Universidade de Brasília
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Ecologia

Biologia reprodutiva, sistema de acasalamento e seleção sexual em *Antilophia galeata* (Aves: Pipridae)

Lia Nahomi Kajiki

Orientadora: Regina Helena Ferraz Macedo

Brasília, DF
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Orientador(a): Dra. Regina H. F. Macedo

Tese apresentada ao Programa de Pós-Graduação em Ecologia da Universidade de Brasília como parte dos requisitos para a obtenção do título de Doutor (a) em Ecologia.

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Antilophia galeata (Lichtenstein, 1823)

Antilophia galeata (Lichtenstein, 1823)

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conheci, e que me ensinou o brilho nos

olhos de um cientista.

"Vença e ajuste o capacete"[1]

Provérbio japonês

---

[1] 勝って兜の緒を締めよ (katte kabuto no o wo shime yo)

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LISTA DE ABREVIATURAS E SIGLAS

AIC – *Akaike Information Criteria* (Critério de Informação Akaike);

CEMAVE – Centro Nacional de Pesquisa e Conservação de Aves Silvestres;

CEUA – Comissão de Ética e Uso Animal;

KDE – *Kernel Density Estimator* (Estimador de Densidade Kernel);

FAL – Fazenda Água Limpa;

GLM – *General Linear Model* (Modelo Linear Geral);

IBAMA – Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis;

ICMBio – Instituto Chico Mendes de Conservação da Biodiversidade;

PCA – *Principal Component Analysis* (Análise de Componentes Principais);

PCR – *Polymerase Chain Reaction* (Reação em Cadeia da Polimerase);

Recor-IBGE – Reserva Ecológica do Instituto Brasileiro de Geografia e Estatística;

SISBIO – Sistema de Autorização e Informação;

SMA – *Standardized Major Axis*;

UnB – Universidade de Brasília;

UV – Ultravioleta.

RESUMO

Uma das vias de diversificação das espécies ocorre via seleção natural; porém, a diversificação de fenótipos pode também ser movida pela variância no sucesso reprodutivo de indivíduos. Nesse caso, pequenas diferenças comportamentais e morfológicas podem fornecer grande vantagem reprodutiva e serem transmitidas às futuras gerações, permitindo condições para a seleção sexual ocorrer. Em uma família de aves neotropicais, a diversificação de caracteres ornamentais aparentemente atingiu condições extremas. Quase todos os membros da Família Pipridae são caracterizados pelo dimorfismo sexual acentuado, onde machos possuem ornamentos extravagantes e elaborados *displays* de corte. Os machos foram liberados do cuidado parental, assim a maioria dos piprídeos possui um sistema de acasalamento poligínico em *lek*. O gênero *Antilophia* se destaca por ter sido considerado monogâmico, porém estudos recentes indicam que tal proposta pode estar equivocada. Assim, este estudo pretende investigar e confirmar o sistema de acasalamento social e genético do Soldadinho (*Antilophia galeata*) (Aves: Pipridae) por meio da investigação de sua biologia reprodutiva, comportamento territorial e relações de parentesco. Para isso, coletamos dados referentes aos parâmetros reprodutivos e de sobrevivência dos ninhos em uma população de *A. galeata* e investigamos se o cuidado é biparental. A ausência de cuidado parental por machos é comum entre piprídeos que fazem *lek*, assim esperávamos encontrar padrão semelhante em *A. galeata* (Capítulo 1). Investigamos o comportamento territorial de machos por meio de uma abordagem experimental para averiguar se eles defendem territórios individuais e fornecemos a descrição do *display* de corte. Esperávamos observar machos defendendo territórios individuais com locais de nidificação, de maneira similar ao seu congênere, *A. bokermanni* (Capítulo 2). Em relação ao sistema de acasalamento, a expectativa era encontrar um padrão de intensa poliginia e poliandria,

como encontrado em seu congênere. Através da análise de parentesco confirmamos o sistema de acasalamento genético da espécie (Capítulo 3) e investigamos se características sexuais secundárias dos machos estão sendo selecionadas pela escolha das fêmeas (Capítulo 4). Neste capítulo, a principal previsão era que ornamentos e canto informariam a condição de machos para fêmeas, e machos que apresentassem esses caracteres sexuais secundários de maneira mais extravagante conseguiriam maior paternidade de ninhadas. Nossos resultados confirmam que não há cuidado biparental na espécie e a fêmea é responsável por todas as etapas do cuidado parental, desde construção do ninho até sua defesa contra predadores. A taxa de predação de ninhos foi estimada em 59%, e foi causada por aves, pequenos mamíferos e primatas. Além disso, a sobrevivência dos ninhos foi maior com a proximidade a corpos d'água e altura do solo. Também demonstramos por meio de experimentos de territorialidade pioneiros com piprídeos que machos de *A. galeata* defendem territórios individuais que contém ninhos, e o *display* se resume a algumas simples posturas e vocalizações de advertência ao redor do território. Com a análise de parentesco, confirmamos que o sucesso reprodutivo entre machos é desigual e encontramos evidências de poliginia. Além disso, 53% das ninhadas apresentaram paternidade múltipla, indicando que fêmeas são poliândricas. Os dados coletados aqui sugerem que *A. galeata* possui um sistema de acasalamento poligínico com evidente poliandria. Os dados não convergem para uma classificação clara do sistema de acasalamento como um *lek* típico, mas sugerimos que *A. galeata* talvez adote displays em *leks* solitários e conserve certos comportamentos atípicos para espécies que fazem *lek*. Por fim, a plumagem da crista e do manto e a duração do canto são indicadores de saúde e condição corporal de machos. Machos com penas vermelhas no manto mais puras, pernas vermelhas da crista menos puras e cantos mais curtos estavam mais saudáveis (menos estresse). O brilho das penas do manto apresentou correlação positiva

com as reservas energéticas. Contudo, contrário às nossas predições, fêmeas preferem machos com plumagem do manto mais fosca, menos contraste acromático e que emitem cantos mais curtos. Por ser a única espécie de piprídeo restrita ao Cerrado, *A. galeata* apresenta comportamentos únicos possivelmente resultantes de um cenário evolutivo particular. Talvez a redução do habitat e falta de área suficiente para displays comunais podem ter afetado o comportamento de machos, que se tornaram mais agressivos e defendem arenas individuais.

Palavras-chave: poligamia, poligínico, poliândrico, piprídeo, região Neotropical, Cerrado, seleção sexual.

ABSTRACT

Species divergence frequently occurs through natural selection; however, variance in reproductive success among individuals may also drive the diversification of phenotypes. In this case, minor behavioral and morphological differences can promote reproductive advantages and may be passed on to future generations, allowing sexual selection to occur. In a family of neotropical birds, the diversification of ornamental characters has reached extreme conditions. Almost all members of the Pipridae family are characterized by marked sexual dimorphism, where males have extravagant ornaments and elaborate courtship displays. Males were released from parental care, so most piprids have a polygynous lek-mating system. The genus *Antilophia* stands out for being considered socially monogamous, but recent studies indicate that this proposition may be wrong. Thus, in this study, we intended to investigate and confirm the social and genetic mating system of the Helmeted Manakin (*Antilophia galeata*) (Aves: Pipridae) by assessing its reproductive biology, territorial behavior, and parentage relations. To this end, we collected data relative to reproductive parameters and nest survival in a population of *A. galeata* and explored whether there is biparental care. The absence of male parental care is widespread among lekking manakins; thus, we expected to find the same pattern in *A. galeata* (Chapter 1). We investigated the territorial behavior of males using an experimental approach to verify whether males defend individual territories and describe their display. We expected to find males defending individual territories with nesting sites, similar to its congener, *A. bokermanni* (Chapter 2). We confirmed the genetic mating system of the species through parentage analysis, and we expected to find a similar pattern as its congener of intense polygyny and widespread polyandry (Chapter 3). Finally, we investigated whether secondary sexual characteristics of males are being selected by female choice (Chapter 4). Our main prediction was that ornaments and vocal

performance would inform the condition of males to females, and males exhibiting these sexual secondary traits more intensely would gain more paternity. The study showed that there is no biparental care in the species, and the female is responsible for all stages of parental care, from nest building to defense of the nest. The nest predation rate was estimated at 59%, caused by birds, small mammals, and primates. Furthermore, nest survival was higher with the proximity to bodies of water and relative to the height above the ground. Through territoriality experiments employed for the first time with a manakin, we demonstrated that *A. galeata* males defend individual territories containing nests. The display boils down to a few simple postures and warning vocalizations around the territory. Through parentage analysis, we found a reproductive skew among males and evidence of polygyny. Furthermore, 53% of the clutches had multiple paternity, suggesting that females are polyandrous. The data collected indicate that *A. galeata* has a polygynous mating system with widespread polyandry. Our data do not converge to a straightforward classification of the mating system as a typical lek. However, we suggest that *A. galeata* may display in solitary leks and retain certain behaviors atypical for lekking species. Finally, crest and mantle plumage and song duration are indicators of male health and body condition. Males with a purer hue of red in the mantle, less pure hue of red in the crest plumages, and performing shorter songs were healthier (less stressed). Mantle feather brightness was positively correlated with energetic reserves. However, contrary to our predictions, females prefer males with duller mantle plumage, less achromatic contrast, and shorter songs. As the only dimorphic manakin restricted to the Cerrado biome, *A. galeata* presents a set of unique behaviors that potentially are a result of a particular evolutionary scenario. Reduced habitat and possibly lack of sufficient area for communal displays may have affected males' behavior, resulting in aggressive males defending individual arenas.

Keywords: polygamy, polygyny, polyandrous, piprid, neotropical region, Cerrado, sexual selection.

PREFÁCIO

Essa tese está dividida em quatro capítulos que em conjunto propõem investigar a biologia reprodutiva e o sistema de acasalamento social e genético do Soldadinho (*Antilophia galeata*). Informações detalhadas sobre história natural e biologia reprodutiva de aves neotropicais baseadas em dados robustos são escassas na literatura. Assim, no primeiro capítulo apresento e descrevo parâmetros reprodutivos de uma população de *A. galeata* investigada por quatro anos no Distrito Federal. Além disso, monitoramos ninhos para confirmar se o cuidado parental é realizado apenas pelas fêmeas e quais parâmetros relativos à construção do ninho afetam seu sucesso.

No segundo capítulo, proponho a discussão das hipóteses de estudos anteriores sobre o sistema de acasalamento da espécie ser monogâmico. Evidências de estudos recentes indicam que a espécie talvez não seja monogâmica, mas tampouco parece ser tipicamente poligínica em *lek* como os outros piprídeos. Essa condição pode estar relacionada à uma possível territorialidade da espécie e à restrição de seu habitat. Para confirmar essas possibilidades, adotamos uma abordagem experimental induzindo resposta territorial e quantificamos o comportamento de defesa dos machos e o tamanho das áreas ocupadas. Além disso, o monitoramento dos machos forneceu informações para a descrição do *display* de corte nessa espécie.

Apesar de *A. galeata* ter sido considerado monogâmico até pouco tempo atrás, estudos recentes com a espécie-irmã *Antilophia bokermanni* revelaram que o sistema de acasalamento dessa espécie não é monogâmico, mas sim poligínico com alta proporção de poliandria. Assim, no terceiro capítulo descrevo o sistema de acasalamento genético de *A. galeata* com base nas análises de paternidade das ninhadas. Incorporando os dados do comportamento de cuidado parental, territorialidade e paternidade das ninhadas, propomos o sistema de acasalamento que melhor caracteriza a espécie.

O elevado desvio reprodutivo encontrado entre machos (Capítulo 3) sugere que os caracteres sexuais secundários poderiam estar sob forte seleção sexual por meio da escolha da fêmea, um traço preponderante entre as outras espécies de piprídeos que fazem *lek*. Assim, no quarto capítulo investigamos se características acústicas do canto e da cor da plumagem de machos funcionam como índices honestos da sua condição imunológica e energética e se fêmeas preferem machos com características sexuais secundárias mais extravagantes.

INTRODUÇÃO GERAL

Referencial teórico

Ao adentrar uma floresta tropical, os cinco sentidos do observador são colocados à prova: a gama de estímulos sensoriais é excepcionalmente diversa, e descrever a experiência se torna um desafio. Ainda mais árdua é a tarefa daqueles que se deparam com uma performance de *display* de corte das pequenas aves neotropicais da Família Pipridae. Assim, Charles C. Nutting tenta descrever a primeira vez que se deparou com o *display* de *Chiroxiphia linearis* na Nicarágua (NUTTING, 1883):

> *"Um dia, enquanto caçava pela densa floresta, o silêncio profundo foi repentinamente interrompido pela nota repetida regularmente do Tangará-rabilongo, e suavemente fazendo meu caminho em direção ao local de onde o som prosseguiu, eu testemunhei uma das performances mais notáveis que o destino me reservou (...).*
>
> *Sobre um galho nu que cobria a trilha a uma distância de cerca de quatro pés do chão, dois Tangarás-rabilongos machos estavam envolvidos em um ato de 'música e dança' que simplesmente me surpreendeu. Os dois pássaros estavam a cerca de um pé e meio de distância, e estavam alternadamente pulando a cerca de dois pés no ar e descendo exatamente sobre o local de onde eles saltaram. O ritmo era tão regular quanto o trabalho de um relógio, um pássaro pulando no instante em que o outro pousava, cada ave acompanhando a melodia de 'to-lé-*do-to-lé-do---to-*lé-do', emitindo a sílaba 'to'*

> *enquanto se agachava para saltar, 'lé' enquanto no ar, e 'do' no pouso".*

As 53 espécies da Família Pipridae têm um sistema de acasalamento poligâmico e são caracterizadas sobretudo pelo intenso dimorfismo sexual na plumagem, acompanhado de elaborados *displays* de corte de machos que incluem diversas manobras acrobáticas, duetos, produção de sons mecânicos, e *displays* coordenados (PRUM, 1990a; b; KIRWAN e GREEN, 2012; WINKLER *et al.*, 2020).

A diversidade de fenótipos se deve, em grande parte, a diferenças nas vantagens adaptativas que determinado grupo de indivíduos adquire sobre o restante numa população (DARWIN, 1859; 1871). Assim, certas características que claramente proporcionam maior vantagem de sobrevivência se mantêm nos organismos, como a variedade de mecanismos de defesa (e.g., garra, espinho, esporão, veneno). Essas características serão herdadas pelas gerações seguintes conferindo-lhes, também, maiores vantagens adaptativas (FUTUYMA e KIRKPATRICK, 2017). Contudo, certos ornamentos, por exemplo, podem ser prejudiciais à sobrevivência e considerados mal adaptados, mas se esses ornamentos aumentam as chances dos indivíduos em adquirir parceiros e se reproduzir, então a seleção natural agindo contra esses ornamentos seria superada (ALCOCK, 2016). A pressão de seleção que atua sobre a variância no sucesso de acasalamento dos indivíduos foi denominada seleção sexual (DARWIN, 1871).

Em espécies poligâmicas admite-se que o dimorfismo sexual atinja níveis extremos em função da pressão da seleção sexual, categorizada em seleção intrassexual (i.e., competição entre membros de um sexo por acesso a membros do outro sexo) e intersexual (i.e., variação na escolha dos parceiros) (DARWIN, 1871; EMLEN e ORING, 1977; ANDERSSON, 1994). Na primeira situação, indivíduos do mesmo sexo competem por acesso a oportunidades de acasalamento: por exemplo, quando machos competem pela monopolização de recursos (e.g., territórios reprodutivos e posição em um *lek*) e dominância social (e.g., performance em *displays*) (ANDERSSON, 1994; ALCOCK, 2016). Em contrapartida, a seleção intersexual decorre quando indivíduos conseguem maior sucesso reprodutivo em função da seleção do sexo oposto. A escolha pode basear-se nos potenciais benefícios materiais adquiridos, como recursos territoriais, ou na aquisição de genes particulares ou combinações de genes que seriam vantajosos para a prole da fêmea exercendo a escolha ("hipótese dos bons genes") (KIRKPATRICK e RYAN, 1991; POMIANKOWSKI e MØLLER, 1995; BROWN, 1997).

A evolução dos ornamentos e *displays* pela escolha da fêmea é geralmente atribuída a duas principais hipóteses e modelos: o modelo de seleção sexual de Fisher e a hipótese da sinalização condicional. No primeiro caso, a preferência das fêmeas seria mantida por meio do reforço positivo da preferência sobre o perfil genético e expressões fenotípicas da prole: ambos os ornamentos do pai e a preferência por esses ornamentos seriam herdados e reforçariam a expressão de sinais sexuais secundários maiores e mais extravagantes ('*runaway sexual selection hypothesis*'; FISHER, 1915; FISHER, 1958). No segundo caso, considera-se que os ornamentos possuem um custo para o macho (i.e., há redução nas vantagens adaptativas), e que os organismos ajustam a expressão desses ornamentos de acordo com sua qualidade ('*condition-dependent signalling hypothesis*'; ZAHAVI, 1975; 1977). Assim, indivíduos de menor qualidade teriam um maior custo adaptativo para expressar o ornamento do que indivíduos de maior qualidade, e esses ornamentos seriam proporcionalmente exibidos em relação à condição fenotípica (FISHER, 1915; ANDERSSON, 1994). Ademais, os ornamentos são condicionais por apresentarem limitações físicas, de desenvolvimento ou fisiológicas nos indivíduos (HAMILTON e ZUK, 1982).

Essencialmente, várias combinações da seleção intrassexual e intersexual determinam o sucesso reprodutivo (ANDERSSON, 1994). Em particular, a seleção intersexual vem sendo intensamente investigada nas aves da família Pipridae, que em sua maioria possuem um sistema poligínico em *lek* (KIRWAN e GREEN, 2012; WINKLER *et al.*, 2020). Nesse sistema de acasalamento, machos são dotados de conspícua ornamentação e compartilham arenas onde realizam elaborados *displays* de corte para atrair e adquirir cópulas com fêmeas visitantes (KIRWAN e GREEN, 2012; HÖGLUND e ALATALO, 2014). Assim, fêmeas impulsionariam a variação dos caracteres sexuais secundários de machos por meio das suas preferências (DARWIN, 1871; ANDERSSON, 1994). Nos piprídeos, sabe-se que caracteres que refletem a preferência da fêmea podem ser relacionados à plumagem (MCDONALD *et al.*, 2001; UY e ENDLER, 2004; STEIN e UY, 2006), idade e dominância social (MCDONALD e POTTS, 1994; LUKIANCHUK e DOUCET, 2014; CARDENAS-POSADA *et al.*, 2018), experiência (DUVAL, 2012), tamanho corporal (PAYNE, 1984; STEIN e UY, 2006), posição da arena no *lek* (SHOREY, 2002), habilidade motora (FUSANI e SCHLINGER, 2012) e tamanho do *lek* (CESTARI *et al.*, 2016).

