GENELAND ($K = 2$). (b) Map illustrating the structure of the clusters identified. Each color represents a different cluster: cluster 1 (gray), cluster 2 (green)

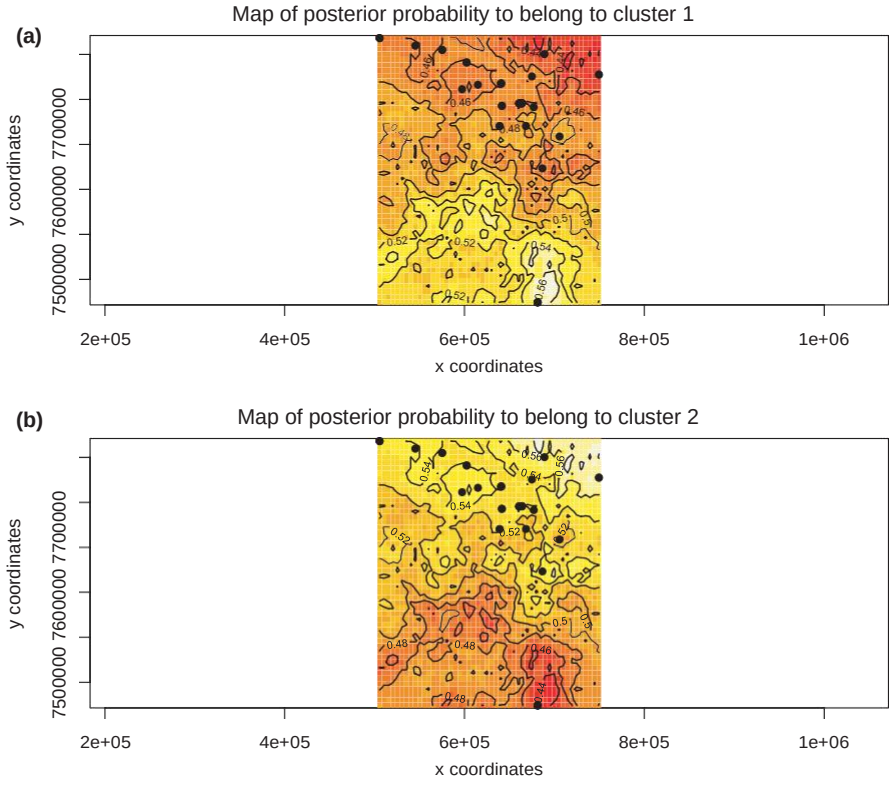

FIGURE 6 (a) Map showing individual attribution and posterior probability of belonging to the cluster 1. (b) Map showing individual attribution and posterior probability of belonging to the cluster 2. Black dots represent the coordinates of the sites where the animals were obtained. Colors indicate regions of high (lightest yellow) and low (darkest red) posterior probability of belonging to the area or the cluster, and the boundary lines represent spatial changes in the probability of population attribution

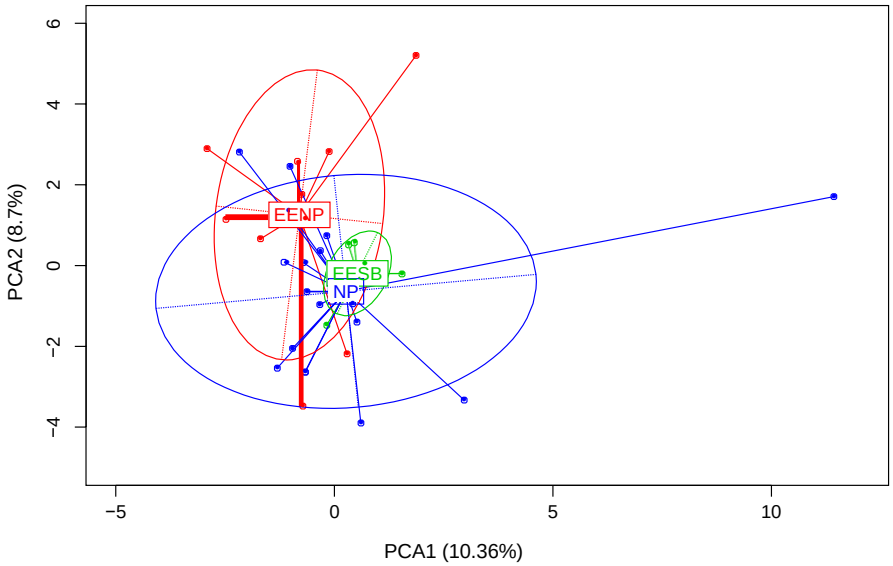

FIGURE 7 PCA result based on the alleles and geographic position of the individuals/populations: Noroeste Paulista Conservation Area population (EENP), Santa Bárbara Conservation Area population (EESB), and isolated individuals sampled in other regions of the northwest area of São Paulo (NP)

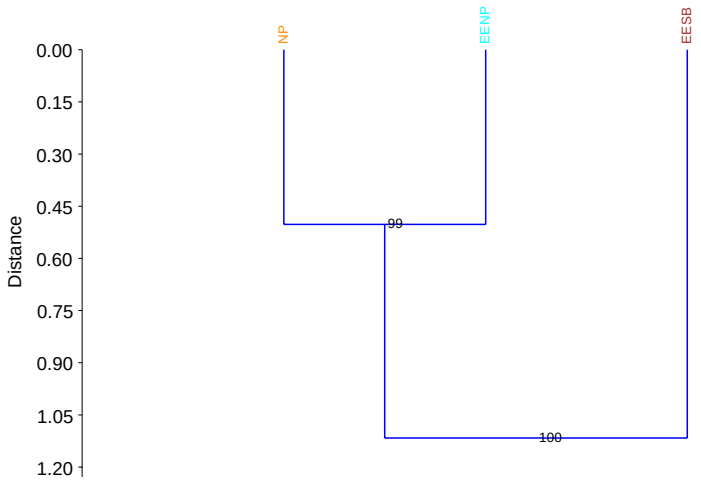

FIGURE 8 Dendrogram (NJ) showing the genetic distance of the populations: Noroeste Paulista Conservation Area population (EENP), Santa Bárbara Conservation Area population (EESB), and isolated individuals sampled in other regions of the northwest area of São Paulo (NP)

heterozygosity (0.7188) and in relation to allelic richness (total number of alleles = 51; mean number of alleles = 8.5 alleles/loci).

Descriptive analyses indicate that all of the loci were highly polymorphic. After the application of Bonferroni's correction, two loci exhibited deviations from Hardy–Weinberg equilibrium, and the tests for linkage disequilibrium were found not to be statistically significant ($p > .05$). Analyses were also performed between individuals found close to the EENP conservation area ($n = 10$) and those found close to the EESB conservation area ($n = 6$) to investigate the genetic diversity and differentiation between the population of the conservation areas. The results reflected greater genetic differentiation between these two groups ($F_{ST} = 0.1739$) and relatively low/moderate F_{IT} (0.1348) and F_{IS} (0.0912) values.

3.4 | Gene flow analysis

Gene flow analyses were carried out for testing levels of gene flow among individuals belonging to different areas, and the results showed that the number of migrants per generation is lower between two areas that are farther away from each other. This reduction in the migration rate at regional levels may have led to population differentiation. Also, we were able to infer migration between the animals that were obtained in the portions of the landscape surrounding the conservation areas and the result indicated a lower level of migration between the populations of the EENP and EESB ($N_m = 0.615729$).

4 | DISCUSSION

4.1 | Genetic diversity of *Myrmecophaga tridactyla*

The use of microsatellite markers in this study aided in the identification of substantial intrapopulation variance, population subdivision ($K = 2$), and high rates of genetic diversity. The genetic structure of the giant anteater populations in this region is best explained by the presence of barriers to gene flow than by geographic distance.

These discoveries, supported by different statistical methods, highlight the importance of small-scale genetic studies to deter- mine whether any barriers are restricting gene flow and, ultimately, to prevent further losses of genetic diversity. Identifying population numbers, levels of genetic diversity, and the degree of connectivity between individuals and populations in an anthropized landscape may provide useful information for conservation strategies and demonstrated the importance of preserving the few habitat fragments that remain (Sullivan et al., 2019; Weiss & Leese, 2016).

Few studies have been performed to evaluate population dynamics and the genetic variability of giant anteater populations (Clozato et al., 2017; Collevatti et al., 2007). Garcia et al. (2005) developed six species-specific microsatellite primers and evalu- ated allelic diversity of markers from fifteen individuals (eight an- imals that had been run over in the states of São Paulo and Mato Grosso, and seven that had been collected in the state of Goiás). They obtained levels of genetic diversity similar to those found in our study (H_o = 0.61, H_e = 0.63, and 26 alleles total), considering the small sample size.

Using the same microsatellite markers, Collevatti et al. (2007) analyzed 27 giant anteaters from Emas National Park (which is also located across the states of São Paulo and Mato Grosso) and found low levels of genetic diversity (mean number of alleles = 3; H_e = 0.482 and H_o = 0.059), as well as a high degree of inbreeding (F_{IT} = 0.879). According to the authors, the low level of diversity in these loci may be the result of the natural history and evolution of *M. tridactyla*, or it may be a consequence of population bottlenecks due to the decades-long history of recurrent fires in the area (Collevatti et al., 2007; Silveira et al., 1999).

The high rates of genetic diversity found were unexpected: The populations are structured, exhibit genetic differentiation, and are located in a fragmented habitat, factors which can decrease genetic diversity (Frankham, 2005). However, this relationship is not always linear; thus, not all large populations will exhibit high genetic diversity, and not all bottlenecked or decimated populations will experience a reduction in genetic diversity (Torres-Florez et al., 2014). Furthermore, changes to genetic diversity associated with habitat fragmentation may be subtle due to recent environmental disturbances (Dixo et al., 2009).

Though some species are highly sensitive to human activity and experience local extinction and/or population reduction when urban development begins, other species are able to persevere as small and relatively isolated populations (Ramalho et al., 2018). As a consequence, in a short period and without the occurrence of extinction, many populations are expected to exhibit fixation more rapidly, but to retain greater overall genetic diversity than a single large population. However, in the long term, when small populations become extinct, the remaining small populations will retain less genetic diversity than the original single large population (Frankham et al., 2002). Therefore, the genetic consequences of habitat fragmentation in a single large population are different from the genetic consequences for various small and isolated population fragments, even in large protected areas.

The genetic patterns observed in this study may be explained by environmental, evolutionary, and biological factors that have led to regional differences in the genetic frequency of isolated populations. Levels of genetic diversity may be maintained or lost through various types of selection, as well as by the effects of genetic drift in selectively neutral mutations in finite populations (Charlesworth et al., 1997; Crow & Kimura, 1970; Gillespie, 1991; Kimura, 1983).

4.2 | Barriers to gene flow

Barriers to gene flow were identified, and it can be inferred that the barriers have reduced connectivity between giant anteater populations and a lack of migrations between fragments located farther away from each other may lead to population differentiation (and likely between populations of many other land species).