Dentre as atualmente reconhecidas 53 espécies da Família Pipridae (WINKLER *et al.*, 2020), um gênero se destaca por aparentemente não se comportar como um típico piprídeo com poliginia em *lek*: o gênero *Antilophia* é composto por duas espécies (*Antilophia galeata* e *A. bokermanni*), e por muito tempo perdurou a ideia de que *A. galeata* possivelmente apresentasse um sistema de acasalamento monogâmico (MARINI e CAVALCANTI, 1992) e com consequente perda do comportamento de *display* em *lek* (PRUM, 1994). Até investigações recentes sugerirem que o comportamento de *lek* foi perdido em algumas espécies na família (RIBEIRO *et al.*, 2015), o gênero *Antilophia* representaria a única instância de perda do comportamento de *lek* e possível reincidência da monogamia em Pipridae, que é considerado o sistema de acasalamento ancestral no clado Cotingidae-Pipridae (PRUM, 1994). A reincidência de um provável sistema social monogâmico na espécie *A. galeata* foi sugerida há quase 30 anos, fundamentada em indicativos de territorialidade, formação de pares sociais, nidificação dentro do território e ausência de *displays* coordenados (MARINI e CAVALCANTI, 1992). Contudo, as observações se limitaram a poucos indivíduos sendo monitorados, restringindo a generalização das hipóteses levantadas para a população (MARINI e CAVALCANTI, 1992).

Um dos traços mais predominantes e evolutivamente associado a aproximadamente 90% das espécies de aves monogâmicas sociais é o cuidado biparental (TRIVERS, 1972. Estudos filogenéticos indicam que o comportamento de *lek* e a correspondente perda do cuidado biparental são conservados uma vez que evoluem (PRUM, 1994). Assim, evidências de ausência de cuidado biparental no gênero *Antilophia* sugerem que *A. galeata* talvez não seja monogâmico; em ambas as espécies, apenas a fêmea foi observada realizando todas as tarefas relacionadas à construção do ninho e cuidado dos filhotes (MARINI, 1992b; GAIOTTI *et al.*, 2019; MARÇAL e LOPES, 2019ª; BRUNO *et al.* 2021).

Outra evidência que contestaria a sugestão de um desvio do sistema poligínico em *lek* em *A. galeata* seria a ausência de territórios contendo recursos importantes para a fêmea (e.g., sítios de nidificação). Apesar de MARÇAL e LOPES (2019b) não terem encontrado evidências de territorialidade em uma população de *A. galeata*, na qual as áreas ocupadas por machos se sobrepuseram amplamente, o mesmo padrão não foi encontrado em *A. bokermanni*. Na espécie-irmã, machos defendem territórios individuais que contêm sítios de nidificação (GAIOTTI *et al.*, 2020). Curiosamente, os machos donos desses territórios não possuíam a paternidade dos ninhos situados em seus próprios territórios. Análises moleculares confirmaram que *A. bokermanni* é poligínica, porém o conjunto de evidências não permitiu uma definição simples do sistema de acasalamento como em outros piprídeos (GAIOTTI *et al.*, 2020). Uma hipótese levantada naquele trabalho é que a limitação de habitat tenha restringido as fêmeas a nidificarem invariavelmente dentro dos territórios, indicando que as espécies podem ter uma certa plasticidade para responder às condições impostas pelo ambiente.

O suposto desvio de um sistema de acasalamento em *lek* típico foi sugerido como consequência de seleção associada a uma preferência evolutiva por um habitat particular dentre os piprídeos: as úmidas matas de galeria no Cerrado (MARINI e CAVALCANTI, 1992; PRUM, 1994). Ao contrário da maioria das espécies na família Pipridae, as duas espécies de *Antilophia* se encontram confinadas em matas úmidas de tamanho restrito, envoltas por vegetação típica de ambientes secos. Durante o Último Máximo Glacial (ca. 22.000 anos atrás) e metade do Holoceno (ca. 6.000 anos atrás), as duas espécies do gênero ocupavam áreas maiores do que a distribuição atual, e as populações ancestrais de *Antilophia* provavelmente já ocupavam corredores de matas úmidas (LUNA *et al*., 2017). Nessa época, o clima era úmido, e foi marcado por subsequente intensificação das secas e pela expansão das savanas do Cerrado (LEDRU, 2002). Disponibilidade de habitat apropriado e níveis baixos de competição são fatores que afetam a distribuição espacial de *leks* (LOISELLE *et al.*, 2007; DURÃES *et al.*, 2008); então, pressupõe-se que a restrição das matas úmidas possa ter exercido pressões seletivas na evolução do sistema de acasalamento em *Antilophia*.

A maioria das espécies na família Pipridae para as quais foi confirmado o sistema de acasalamento poligínico em *lek* apresenta acentuado dimorfismo sexual acompanhado de elaborados *displays* de corte (KIRWAN e GREEN, 2012). Sistemas de acasalamento são uma importante força evolutiva que podem definir padrões de dimorfismo sexual

(PAYNE, 1984), e assim como a intensa seleção sexual encontrada em espécies que fazem *lek* pode resultar na evolução de ornamentos extravagantes, o inverso também ocorre. Em *Xenopipo holochlora* é possível que um relaxamento na pressão de seleção sexual possa ter resultado na perda do comportamento de *lek* e na ausência de dicromatismo sexual (RIBEIRO *et al.*, 2015). Nesse estudo, não foram encontradas evidências que machos se juntem em arenas ou realizem qualquer *display* de corte, e muito raramente vocalizavam a uma distância dentro do alcance visível ou auditivo de outros machos. Inicialmente, também foi sugerido que *A. galeata* tivesse perdido o *display* de corte, contudo relatos de frequentes perseguições circulares executadas por machos nas duas espécies de *Antilophia* (MARINI e CAVALCANTI, 1992; SICK, 1997; GAIOTTI, 2016; MARÇAL, 2017; GAIOTTI *et al.*, 2020) colocam em dúvida se a espécie realmente perdeu o comportamento de *display*. Além disso, o acentuado dimorfismo sexual indica que a pressão de seleção sexual nos machos deve ser intensa, mas não há estudos que comprovem desvio reprodutivo entre machos de *A. galeata*.

Espécie de estudo

O Soldadinho (*Antilophia galeata*) por muito tempo foi considerado a única espécie do gênero *Antilophia*, até seu congênere *Antilophia bokermanni* ser descrito (COELHO e SILVA, 1998). As duas espécies não são simpátricas; *A. galeata* é amplamente distribuído pelo Cerrado, abrangendo grande parte do Brasil central até nordeste da Bolívia e Paraguai (WINKLER *et al.*, 2020), enquanto que *A. bokermanni* é restrito à Chapada do Araripe no Ceará (KIRWAN *et al.*, 2020). As duas espécies são caracterizadas por possuir uma crista vermelho escarlate proeminente que se eriça para frente, como um topete, e possuem manto na mesma cor; contudo, se distinguem por terem o restante do corpo coberto por plumagem preta (*A. galeata*) ou branca (*A.*

bokermanni). Indivíduos machos adultos de *A. galeata* são inconfundíveis e de espantosa beleza (Fig. 1; KIRWAN e GREEN, 2012). As fêmeas, como em outras espécies da Família Pipridae, não possuem tanta distinção, são um pouco menores com a plumagem do corpo verde oliva ou amarelada, e possuem apenas um indício de crista (Fig. 1). Esse piprídeo de tamanho considerável (13,9 a 17,5 cm) habita o estrato baixo e médio das matas ao longo de cursos d'água.

A espécie regularmente se junta a bandos mistos fora da estação reprodutiva (SILVA e ONIKI, 1988; TUBELIS, 2004; 2007). Apesar de sua dieta ser composta majoritariamente por frutos de diversas espécies (e.g., *Rapanea lancifolia* – Myrsinaceae, *Davilla rugosa* – Dilleniaceae, *Nectandra oppositifolia* – Lauraceae, *Miconia* spp. – Melastomataceae), regularmente se alimenta de artrópodes (MARINI, 1992a; SILVA e MELO, 2011). A vocalização do macho é composta por uma sequência surpreendentemente musical e rápida de *whip-dip, wih-deh-deh-dehdidip* (Ridgely & Tudor 2009) audível a uma distância de 100 m. Ninhos e ovos da espécie foram descritos por IHERING (1900, 1902), depois por MARINI (1992b), MARÇAL e LOPES (2019a) e BRUNO *et al*. (2021). No Planalto Central se reproduz principalmente entre agosto e dezembro, e a ninhada normalmente é composta por dois ovos (MARINI, 1992b; MARINI *et al*., 1997; MARÇAL e LOPES, 2019ª; BRUNO *et al*. 2021). Os ninhos são construídos em uma forquilha a uma altura de 50 cm a 10 m acima do solo, têm o formato de copo ou semiesfera abertos, constituídos por finos ramos entrelaçados por hifas ou teias de aranha, com folhas camuflando o exterior (KIRWAN e GREEN, 2012).

Figura 1 Indivíduos de Soldadinho (*Antilophia galeata*) macho (à esquerda) e fêmea (à direita). Fotos de Lia N. Kajiki.

Área de estudo

O estudo foi realizado na "Fazenda Água Limpa" (FAL), uma fazenda experimental pertencente à Universidade de Brasília e que possui aproximadamente 4.500 ha. A FAL situa-se a ca. 20 km ao sul da cidade de Brasília na Área de Proteção Ambiental (APA) das Bacias do Gama e Cabeça de Veado, entre as coordenadas 15° 56' a 15° 59' S e 47° 55' a 47° 58' O (ALMEIDA, 2013). Limita-se ao norte com o Ribeirão do Gama e o Núcleo Rural da Vargem Bonita, ao sul com a BR-251 (rodovia que conecta Brasília a Unaí/MG), ao leste com o Córrego Taquara e Reserva Biológica do IBGE, e a oeste com a estrada de ferro. A fitofisionomia predominante na FAL é o Cerrado *sensu stricto,* com vastas áreas de Campo sujo e Campo limpo (ALMEIDA, 2013). Conforme a classificação Köppen – Geiger, o clima da região é classificado como Aw, com duas estações bem definidas: uma chuvosa (outubro a abril) e outra seca (maio a setembro) (NIMER, 1989; DA SILVA *et al.*, 2008). A precipitação média anual é de 1.400 mm, contudo na estação seca a umidade relativa pode cair a menos de 15% e a precipitação a menos de 100 mm (KLINK e MACHADO, 2005). A área de estudo compreendeu as matas de galeria que percorrem os córregos Capetinga, da Onça e Ribeirão do Gama, totalizando em torno de 120 ha de mata amostrada (Fig. 2).

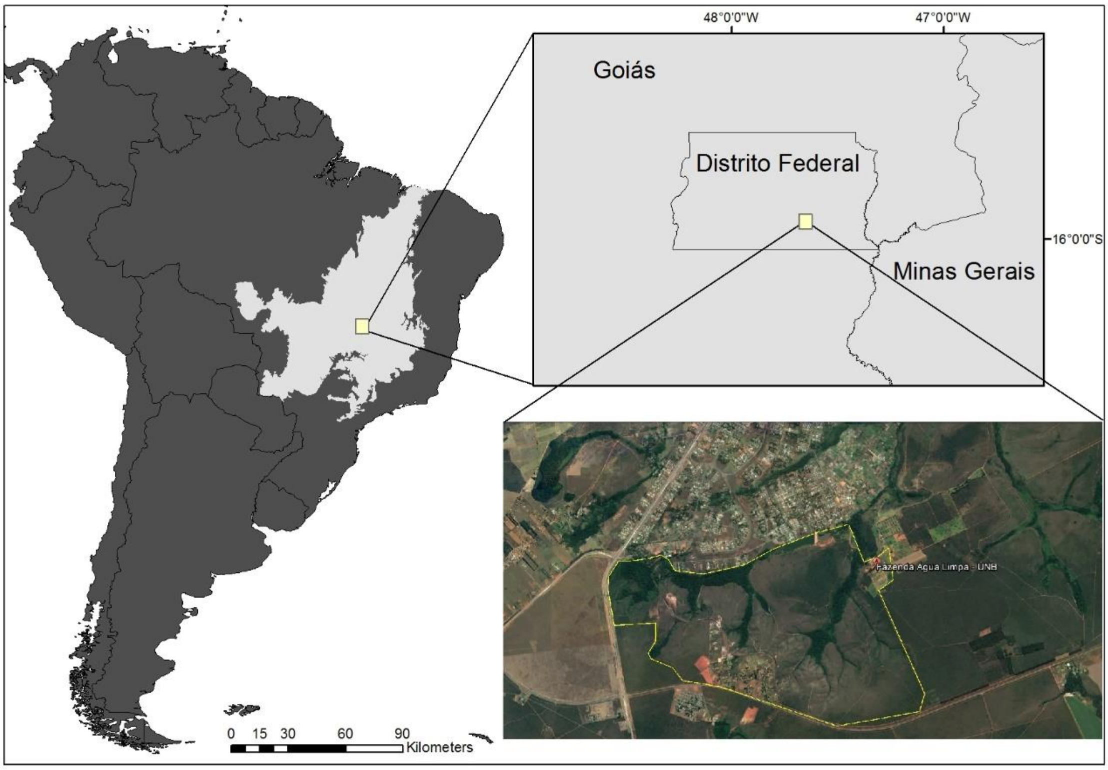

Figura 2 Mapa indicando a área de estudo (destacado em amarelo na imagem de satélite) situada na Fazenda Água Limpa. As coletas ocorreram nas matas de galeria, representadas em verde escuro na imagem de satélite.

Objetivos da tese

Objetivo geral

Investigar o sistema de acasalamento social e genético do Soldadinho (*Antilophia galeata*) (Aves: Pipridae) através do estudo de sua biologia reprodutiva.

Objetivos específicos

1. Descrever os parâmetros reprodutivos, e averiguar os fatores que afetam a sobrevivência de ninhos em uma população de *A. galeata* no Distrito Federal;
    a. Descrever o ninho e suas dimensões, local do ninho;
    b. Descrever os ovos, o tamanho da ninhada, a construção do ninho, o período de incubação e provisionamento de filhotes;
    c. Confirmar se o cuidado é biparental ou uniparental;
    d. Quantificar a taxa de predação dos ninhos e identificar os predadores;
    e. Averiguar fatores que afetam a sobrevivência de ninhos.
2. Investigar o comportamento territorial em machos de *A. galeata*;
    a. Confirmar se machos defendem territórios individuais;
    b. Quantificar o tamanho de territórios, caso existam;
    c. Averiguar se territórios, caso existam, contêm sítios de nidificação;
    d. Descrever o *display* de corte, caso confirmada a existência.
3. Descrever o sistema de acasalamento genético de *A. galeata*.
    a. Averiguar se o sucesso reprodutivo entre machos é desigual;
    b. Averiguar se machos copulam com mais de uma fêmea durante uma estação reprodutiva;

    c. Averiguar se ninhadas apresentam paternidade múltipla.

4. Investigar se caracteres sexuais secundários de machos refletem sua qualidade, e se estão sob seleção sexual.
    a. Identificar as variáveis acústicas do canto de machos e de cor da plumagem que correlacionam com condição corporal e imunológica;
    b. Averiguar se machos que exibem essas características que indicam condição são preferidos por fêmeas.

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Bioética

Este trabalho foi protocolado (Protocolo n° 54/2019) e autorizado pela Comissão de Ética no Uso Animal (CEUA) do Instituto de Ciências Biológicas da Universidade de Brasília (Processo 23106.051860/2019-47) (Anexo 1).

Licenças

O Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) por meio do Sistema de Autorização e Informação em Biodiversidade (SISBIO), emitiu autorizações para a realização deste projeto (nº 58094-1, 58094-3 e 58094-4) (Anexo 2). As atividades de captura e anilhamento foram realizadas sob as autorizações nº 4221/1, 4221/2 e 4221/3 (Anexo 3) expedidas pelo Centro Nacional de Pesquisa e Conservação de Aves Silvestres (CEMAVE). A atividade de acesso ao Patrimônio Genético foi cadastrada no SisGen em atendimento ao previsto na Lei n°13.123/2015 e seus regulamentos.

Formatação da tese

Os capítulos 1, 2 e 4 estão formatados como artigos científicos seguindo as regras de formatação dos respectivos periódicos escolhidos. Os capítulos 1 e 2 estão formatados de acordo com as normas das revistas científicas *Journal of Field Ornithology* e *Emu – Austral Ornithology*, respectivamente. O capítulo 4 está formatado nas normas do periódico *Animal Behaviour*. O restante da tese e o capítulo 3 estão formatados de acordo com as normas da Agência Brasileira de Normas Técnicas (ABNT) para teses e dissertações.

CAPÍTULO 1

Breeding biology and nest survival of an endemic manakin in the Cerrado savanna of central Brazil

Uma versão deste capítulo foi submetido para publicação em *Journal of Field Ornithology* e está redigido de acordo com as normas de formatação da revista.

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Breeding biology and nest survival of an endemic manakin in the Cerrado savanna of central Brazil

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Abstract

Breeding biology data are essential for understanding avian life history, ecology, evolution, and behavior. Nevertheless, there are massive information gaps about basic breeding biology for most birds, especially in the Tropics and Neotropics. Among the Pipridae family, the Helmeted Manakin (*Antilophia galeata*) is the only dimorphic manakin endemic to the Cerrado biome. The species was suggested as a rare case of monogamy within the family, but its mating system remains unconfirmed. In this study, we provide detailed information on the breeding biology of a population of the Helmeted Manakin in the Cerrado of central Brazil and discuss the factors that affect nest survival rates and nest site selection. We tested whether proximity to streams, nest height, parental visitation rate, nesting stage, and male presence affected the daily survival rate of Helmeted Manakins' nests. We predicted that sites closer to watercourses would be preferred for nest construction, and that nest survival would be higher at these sites than at nest sites located in the middle of the gallery forest. We found that females are solely responsible for all nesting duties, which include nest construction (12 ± 2.1 days), incubation (16 ± 3 days), and nestling care (18.3 ± 1.5 days). Clutches have two eggs, which hatch asynchronously. The breeding season is long (~ 7 months) and extends from the second half of July to the second half of February. Nests are built close to and far from watercourses equally, but nest survival was higher with proximity to streams and at greater heights. Female visitation rate, nesting stage, and male presence did not influence nest survival. Nest predation rate was estimated at ca. 59%, with most events due to primates, and we provide the first documentation of nest predation by the Bearded Capuchin monkey (*Sapajus libidinosus*).

Key-words: DSR, MARK, nest failure, nesting success, Pipridae, reproduction

Introduction

Birds are one of the few groups of animals for which total species richness is purported to be known, and the biodiversity of nearly 11,000 species (Billerman et al. 2020) is associated with a corresponding variety and complexity of reproductive strategies (Winkler 2016). Information on breeding parameters and predation rates is of paramount importance for understanding broader aspects of avian life history and its interaction with ecological, behavioral, and evolutionary aspects (Bartholomew 1986, Martin 1995, Martin 1996, Martin 2004, Jamieson 2007). Such information is particularly valuable for population management of species showing either population decline or excessive proliferation and for conservation or pest control measures, respectively (Elphick et al. 2007).

Although data on the breeding biology of birds have been accumulating for centuries, there are pervasive gaps in knowledge, especially for certain biogeographical regions, such as the Neotropics (Xiao et al. 2017). The Neotropical realm is one of the most species-rich regions in the world (Pennington et al. 2015, Antonelli et al. 2018), yet information on avian breeding biology is well known for less than a fourth (19.2%) of its bird diversity, and it is poorly known for nearly half (47.4%) of tropical forest birds (Xiao et al. 2017).