The results of Bayesian analyzes, edge detection method, and PCA were slightly different. Nevertheless, these results showed the separation of the EENP individuals and EESB individuals. Also, the number of migrants per generation calculated for the populations of the conservation areas (N_m = 0.615729) indicates the subdivision of the population since rates lower than one migrant per generation are insufficient for counteracting the effects of genetic drift (Slatkin, 1993). Inferring about the cause of the barrier was not possible due to the characteristics of species, population dynamics, and even the fragmentation period in the area, that make it difficult to distinguish between recent and historical signatures of interrupted gene flow in our experimental scheme (Dixo et al., 2009). The main discrepancy found in the analyses was the results of the PCA and the other analyses such as NJ and Bayesian methods, that differently grouped the individuals sampled throughout the northwest of São Paulo (NP). This difference may be the result of the experimental sampling used in the present study and must be carefully considered in the identification of clusters when the genetic difference is low. On the other hand, the methods presented here proved to be effective in the differentiation of clusters in populations with moderate/high genetic differences, as in the case of the EESB and EENP individuals, even with few individuals sampled per population.

In general, the current results show that barriers have prevented gene flow at the regional level, but migrations may have occurred at shorter distances and may have increased intrapopulation variation at the local level contributing to the sustained genetic diversity observed herein (Barton, 2008). Following this logic, differences between the most geographically distant populations of São Paulo State would have increased over time and led to cluster differentiation, but with substantial genetic variation at short distances due to probable short-distance migrations.

Population subdivision may also lead to a geographic structure that affects allele frequencies in space and proportions of different genotypes in local populations (Garnier-Géré & Chikhi, 2013). Thus, gene flow does not depend on distance alone, but also on the nature

of the landscape surrounding and between the populations; the separation of populations may have favored the retention of genetic variants in small and isolated populations (Templeton et al., 2001).

4.3 | Biological characteristics of the species

In addition to evolutionary and environmental factors, certain species' biological characteristics may influence the degree of genetic diversity loss in populations; for example, a long life span seems to contribute to sustained genetic variability in wild populations (Torres-Florez et al., 2014). The processes that lead to the loss of genetic diversity may be buffered by intrinsic biological characteristics, and long generation times may result in small populations that seem genetically diverse despite periods of substantial decline (Hailer et al., 2006).

The estimated lifespan of the giant anteater is more than 15 years in the wild and 20–30 years in captivity. The species' generation time is estimated to be approximately seven years (Gaudin et al., 2018; Knott et al., 2013; Medri et al., 2006; Miranda et al., 2014; Nowak, 2018). Studies on long-living mammals have identified high rates of genetic variability in many small and isolated populations; in some cases, even species that are almost extinct still exhibit high genetic diversity (Dinerstein & Mccracken, 1990; Swart et al., 1994; Taylor et al., 1994; Zavala-Páramo et al., 2017).

Species with shorter generation times and smaller body sizes typically tend to experience faster genetic responses to man-made barriers (Epps et al., 2005). Therefore, a loss in genetic diversity in a long-living species cannot be easily detected in a short period of time, and this factor should be considered when evaluating the species' risk of extinction. Thus, it is plausible that the long life span of the giant anteater combined with its demographic and evolutionary history delayed the loss of the genetic diversity in these populations.

Other hypotheses to explain the processes that have maintained genetic diversity in these populations should not be discarded, but regardless of the hypotheses and theories that explain population patterns, the analyses performed herein revealed that giant anteaters in the state of São Paulo should be a priority in conservation measures due to their high genetic diversity and variability.

4.4 | Conservation issues

Genetic criteria and allelic richness in particular are crucial tools for selecting candidate populations of wild species to be prioritized for conservation (Petit et al., 2008). Thus, despite this persistently high genetic variability, factors such as habitat fragmentation, habitat destruction, and demographic instability may lead wild populations to extinction before the genetic response to environmental impact becomes clear, particularly in long-living species (Lacy, 1997).

Conservation plans are therefore essential to increase the chances of giant anteater survival and to enable the conservation of genetic resources for subsequent species recovery (Théry, 2011).

Due to the use of convenience sampling in this study, the degree of genetic diversity observed may be the result of the use of various study sites with one or more individuals; but in any case, these individuals as a whole represent substantial genetic richness for a species living in an anthropized region. The findings reported herein indicate that management plans must be implemented at local and regional levels because, in the context of intense human activity, the giant anteater population is likely to be limited to a few individuals isolated in the remaining suitable habitats around the state (Bertassoni et al., 2019).

The frequency at which giant anteaters are run over on local highways shows that road ecology studies are necessary to determine local mitigating measures to consider when implementing fauna passage systems; the ultimate goal of this research and these policy measures should be to prevent the decline of animal populations, including that of the giant anteater (Teixeira et al., 2017).

Finally, conservation genetic studies on small geographic scales may provide important information for determining the status of threatened species populations. It is also crucial that interactions between scientists and policymakers improve so that the results of genetic and ecological research can be applied in the establishment of effective conservation strategies (Montgelard et al., 2014; Vernesi et al., 2008).

5 | FINAL CONSIDERATIONS

This study has shown that population of *M. tridactyla* in the state of São Paulo, including the conservation areas, maintain high levels of genetic diversity. Though the sample size is relatively small, it seems to be the largest sample size in conservation genetics studies performed on the giant anteater at the regional level available in the literature.

The results emphasize the importance of fragmented areas for the maintenance of genetic diversity in anthropogenically modified landscapes and support the creation of adequate conservation plans that can work to prevent the local extinction of genetically important populations.

Environmental causes such as roadkills and the increasing agricultural borders can lead populations to local extinctions before a perceptible genetic response to contemporary habitat fragmentation. Thus, the populations in the state of São Paulo should take priority in the conservation of the genetic resources of the species.

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CONFLIC T OF INTEREST

None declared.