Nest predation is a primary driver of birds nest failure (Ricklefs 1969, Skutch 1985) and can therefore be considered a selective force with broad implications for life history traits and reproductive behaviors, such as nest site selection and parental activity (Martin 1995, Conway and Martin 2000, Martin et al. 2000, Ricklefs and Brawn 2013). For example, it has been demonstrated that nest height is an important variable in nest site selection, and this parameter is frequently inversely correlated with nest survival rate (Hatchwell et al. 1999, Colombelli-Negrel and Kleindorfer 2009, Guan et al. 2018). Further important characteristics with implications for nest survival include the behavior of building nests over watercourses (Ocampo and Londoño 2014, Perrella et al. 2021) and parental activity at the nest (Skutch 1949, Skutch 1985, Martin et al. 2000).

The manakins (family Pipridae), remarkable for their conspicuous plumage patterns and complex courtship behaviors, have been intensely studied for decades, especially regarding specific aspects of their courtship displays (Snow and Snow 1985, Prum 1990, McDonald and Potts 1994, Duval 2007, Anciães and Prum 2008, Ribeiro et al. 2019, Janisch et al. 2020, Tolentino and Anciães 2020) (review in Kirwan and Green 2012). However, less attention has been given to descriptions of significant characteristics of their breeding biology. Basic information, such as nest and egg descriptions, is available for most of the species in the family (Kirwan and Green 2012). Nest and egg descriptions for the Helmeted Manakin can date back to the 19th century (Allen 1893). However, details relative to nest construction, incubation and nestling periods, nest survival, and predation rates are limited for most species of the family. Some gaps are, however, being filled; recent work has provided valuable data about the breeding biology of, for example, the Blue Manakin (*Chiroxiphia caudata*; Zima et al. 2017), Pale-bellied Tyrant-Manakin (*Neopelma pallescens*; Ferreira and Lopes 2018), Araripe Manakin (*Antilophia bokermanni*; Gaiotti et al. 2019) and Helmeted Manakin (*Antilophia galeata*; Marçal and Lopes 2019a, Bruno et al. 2021). Previous studies with the Helmeted Manakin provided valuable but limited information, with a reduced sample size (< 12 nests). Thus, there remains an indisputable need for comprehensive investigations on the breeding biology of Neotropical birds, even when considering well-known clades such as that of the manakins.

The Helmeted Manakin, a sexually dimorphic manakin resident in the gallery forests within the Cerrado biome of Brazil, presents an example of how scant information about basic breeding biology can affect researchers' inferences about broader aspects of behavior and life history. Nearly three decades ago, a small study of the Helmeted Manakin yielded behavioral observations of a few individuals (four adult males and two adult females) (Marini and Cavalcanti 1992). Since these first preliminary observations were conducted, manakins within the *Antilophia* genus were considered the sole exception to polygamous mating systems within the Pipridae family (Prum 1994) (but see Marçal and Lopes 2019b). This hypothesis, however, is inconsistent with recent behavioral and genetic data showing that the sister species of the Helmeted Manakin, the Araripe Manakin (*Antilophia bokermanni*), is not genetically monogamous but has an atypical mating system (Gaiotti et al. 2020). More precisely, close monitoring of dozens of Araripe Manakin nests revealed that only females performed all activities related to nesting, an indication of a polygamous mating system (Gaiotti et al. 2019). This study suggests that more data are needed to verify the true nature of the Helmeted Manakin's mating system as well.

In this study, we provide information on the Helmeted Manakin's breeding biology and related aspects of natural history, working at the same site in central Brazil where the earliest study with the species was conducted more than 30 years ago (Marini and Cavalcanti 1992). Our specific objectives are to 1) provide detailed descriptive information concerning nesting parameters, 2) estimate the species' nest survival rates, 3) document ecological and behavioral factors affecting the species' nest survival, and 4) identify the species' main nest predators.

Methods

Field site

We studied a population of Helmeted Manakins for four years (2017 – 2020) at the “Fazenda Água Limpa” (FAL; 15º 56’S to 15º 59’S, 47º 55’W to 47º 58’W), a 4500-ha experimental farm that belongs to the University of Brasília, in Distrito Federal, Brazil. The farm is located at ca. 1100 m a.s.l. and is mainly characterized by Cerrado *sensu stricto*, a type of savanna landscape with narrow strips of gallery forests along rivers and streams. The weather is highly seasonal, with a rainy season from October to April and a dry season from May to September (Almeida et al. 2014). The average annual precipitation at the study site is 1450 mm (Parron et al. 1998). The breeding activity of birds in the Cerrado is highly limited by this seasonality (Marini et al. 2012). Thus, we used total monthly precipitation (mm/month) across each season to evaluate whether the Helmeted Manakin breeding activity was associated with the rainy season and the amount of rain (Fig. 1). Rainfall data were obtained from the governmental online database Hidroweb (http://www.snirh.gov.br/hidroweb/apresentacao).

General field methods

We captured Helmeted Manakins with mist nets in the gallery forests along Capetinga, Onça, and Gama rivers. All captured birds were banded with a uniquely coded aluminum band provided by the Centro Nacional de Pesquisa para Conservação de Aves Silvestres (CEMAVE), the Brazilian agency responsible for bird banding regulation, and a unique combination of three plastic colored bands. The combinations were generated with the

“Variable frequency” algorithm in the GenTag R package (Biagolini-Jr. and Macedo 2019). We randomly chose combinations given the availability of bands of each color.

Like other members of the Pipridae family, the Helmeted Manakin is extremely dichromatic. Adult male plumage is mostly black, contrasting with a bright crimson frontal crest and mantle. Females have a less pronounced frontal crest, and body plumage is olive overall (Kirwan and Green 2012). Juveniles are very similar to females and probably acquire adult plumage in their third year of life (Snow and de Juana 2020). Thus, most of the time, it is impossible to determine the sex of individuals in green plumage in the field, and molecular sexing is needed for confirmation purposes. Blood samples (~ 50 µl) were collected through brachial venipuncture and stored in 99% ethanol at 4ºC. Molecular sexing was conducted following Griffiths et al. (1998) using PCR (polymerase chain reaction) analyses with P2/P8 primers (Griffiths et al. 1998, Çakmak et al. 2017).

Nest searching and monitoring

We searched for nests between August and January of three breeding seasons (2017 – 2020) and in September 2020 during a shortened fieldwork season. We located nests by searching vegetation within areas where males were continuously observed and along the entire length of rivers and by following birds carrying food or nest material. Females often re-nest in a particular area (LNK, pers. obs.); thus, we searched more intensely in a 25 m radius from the nest in the following months and breeding seasons when a nest was found. Subsequently, we georeferenced their locations with a GPS and monitored them every three days. We video-recorded active nests with camera traps (®Bushnell Trophy Cam HD) installed 1–2 m from the nest. Video recordings took place during nest construction, incubation, and nestling stages. A nest was considered abandoned when construction was interrupted, when eggs were not laid, or when no adult activity was detected during incubation or nestling stages for an entire day. Nest predation and predators were often detected with the aid of camera traps. However, we also considered predation to have occurred when we found destroyed nests, broken or missing eggs, or that nestlings were missing before their expected fledging date.

Nest, egg and nestling description

We followed Hansell's (2000) proposal for standardizing nest description and measured nest diameter, nest depth, cup diameter, and cup depth (Fig. 2) with calipers. We also measured the height of each nest relative to the ground or the water surface, in the cases when it was located over streams, with a laser distance measurer (®Leica DISTO E7100i, accuracy of ± 1.5 mm within 0.2 – 5 m with a confidence level of 95%). Egg width and length were measured with calipers and egg mass with a 30 g (accuracy 0.25 g) ®Pesola spring scale. The nest construction period lasted from the day the nest was found until the first egg was laid. We estimated the construction period only for nests found in the initial stages when dry leaves had not been deposited yet, and which were subsequently confirmed as active nests. The end of the construction period was established by verifying that females were no longer carrying nest material to the nest based on the camera trap recordings or encountering an egg in the nest in subsequent visits. The incubation period was defined as the number of days from clutch completion to the hatching of the last egg. We estimated incubation periods only for nests found during construction or those with a single egg in the initial stages of development, with ~ 2 days old. The developmental stage of eggs was verified using the candling technique. The nestling period lasted from the hatching of the first egg until fledging of the last chick. We estimated the nestling period for nests found before hatching started or on the day the first egg hatched and that were successful (at least one chick fledged). When nestlings were 14 days old, they were banded with metal and color bands in the same way as adults. However, we intentionally chose sequences that had paler or less conspicuous colors (e.g., grey and black) to prevent females from attempting to remove the bands (LNK, pers. obs.). The total breeding period was estimated based on the earliest active nest found and the last day of activity in the last nest found.

Nest attendance, predators and parental care

To assess parental care patterns and whether the adult movement in and around the nests could be linked with nest predation, we quantified nest attendance and adult activity by deploying camera traps near nests. Camera traps were set to record after any movement was detected around the nest and automatically adjusted the length of the videos (between 5 to 30 s) during daytime and nighttime (24 hr cycle). We measured attendance (i.e., percentage of time parents attend the nest during an observation period), on-bout length (i.e., the duration of a single period of adult attendance), off-bout length (i.e., the time adults were absent from the nest between on-bouts), and visit rate (i.e., the number of visits adults made to their nests per unit of time) considering only daytime recordings (12 hr cycle, from 06:00 to 18:00 h). Male activity close to nests was calculated based on vocal occurrence recorded by camera traps but not upon visual records. This measurement was obtained from nests located in areas where a banded adult male was frequently observed. Although not all males close to these nests were banded, an individual would typically use the same singing perches, as described for the Araripe Manakin (Gaiotti et al. 2020), and we speculate that the same individual males consistently occupied the same areas. However, it was not possible to confirm whether the songs indirectly recorded by the camera traps belonged to the same male. We counted the number of songs in relation to the total length of recordings for each nest and obtained a measure of male song rate in the immediate vicinity of each nest. The camera traps could not record uninterruptedly; thus, the measures mentioned above were calculated in relation to the total length of recorded videos for each nest (mean ± SD; 42.5 ± 37.5 min). The same camera traps were used to record predation events.

We quantified parental care at nests by analyzing videos recorded with the camera traps. Behaviors recorded in each video were assigned to one of the following categories: brooding (incubating/sitting), agonistic (vigilant/defending), feeding, and cleaning. Because camera traps recorded activity during 24-hours and females sleep on the nest, we did not include brooding while sleeping in the analysis. We calculated the percentage of each behavior in relation to the total number of videos recorded for each nest, and we compared female effort between nests with one and two nestlings.

Statistics

We used Mann-Whitney *U*-tests to compare nest height between nests located above streams and far from streams (i.e., over 1 m away from the stream bank) and evaluate the relationship between nest height and survival. We also used a Mann-Whitney *U*-test to compare female effort between nests with one and two nestlings. The effects of distance to streams in nest survival were assessed with Fisher’s exact test. We calculated nest success rates using the daily-survival estimator in program MARK (White and Burnham 1999). Daily survival rate (DSR) was used to estimate cumulative probabilities for nest survival. We pooled samples from all breeding seasons to improve the effectiveness of analysis, and we tested whether DSR varied as a quadratic, linear or constant function over the breeding season. We generated models to evaluate if co-variables affect DSR, including nest height relative to the ground, distance to water, male activity (i.e., percentage of males actively singing), female visit rate (i.e., number of visits to the nest, hr^{-1}), and nest manipulation. We compared models based on AIC_c, which correct small sample sizes (Burnham and Anderson 2002). Following standard convention, the model with the lowest AIC_c value was considered to have the best fit; models with $\Delta AIC_c < 4$ were considered with good fit (Burnham et al. 2011). We calculated DSR for incubation and nestling periods separately and both stages combined because survival rates usually differ between these stages (Martin et al. 2000). DSR analyses were divided into two sections: 1) including only the first three co-variables (nest height, distance to water, and presence of males) when calculating DSR for incubation and nestling periods separately, and 2) including all co-variables when calculating DSR for incubation and nestling periods combined. We used this approach for the DSR analyses because data collected for each variable differed among nesting stages. We also used the Mayfield method to calculate the probabilities of egg hatching and nest failure during incubation and nestling stages (Mayfield 1975).

During the first two breeding seasons (2017/2018 and 2018/2019), nearly all active nests found with eggs were predated (85.7%, $N = 7$). Therefore, during the third field season (2019/2020), we adopted another sampling regime to meet other goals of our research (i.e., blood collection from nestlings for paternity analyses). We replaced real eggs with plasticine replicas made with air-dry clay, which were immediately accepted by females and did not change their incubation behavior, consistent with prior experiments with other manakins (Tori et al. 2006). The plasticine replicas were left in the nest until the real eggs hatched in an incubator (5–20 days), after which hatchlings were returned to their natal nests. If the natal nest failed before the eggs hatched in the laboratory, the artificially incubated eggs were sacrificed for genetic sampling. We generated models and ran DSR analyses accounting for these two nest treatments (manipulated and non-manipulated) for the dataset with incubation and nestling periods combined. Other calculations related to the nest, such as incubation period, activity, and hatching probability, did not include manipulated nests.

Results

Breeding season

We captured and banded 254 Helmeted Manakins (138 males, 113 females, and three greenish birds that could not be sexed with molecular analysis). We found 252 nests (107, 77, and 60 in the 2017/2018, 2018/2019, and 2019/2020 breeding seasons, respectively; four nests opportunistically in September 2020), of which 58 (23.02%) became active and were monitored. These nests belonged to at least 21 distinct females. Among the active nests, 19 were found during construction, 29 after the first egg was laid, and ten after the first nestling hatched. Of the 58 active nests found, 27 had clutches removed for paternity analysis. Of the remaining nests, 23 (74.19%) were unsuccessful: two were abandoned during construction, four were abandoned during incubation and nestling periods, nine were predated during the incubation period, seven were predated during the nestling stage, and one was lost because of heavy rain.

During the first breeding season (2017/2018), we found the first active nest with two eggs on 1 September 2017, and the last active nest with two eggs survived until 7 February 2018. In the next breeding season (2018/2019), we found the first active nest with two nestlings (~ 7 days old) on 1 September 2018, and the last active nest found with nestlings survived until 23 December 2018. During the third breeding season (2019/2020), we found the first active nest under construction on 13 August 2019, and the last active nest with a nestling was abandoned on 15 January 2020. During the shortened fieldwork season in September 2020, the first active nest was found under construction on 10 September 2020, and the last active nest under construction was monitored until 13 October 2020. Furthermore, three females with brood patches were captured in the last week of July 2018. We estimate the Helmeted Manakin nesting cycle, from incubation to fledging, to be ~ 31 days (mean ± SD = 31 ± 2 days, $N = 13$ nests). Therefore, the breeding season of the Helmeted Manakin in our study area can extend for approximately seven months, from the second half of July to the second half of February.

Rainfall appears to play a central role in determining the start of nesting and egg hatching (Fig. 1). In the first two breeding seasons, active nests increased in number with the start of the rainy season. In all three breeding seasons, the number of nests with nestlings was higher in the middle of the rainy season, between December and January. The exception was the third breeding season, in which nesting activity appeared to have initiated regardless of precipitation levels (Fig. 1). In contrast, the end date of nesting activity remained nearly the same across the three breeding seasons. Around mid-January, which corresponds to the middle of the wet season, birds no longer initiated new nests after fledging or nest failure.

Description of nests, nest site selection and nest construction

Average measurements of nests were as follows: nest diameter = 64.86 ± 6.86 mm (range = 49.60–80.10 mm; $N = 158$), nest depth = 45.69 ± 7.94 mm (range = 28.40–79.00 mm;

N = 150), cup diameter = 54.42 ± 5.31 mm (range = 34.30–74.60 mm; *N* = 156) and cup depth = 34.74 ± 5.21 mm (range = 23.80–54.00 mm; *N* = 142). All nests were found between forked branches in the lower and middle strata of the gallery forests, and manakins built nearly half (*N* = 117) of the nests over water and the rest at 52.22 ± 67.90 m (range = 0.1 – 345 m; *N* = 130) from the edge of the streams. The height of nests built over water (161.29 ± 66.08 cm; range = 33 – 451 cm; *N* = 107) and away from streams (153.69 ± 70.29 cm; range = 50.35 – 475 cm; *N* = 125) did not differ (*N* = 232, *U* = 7629.5, *P* = 0.06466), and the pooled average height was 157.20 ± 68.48 cm (range = 33–475 cm; *N* = 232). Nearly half of the active nests (*N* = 20) were located within a male's potential territory, and male activity close to these nests averaged 0.58 ± 0.52 songs/min (range = 0.03–1.97 songs/min) in 733.76 min of video recordings.

In 384.92 min video recordings of 10 nests under construction, females were solely responsible for the construction process and similar to other manakins, females do not reuse nests. Nests are open baskets made of dry leaves, twigs, and leaf petioles finely arranged and attached to the forked branches with a considerable amount of spider silk (Fig. 3c). The nest is first shaped with some dry spider silk weaved from one side to the other side of the forked branches, and a few small dry leaves are deposited, forming a platform (Fig. 3a). The bird sits on the platform and stretches it downward, expanding it into a cup. In the following 12 ± 2.1 days (range = 10–16 days; *N* = 5), the female actively works on the entanglement of dry leaves and sticks in the threads of dry silk to complete the open cup nest (Fig. 3a-c). In each visit, the female sits on the nest platform and employs vigorous breast movements to push the material inside and shape the open cup, along with fast side-to-side movements with the beak to draw out and fasten the silk.

Description of eggs, incubation, and nestlings

We monitored 12 non-manipulated nests during the incubation period. Eggs have a long oval shape, with a beige background covered with irregularly distributed dark-brown spots, sometimes concentrated at the larger pole (Fig. 3c). Eggs (*N* = 37) had a mean length of 23.09 ± 1.28 mm (range = 20.60–25.45 mm) and width of 16.02 ± 0.52 mm (range = 14.90–17.00 mm). Clutch size varied between one and two eggs, but most nests had two eggs (87.93%). After completing nest construction, females (*N* = 9) took from one to four days to lay the first egg. One nest found in the third season took nearly a month to become active: it was found in the early stages of construction, on 19 November 2019, and after eight days, it was nearly complete. However, the camera trap did not record any activity in the nest after that, and we thought it was abandoned. On 27 December 2019, we found two fresh eggs (around two days old) in this nest, indicating that the female delayed egg-laying for approximately 27 days. Another possibility is that the nest was abandoned after completed and taken over by another female. This could not be confirmed because we only attempted to capture and band females after eggs were laid to avoid abandonment of the nest. In most cases, the second egg is laid two days after the first egg (*N* = 7), but females can lay their two eggs in consecutive days (*N* = 2).

Incubation is performed exclusively by females (Fig. 3d), and the mean duration of the incubation period was 16 ± 3 days (range = 11–20 days; *N* = 11). Camera traps recorded one female starting incubation after the second egg was laid, and in four nests, incubation started right after the first egg was laid. In this latter situation, incubation activity was irregular until the second egg was laid. Of 31 active nests, 14 (45.16%) reached the nestling stage, generating a total of 22 nestlings, and nearly half of these nestlings (*N* = 10) reached the fledgling stage. Hatching was asynchronous for most Helmeted Manakin nestlings in the same clutch (55.5% of nine nests with two nestlings hatched one day apart, and the rest hatched on the same day). The average duration of the nestling period was 18.3 ± 1.5 days (range = 16–20 days; *N* = 3). We identified two renesting events during the third breeding season, in which females lost their nest and built a new one a month later.