AUTHOR CONTRIBUTION

Ricardo Sartori: Data curation (equal); Formal analysis (equal); Investigation (lead); Methodology (lead); Project administration (equal); Resources (equal); Software (lead); Visualization (equal); Writing-original draft (supporting); Writing-review & editing (lead). **Alessandro Garcia Lopes:** Methodology (equal); Visualization (equal); Writing-review & editing (equal). **Luiz Paulo Nogueira Aires:** Methodology (equal); Visualization (equal); Writing-review & editing (equal). **Rita de Cassia Bianchi:** Formal analysis (equal); Funding acquisition (equal); Validation (equal); Visualization (equal); Writing-review & editing (equal). **Cinara Cássia Brandão:** Formal analysis (equal); Supervision (supporting); Validation (equal); Visualization (equal); Writing-review & editing (equal). **Adriana Coletto Morales:** Formal analysis (equal); Validation (equal); Visualization (equal); Writing-review & editing (equal). **Lilian Castiglioni:** Conceptualization (lead); Data curation (equal); Formal analysis (equal); Funding acquisition (lead); Project administration (lead); Resources (equal); Supervision (lead); Validation (lead); Visualization (equal); Writing-original draft (lead); Writing-review & editing (equal).

DATA AVAIL ABILIT Y STATEMENT

Sampling locations and microsatellite genotypes are archived in the Dryad Data Repository (https://doi.org/10.5061/dryad.cfxpnvx31).

ORCID

Ricardo Quiterio Sartori https://orcid.org/0000-0003-4904-2853
Luiz Paulo Nogueira Aires https://orcid.org/0000-0003-3229-9396
Cinara Cássia Brandão de Mattos https://orcid.org/0000-0002-4836-3113

R EFER EN CE S

Arif, I. A., Khan, H. A., Shobrak, M., Al Homaidan, A. A., Al Sadoon, M., Al Farhan, A. H., & Bahkali, A. H. (2010). Interpretation of electrophoretograms of seven microsatellite loci to determine the genetic diversity of the Arabian Oryx. *Genetics and Molecular Research*, *9*, 259–265. https://doi.org/10.4238/vol9-1gmr714

Barton, N. H. (2008). The effect of a barrier to gene flow on patterns of geographic variation. *Genetics Research*, *90*(1), 139–149. https://doi.org/10.1017/S0016672307009081

Barton, N. H., & Slatkin, M. A. (1986). Quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity*, *56*, 409–415.

Bertassoni, A., Costa, R. T., Gouvea, J. A., Bianchi, R. D. C., Ribeiro, J. W., Vancine, M. H., & Ribeiro, M. C. (2019). Land-use changes and the expansion of biofuel crops threaten the giant anteater in south-eastern Brazil. *Journal of Mammalogy*, *100*(2), 435–444. https://doi.org/10.1093/jmammal/gyz042

Blair, C., Weigel, D. E., Balazik, M., Keeley, A. T. H., Walker, F. M., Landguth, E., Cushman, S., Murphy, M., Waits, L., & Balkenhol, N. (2012). A simulation-based evaluation of methods for inferring linear barriers to gene flow. *Molecular Ecology Resources*, *12*(5), 822–833. https://doi.org/10.1111/j.1755-0998.2012.03151.x

Bowlby, H. D., Fleming, I. A., & Gibson, A. J. F. (2016). *Conservation Genetics*, *17*, 823.

Butchart, S. H. M., Walpole, M., Collen, B., Strien, A., Scharlemann, J. P. W., Almond, R. E. A., Baillie, J. E. M., Bomhard, B., Brown, C., Bruno, J., Carpenter, K. E., Carr, G. M., Chanson, J., Chenery, A. M., Csirke, J., Davidson, N. C., Dentener, F., Foster, M., Galli, A., … Watson, R. (2010). Global biodiversity: Indicators of recent declines. *Science*, *328*, 1164.

Canale, G. R., Peres, C. A., Guidorizzi, C. E., Gatto, C. A. F., & Kierulff, M. C. M. (2012). Pervasive defaunation of forest remnants in a tropical biodiversity hotspot. *PLoS One*, *7*(8). https://doi.org/10.1371/journal.pone.0041671

Carvalho, F. M. V., De Marco, P., & Ferreira, L. G. (2009). The Cerrado into-pieces: Habitat fragmentation as a function of landscape use in the savannas of central Brazil. *Biol. Conser*, *142*, 1392–1403. https://doi.org/10.1016/j.biocon.2009.01.031

Charlesworth, B., Nordborg, M., & Charlesworth, D. (1997). The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided popula- tions. *Genetical Research*, *70*(2), 155–174.

Clozato, C. L., Miranda, F. R., Lara-Ruiz, P., Collevatti, R. G., & Santos, F. R. (2017). Population structure and genetic diversity of the giant anteater (*Myrmecophaga tridactyla:* Myrmecophagidae, Pilosa) in Brazil. *Genetics and Molecular Biology*, *40*, 1. https://doi.org/10.1590/1678-4685-gmb-2016-0104

Collevatti, R. G., Leite, K. C. E., Miranda, G. H. B., & Rodrigues, F. H. G. (2007). Evidence of high inbreeding in a population of the endangered giant anteater, *Myrmecophaga tridactyla* (Myrmecophagidae), from Emas National Park. *Brazil. Genetics and Molecular Biology*, *30*(1), 112–120. https://doi.org/10.1590/S1415-47572007000100020

Crow, J. F., & Kimura, M. (1970). *An introduction to population genetics theory*. Harper and Row.

Dempster, A. P., Laird, N. M., & Rubin, D. B. (1977). Maximum likelihood from incomplete data via the EM algorithm. *Journal of the Royal Statistical Society. Series B*, *39*(1), 1–38.