Parental care and nest attendance

A total of 1310 min of video recordings of 30 nests (10 in the construction stage, 16 with eggs, and 14 with nestlings) revealed that the female is the only one involved in nest construction, incubating the eggs, provisioning the chicks, cleaning, and defending the nest. We observed that females actively defended nests against different intruders, such as a Swallow Tanager (*Tersina viridis*), a Flavescent Warbler (*Myiothlypis flaveola*), and a White-necked Thrush (*Turdus albicollis*). In this latter case, the female evicted the intruder by grabbing it by the beak and pulling it down from the nest. While monitoring nests, LNK also observed the females performing a distraction display. On three occasions, the females flew closely at eye level to the observer and maintained hovering for a few seconds; after capturing the observer's attention, they continued hovering in a short distance to the nest and performing an erratic flight. It looked like the female was ill during the entire distraction display, possibly in an attempt to draw the attention away from the nest.

Mean nest attendance (percentage of time on the nest) overall was 54.05 ± 28.51% (range = 4.25–100%, *N* = 26); during the nest construction stage it was 41.12 ± 33.53% (range = 1.88–100%, *N* = 10); during the egg stage it was 64.92 ± 19.25% (range = 24.51–100%, *N* = 15), and during the nestling stage it was 58.33 ± 25.28% (range = 9.56–89.29%, *N* = 14).

We analyzed 600 min recordings of 12 nests (nine nests with two nestlings and three nests with one nestling), and females spent most of the time at the nest feeding the nestlings. The behavior “feeding” had the highest average percentage (37%; 1 nestling = 16.1% and 2 nestlings = 43.9%), followed by “brooding” (26.3%; 1 nestling = 11.5% and 2 nestlings = 43.9%), “vigilant/defending” (13.1%; 1 nestling = 22.8% and 2 nestlings = 9.9%) and “cleaning” (5.1%; 1 nestling = 1.7% and 2 nestlings = 6.2%). There were no differences in total effort invested by females for any of the behaviors analyzed between nests with one and two nestlings (brooding: $U = 10$, $P = 0.6$, $r = 0.19$; cleaning: $U = 13$, $P = 1.00$, $r = 0.03$; feeding: $U = 5$, $P = 0.15$, $r = 0.45$; vigilant/defending: $U = 18$, $P = 0.48$, $r = 0.24$).

Nest manipulation effects

We replaced the eggs of nine active nests (29%) with plasticine eggs. DSR for manipulated and non-manipulated nests were similar (MARK: manipulated, 0.925 ± 0.037, non-manipulated, 0.924 ± 0.0235. Models that incorporated manipulation as a covariate performed worse overall (S(manipulation): $\Delta AIC_c=3.3043$); (S(manipulation+height+water+male+fvisit): $\Delta AIC_c=7.9309$) compared to the general model S(.), indicating that this variable had a low effect on nest survival.

Nest survival

Unsuccessful nests were either predated ($N = 18$), abandoned ($N = 7$), or destroyed by heavy rain ($N = 1$). Predation occurred during incubation ($N = 11$) and nestling ($N = 7$) stages, and females abandoned nests during construction ($N = 2$), incubation ($N = 2$) and nestling ($N = 3$) stages. Overall, the nest predation rate is estimated to be 58.6%. Although not directly recorded in a predation event, the video recordings revealed four potential nest predators: the Barred Forest-Falcon (*Micrastur ruficollis*) (Fig. 4a), the White-necked Thrush, the Swallow Tanager, and the Flavescent Warbler. The Barred Forest-Falcon appeared in a recording of an empty nest; the White-necked Thrush, Swallow Tanager, and Flavescent Warbler were recorded being repelled by females on active nests. We directly identified four predators of Helmeted Manakin nests: the Yellow-ridged Channel-billed Toucan (*Ramphastos vitellinus pintoi*) was recorded predating two nests with eggs (Fig. 4b); Bearded Capuchin monkeys (*Sapajus libidinosus*) predated a nest with eggs (Fig.4c) and a nest with nestlings; an opossum (*Gracilinanus* sp.) was recorded predating a nest with eggs (Fig. 4d), and a Grey-headed Kite (*Leptodon cayanensis*) predated two nests with nestlings. Partial predation was recorded in three nests, in which the predator took from a couple of hours to two days to predate the second egg. We also video-recorded one banded female ejecting a potentially infertile egg from her nest in the first breeding season.

According to the Mayfield method (Mayfield 1975), the probability of egg hatching was 48%, with a failure probability of 84.81% across the incubation period. For the nestling period, the probability of success was 29.26%. When incubation and nestling periods were combined, the most parsimonious model of DSR for the first section of analysis included constant time (Tc) and nest height (height), with 19.33% evidence of being the best model (Table 1). Other models had ΔAIC_c values lower than 2.0, but evidence of being good models was lower (< 11%, Table 1). The estimated regression coefficient for nest height was positive with a low effect (SE = 0.005), indicating higher nests to be more successful.

When analyzed separately, models that best adjusted the DSR for the incubation and nestling periods were slightly similar. The most parsimonious model of DSR for the incubation period included constant time (Tc) and nest height (height), with the lowest ΔAIC_c and 20.21% evidence of being a good model (Table 2). The model including the co-variable "distance to water" presented a ΔAIC_c value lower than 2.0 and 12.78% evidence of being a good model, and that co-variable had a negative estimated regression coefficient with low effect (SE = 0.008). This suggests that distance to water bodies may negatively affect DSR during the incubation period (Table 2). The most parsimonious model only included constant time (Tc) during the nestling period, with 21.34% evidence of being a good model (Table 3). The second most parsimonious model included constant time (Tc) and nest height, suggesting that the latter may affect the DSR during the nestling period (Table 3).

Predated and non-predated nests differed in nest height ($U = 150.5$, $P = 0.012$, estimate = 0.48) and distance from streams ($P = 0.024$, estimate = 0.13), indicating that higher nests located closer to streams survived more than lower nests located farther from streams.

Discussion

After a gap of nearly 30 years since the first investigations on the Helmeted Manakin were conducted, our study provides novel and detailed information about this species' breeding biology and natural history in gallery forests of the Cerrado biome in central Brazil. A population was monitored during three breeding seasons, and here we provide information on breeding parameters, nest site selection, parental care, nest survival, and predation rates.

Our results indicate that the Helmeted Manakin breeding season duration is the longest reported for the species, but still similar to the ones found for different populations investigated previously (6 months in Distrito Federal, Marini 1992; 5 months in Minas Gerais, Marçal and Lopes 2019a; 5 months in São Paulo, Bruno et al. 2021) and to birds in Amazonian savannas (Sanaiotti and Cintra 2001). The 7-months breeding season is considerably longer when compared to the 3–4 month typical breeding season of birds in the Cerrado savanna (Marini et al. 2012) and the Neotropics (Wikelski et al. 2003, Marini et al. 2012). However, the breeding season length of Neotropical birds may vary considerably among species (Marini et al. 2012). This long breeding season may be a characteristic shared with other members of the Pipridae family, which inhabit less seasonal equatorial forests. The 31 days nesting cycle (incubation and nestling stages) is similar to that of other manakins (Kirwan and Green 2012, Zima et al. 2017, Gaiotti et al. 2019, Marçal and Lopes 2019a, Bruno et al. 2021), but slightly longer than that of open cup nesters (Auer et al. 2007, Brawn et al. 2011, Marques-Santos et al. 2015). Daytime nest attendance rates of 65% and 58% for incubation and nestling periods, respectively, fall within the ranges reported for other tropical inner-forest birds with open-cup nests (Martin 2002, Ricklefs and Brawn 2013).

In general, tropical species present protracted nesting periods at the cost of higher nest predation risk (Skutch 1985, Conway and Martin 2000, Martin et al. 2000, Martin et al. 2015), and this could be associated with low nest attendance as a strategy adopted by birds with lower adult mortality (Martin 2002). Parents may enhance their survival at the expense of clutch survival by reducing daily time spent on the nest. However, the more prolonged incubation could enhance the quality of embryos (Ricklefs et al. 2017). This may be the case of the Helmeted Manakin, a species with great longevity (Silva-Jr et al. 2020), considerably high nest predation rates (58.6% in this study), and a high proportion of nest failure (73% in Marçal and Lopes 2019a and 63.6% in Bruno et al. 2021)

The beginning of the Helmeted Manakin's breeding season appears to be correlated to precipitation, with the nestling stage coinciding with high levels of rainfall. It is widely recognized that the start of birds' breeding seasons is closely associated with food abundance, and usually, there is some synchrony of rearing young when suitable food for them is most abundant (Lack 1954, Marini and Durães 2001, Gomes et al. 2010). The Helmeted Manakin fits this pattern, as they feed their young mainly on fruits and some arthropods (LNK, pers. obs.), found abundantly during the rainy season in the Cerrado (Funch et al. 2002, Silva et al. 2011, Roque et al. 2013, Mendoza et al. 2017, Maruyama et al. 2019, Pires and de Melo 2020). This same pattern was described with other populations of the species (Marini 1992, Marçal and Lopes 2019a and Bruno et al. 2021), for its congener, the Araripe Manakin (Gaiotti et al. 2019), as well as for other Neotropical birds (Wikelski et al. 2003, Marini et al. 2012).

Unlike the onset of egg laying, the conclusion of Helmeted Manakin's nesting activity does not appear to follow the rainfall regime; we did not find any active nests during the last two months of the rainy season (i.e., March and April). Other Neotropical birds present a similar pattern (e.g., Spotted Antbird *Hylophylax naevioides*, Styrsky and Brawn 2011). A possibility is that females prioritize looking after demanding juveniles rather than initiating new nests. Furthermore, in the gallery forests of Cerrado, insect abundance peaks during the transition period from the end of the dry season to the start of the rainy season, followed by a progressive decline in the middle of the rainy season (Silva et al. 2011). Thus, the availability of food resources for nestlings may have a more considerable impact on the end of Helmeted Manakin nesting activity than the climate conditions.

The second wet season (2018-2019) was longer than the other seasons, followed by atypical nesting activity in the third breeding season (2019-2020), with a high number of nests initiated nearly a month before the wet season began in 2019 (Fig. 1). Possibly, breeding activity in that year was influenced by anomalous climatic events, such as the El Niño Southern Oscillation. A mild El Niño event occurred from October 2018 to May 2019, characterized by enhanced precipitation and temperature levels (Petrova et al. 2020). Previous studies detected similar climatic effects in central Brazil due to this phenomenon (Moreira de Araújo et al. 2012). This potentially has accelerated egg-laying, as reported for other Neotropical birds (Grant et al. 2000, Styrsky and Brawn 2011, Hidalgo Aranzamendi et al. 2019).

Females undertook all duties related to the nest, similar to other members of the Pipridae family. The same pattern was found in recent studies with other populations of the Helmeted Manakin, but the sample size was either reduced (12 active nests; Marçal and Lopes 2019a) or individuals were unbanded and lacked sex confirmation with molecular analysis (Bruno et al. 2021). The absence of male parental care is a prevalent characteristic of polygamous mating systems (Alcock 2016), reinforcing the hypothesis that the Helmeted Manakin may have a non-monogamous mating system (Marçal and Lopes 2019b). However, confirmation of the genetic mating system awaits molecular analyses.

Female nest attendance of about 65% during the incubation period was similar to that of other tropical forest passerines with female-only incubation (Tieleman et al. 2004, Ricklefs and Brawn 2013). The value is lower when compared with bi-parental incubators

(Dusky Antbird *Cercomacroides tyrannina*: 84.4%, Spotted Antbird *Hylophylax naevioides*: 73.4%; Ricklefs and Brawn 2013). Despite this, the proportion of nest attendance does not affect egg temperature (Tieleman et al. 2004), and the incubation period is still longer than that of temperate species (Chalfoun and Martin 2007). The strategy seems counter-intuitive considering that more extended incubation periods expose the clutch to greater time-dependent mortality from nest predators, especially in tropical regions with high nest predation rates. It is more likely that the association of prolonged incubation periods and parental attendance is linked to a strategy of higher-quality embryo development (Ricklefs and Brawn 2013, Ricklefs et al. 2017).

Microhabitat characteristics, such as the presence or proximity to bodies of water, are important factors affecting birds' nest site selection (Ocampo and Londoño 2014, Perrella et al. 2021). The behavior of building nests over or near bodies of water is reasonably typical among manakins (Snow 1962, Alvarez Alonso 2001, Christian 2001, Greeney and Gelis 2007, Hidalgo et al. 2008, Hazlehurst and Londoño 2012, Kirwan and Green 2012). For some, such as the Blue Manakin (*Chiroxiphia caudata*), nests are preferentially built on sites closer to streams (Zima et al. 2017). Although closely related to the *Chiroxiphia* genus (Leite et al. 2021), the Helmeted Manakins in our study site appeared not to show any preference for building nests near streams. In fact, in another population located away from the species distribution in the Cerrado biome, nests were mainly built far from bodies of water (Marçal and Lopes 2019a). Additionally, we found that proximity to bodies of water had no substantial effect on DSR. However, there was a slight trend of lower DSR values for nests farther away from streams than nests closer to streams during the incubation period. Furthermore, there was a significant correlation between predation and the distance of nests from streams.

A potential explanation relies on the particular landscape configuration in the Cerrado biome, where multiple discontinuous habitats naturally occur within short distances. Predation rates can vary within small areas due to differences in predator assemblages, both limited by barriers (Sieving 1992, Ocampo and Londoño 2014) or increased by proximity to other habitats – a phenomenon known as the “edge effect” (Gates and Gysel 1978, Batáry and Báldi 2004, Sosa and de Casenave 2017). Gallery forests are narrow strips of humid forest surrounded by savanna-like vegetation; thus, nests located farther from streams and bodies of water inside the forest are inevitably closer to the forest-savanna edge and potentially more susceptible to higher predation rates. Studies on natural nests predation in tropical forests show an overall lack of edge effect (Vetter et al. 2013, Newmark and Stanley, Marini 2017). However, the nature of the relation between nest predation and distance of nest to edge can vary considerably among species (Newmark and Stanley 2011), emphasizing caution when interpreting general patterns of nest survival based on single or several species.

Our results demonstrate that nest height may influence nest success, and the best models included nest height (see Tables 1 and 2). According to our results, although most nests were located ~ 2 m from the ground, higher nests experienced higher survival estimates, which contradicts the predominant idea that higher nests are more exposed to predators and have lower survival rates (Hatchwell et al. 1999, Colombelli-Negrel and Kleindorfer 2009, Ocampo and Londoño 2014, Sosa and de Casenave 2017, Guan et al. 2018). However, higher nests survived more than nests closer to the ground in areas where primates are the predominant nest predators (Kaisin et al. 2018). Capuchins are significant nest predators among primates (Rose 1994, Rose 1997), and in our study area, Bearded Capuchins (*S. libidinosus*) were responsible for nearly a third of the identified predation events. This species is primarily arboreal, and individuals were frequently encountered traveling through the forest canopy at ca. 3 m height (LNK, pers. obs.). Despite this, they are known to forage, move and play on the ground frequently (de A. Moura and Lee 2004, Wright et al. 2019), potentially increasing the encounter rate with bird nests located closer to the forest floor. Thus, Helmeted Manakin nests built at higher levels may be less visible to the primates inhabiting the forests in our study site.

Vertebrate predation is common among primates (Butynski 1982), and most capuchin species have been reported to predate birds occasionally (Freese and Oppenheimer 1981) and birds' nests (Fedigan 1990, Rose 1997, Tarwater 2008, Canale and Bernardo 2016, Cockle et al. 2016, Menezes and Marini 2017, Ribeiro-Silva et al. 2018). Here we provide the first documentation of the near-threatened Bearded Capuchin monkey (*S. libidinosus*) predating birds' eggs and nestlings (Fig. 4c), contributing to the scattered records and knowledge about the omnivorous diet of capuchin monkeys. Most predation in our study site occurred during the daytime, similarly to other studies (Libsch et al. 2008). However, we provide documentation of a night predation event by an opossum *Gracilinanus* sp., contributing to the few records of such events. These accounts highlight the importance of employing camera traps or other video technologies that enable remote monitoring of nocturnal activity at birds' nests, possibly increasing the detection of nocturnal nest predators, deemed rare.

Conclusion

This study provides novel and detailed data related to the Helmeted Manakin breeding biology, nest site selection, survival, and predation rates. Our results indicate that nest survival of the Helmeted Manakin is mainly affected by nest height and distance from watercourses within the gallery forest and is potentially explained by the predator assemblage in our study area. It remains to be investigated whether potential differences in habitat quality, such as availability of food resources, affect nest site selection in the species. Birds and mammals predated Helmeted Manakin nests, and primates were responsible for a third of the predation events. Furthermore, we confirm that Helmeted Manakin males do not participate in offspring care, similarly to its sister species, the Araripe Manakin, and other members of the Pipridae family. The confirmation of uniparental care in the Helmeted Manakin challenges a previous hypothesis of monogamy for the species, but this can only be confirmed with molecular paternity analysis.

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TABLES

Table 1. Results from models selection using Akaike information criteria for small sample sizes (AIC_C) for daily survival rate DSR (incubation and nestlings; *N* = 18 nests) for Helmeted Manakin nests in Brasília, Distrito Federal, Brazil. The co-variables nest height (height), distance from water (water), percentage of male activity (male), female visit rate (fvisit), and nest manipulation (manip) were included in the analysis. Scenarios ran with constant time (Tc), linear time (T linear), and quadratic time (Tq). Num. par. is the number of parameters used in each model.

Model	AIC_c	ΔAICc	AICc weight	Model likelihood	Num. par.	Deviation
{Tc + height}	98.398	0	0.1933	1	2	94.330
{Tc}	99.678	1.280	0.1019	0.527	1	97.655
{Tc + height + fvisit}	99.996	1.598	0.0869	0.450	3	93.859
{Tc + height + male}	100.445	2.047	0.0695	0.359	3	94.308
{Tc + height + manip}	100.450	2.052	0.0693	0.359	3	94.313
{Tc + height + water}	100.463	2.065	0.0688	0.356	3	94.326
{T linear + height}	100.464	2.066	0.0688	0.356	3	94.327
{Tc + stage + height}	100.467	2.069	0.0687	0.356	3	94.330
{Tc + fvisit}	100.562	2.163	0.0655	0.339	2	96.493

{T linear}	101.503	3.105	0.0409	0.212	2	97.435
{Tc + stage}	101.620	3.221	0.0386	0.200	2	97.551
{Tc + manip}	101.702	3.304	0.0370	0.192	2	97.634
{Tc + water}	101.707	3.309	0.0370	0.191	2	97.639
{Tc + height + water + fvisit}	102.089	3.691	0.0305	0.158	4	93.859
{Tc + water + fvisit}	102.630	4.232	0.0233	0.121	3	96.493

Table 2. Results from models selection using Akaike information criteria for small sample sizes (AIC_c) for the incubation period daily survival rate DSR (*N* = 15 nests) for the Helmeted Manakin nests in Brasília, Distrito Federal, Brazil. The co-variables nest height (height), distance from water (water) and presence of males (male) were included in the analysis. Scenarios ran with constant time (Tc), linear time (T linear), and quadratic time (Tq). Num. par. is the number of parameters used in each model.