Dinerstein, E., & McCracken, G. F. (1990). Endangered greater one-horned rhinoceros carry high levels of genetic variation. *Conservation Biology*, *4*(4), 417–422. https://doi.org/10.1111/j.1523-1739.1990.tb00316.x

Diniz, M. F., & Brito, D. (2013). Threats to giant anteater, *Myrmecophaga tridactyla* (Pilosa: Myrmecophagidae), viability in a protected Cerrado remnant encroached by urban expansion in central Brazil. *Zoologia*, *30*, 151–156.

Diniz, M. F., & Brito, D. (2015). Protected areas effectiveness in maintaining viable giant anteater (*Myrmecophaga tridactyla*) populations in an agricultural frontier. *Revista Natureza E Conservação*, *13*(2), 145–151. https://doi.org/10.1016/j.ncon.2015.08.001

Dixo, M., Metzger, J. P., Morgante, J. S., & Zamudio, K. R. (2009). Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. *Biological Conservation*, *142*(8), 1560–1569. https://doi.org/10.1016/j.biocon.2008.11.016

Don, R., Cox, P., Wainwright, B., Baker, K., & Mattick, J. S. (1991). Touchdown PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research*, *19*(14), 4008. https://doi.org/10.1093/nar/19.14.4008

Durigan, G., Siqueira, M. F., & Franco, G. A. D. C. (2007). Threats to the Cerrado remnants of the state of São Paulo, Brazil. *Scientia Agricola, Piracicaba, Brazil*, *64*(4), 355–363. https://doi.org/10.1590/S0103-90162007000400006

Earl, D. A., & Vonholdt, B. M. (2012). Structure Harvester: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*, 359–361. https://doi.org/10.1007/s12686-011-9548-7

Epps, C. W., Palsboll, P. J., Wehausen, J. D., Roderick, G. K., Ramey, R. R., & McCullough, D. R. (2005). Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn shee. *Ecology Letters*, *8*(10), 1029–1038.

Evanno, G., Regnaut, S., & Goudet, J. (2005). (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Fahrig, L. (2003). Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 487–515.

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, *164*(4), 1567–1587.

Frankham, R. (2005). Genetics and extinction. *Biological Conservation*, *126*, 131–140. https://doi.org/10.1016/j.biocon.2005.05.002

Frankham, R., Ballou, J. D., & Briscoe, D. A. (2002). *Introduction to conservation genetics*. Cambridge University Press.

Garcia, J. E., Vilas Boas, L. A., Lemos, M. V. F., de Macedo Lemos, E. G., & Contel, E. P. B. (2005). Identification of microsatellite DNA markers for the giant anteater *Myrmecophaga tridactyla*. *Journal of Heredity*, *96*, 600–602. https://doi.org/10.1093/jhered/esi089

Garnier-Géré, P., & Chikhi, L. (2013). Population subdivision, Hardy–Weinberg equilibrium and the wahlund effect. In: *Encyclopedia of Life Sciences (eLS)*. Hoboken: John Wiley & Sons, Ltd (Ed.). https://doi.org/10.1002/9780470015902.a0005446.pub3

Gaudin, T. J., Hicks, P., & di Blanco, Y. (2018). *Myrmecophaga tridactyla* (Pilosa: Myrmecophagidae). *Mammalian Species*, *50*(956), 1–13. https://doi.org/10.1093/mspecies/sey001

Gillespie, J. H. (1991). *The causes of molecular evolution*. Oxford University Press.

Guillot, G. (2008). Inference of structure in subdivided populations at low levels of genetic differentiation-the correlated allele frequencies model revisited. *Bioinformatics*, *24*(19), 2222–2228. https://doi.org/10.1093/bioinformatics/btn419

Guillot, G., Leblois, R., Coulon, A., & Frantz, A. C. (2009). Statistical methods in spatial genetics. *Molecular Ecology*, *18*, 4734–4756.

Guillot, G., Mortier, F., & Estoup, A. (2005). GENELAND: A computer package for landscape genetics. *Molecular Ecology Notes*, *5*, 712–715. https://doi.org/10.1111/j.1471-8286.2005.01031.x

Guillot, G., Santos, F., & Estoup, A. (2011) Population genetics analysis using R and the Geneland program. https://orbit.dtu.dk/en/publications/population-genetics-analysis-using-r-and-the-geneland-program

Hailer, F., Helander, B., Folkestad, A. O., Ganusevich, S. A., Garstad, S., Hauff, P., Koren, C., Nygård, T., Volke, V., Vilà, C., & Ellegren, H. (2006). Bottlenecked but long-lived: High genetic diversity retained in white-tailed eagles upon recovery from population decline. *Biology Letters*, *22*(2), 316–319. https://doi.org/10.1098/rsbl.2006.0453

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, *4*(1), 9.

Hijmans, R. J., Guarino, L., Bussink, C., Mathur, P., Cruz, M., Barrentes, I., Rojas, E. (2012) DIVA-GIS 7.5. A geographic information system for the analysis of species distribution data. http://www.diva-gis.or

IF – INSTITUTO FLORESTAL (2011). *Plano de Manejo da Estação Ecológica de Santa Bárbara*. Governo do Estado de São Paulo.

IF – INSTITUTO FLORESTAL. (2014). *Proposta de criação da floresta estadual do Noroeste Paulista*. Governo do Estado de São Paulo.

Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*, 94. https://doi.org/10.1186/1471-2156-11-94

Jombart, T., Pontier, D., & Dufour, A. B. (2009). Genetic markers in the playground of multivariate analysis. *Heredity (Edinb)*, *102*(4), 330–341. https://doi.org/10.1038/hdy.2008.130

Keinath, D. A., Doak, D. F., Hodges, K. E., Prugh, L. R., Fagan, W., Sekercioglu, C. H., Buchart, S. H. M., & Kauffman, M. (2017). A global analysis of traits predicting species sensitivity to habitat fragmentation. *Global Ecology and Biogeography*, *26*, 115–127. https://doi.org/10.1111/geb.12509

Kimura, M. (1983). *The neutral theory of molecular evolution*. Cambridge University Press.

Knott, K. K., Roberts, B. M., Maly, M. A., Vance, C. K., DeBeachaump, J., Majors, J., Riger, P., DeCaluwe, H., & Kouba, A. J. (2013). Fecal estrogen, progestagen, and glucocorticoid metabolites during the estrous cycle and pregnancy in the giant anteater (*Myrmecophaga tridactyla*): Evidence for delayed implantation. *Reproductive Biology and Endocrinology*, *11*,83. https://doi.org/10.1186/1477-7827-11-83

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*(5), 1179–1191.

Korbie, D. J., & Mattick, J. S. (2008). TouchDown PCR for increased specificity and sensitivity in PCR amplification. *Nature Protocols*, *3*, 1452–1456.

Kronka, F. J. N., Nalon, M. A., Matsukuma, C. K.,Kanashiro, M. M., Shin-Ike, M. S., Pavão, M., Durigan, G., Lima, L. M. P. R., Guillaumon, J. R., Baitello, J. B., Borgo, S. C., Manetti, L. A., Barradas, A. M. F., Fukuda, J. C., Shida, C. N., Barbosa, O., Soares, A. P. (2005). *Inventário florestal da vegetação natural do estado de São Paulo* (200 p). Secretaria do Meio Ambiente; Instituto Florestal; Imprensa Oficial.

Lacy, R. C. (1997). Importance of genetic variation to the viability of mammalian populations. *Journal of Mammalogy*, *78*(2), 320–335. https://doi.org/10.2307/1382885

Landguth, E. L., Fedy, B. C., OYLER-McCANCE, S. J., Garey, A. L., Emel, S. L., Mumma, M., Wagner, H. H., Fortin, M.-J., & Cushman, S. A. (2012). Effects of sample size, number of markers, and allelic richness on the detection of spatial genetic pattern. *Molecular Ecology Resources*, *12*, 276–284. https://doi.org/10.1111/j.1755-0998.2011.03077.x

Levy, E., Kennington, W., Tomkins, J., & Lebas, N. (2012). Phylogeography and population genetic structure of the ornate dragon lizard, *Ctenophorus ornatus*. *PLoS One*, *7*, e46351. https://doi.org/10.1371/journal.pone.0046351

Manel, S., Schwartz, M. K., Luikart, G., & Taberlet, P. (2003). Landscape genetics: Combining landscape ecology and population genetics. *Trends in Ecology and Evolution*, *18*(4). https://doi.org/10.1016/S0169-5347(03)00008-9

Mank, J., & Avise, J. (2004). Individual organisms as units of analysis: Bayesian-clustering alternatives in population genetics. *Genetical Research*, *84*(43). https://doi.org/10.1017/S0016672304007190

Manni, F., Guerard, E., & Heyer, E. (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected

by using Monmonier's algorithm. *Human Biology*, *76*(2), 173–190. https://doi.org/10.1353/hub.2004.0034

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research, Birmingham*, *27*(2), 209–220.

Medri, I. M., Mourão, G., Rodriguez, F. (2006). Ordem Xenarthra. In N. R. Reis, A. L. Perachi, W. A. Pedro, and I. P. Lima, (Eds.), *Mamíferos do Brasil* (pp. 71–99). Universidade Estadual de Londrina.

Miller, M. P. (2005). Alleles in space (AIS): Computer software for the joint analysis of interindividual spatial and genetic information. *Journal of Heredity*, *96*(6), 722–724. https://doi.org/10.1093/jhere d/esi119

Miranda, F., Bertassoni, A., & Abba, A. M. (2014) Myrmecophaga tridactyla. The IUCN Red List of Threatened Species.

Miranda, F. R., Chiarello, A. G., Röhe, F. et al (2015). *Avaliação do Risco de Extinção de* Myrmecophaga tridactyla *Linnaeus*, *1758 no Brasil*. Processo de avaliação do risco de extinção da fauna brasileira. http://www.icmbio.gov.br/portal/biodiversidade/fauna-brasileira/lista-de-especies/7049-mamiferos-myrmecophaga-tridactyla-taman dua-bandeira.html

Monmonier, M. S. (1973). Maximum-difference barriers: An alternative numerical regionalization method. *Geographical Analysis*, *3*, 245–261. https://doi.org/10.1111/j.1538-4632.1973.tb01011.x

Montgelard, C., Zenboudji, S., Ferchaud, A.-L., Arnal, V., & van Vuuren, B. J. (2014). Landscape genetics in mammals. *Mammalia*, *78*(2). https://doi.org/10.1515/mammalia-2012-0142

Morris, W. F., Pfister, C. A., Tuljapurkar, S., Haridas, C. V., Boggs, C. L., Boyce, M. S., Bruna, E. M., Church, D. R., Coulson, T., Doak, D. F., Forsyth, S., Gaillard, J.-M., Horvitz, C. C., Kalisz, S., Kendall, B. E., Knight, T. M., Lee, C. T., & Menges, E. S. (2008). Longevity can buffer plant and animal populations against changing climatic variability. *Ecology*, *89*, 19–25. https://doi.org/10.1890/07-0774.1

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*, 853–858. https://doi.org/10.1038/35002501

Nowak, R. M. (2018). *Walker's Mammals of the World: Monotremes, Marsupials, Afrotherians and Sundatherians*, 7th ed. Johns Hopkins University Press.