Model	AIC_c	ΔAIC_c	AIC_c weight	Model likelihood	Num. par.	Deviation
{Tc + height}	55.369	0	0.2021	1	2	51.24
{Tc + height + water}	56.287	0.918	0.1278	0.632	3	50.03
{T linear + height + water}	56.800	1.430	0.0989	0.489	4	48.37
{T linear + height}	57.185	1.816	0.0815	0.403	3	50.93
{T quadratic + height}	57.610	2.241	0.0659	0.326	4	49.18
{Tc}	57.710	2.341	0.0627	0.310	1	55.67
{T quadratic + height + water}	57.794	2.424	0.0602	0.298	5	47.14
{T quadratic + height + water}	57.794	2.424	0.0602	0.298	5	47.14
{Tc + height + water + male}	57.999	2.630	0.0543	0.269	4	49.57

{Tc + water}	58.029	2.660	0.0535	0.265	2	53.90
{T linear + water}	58.604	3.235	0.0401	0.198	3	52.35
{T quadratic}	58.943	3.574	0.0339	0.168	3	52.69
{T linear}	59.514	4.144	0.0255	0.126	2	55.39
{Tc + male}	59.605	4.236	0.0243	0.120	2	55.48
{T linear + male}	61.533	6.164	0.0093	0.046	3	55.28

Table 3. Results from models selection using Akaike information criteria for small sample sizes (AIC_c) for the nestling period daily survival rate DSR (*N* = 18 nests) for the Helmeted Manakin nests in Brasília, Distrito Federal, Brazil. The co-variables nest height (height), distance from water (water) and presence of males (male) were included in the analysis. Scenarios ran with constant time (Tc), linear time (T linear), and quadratic time (Tq). Num. par. is the number of parameters used in each model.

Model	AIC_c	ΔAIC_c	AIC_c weight	Model likelihood	Num. par.	Deviation
{Tc}	65.848	0	0.213	1	1	63.81
{Tc + height}	66.362	0.513	0.165	0.774	2	62.26
{Tc + male}	67.599	1.751	0.089	0.417	2	63.50
{T linear}	67.740	1.892	0.083	0.388	2	63.64
{Tc + water}	67.915	2.067	0.076	0.356	2	63.81
{T linear + height}	68.254	2.406	0.064	0.300	3	62.05
{Tc + height + male}	68.399	2.551	0.060	0.279	3	62.19
{Tc + height + water}	68.465	2.617	0.058	0.270	3	62.26
{T quadratic}	69.160	3.312	0.041	0.191	3	62.96
{T linear + male}	69.669	3.821	0.032	0.148	3	63.46
{Tc + water + male}	69.672	3.824	0.032	0.148	3	63.47
{T linear + water}	69.798	3.950	0.030	0.139	3	63.59

{T quadratic + height}	70.277	4.428	0.023	0.109	4	61.93
{Tc + height + water + male}	70.535	4.687	0.020	0.096	4	62.19
{T quadratic + male}	71.145	5.296	0.015	0.071	4	62.80

Figures

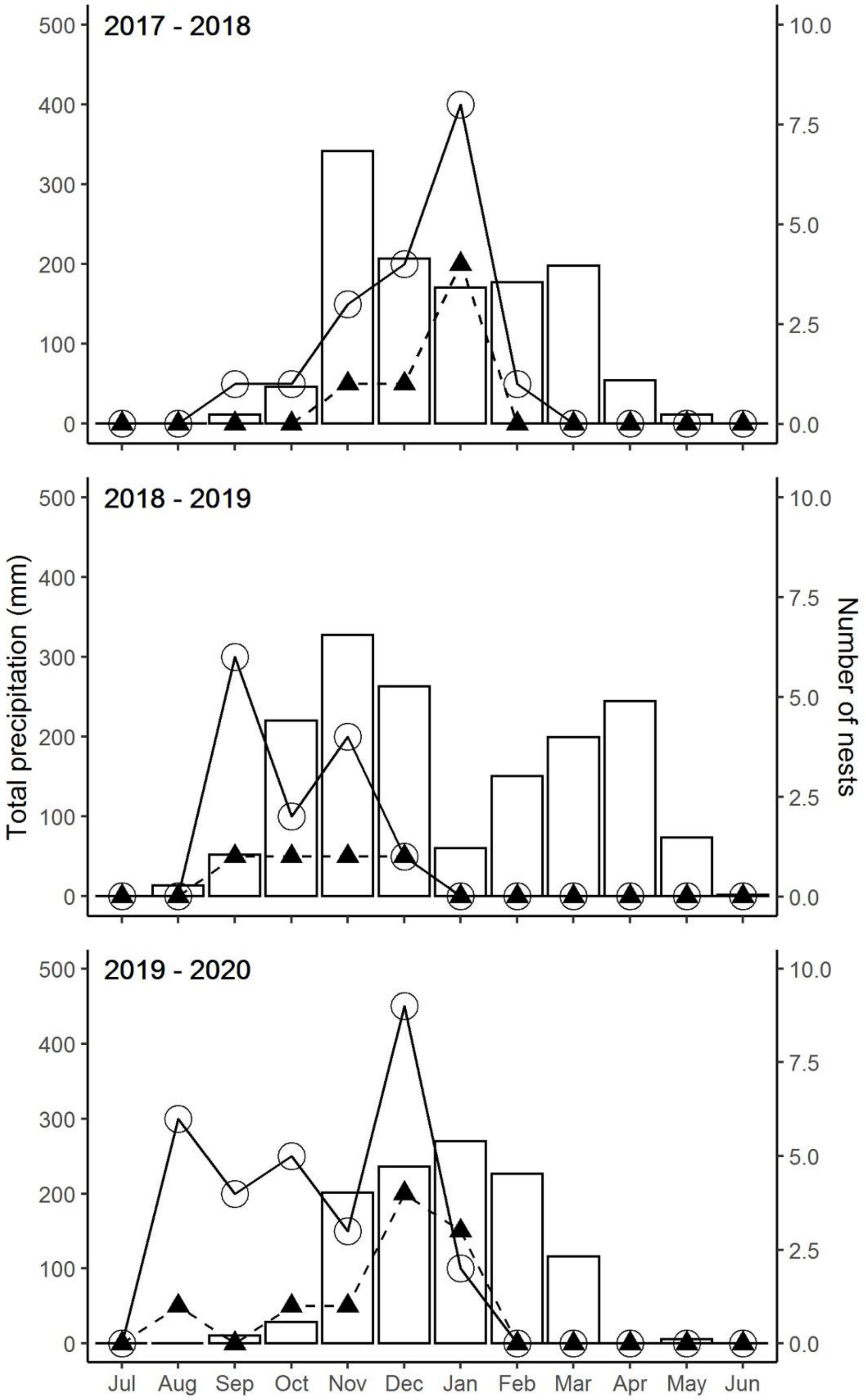

Figure 1 Seasonal variation in number of actives nests (circles) and nests with nestlings (triangles) of the Helmeted Manakin found across three breeding seasons compared to rainfall regime (bars) at the Fazenda Água Limpa, Brasília, Distrito Federal, Brazil.

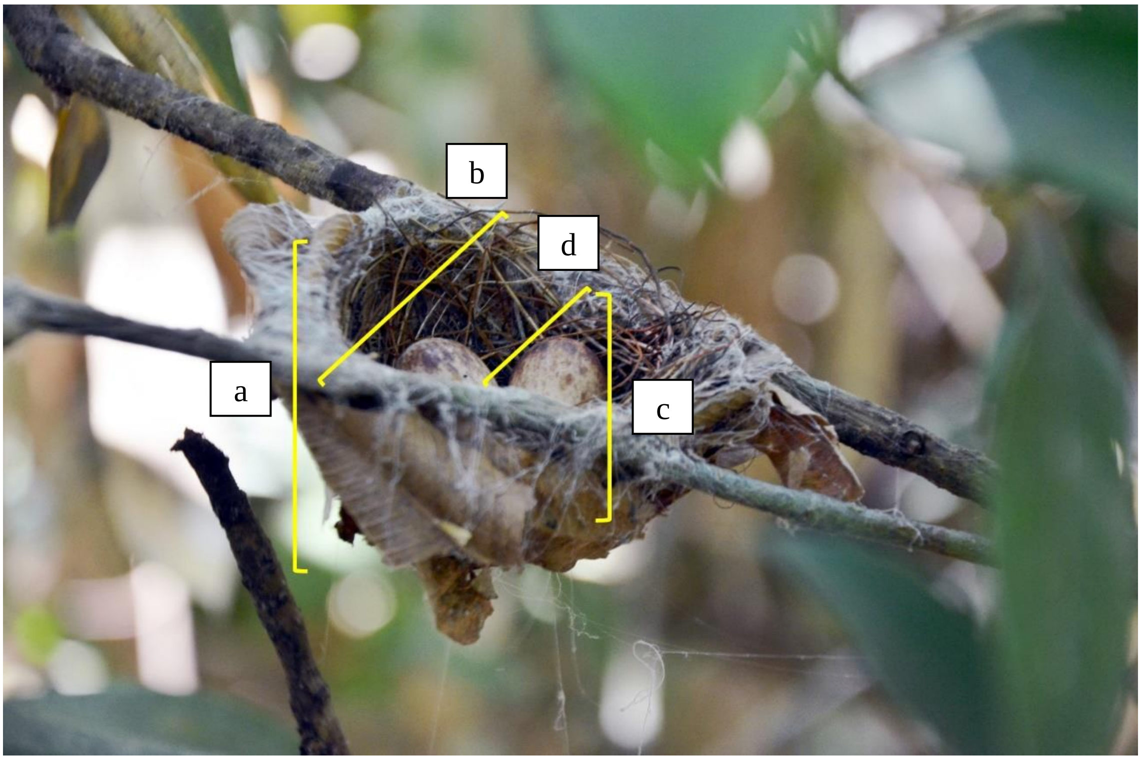

Figure 2 We took four measurements of the nest following Hansell's (2000): nest depth (a), nest diameter (b), cup depth (c), and cup diameter (d). Photo: Lia N. Kajiki.

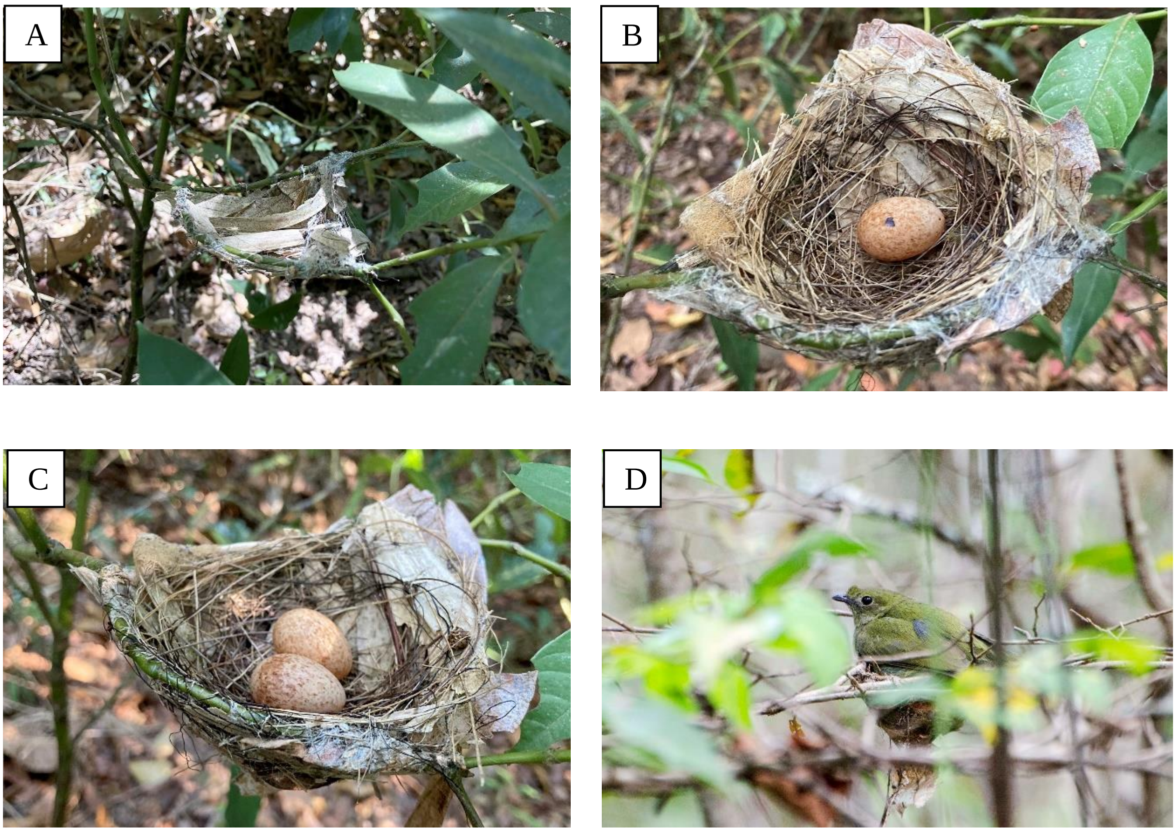

Figure 3 The construction stages of a Helmeted Manakin nest found on 10 September 2020. Initially, the nest presents spider silk web and a few dry leaves (a), after 10 days the nest already has a considerable amount of dry leaves and spider web attached to the forked branches and the first egg is laid (b). Finally, after two days the second egg is laid (c). Females conduct all the parental care related to the offspring (d). Photos: Lia N. Kajiki.


A
Bushnell
64°F17°C
12-05-2018 15:22:06
B
Bushnell
09-11-2018 11:17:05
C
Bushnell
59°F15°C
08-20-2019 14:15:54

Figure 4 Predation was the main cause of Helmed Manakin nest failure. A potential nest predator is the Barred Forest-Falcon (*Micrastur ruficollis*) recorded visiting an empty nest (a). The Yellow-ridged Channel-billed Toucan (*Ramphastos vitellinus pintoi*) (b) and Bearded Capuchin monkeys (*Sapajus libidinosus*) (c) were common nest predators and we recorded a night predation event by an opossum (*Gracilinanus* sp.) (d). Photos: Lia N. Kajiki.

CAPÍTULO 2

The false acrobat: territoriality and solitary display in the Helmeted Manakin (*Antilophia galeata*)

Uma versão deste capítulo foi submetido para publicação em *Emu – Austral Ornithology* e está redigido de acordo com as normas de formatação da revista.

The false acrobat: territoriality and solitary display in the Helmeted Manakin (*Antilophia galeata*)

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Abstract

Neotropical manakins in the Family Pipridae typically exhibit polygynous lekking systems where males do not provide paternal care or elaborate courtship displays. Despite familial patterns, some historical field observations have suggested that the Helmeted Manakin (*Antilophia galeata*) has evolved to be distinct: territoriality rather than lekking, and a socially monogamous mating system. Recent investigations show that the species' only congener, the Araripe Manakin (*Antilophia bokermanni*), likewise exhibits an atypical mating system for the Family Pipridae, being polygynous, defending exclusive territories with nesting sites, but unable to monopolize paternity within their territories. Our objective in this study was to verify whether territorial behaviour also occurs in the Helmeted Manakin and provide a preliminary assessment of this species' hypothesized social monogamy. We demonstrate that males in this species indeed hold individual territories with nesting sites but do not provide parental care, similar to its congener. Our results also suggest that the Helmeted Manakin has a polygamous mating system. Finally, we provide a description of the male display.

Keywords: aggressiveness; breeding behaviour; home range; lekking; display; experiment

Introduction

Mating system diversity has mainly been investigated within an ecology-based framework, grounded mainly upon the capacity of one sex, usually males, to monopolize territories, resources, and mates, which is directly related to the degree of parental care required for successfully rearing offspring (Emlen and Oring 1977). In resource defence polygyny or monogamy, males may exclude other males from resource-rich sites, consequently attracting and mating with one or more females. Such males typically achieve higher reproductive success compared to those either lacking or defending lower-quality territories (Emlen and Oring 1977).

However, resources can also be abundant and widely distributed, which would prevent their control by a single individual. In such a context, males may aggregate and display in arenas devoid of resources, to where females are attracted primarily for mating. Thus, these arenas contain no other resources than the males themselves. This form of aggregation is commonly referred to as a lek (Beehler 1987; Höglund and Alatalo 2014).

The lek system has been described for various vertebrate and invertebrate species, but it is best known in birds (Höglund and Alatalo 2014). The lek mating system prevails in manakins (family Pipridae), a family of Neotropical birds known for their striking ornaments and complex courtship displays (Lovette and Fitzpatrick 2016). The lekking behaviour is hypothesized to be the ancestral trait in the Pipridae family and potentially facilitated the evolution of courtship displays and sexual dimorphism among most manakin species (Prum 1990; Prum 1994). Some manakin species have been thoroughly investigated, especially the *Chiroxiphia* (Duval 2007; Ribeiro *et al.* 2019) and *Manacus* genera (Cestari and Pizo 2012; Fusani and Schlinger 2012), which have very stereotyped and complex courtship displays. A few species in the family do not exhibit the typical lekking configuration, and despite their potential to shed light on the evolution of mating systems and sexual selection among manakins, they have been relatively overlooked.

The Helmeted Manakin (*Antilophia galeata*) is one such species. Despite its striking sexual dimorphism, it has long been considered to have deviated from the family-wide lekking pattern and evolved towards a possible social monogamy, given observations of pair-bonding behaviour and individual territories (Marini 1992b; Marini and Cavalcanti 1992). The species' divergence from the typical lekking system was suggested as resulting from selection associated with an evolutionary preference for a unique habitat among piprids, that of humid gallery forests within the savanna Cerrado biome of central Brazil (Marini and Cavalcanti 1992; Prum 1994). With 15-20 m high canopies, gallery forests line the watercourses and are surrounded by an open savanna-like vegetation matrix. They represent only 5% of the Cerrado biome and usually do not extend more than 50 m from the watercourses. Despite their limited areas, these forests contribute to most of the biodiversity in the Cerrado, harbouring many unique and rare species (Ratter *et al.* 1997). Gallery forest habitat is much more restricted compared to the typical equatorial forests occupied by most manakins, and essential resources such as nesting sites and fruiting trees may thus have patchy distributions and limited availability.

Recently, a study with a population of Helmeted Manakin located far from the Cerrado biome centre challenged the proposed monogamy and absence of lekking behaviour in the species based on eight males' "breeding home ranges" that overlapped extensively (Marçal and Lopes 2019b). The authors concluded that this overlap demonstrated a lack of territories. However, the data analysed was based solely on locations of singing perches, and home ranges were potentially underestimated, indicating that smaller exclusive core areas (i.e., territories) could have been overlooked. Furthermore, home ranges were compared to display sites, and an apparent lack of concentration of resources in these areas was associated with non-resourceful arenas. However, data on resources that could be important for females was not systematically collected. Thus, whether males defend resourceful territories, an essential aspect of males' breeding behaviour associated with mating systems, remains largely untested.