Oksanen, J., Blanchet, F. G., Michael, F., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P. R., O'hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H. (2019). vegan: Community Ecology Package. R package version 2.5.4.

Patterson, N., Price, A. L., & Reich, D. (2006). Population structure and eigenanalysis. *PLoS Genetics*, *2*, 2074–2093. https://doi.org/10.1371/journal.pgen.0020190

Petit, R. J., el Mousadik, A., & Pons, O. (2008). Identifying Populations for Conservation on the Basis of Genetic Markers. *Conservation Biology*, *12*(4), 844–855. https://doi.org/10.1111/j.1523-1739.1998. 96489.x

Pritchard, J. K., Stephens M., Donnelly P., (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

Prunier, J. G., Kaufmann, B., Fenet, S., Picard, D., Pompanon, F., Joly, P., & Lena, J. P. (2013). Optimizing the trade-off between spatial and genetic sampling efforts in patchy populations: Towards a better assessment of functional connectivity using an individual-based sampling scheme. *Molecular Ecology*, *22*(22), 5516–5530. https://doi.org/10.1111/mec.12499

R Core Team. (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Ramalho, C. E., Ottewell, K. M., Chambers, B. K., Yates, C. J., Wilson, B. A., Bencini, R., & Barrett, G. (2018). Demographic and genetic viabil- ity of a medium-sized ground-dwelling mammal in a fire-prone, rap- idly urbanizing landscape. *PLoS One*, *13*(2). https://doi.org/10.1371/journal.pone.0191190

Raymond, M., & Rousset, F. (1995). GENEPOP (Version 1.2): Population-genetics software for exact tests and ecumenicism. *Journal of Heredity*, *86*, 248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Rocha, E. C., Brito, D., Silva, P. M., Silva, J., Bernardo, P. V. S., & Juen, L. (2018). Effects of habitat fragmentation on the persistence of medium and large mammal species in the Brazilian Savanna of Goiás State. *Biota Neotropica*, *18*(3). https://doi.org/10.1590/1676-0611-bn-2017-0483

Safner, T., Miller, M.P., Mcrae, B.H., Fortin, M.J., Manel, S. (2011). Comparison of Bayesian clustering and edge detection methods for inferring boundaries in landscape genetics. *International Journal of Molecular Sciences*, *12*, 865–889.

Saitou, N., Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees, *Molecular Biology and Evolution*, *4*, (4), 406–425, https://doi.org/10.1093/oxfordjournals.molbev.a040454.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*, *18*(2), 233–234. https://doi.org/10.1038/72708

Shirk, A. J., Landguth, E. L., & Cushman, S. A. (2017). A comparison of individual-based genetic distance metrics for landscape genetics. *Molecular Ecology Resources*, *17*(6), 1308–1317. https://doi.org/10.1111/1755-0998.12684

Silveira, L., Henrique, F., Rodrigues, G., de Almeida Jácomo, A. T., & Filho, J. A. F. D. (1999). Impact of wildfires on the megafauna of Emas National Park, Central Brazil. *Oryx*, *33*, 108–114. https://doi.org/10.1046/j.1365-3008.1999.00039.x

Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, *47*(1), 264–279. https://doi.org/10.1111/j.1558-5646.1993.tb01215.x

Sokal, R. R. (1979). Testing statistical significance of geographic variation patterns. *Systematic Zoology*, *Washington*, *28*(2), 227–232.

Sullivan, E. R., Barker, C., Powell, I., & Ashton, P. A. (2019). Genetic diversity and connectivity in fragmented populations of *Rhinanthus minor* in two regions with contrasting land-use. *Biodiversity and Conservation*, *28*, 3159–3181.

Swart, M. K. J., Ferguson, J. W. H., Toit, R., & Flamand, J. R. B. (1994). Substantial Genetic Variation in Southern African Black Rhinoceros (*Diceros bicomis*). *Journal of Heredity*, *85*(4), 261–266.

Taylor, A. C., Sherwin, W. B., & Wayne, R. K. (1994). Genetic variation of microsatellite loci in a bottlenecked species: The northern hairy-nosed wombat *Lasiorhinus krefftii*. *Molecular Ecology*, *3*(4), 277–290.

Teixeira, Z. F., Kindel, A., Hartz, S. M., Mitchell, S., & Fahrig, L. (2017). When road-kill hotspots do not indicate the best sites for road-kill mitigation. *Journal of Applied Ecology*, *54*(5), 1544–1551.

Templeton, A. R., Robertson, R. J., Brisson, J., & Strasburg, J. (2001) Disrupting evolutionary processes: The effect of habitat fragmentation on collared lizards in the Missouri Ozarks. *Proceedings of the National Academy of Sciences of USA - PNAS*, *98*(10), 5426–5432.

Théry, N. M. (2011). Conservation of natural areas in São Paulo. *Estudos Avançados*, *25*, 175–188.

Torres-Florez, J. P., Hucke-Gaete, R., Rosenbaum, H., & Figueroa, C. C. (2014). High genetic diversity in a small population: The case of Chilean blue whales. *Ecology and Evolution*, *4*(8), 1398–1412.

Vernesi, C., Bruford, M. W., Bertorelle, G., Pecchioli, E., Rizzoli, A., & Hauffe, H. C. (2008). Where's the conservation in conservation genetics? *Conservation Biology*, *22*(3), 802–804.

Wan, Q. H., Wu, H., Fujihara, T., & Fang, S. G. (2004). Which genetic marker for which conservation genetics issue? *Electrophoresis*, *25*, 2165–2176.

Wang, J. (2017). The computer program structure for assigning individu- als to populations: Easy to use but easier to misuse. *Molecular Ecology Resources*. *17*: 981–990 . https://doi.org/10.1111/1755-0998.12650

Weir, B. S., & Cockerham, C. C. (1984). Estimating F statistics for the analysis of population structure. *Evolution*, *38*, 1358–1370. https://doi.org/10.2307/2408641

Weiss, M., & Leese, F. (2016). Widely distributed and regionally isolated! Drivers of genetic structure in *Gammarus fossarum* in a human-impacted landscape. *BMC Evolutionary Biology*, *16*(153). https://doi.org/10.1186/s12862-016-0723-z

Wolff, J. O., Schauber, E. M., & Edge, W. D. (1997). Effects of habitat loss and fragmentation on the behavior and demography of Gray-tailed voles. *Biological Conservation*, *11*, 945–956.

Zavala-Páramo, A. S., Oyama, K., Mendoza, E., Zavala-Páramo, M. G., Pollinger, J., & Smith, T. B. (2017). Genetic variability in captive individuals of the endangered species *Tapirus bairdii* in Mexico. *Mexican Journal of Biodiversity*, *88*(2), 480–484. https://doi.org/10.1016/j.rmb.2017.03.002

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ANEXO B

Autorização do Comitê de Ética no Uso de Animais (Faculdade de Medicina de São José do Rio Preto - FAMERP).

Projeto de Pesquisa realizado no laboratório de Imunogenética, a partir de amostras destinadas ao projeto desenvolvido previamente.

CEUA
Comissão de Ética no Uso de Animais

Comissão de Ética no Uso de Animais
Faculdade de Medicina de São José do Rio Preto - FAMERP
Autarquia Estadual – Lei nº 8899 de 27/09/94
(Reconhecida pelo Decreto Federal nº 74.179 de 14/06/74)

LICENÇA CEUA 01/2015
São José do Rio Preto 30 de Março de 2015.

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

A Comissão de Ética no Uso de Animais da Faculdade de Medicina de São José do Rio Preto – CEUA/FAMERP, em reunião, analisou o Protocolo FAMERP nº: **001-003298/2014** referente ao projeto intitulado **"Caracterização Genética de Toxoplasma gondii em Myrmecophaga Tridactyla(Linnaeus, 1978)"**, sob responsabilidade do Profa. Dra. Lilian Castiglioni e deliberou que o mesmo está de acordo com os princípios éticos estabelecidos na Lei nº 11.794/2008 e na Resolução nº 714/2002, concedendo a presente licença.

Atenção: **Até 30 dias após a finalização do projeto**, o pesquisador deverá preencher o Formulário do Relatório Final disponivel no site e enviar à CEUA. O descumprimento desta obrigação poderá prejudicar o andamento de futuras solicitações.

Ilmo. Sr
Profa. Dra. Lilian Castiglioni
Pesquisador Responsável pelo Projeto

Prof. Dr. Júlio César André
Presidente CEUA

ANEXO C

Parceria realizada com o Zoológico Municipal de São José do Rio Preto (Secretaria do Meio Ambiente e Urbanismo - SMAURB).

A colaboração visa a avaliação genética de tamanduás-bandeira no Zoológico, para iniciar a criação de uma base de dados e caracterizar os animais cativos, a fim de auxiliar na conservação da espécie.

TERMO DE PARCERIA PARA ANÁLISES EM TAMANDUÁS-BANDEIRA NO ZOOLÓGICO MUNICIPAL DE SÃO JOSÉ DO RIO PRETO

Termo de Parceria, que entre si celebram a Profa. Dra. Lilian Castiglioni e Prof. Msc. Ricardo Sartori e o Zoológico Municipal de São José do Rio Preto, visando a mútua cooperação técnica para viabilizar as avaliações genéticas dos indivíduos cativos da espécie *Myrmecophaga tridactyla* no Zoológico de São José do Rio Preto.

Os proponentes, com pesquisa desenvolvida na FAMERP, Avenida Brigadeiro Faria Lima, 5416, Vila São Pedro, São José do Rio Preto - SP, 15090-000, e o Zoológico Municipal de São José do Rio Preto situado na Rua José Deguer, S/N, Jardim Nazareth, São José do Rio Preto – SP, 15050-190, neste ato representado pelo seu Responsável, Samuel Villanova Vieira, resolvem celebrar o presente termo de Parceria, de acordo com as seguintes cláusulas e condições a seguir especificadas:

Cláusula Primeira: Das considerações
O presente termo de parceria visa a cooperação entre os partícipes para a realização de análises genéticas em indivíduos cativos, para apoio ao programa de reprodução e manejo do Zoológico Municipal.

Cláusula Primeira:
Este Termo de parceria vigorará durante o período de Execução do Projeto de Pesquisa "Análises moleculares para a Conservação de tamanduás-bandeira remanescentes no oeste do estado de São Paulo", desenvolvido pelos proponentes.

Cláusula Segunda: Do objeto
O presente instrumento tem por objeto, viabilizar a colheita de material para avaliar as características genéticas dos indivíduos nascidos em cativeiro, a fim de criar um banco de dados inicial para auxílio em programa de reprodução.

Cláusula Segunda:
Este termo poderá ser rescindido pelos partícipes, a qualquer momento, desde que haja comunicação prévia de, no mínimo; trinta dias, no caso de descumprimento de qualquer uma de suas cláusulas ou condições.

E por estarem justos e de acordo, assinam o presente termo de parceria em duas vias de igual teor e forma.

São José do Rio Preto, 20 de dezembro de 2021

[redacted]

Lilian Castiglioni
Orientadora

[redacted]

Ricardo Sartori
Orientado

[redacted]

Samuel Villanova Vieira
Responsável - Zoológico