Another recent study conducted with the Helmeted Manakin's only congener, the Araripe Manakin (*Antilophia bokermanni*), found genetic evidence that the species is not monogamous as previously thought for the *Antilophia* genus but instead exhibits an atypical mating system among manakins. As with other manakin species, Araripe Manakin males are polygynous and do not perform parental care. However, unlike most other manakin species, they defend individual territories where females nest. Curiously, males sire only 7% of offspring produced in their territories. This puzzling array of behavioural traits did not allow a straightforward description of the species' mating system (Gaiotti *et al.* 2020).

The two *Antilophia* species, although strikingly different in plumage colours, are very similar in morphology, behaviour, and genetic profiles (Marini 1992a; Marini 1992b; Coelho and Silva 1998; Gaiotti *et al.* 2017; Luna *et al.* 2017). The divergence between the two populations is estimated to have occurred 24,000 – 1,500 years ago (Luna *et al.* 2017). However, while the entire population of the Araripe Manakin occupies a limited area of approximately 30 km^2 of humid forest amid a xeric area (Coelho and Silva 1998), the Helmeted Manakin is widely distributed across its range, within gallery forests in the Cerrado biome (Snow and de Juana 2020). Thus, the two species differ in terms of habitat availability, potentially affecting their population dynamics in different ways, presumably affecting territorial size and breeding behaviour (Schoener 1968).

Our aim in this study was to investigate aspects of the Helmeted Manakin's breeding behaviour with increased precision than was achieved previously. Foremost among our interests was the issue of whether males exhibit some form of resource-based territoriality, an essential attribute of social monogamy. Although the lekking mating system prevails in the Pipridae family, the *Antilophia* species present a unique evolutionary history and behaviours that do not appear to fit the traditional lek. We used an experimental approach combining the display of a taxidermied decoy and a playback protocol to investigate males' behavior. Given the phylogenetic proximity of the two *Antilophia* species, we explored the possibility that the Helmeted Manakin's general breeding behaviour is analogous to that of the Araripe Manakin. Thus, we considered it highly probable that Helmeted Manakin males defend individual territories with nesting sites. Here, we define territory as a defended and exclusive area where breeding typically occurs. Furthermore, based on video records of parental care and observations of male behaviour, we sought to provide preliminary information to evaluate assumptions (e.g., males do not participate in parental care) relative to the proposed social monogamy in the species.

Methods

Study site and species

This study was conducted within the 'Fazenda Água Limpa' (15° 56' 56.1"S, 47° 56' 02.6"W), an experimental farm that belongs to the University of Brasília, Brasília, Distrito Federal, Brazil. The study site is Cerrado *sensu stricto* vegetation, at 1,100 m a.s.l. The local climate has two well-defined seasons, one that is rainy (from October to April) and another that is dry (from May to September) (Almeida *et al*. 2014), with average annual precipitation of 1,450 mm (Parron *et al.* 1998). Our fieldwork was conducted within gallery forests that covered an area of approximately 120 ha.

The Helmeted Manakin is a sexually dimorphic and mainly frugivorous, large (≈ 21 g) manakin (Kirwan and Green 2012) that ranges from central Brazil to northeastern Bolivia and Paraguay (Snow and de Juana 2020). Individuals are usually observed singly or in pairs, mainly occupying gallery forest woody vegetation’s lower to middle strata (Marini 1992a; Kirwan and Green 2012). Males sing throughout the year, but they peak in vocalizations before and during the breeding season in central Brazil, from around August to December (Marini 1992b). The Helmeted Manakin is strikingly dichromatic: males have a bright crimson frontal crest and mantle, and the rest of the plumage is black; females have a less pronounced frontal crest and body plumage is olive overall (Kirwan and Green 2012). Juveniles closely resemble females and probably acquire adult plumage in their third year of life (Snow and de Juana 2020). Therefore, sexing of individuals in green plumage cannot be visually determined in the field, and molecular sexing is needed for confirmation purposes.

Nests are open-cup structures suspended between two twigs (Figure 1) and usually placed 0.5 – 10.0 m above the ground. They are made with small rootlets, lined with fungal hyphae, spider silk, and leaves to camouflage the outer side (Marini 1992b; Kirwan and Green 2012; Marçal and Lopes 2019a; Bruno *et al.* 2021; Capítulo 1). Females lay two eggs, beige with dark stripes and spots concentrated at the larger end (Kirwan and Green 2012) (Figure 1). Eggs hatch after an average of 18.5 days of incubation, and juveniles fledge after 18.3 ± 1.15 days (Marçal and Lopes 2019a). A past study conducted in our field area, but with a reduced number of observations, suggested that males hold individual territories and may be socially monogamous (Marini and Cavalcanti 1992). Additionally, territoriality was documented for the Helmeted Manakin’s sole sister species, the Araripe Manakin (Gaiotti *et al.* 2020). However, a recent study in another region of Brazil concluded that the Helmeted Manakin does not hold territories (Marçal and Lopes 2019b).

General field methods

We mist-netted and banded Helmeted Manakins in the study area from July 2017 to September 2020. We used a numbered aluminium band (provided by the National Centre of Research and Bird Conservation - CEMAVE, ICMBio, Brazil) and a unique combination of three coloured plastic bands for each individual (research permits SISBIO 58094-3 and CEMAVE 4221). Whenever possible, we captured unbanded nesting females by setting up a mist-net in the area around the nest. Blood samples (~ 50 µl) were collected through brachial venipuncture and stored in 99% ethanol at 4°C. Molecular sexing was conducted following Griffiths *et al.* (1998) using PCR (polymerase chain reaction) analyses with P2/P8 primers (Griffiths *et al.* 1998; Çakmak *et al*. 2017).

Between August 2018 and January 2020, we opportunistically monitored marked birds with binoculars and collected individuals' locations in temporal bouts of approximately 30 min, during which the male was followed via direct observation. Every new perch location was recorded with a GPS (®Garmin eTrex 10) if the distance from the previous perch was at least 10 m. The interval between bout sessions for a marked individual occurred within approximately two weeks to avoid autocorrelation.

At the beginning of the 2018 – 2019 breeding season, we identified possible territories of banded adult males. This first assessment was based upon records where we had georeferenced the perching locations of the same individuals in the same sites across the 2017 – 2018 breeding season. We recorded at least five locations for each male, thus allowing us to estimate a possible territory for each individual using the minimum convex polygon (MCP territories hereafter) method described by Mohr (1947). Using these initial estimates, we then conducted territorial defence experiments (see below) to verify whether areas where males were recorded repeatedly actually coincided with areas they would defend. Subsequently, using data from the territorial defence experiments and the monitoring of marked birds, we used the Kernel Density Estimation technique to determine marked males' home ranges and territories (see details below).

Territorial defence experiments

For the experiments, which took place between October 2018 and April 2019, we used a taxidermied decoy of an adult male Helmeted Manakin in mature plumage. We conducted from four to nine experiments in each of the eight MCP territories, during which we displayed the decoy at the height of 2 m associated with a 10 min song playback protocol. The playback protocol consisted of repetitions of a 1 min main song (the distinctive and rollicking song described by Ridgely and Tudor 2009; downloaded from the Xeno-canto database) of the Helmeted Manakin followed by an interval of 30 s of silence. We conducted the experiments consistently at three locations relative to the potential territory: (1) central point of potential territory; (2) closest point to the prior point where the gallery forest bordered the savanna, whether or not that was within the putative territory; and (3) an intermediate location between the two points described above. We conducted only one experiment at each potential territory per day to decrease the likelihood that males would become familiarized with the playback, which could interfere with their responsiveness. At least two observers took notes simultaneously, describing the approach of any banded Helmeted Manakins relative to the decoy and, whenever possible, video-recording interactions until the individuals left. Approaches were considered whenever an individual not observed before the experiment flew into the area within a 5 m radius from the decoy. We considered cases when males came within 2 m or less of the decoy or performed agonistic behaviour (e.g., flying close over the decoy) as defensive behaviour.

Home range and territory estimates

We used a Kernel Density Estimator technique (KDE) (Laver and Kelly 2008) to estimate home ranges and territory areas (hereafter "kernel-estimated" or simply "estimated territory") of the Helmeted Manakin, based upon the defensive behaviour data collected in the field experiments and the opportunistic observations of the eight focal males. The experiments generated a series of georeferenced points for each focal individual where the male was observed at less than 2 m from the introduced decoy. These points and the ones georeferenced during opportunistic monitoring of focal males were analysed together with KDE. The KDE method estimates the Utilization Distribution (UD hereafter), which is the bivariate function that provides the probability density that an animal is found at a certain point according to its geographical coordinates (Worton 1995). We used an adaptive kernel density estimator (local bandwidths) with a bivariate normal kernel function (Worton 1995). We adapted the rule-based *ad hoc* bandwidth approach suggested by Kie (2013) to obtain the smoothing parameter for each individual. The rule-based *ad hoc* bandwidth outperforms reference and least-squares cross-validation (Kie 2013), two of the most commonly used bandwidths in home range studies. We sequentially reduced the reference bandwidth (h_{ref}) in 0.10 increments (0.9 h_{ref}, 0.8 h_{ref}, 0.7 h_{ref},… 0.1 h_{ref}) to the smallest increment that resulted in a contiguous polygon. When fragmented estimates were generated with reference bandwidth, we chose this parameter and did not reduce it.

The kernel UD for each individual was used to estimate and delineate the intensity of use across each male's home range. The kernel UD was plotted with sequential isopleths that connected areas of equal use (as illustrated in Figure 2). The likelihood of encountering a male in a particular area is represented by isopleth values. Home range boundaries were set at the 95th density isopleth. We visually inspected UDs to establish territories' boundaries at the minimum isopleth that 1) included all of the locations in which there was a response to the territorial defence experiments; 2) excluded more than half of the locations in which there was no response to the territorial defence experiment; and 3) resulted in a contiguous area. We visually assessed the graphs in each reduction step. We prioritized the first condition because we assumed the localities that evoked a response from the Helmeted Manakins represent areas they defend. Therefore, these points should be included as part of the territories.

We calculated the percentage of the home range that is covered by the defended territory (% home range), percentage of total locations within the territory (% use), and a relative measure of the intensity of use (% use / % home range) as described by Samuel *et al*. (1985). Furthermore, we assessed site fidelity and home range asymptotes for each individual. We included all georeferenced points from experiments and monitoring to evaluate whether males exhibited fidelity to a given area across the sampling period and to check whether the number of total locations used in the analyses was adequate for estimating home range (Swihart and Slade 1997).

We used the packages 'adehabitatHR' (Calenge 2006) for estimating home range and territory, and 'rhr' to assess site fidelity and home range asymptotes (Signer and Balkenhol 2015) on RStudio 1.4.1103 (RStudio, Inc., Boston, MA, USA) running R version 3.6.3.

Nest monitoring

Between August and January of three breeding seasons (2017 – 2020), we searched for

Helmeted Manakin nests within the study area. In the first breeding season, we searched for nests by randomly walking through the full extension of the gallery forest and wading across streams while searching visually in the lower strata of the vegetation. In the following breeding seasons, we continued searching the woodlands randomly but searched more intensely in those areas where nests had been found in previous years. Once found, the locations of nests were georeferenced with a GPS and monitored every three days. We video-recorded nests with camera traps (®Bushnell Trophy Cam HD) installed 1 – 2 m from the nest. Nests were considered active when found with one or two eggs or nestlings, with building activity, or when camera traps recorded parental activity at the nest. Nests were considered inactive when found empty and without any parental activity observed or recorded by camera traps ten days after being found. Video recordings took place during nest building, incubation, and nestling stages.

Results

We captured and banded 254 individuals, of which 138 were males, 113 were females, and three were green individuals that could not be sexed with molecular analysis. We focused on eight possible individual territories in our study site, which in our preliminary estimates with MCP ranged from 0.01 to 0.11 ha (Table 1). Males in definitive plumage appeared to be occupying these MCP territories. Although half of these individuals exhibited site fidelity, overall males' locations overlapped across small areas in each territory estimated with Kernel, suggesting fidelity to the area during the sampling period (Figure 3). Furthermore, none of the males moved their territories during the sampling period. None of the home ranges approached an asymptote, indicating that the observed home ranges were underestimated or influenced by possible seasonal movements.

Territorial defence experiments

In 55 experiments conducted across the eight MCP territories, males approached the decoy 20 times (36.4% of all experiments; Table 2). Interactions varied greatly in duration and intensity. Individuals took 38 to 460 s to respond to the playback-decoy events, and average interaction with the decoy lasted 398 s. Typically, birds first responded by vocalizing the main distinctive and rollicking song (e.g., 'whip-dip, whih-deh-deh-dédidip' described in Ridgely and Tudor 2009) and then silently approached the decoy. Occasionally, males also produced the 'wreee?' call near the decoy. Agonistic responses included constant observation of the decoy by the approaching bird while moving closer to the decoy and finally perching or moving quickly around the decoy within two meters. In 45% of experiments with a response, males attempted to attack the decoy by flying very close over it or hovering next to it (see Supplementary Video S1). Despite close flights, males never made physical contact with the decoy. In four territories, individuals other than the resident male (both banded and unbanded) in several moulting stages (immature males), as well as green ones (males or females), also responded to the playback experiments by approaching the area but did not come close to the decoy. Thus, data from the territorial defence experiments and the monitoring of marked birds indicate that males indeed occupy and defend individual territories.

Display description

During the experiments and monitoring of adults, we observed that most males were within auditory, but not visual range, of each other and were intolerant of the presence of other males in adult plumage. Some individuals frequently moved within their territories, usually perching on the same trees and following the same order. We observed some males performing two stereotyped movements while completing the circuit within the territory: 1) horizontal posture while perched, in which the male stretches the body horizontally and leans forward, with wings folded against the body, flicking them rapidly with short movements for 2-3 times, and red crest pointed forward; and 2) upward posture while perched, in which the male stretches the neck and body upwards (see Supplementary Video S2).

In the territorial defence experiments conducted in territories 1, 4, and 7, the adult male did not attack the decoy. Instead, the male initiated a fast circular chase with other individuals high in the canopy, eventually getting close to the ground (observations by LNK). These chases often occurred at the borders of the male's territory. Females were never observed during these circular chases among males.

Home range and territory

Most of the area of each male's MCP territory was included in the kernel-estimated territory area (mean = 78.80%, range = 11.98 – 100.00%) (Table 1). Kernel density estimates of home range size for the Helmeted Manakin varied considerably (mean ± SE, 5.70 ± 9.60 ha; range = 0.27 – 32.22 ha, untransformed values; Table 1). Kernel-estimated territory areas ranged from 0.12 to 2.29 ha (mean ± SE, 0.85 ± 0.77 ha, untransformed values; Table 1) and represented relatively small parts of the home range (mean = 29.60%, range = 7.10 – 53.87%; Table 3). Territories were intensely used as shown by the relatively large values of intensity of use (mean = 4.10; range = 1.86 – 9.86; Table 3) and the percentage of total locations within territories (mean = 89.22%, range = 66.67 – 100%; Table 3).

Nest monitoring

Of 221 nests found during the study, 37 (16.74%) were located within the boundaries of the eight kernel-estimated territories, and nearly a third of these (N = 10) were active (Figure 4). Active nests found within territories investigated here represent nearly a fourth of total active nests found across the entire study area (N = 46), which may have occurred within other male territories that we did not map. The kernel-estimated territories usually had at least one active nest within them, but none of the territories had simultaneously active nests. Two of the eight territories lacked any active nests within their limits (Table 3) (Figure 4). Although the vegetation is similar in the entire area where nest searching was conducted, we did not find nests in some extensive parts of the forest with many potential support plants for nests.

We video-recorded 39 nests for a total of 894 min, which included activities of nest building (N = 8 nests), clutch incubation (N = 14 nests), and brooding of nestlings (N = 22 nests). Only females were recorded building the nest, incubating, and performing parental care duties, such as cleaning the nest, brooding and feeding nestlings, and remaining vigilant near the nest. Furthermore, females actively defended nests against intruders, such as a White-necked Thrush (*Turdus albicollis*) and a Swallow Tanager (*Tersina viridis*).

Discussion

Our objectives in this study were two-fold: (1) to examine the assumption that the Helmeted Manakin is a territorial species; and (2) that they deviate from the typical manakin polygyny and are monogamous (Prum 1990; Kirwan and Green 2012). The sparse field observations of over three decades ago (Marini 1992b; Marini and Cavalcanti 1992) described possible pair bonding in a small sample of eight individuals in addition to the occurrence of four females nesting within what appeared to be male territories. These field observations provided the basis for speculation that the species could have evolved a non-polygamous mating system, which would be a rarity among manakins (Marini and Cavalcanti 1992; Prum 1994; Kirwan and Green 2012). Here we provide results based on an experimental study suggesting that Helmeted Manakin males hold individual territories, contrary to what was found by Marçal and Lopes (2019b), and active nests are located within or at the boundaries of these territories. Our observations provide insights into the species' probable display and yield further information about its mating system. We also investigated parental care with video recordings, which show no biparental care of nestlings, a pattern typically associated with polygamous avian breeding.

The home range size estimates for the Helmeted Manakin males in our study (mean ± SE, 5.70 ± 9.60 ha) were nearly three times smaller than estimates from a recent report (mean ± SE, 16.48 ± 9.03 ha, N = 8; Marçal and Lopes 2019b). Although home ranges did not approach an asymptote in our study, indicating that the values are underestimated, home range asymptote is not often found in empirical studies, even with large samples (Gautestad and Mysterud 1995). Estimates with KDE can be largely affected by the smoothing parameter choice and number of samples (Laver and Kelly 2008; Kie 2013). Reference bandwidths, which are extensively adopted, may overestimate home ranges if data do not originate from unimodal distributions (Gitzen and Millspaugh 2003; Signer and Balkenhol 2015). Marçal and Lopes (2019b) used this smoothing parameter for all their estimates without a clear justification, and these methodological choices may have produced results that differ markedly from ours.

Home ranges of the Helmeted Manakin found by Marçal and Lopes (2019b) overlapped extensively, leading to their conclusion that the species does not hold individual territories during the breeding season and, thus, is more likely to be polygamous. These results have to be interpreted with caution because the authors did not conduct genetic parentage analysis to evaluate the mating system and estimated territories of eight individuals using the location of singing perches without playback experiments for confirmation purposes. It is essential to highlight that the concepts of home range and territory are often confused and could be interpreted more or less loosely. Here, we use Burt's (1943) definition of home range as the 'area traversed by an individual in its normal activity of food gathering, mating and caring for young'. We define territory as a defended (Noble 1939; Nice 1941) and an exclusive area (Pitelka 1959) where breeding activities are typically concentrated (Brown 1964). Thus, there may be considerable overlap between home ranges of different males, and an individual's territory could be contained within another's home range, as long as intrusion within the territory was not tolerated. The concept of territoriality may also comprise different behavioural contexts, and the term has been applied to lekking arenas, which are non-exclusive areas where no nesting occurs. There are several types of behaviours used by animals to defend a territory, such as singing (Hasselquist and Bensch 2008), but none is more straightforward than chasing off intruders (Alcock 2016). The territorial defence experiments we conducted in our study are essential to discriminate between home range and territory boundaries. Thus, our experiments allow us to confirm that the Helmeted Manakin does indeed exhibit male individual territoriality, that these smaller territories are overall intensely used and encompassed within their more extensive home ranges, and that females may nest within territorial boundaries.

Nonetheless, these different patterns in the territorial behaviour of the Helmeted Manakin found in the Marçal and Lopes (2019b) and our study may reflect a variation between populations. Territorial behaviour could vary as a function of site quality, moving ability, intruder pressure, density, and defence costs, affecting territory sizes, shapes, and placement (Eason 1992, Adams 2001, Formica et al. 2004). Marçal and Lopes (2019b) sampled a fraction of a large rectangular 180 ha fragment of semi-deciduous secondary forest located at the outer limit of the Cerrado biome range. In contrast, our study site of ~ 120 ha is distributed over two main strips of a forest with fewer than 250 m of width (Figure 4) located in the core of the Cerrado biome.

Few manakin species experience such extreme spatial limitations imposed by suitable habitat availability as that of the Helmeted Manakin. Other species in a similar condition include the critically endangered Araripe Manakin and a few species (e.g., Black Manakin *Xenopipo atronitens* and Yellow-crested Manakin *Heterocercus flavivertex*) restricted to amazonian non-forested habitats surrounded by a forested matrix (Kirwan and Green 2012; Capurucho *et al*. 2013). The spatial distribution of leks for some manakin species follows a highly regular pattern, suggesting that some factors, such as reduction of competition (Durães *et al*. 2008) and lekking habitat suitability (Loiselle *et al*. 2007), affect lek settlement. Thus, it is possible that the shape of the forested area, which stretches out like long corridors along the watercourses, establishes a spatial limitation for the distribution and sizes of Helmeted Manakin's territories. Males are much more sedentary than females in manakin species. These forested corridors may also limit the rate of male encounters, favoring the establishment of smaller and defendable territories in our study site. Future studies investigating Helmeted Manakin's territorial behavior would benefit from considering factors related to the spatial configuration that could directly affect territory settlement and size, such as habitat suitability, the existence of barriers, the shape of the forest fragment, number and distance to neighbouring territorial males.

Territory size for the Helmeted Manakin (mean = 0.85 ± 0.77 ha) is similar to the ~ 1 ha estimated by Marini and Cavalcanti (1992) and approximately seven times larger than the territory size estimated for the Araripe Manakin (mean = 0.12 ± 0.01 ha; Gaiotti *et al*. 2020). In their study, the authors estimated territory areas using the popular MCP, which is widely used for home range estimates, but has several limitations (e.g., highly affected by sample size and locations on the periphery of a boundary) (Worton 1987; Laver and Kelly 2008). Our results suggest that the potential territories for the Helmeted Manakin estimated with MCP (mean = 0.07 ± 0.04 ha; range = 0.01 – 0.11 ha) were conservative estimates of the probably more accurate territory sizes estimated with the kernel method. Thus, the territory sizes for the Araripe Manakin may be underestimated. However, another possibility is that the extreme limitations of the habitat occupied by that species (Gaiotti *et al.* 2020) has led to very small territories.

Our results support Marini and Cavalcanti's (1992) suggestion that Helmeted Manakin males defend individual territories. However, we speculate that these territories may function as solitary arenas instead of indicating a resourceful territory associated with monogamous mating systems. Contrary to most lekking manakin species with complex courtship displays, the Helmeted Manakin seems to have a simple male display in the form of frequent vocalizations on a few singing perches associated with a few unremarkable postures. Previous studies also reported male-male chasing behaviour in the Helmeted Manakin (Sick 1967, Marini 1992b, Marçal and Lopes 2019b). These energetic chases among males, considered part of their display repertoire, have been reported in other manakin species, such as the Black Manakin (*Xenopipo atronitens*; Sick 1967), Yellow-crowned Manakin (*Heterocercus flavivertex*; Prum *et al*. 1996), Orange-crowned Manakin (*Heterocercus aurantiivertex*; Alonso 2000), Band-tailed Manakin (*Pipra fasciicauda*; Robbins 1983), and White-bibbed Manakin (*Corapipo leucorrhoa*; Rosselli *et al.* 2002). We noticed that the chasing behaviour did not occur regularly or in the same locations, so we find it more likely that chases are responses to territorial intrusion attempts rather than a part of the courtship repertoire. We did not observe physical attacks among males, but Marini (1992) occasionally observed a male pecking the back of the chased bird, and Gaiotti (2016) described four Araripe Manakin physical disputes on the ground.

An unspectacular male display is also a characteristic of the Black Manakin, which shares features with the Helmeted Manakin, such as sexual dimorphism and restriction to the limited gallery forests in the non-forested areas of the Amazon biome (Kirwan and Green 2012; Capurucho *et al*. 2013). The loss of complex courtship displays in both species could be associated with the relaxation of sexual selection pressure on males, parallel to the loss of sexual plumage dimorphism in unornamented manakins. In their study, Ribeiro *et al*. (2015) found that Green Manakin (*Xenopipo holochlora*) males hold solitary territories but do not form classical or exploded leks; they suggested that it is unlikely that females nest inside males territories. Despite the behavioural similarities between the Helmeted Manakin and a few others that share these attributes, our conjectures regarding the function of these displays must be interpreted with caution, as they were not investigated systematically.

The spatial configuration of the male territories, in which males are located a few hundred meters away and in hearing range from each other, resembles exploded (Bradbury 1981; Beehler and Foster 1988) and solitary (Prum 1994) leks found in some manakins, such as the Orange-crowned Manakin (*Heterocercus aurantiivertex*) (Alonso 2000). Helmeted Manakin males' aggregation appears to fit in a continuum between the two types of leks. Males are clustered in a specific and limited habitat where they display but are loosely grouped across the gallery forest. At the same time, males display by singing in advertising perches in solitary display arenas.

The aggressive behaviour in Helmeted Manakin males could be related to a female preference for fighting ability or demonstrated dominance. In this scenario, males' aggressiveness towards intruders in their territories could 1) be a response to potential display disruption by other males and 2) demonstrate dominance to visiting females. Because disruptions during courtship displays in cooperative and non-cooperative lekking birds can decrease male mating success (Trail 1985, Saether et al. 1999), it can be considered a reasonable cost for close spatial aggregation. The additional extreme habitat limitation could increase disruptions and result in Helmeted Manakin males displaying solitarily.

Our observations and data from nest monitoring further support the proposition that the Helmeted Manakin has a polygamous mating system. We recorded the activity of 39 nests in nearly 15 h of video recording. Only females built nests and performed all parental care, and similar to previous studies, we did not record any male activity at nests. The absence of parental care by male Helmeted Manakins might indicate evolutionary emancipation from offspring care and thus would provide an opportunity for males to take full advantage of the environmental potential for polygamy, similar to other members of the Pipridae family.

The Helmeted Manakin is much more widespread geographically when compared with its congener, the Araripe Manakin. Additionally, it is not restricted to an enclave of riparian forest surrounded by a xeric habitat (Gaiotti *et al*. 2020). However, the population in our study was not widely distributed across the ~120 ha of gallery forest sampled, and some individuals were recaptured consistently in the same restricted areas. Furthermore, there were extensive portions of the gallery forest where we often observed Helmeted Manakins but never found nests. Therefore, the lack of available nesting sites is probably not a factor leading female Helmeted Manakins to nest close to or within males' territories, as was suggested for the Araripe Manakin (Gaiotti *et al*. 2020). In light of these observations, the Helmeted Manakin most possibly has a polygamous mating system. However, the evidence collected so far does not allow us to classify it as a typical lekking mating system. Instead, our results indicate that the Helmeted Manakin possibly presents an atypical mating system characterized by male emancipation from parental care but defended territories.

The Helmeted Manakin is the only dimorphic manakin endemic to the Cerrado biome, suggested as perhaps the only non-polygamous and non-lekking species in the Pipridae family (Marini and Cavalcanti 1992; Prum 1994). Here we provide data from an experiment, observations on its probable display, and records of parental care that challenge these ideas. As far as we know, our territorial defence experiment is the first one ever conducted with any manakin and allows us to confirm that Helmeted Manakin males defend individual territories. Data on nesting behaviour revealed a similar pattern of nest distribution and parental care as its congener, the Araripe Manakin (Gaiotti *et al*. 2020). That is, nests are frequently found within or very close to male territories, and females provide all parental care. Further assessment of male territory characteristics should be conducted to explore whether these areas provide better nesting sites for females. Finally, parental care data suggest the Helmeted Manakin has a polygamous mating system, but genetic parentage data are necessary to confirm the genetic mating system of the species.

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Disclosure statement

The authors reported no potential competing interest.

Data deposition

The data that support the findings of this study will be openly available in Mendeley Data at http://dx.doi.org/10.17632/cv4h8d8w2k.1 (DOI reserved but not published). A preview version of the dataset to be published is available at https://data.mendeley.com/datasets/cv4h8d8w2k/draft?a=8ef01799-4476-49cd-a9f8-b1951a181284.

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TABLES

Table 1. Helmeted Manakin home range and territory sizes calculated by minimum convex polygon (MCP) and kernel methods. Number of locations (*n*) used to generate kernel estimates and smoothing parameter (*h*) are shown, as well as the method to choose *h*. The percentage of home ranges that correspond to territories is shown. The overlap represents the percentage of the potential territories (MCP) that is contained within the kernel territory boundary.

		MCP	Kernel method				
Territory	*n*	Potential territory (ha)	Method for *h*	*h*	Home range (ha)	Territory (ha)	Overlap (%)
1	27	0.05	reference	19.14	0.84	0.22	11.98
2	5	0.06	*Ad hoc*	9.47	0.27	0.12	100
3	24	0.06	reference	21.19	2.61	1.20	100
4	10	0.11	*Ad hoc*	79.55	32.22	2.29	100
5	8	0.11	*Ad hoc*	32.05	6.31	1.99	100
6	16	0.02	*Ad hoc*	10.50	0.89	0.28	100
7	5	0.11	reference	23.23	1.94	0.53	18.45
8	7	0.01	reference	9.10	0.51	0.17	100

Table 2. General description of experiments conducted for eight potential territories of the Helmeted Manakin in central Brazil, from 9 October 2018 to 16 April 2019.

Territory	Number of trials	Period	Type of response (%)	
			Approach	Aggressive
1	8	16 Oct 2018 – 16 Apr 2019	50.0	25.0
2	7	15 Oct 2018 – 29 Nov 2018	12.5	0
3	6	15 Oct 2018 – 16 Apr 2019	16.6	0
4	6	9 Oct 2018 – 30 Jan 2019	66.6	33.3
5	7	18 Nov 2018 – 30 Jan 2019	42.9	14.3
6	9	23 Oct 2018 – 4 Feb 2019	11.1	11.1
7	4	18 Nov 2018 – 30 Jan 2019	75.0	50.0
8	8	23 Oct 2018 – 16 Apr 2019	37.5	12.5

Table 3. Helmeted Manakin territories and descriptive characteristics including: number of nests within territory (active nests shown in parentheses); percentage of home range area occupied by the territory; percentage of use, based on total locations contained within the territory; and intensity, a measure of use intensity within the territory (% use / % of home range).

Territory	No. total nests[1]	% home range	% use	Intensity
1	2 (2)	33.73	66.67	2.20
2	0	28.66	100	3.49
3	5 (2)	46.12	95.83	2.08
4	8 (3)	7.10	70.00	9.86
5	14 (1)	31.53	100	3.17
6	1	31.10	81.25	2.61
7	2 (1)	27.40	100	3.65
8	5 (1)	53.87	100	1.86

[1] Nests were active non-synchronously

FIGURES

Figure 1. An active Helmeted Manakin nest at ca. 84 cm above water surface photographed on 27 December 2019 (photo: Lia N. Kajiki).

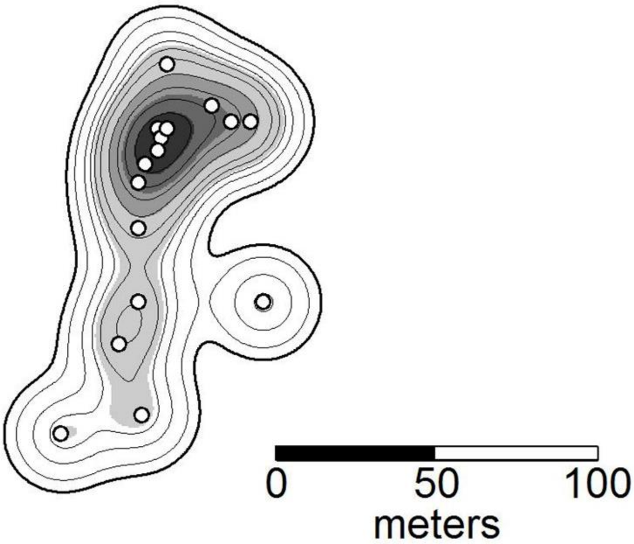

Figure 2. Utilization Distribution for Helmeted Manakin male #6 generated with adaptive kernel density estimator. White circles (N = 16) represent all locations (monitoring and experiments) collected for this individual. The outermost thicker line represents the 95th density isopleth and home range boundary. The shaded area represents the territory (core area), delimited at the 60th isopleth for this individual, with darker shading denoting higher intensity of use.

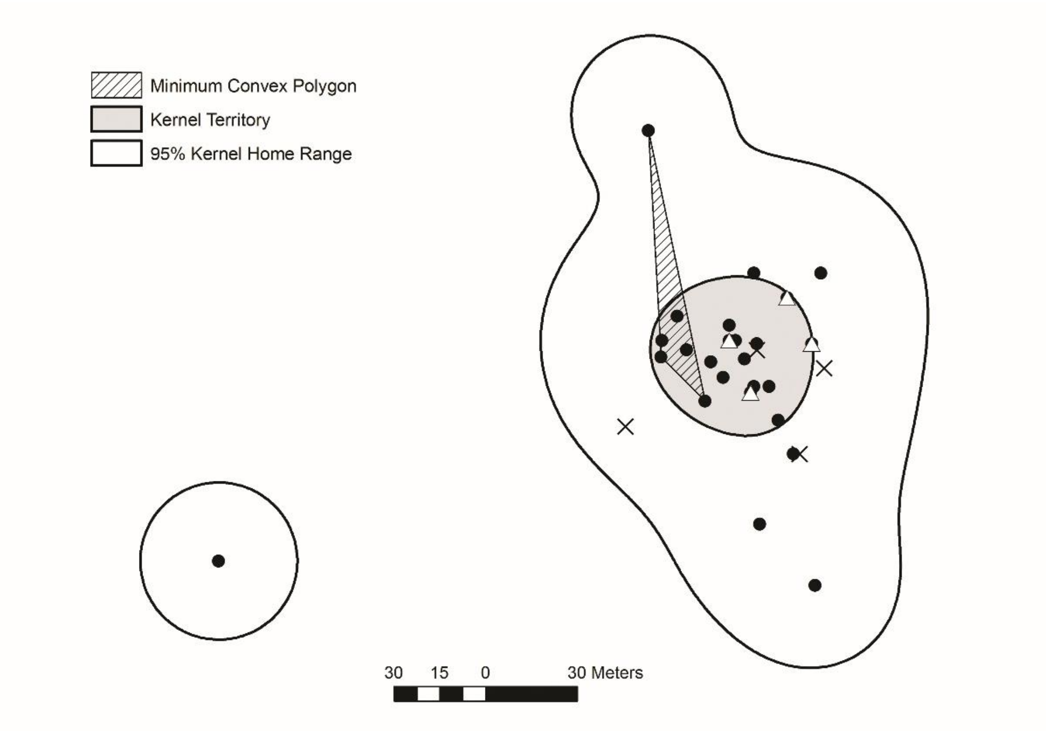

Figure 3. Locations collected for Helmeted Manakin male #1 during the sampling period. Most of the locations are concentrated within a small area, suggesting site fidelity. The outermost line is the home range boundary at the 95th isopleth. The diagonal hatch area represents the potential territory (MCP). The grey area is the actual territory (core area) with a boundary at the 40th isopleth. Dots are Helmeted Manakin locations, triangles are locations with response to the territorial experiment, and ‘X’ are locations without response to the territorial experiment.

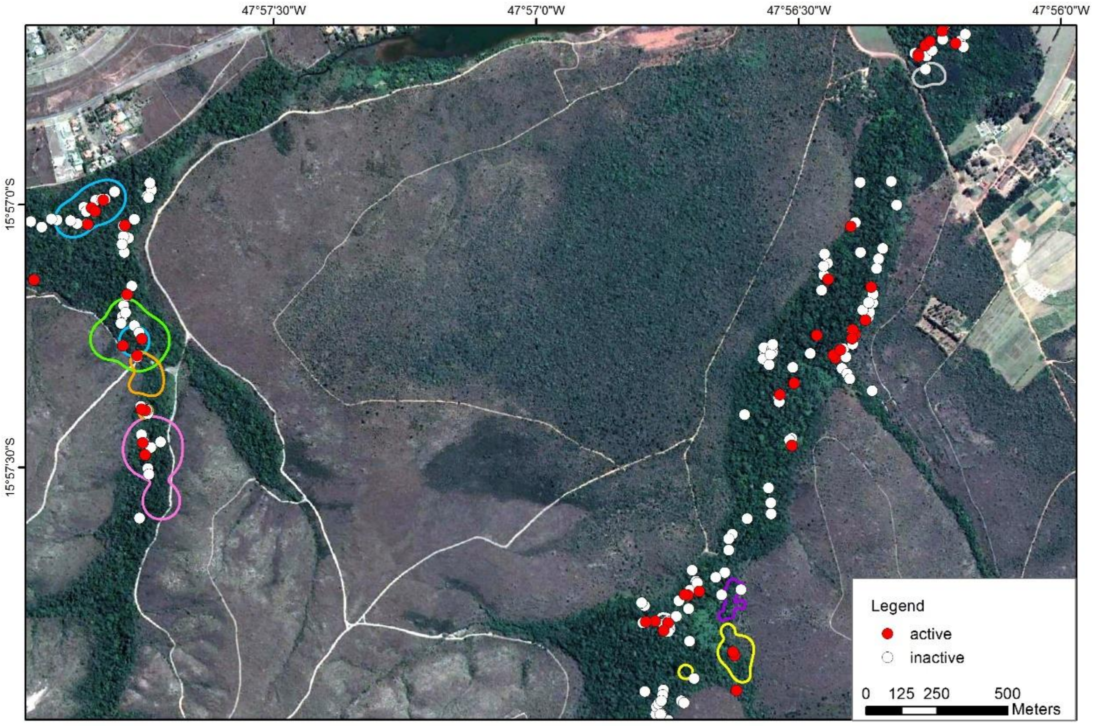

Figure 4. Map of the section of gallery forest at the 'Fazenda Água Limpa' where this study was conducted, with territories delimited in blue, green, orange, violet, yellow, white, purple, and grey. Active and inactive nests found between 2017 – 2020 are represented as red and white circles, respectively.

Supplemental online material

Video S1. Helmeted Manakin male number 1 agonistic response to a territorial defence experiment on 23 October 2018 in the ‘Fazenda Água Limpa’ study site. Video recorded by Samara de Albuquerque Teixeira.

Video S2. A Helmeted Manakin male recorded in one of the preferred perches of a tree in its territory, performing the characteristic advertisement song along with horizontal and upward postures on 4 October 2017 in the ‘Fazenda Água Limpa’ study site. Video recorded by Lia Nahomi Kajiki.

CAPÍTULO 3

O sistema de acasalamento de *Antilophia galeata* (AVES: Pipridae)

Uma versão deste capítulo será preparada como manuscrito e submetida para publicação em *Journal of Avian Biology*.

RESUMO

Espécies de aves da família Pipridae são conhecidas pela vistosa plumagem e elaborados displays de corte, traços de ornamentação que possivelmente evoluíram por seleção sexual intermediado pela escolha da fêmea. Estudos inicialmente especularam que o gênero *Antilophia* fosse socialmente monogâmico e tivesse perdido o comportamento de *lek* típico de piprídeos. No entanto, evidências da ausência de cuidado parental do macho de *Antilophia galeata* e observações do que poderia ser um traço remanescente de *display* de corte sugerem que o sistema de acasalamento de *A. galeata* pode ser do tipo poligínico em *lek*. Porém, machos defendem territórios, o que é incomum entre as outras espécies da família. O objetivo desse estudo foi documentar o sistema de acasalamento genético de *A. galeata* e averiguar se o comportamento territorial se associa à paternidade de ninhadas. A paternidade das ninhadas foi determinada com análise de fragmentos de microssatélites e os territórios foram delimitados com experimentos de playback. Um terço dos machos genotipados obteve a paternidade de ninhegos em ninhos amostrados, e encontramos que em 55% das ninhadas os filhotes de uma mesma ninhada foram gerados por machos diferentes. Um total de 13 machos geraram filhotes em 11 ninhos, mas nenhum destes se encontrava dentro dos territórios destes machos. A análise de paternidade mostrou que 69% dos machos genotipados tiveram um ou dois filhotes em uma mesma ninhada, e quase um terço dos machos geraram mais de um filhote com fêmeas diferentes. Os resultados indicam que a população de *A. galeata* investigada é poligínica, com elevada proporção de poliandria entre fêmeas. Assim, esse estudo sugere que *A. galeata* é poligínico em *lek*, no qual machos realizam *displays* solitários.

Palavras-chave: poliginia, poliandria, paternidade múltipla, territorialidade, soldadinho, Cerrado, endêmico, parentesco

INTRODUÇÃO

Indivíduos em uma população podem diferir fortemente quanto ao seu sucesso reprodutivo, e a competição se intensifica em sistemas poligâmicos, que surgem quando as condições ambientais criam o potencial para a poligamia (EMLEN e ORING, 1977; PAYNE, 1984; ALCOCK, 2005). A evolução de um sistema poligâmico de acasalamento tem duas pré-condições: 1) um dos sexos consegue monopolizar o acesso aos parceiros ou aos recursos necessários aos parceiros (CAREY, 1991) e 2) um dos sexos deve conseguir emancipar-se do cuidado parental (ORIANS, 1969). Quando o ambiente ou condições comportamentais favorecem a aglomeração de fêmeas, e machos são capazes de monopolizá-las, surge a oportunidade para a poliginia. Quando nenhum dos sexos é capaz de utilizar o potencial ambiental para a poligamia (i.e., cuidado parental de ambos os pais é necessário para a criação bem-sucedida dos filhotes), então a monogamia prevalece (EMLEN e ORING, 1977).

A poliginia pode evoluir por três diferentes vias: 1) defesa de recursos, 2) defesa de fêmeas, e 3) hierarquia de dominância entre machos (i.e., *lek*) (ALCOCK 2005). A poliginia pela defesa de recursos é baseada na capacidade de machos monopolizarem recursos importantes que estão distribuídos de maneira heterogênea no ambiente. Se esses recursos forem essenciais para a reprodução das fêmeas, então o sucesso reprodutivo de machos poderá ser condicionado pela competição entre eles para monopólio dos recursos e pela escolha da fêmea (CAREY, 1991). A poliginia por meio de defesa de fêmeas (ou haréns) ocorre quando machos monopolizam grupos de fêmeas que já se encontram agrupadas por outras razões além de recursos. Por fim, quando o ambiente não fornece oportunidade para o controle de parceiros ou recursos e o macho é emancipado do cuidado parental ocorre a poliginia pela dominância de machos, também conhecida como poliginia em *lek* (EMLEN e ORING, 1977; PAYNE, 1984).

Na poliginia em *lek*, machos se agrupam em arenas de *display* para competir entre si por posições de dominância (HÖGLUND e ALATALO, 2014). Há defesa de territórios na forma das arenas de *display*, contudo elas não fornecem nenhum tipo de recurso (e.g., alimento ou local de nidificação) às fêmeas (BROWN, 1964). Assim, sugere-se que as fêmeas escolham os machos unicamente com base no seu status social ou algum aspecto da sua ornamentação que seriam indicados pelos displays ou fenótipos dos machos, resultando em elevado desvio reprodutivo, no qual apenas alguns machos obtêm sucesso reprodutivo (MACKENZIE *et al.*, 1995; FISKE *et al.*, 1998; STEIN e UY, 2006). As arenas normalmente são compartilhadas entre machos de diferentes status social, e sugere-se que executar *displays* em arenas comunais é favorável tanto para machos dominantes quanto para machos de menor status social, que se beneficiam desses agrupamentos por serem mais chamativos do que *displays* solitários ou pelo potencial de herdarem a dominância na arena no futuro (BEEHLER e FOSTER, 1988).

Muitos estudos que investigam a interação entre espécies e seus recursos no contexto da seleção sexual se beneficiaram da classificação simples e intuitiva dos sistemas de acasalamento proposta no clássico trabalho de EMLEN e ORING (1977). Contudo, observa-se que tal categorização não deve ser adotada invariavelmente: no caso de estudos sobre a poliginia, pesquisadores se esforçaram em encaixar as espécies em uma das categorias (i.e., defesa de recursos, de fêmeas ou *lek*), porém muitas vezes acabaram em categorizações ambíguas (OSTFELD, 1987). Além disso, organismos demonstram uma certa plasticidade com relação a variações no ambiente e possivelmente adotam respostas que podem se encaixar em mais de um sistema (ALCOCK, 2016). A ferreirinha-comum (*Prunella modularis*) exemplifica bem o caso: essa espécie de ave apresenta sistema de acasalamento variável, onde parte da população é socialmente monogâmica, outra é poliândrica e o restante é poliginiândrica (BIRKHEAD, 1981; SANTOS e NAKAGAWA, 2013).

Uma considerável parte da confusão pode surgir a partir da discussão sobre o conceito da territorialidade. Adquirir e defender territórios é bastante custoso energeticamente, e por isso apenas é viável quando os benefícios na aptidão dos indivíduos superam os custos (BROWN, 1964; ALCOCK, 2005), como no caso de aumentar as chances de sucesso reprodutivo (MACKENZIE *et al.*, 1995). Territórios são áreas que animais defendem contra intrusos (ALCOCK, 2005) e que servem como sítios reprodutivos onde podem obter alimento e proteção (O’DONALD, 1977). Esses atributos já foram utilizados para quantificar a qualidade de territórios, e eles podem estar relacionados com a própria qualidade dos machos que os detêm. Por exemplo, em algumas espécies machos de maior tamanho são socialmente dominantes e detêm territórios maiores (PAYNE, 1984; MACKENZIE *et al.*, 1995) ou com recursos que são importantes para fêmeas (MAHER e LOTT, 2000). Assim, torna-se uma tarefa árdua encaixar os organismos em categorias de sistema de acasalamento (O’DONALD, 1977) quando os critérios largamente se baseiam em conceitos relacionados a territorialidade.

Apesar da família Pipridae ser amplamente conhecida por adotar o sistema de acasalamento poligínico em *lek* (PRUM, 1990; KIRWAN e GREEN, 2012; HÖGLUND e ALATALO, 2014), um gênero se destaca por aparentemente não se encaixar totalmente nessa categoria. *Antilophia* é um gênero composto atualmente por duas espécies (*Antilophia galeata* e *A. bokermanni*), e por muito tempo a proposta de uma possível monogamia social em *A. galeata* (MARINI e CAVALCANTI, 1992) foi aceita e pouco investigada. Apesar do reduzido tamanho amostral, os autores encontraram indicativos de pareamento social, territorialidade, e um ninho foi encontrado em um provável território. Além disso, não encontraram evidências de machos aglomerados em arenas, como ocorre tipicamente nos *leks*. O estudo concluiu que *A. galeata* possivelmente teria perdido o comportamento de *lek* e a monogamia poderia ter reincidido no clado (MARINI e CAVALCANTI, 1992; PRUM, 1994).

Contudo, estudos recentes sugerem que *A. galeata* não deve ser socialmente monogâmica: apenas a fêmea realiza o cuidado parental em todas as fases de desenvolvimento dos filhotes (Capítulo 1; MARÇAL e LOPES, 2019a; BRUNO *et al*., 2021), e apesar de defender territórios individuais e terem sido encontrados ninhos ativos nesses territórios, como em *A. bokermanni* (GAIOTTI et al., 2020), é desconhecida a paternidade das ninhadas nos territórios de *A. galeata* (Capítulo 2). Alguns machos de *A. bokermanni* tiveram a paternidade genética confirmada para mais de uma ninhada, indicando que machos copularam com mais de uma fêmea (GAIOTTI *et al.*, 2020). Além disso, em mais de 90% das ninhadas os donos dos territórios onde o ninho estava localizado não tinham vínculo de paternidade com as proles. GAIOTTI (2016) reportou ter observado *displays* simples, potencialmente de corte, nos quais machos realizavam voos circulares fora dos territórios individuais. Um padrão similar de voo foi observado em *A. galeata* por Marini (1992) e Marçal (2017). As duas espécies do gênero *Antilophia* são consideradas espécies-irmãs, que divergiram muito recentemente (24.000 – 1.500 anos atrás) na escala evolutiva (Luna et al. 2017), e por isso espera-se que se comportem de maneira similar.

Assim, o objetivo desse trabalho foi investigar e descrever o sistema de acasalamento social e genético de *A. galeata*, e compreender como o comportamento territorial está associado ao sistema de acasalamento da espécie. Nossa hipótese de trabalho é de que, assim como a espécie irmã, *A. galeata* tem um sistema de acasalamento poligínico, tal como outras espécies da família. Contudo, de maneira semelhante a *A.*

bokermanni, esperamos encontrar um sistema de acasalamento com desvios do modelo mais tradicional de *lek*.

MÉTODOS

Foram realizadas capturas com redes de neblina entre 2017 e 2020 na “Fazenda Água Limpa” (FAL), fazenda experimental que pertence à Universidade de Brasília e possui aproximadamente 4.500 ha. As coletas de dados foram realizadas nas matas de galeria que permeiam dois córregos principais na FAL: o Córrego Capetinga e o Córrego da Onça; assim, a área de estudo correspondeu a aproximadamente 120 ha. As aves capturadas foram anilhadas com uma anilha metálica do CEMAVE (Centro Nacional de Pesquisa e Conservação de Aves Silvestres) e com uma combinação de três anilhas coloridas de plástico (autorização SISBIO n° 58094-3 e CEMAVE n° 4221). Redes de neblina 12 x 3m foram instaladas em vários pontos da mata de galeria na área de estudo, principalmente em locais próximos a corpos d’água. Ocasionalmente a técnica do *playback* foi utilizada para facilitar a captura de indivíduos, principalmente de machos que consistentemente eram avistados em determinadas áreas. Apenas a fêmea realiza o cuidado parental em *A. galeata* (Capítulo 1; MARÇAL e LOPES, 2019a; BRUNO *et al.*, 2021), por isso tentamos capturar fêmeas enquanto estavam associadas a seus ninhos. Para isso, instalamos 1 – 3 redes de neblina ao redor do ninho, deixando uma distância de 1 – 2m entre a rede e o ninho. Essas tentativas de capturas apenas foram empregadas quando o ninho tinha filhotes, ou se tivesse ovos, apenas quando estes estavam em um estágio avançado de desenvolvimento (a partir do 8° – 10° dia).

Machos passam por pelo menos dois estágios de muda até atingirem a plumagem definitiva adulta. Machos jovens possuem a plumagem muito similar às fêmeas (MARINI, 1989; KIRWAN e GREEN, 2012) e são indistinguíveis na visão de aves (KAJIKI e MACEDO dados não publicados). Machos podem permanecer nessa plumagem por pelo menos 8 meses até começarem a adquirir penas características da plumagem definitiva, estágio caracterizado por uma plumagem pré-definitiva. Em seguida, machos levam pelo menos outros 9 meses para adquirirem a plumagem adulta definitiva (KAJIKI e MACEDO dados não publicados).

Realizamos buscas de ninhos em três estações reprodutivas, entre Agosto e Fevereiro: 2017 – 2018 (1ª estação), 2018 – 2019 (2ª estação) e 2019 – 2020 (3ª estação). As buscas foram realizadas ao longo dos córregos e corpos d’água e por toda extensão da mata de galeria, observando todo estrato florestal até 8 – 10m de altura. Uma vez encontrado, o ninho tinha sua localização georreferenciada com um GPS Garmin® eTrex10. A partir da segunda estação reprodutiva, a busca de ninhos foi intensificada no entorno (raio de ca. 25m) do local de ninhos encontrados em estações anteriores.

Coletamos uma amostra de sangue (ca. 15 – 50µl) de adultos, jovens e filhotes. A coleta de sangue foi feita por meio da punção da veia braquial com agulha (0,55 x 20mm, Sterile®) e tubos capilares com heparina (Perfecta®). O sangue foi armazenado em álcool 99,7% e mantido congelado (-3° C). A coleta de sangue em filhotes foi realizada a partir do 5° - 6° dia de idade, com o mesmo procedimento aplicado aos adultos, mas com a punção na parte distal interna do tarso.

A predação de ninhos na área de estudo é elevada (Capítulo 1), por isso foram encontrados poucos ninhos com ninhadas completas na primeira e segunda estações reprodutivas. Na terceira estação reprodutiva, manipulamos a sobrevivência de ninhos substituindo os ovos por réplicas feitas com argila seca, e incubando os ovos artificialmente (Capítulo 1). Quando os filhotes nasciam, eram imediatamente retornados aos seus ninhos. Assim, analisamos e determinamos a paternidade de ninhegos pertencentes a ninhadas completas encontrados na terceira estação reprodutiva (2019 – 2020).

Durante o início da terceira estação reprodutiva, foram conduzidos experimentos de territorialidade que consistiam na exibição de um modelo taxidermizado de um indivíduo macho em plumagem adulta acompanhado de um protocolo de playback. Os experimentos foram realizados com oito machos em plumagem adulta, que nos permitiram confirmar se machos são territoriais ou não. Detalhes do experimento e das análises para estimar a área dos territórios podem ser encontrados no Capítulo 2.

Análises moleculares

Sexagem

A plumagem de machos jovens e fêmeas é muito similar, por isso é necessário empregar técnicas moleculares para confirmar o sexo desses indivíduos. A extração de DNA seguiu o protocolo de fenol:clorofórmio e precipitação com etanol (SAMBROOK *et al.*, 1989).

Para a sexagem, seguimos GRIFFITHS *et al.* (1998) e utilizamos os primers P2/P8 (ÇAKMAK *et al.*, 2017) em reações de cadeia de polimerase (PCR). As reações foram preparadas utilizando 4,86µl de água ultrapura, 1µl de tampão para PCR 10x (Invitrogen), 1µl de dNTPs (25mM), 1µl do primer P2 (10µM), 1µl do primer P8 (10µM), 0,5 U Taq polimerase (5 U/µl, Invitrogen) e 1,5µl de DNA. As condições da PCR utilizadas foram as seguintes: 1 ciclo a 95°C (5min) seguido por 30 ciclos a 95°C (30s) / 47°C (30s) / 72°C (30s), seguido por uma extensão a 72°C por 7min. O volume total de cada reação foi misturado com 2µl de GelRed e 2µl de tampão de eletroforese, e revelado em gel de agarose (3%), com a fonte configurada em 100V por 5min e 45V por 60min.

Análises de paternidade

Utilizamos 12 pares de microssatélites (ver Tabela S3, Informações Suplementares, Capítulo 4) para genotipar as amostras de sangue de 22 ninhegos pertencentes a 11 ninhadas, e nove fêmeas associadas a esses ninhos na terceira estação reprodutiva. Foram genotipados 45 machos (jovens e adultos) capturados a um raio de aproximadamente 250 m ao redor das ninhadas genotipadas ao longo das três estações reprodutivas do estudo.

As amplificações de PCR foram realizadas em volumes de reação de 11,5 µl usando 50 ng de DNA genômico, 1 pmol de primer *forward* com a sequência M13 (5’ – 3’: CAC GAC GTT GTA AAA CGA), 3 pmoles de primer *reverse* não marcado, 2 pmoles de primer M13 marcado com os corantes 6-FAM, VIC ou NED (Applied Biosystems), 1,2 µl de tampão PCR 10x (Invitrogen), 0,4 µl de dNTPs (25 mM), 0,4 µl de $MgCl_2$ (25mM) e 0,5 U de Taq polimerase (5 U / µl, Invitrogen). As condições gerais da PCR foram as seguintes: 94°C (7 min), 35 ciclos a 94°C (30 s) / temperatura de anelamento (Informações Suplementares Capítulo 4 Tabela S3; 30 s) / 72°C (30 s), seguido por uma extensão a 72°C por 10 min. Os amplicons de PCR de um a três loci marcados com diferentes corantes foram combinados em proporções de diluição apropriadas com água ultrapura, formamida e um padrão de tamanho (ROX 500; Perkin-Elmer) para genotipar em um sequenciador capilar automatizado ABI 3730.

Os tamanhos dos alelos foram determinados manualmente utilizando o programa GeneMarker 2.6.3. Usamos o programa CERVUS 3.0.0 (KALINOWSKI *et al.*, 2007) para atribuir a paternidade e maternidade dos ninhegos. Calculamos a diversidade alélica, heterozigosidade observada (Ho) e esperada (He), probabilidade de não exclusão para o primeiro (NE-1P) e segundo pai candidato (NE-2P), probabilidade de equilíbrio de Hardy-Weinberg (HW) e frequência de alelos nulos (Fnull). Obtivemos frequências alélicas de 77 indivíduos, e o erro de digitação foi baixo (~ 7,8%) para a maioria dos marcadores usados, exceto para loci Abom12. A identidade das mães biológicas de todas as ninhadas analisadas era conhecida por terem sido registradas durante o monitoramento dos ninhos ou capturadas enquanto cuidavam dos ninhegos. Presumimos que todos os machos poderiam ser pais candidatos e, portanto, incluímos indivíduos capturados na plumagem masculina adulta ou sexados como machos com análise molecular.

Foram genotipados 45 machos candidatos que foram completamente genotipados nos 12 loci. Atribuímos parentesco usando níveis de confiança relaxados (50%) e estritos (95%) calculando as pontuações da razão de verossimilhança (*likelihood ratio score* – LOD) bem como usando uma abordagem de "evidência total". Esta abordagem usa simulações para determinar as diferenças entre os pais candidatos mais prováveis e produzir níveis de confiança nas atribuições (JONES et al., 2010). Primeiro, selecionamos o macho da população com a maior pontuação LOD do trio como o pai mais provável. A pontuação LOD do trio é a razão de probabilidade logarítmica para uma relação pai-filho entre o pai candidato e o filho, dado o pai conhecido. Em segundo lugar, usamos uma abordagem de "evidência total" para cada atribuição de paternidade para determinar se a atribuição de CERVUS era razoável (WEBSTER *et al.*, 2004; RYDER *et al.*, 2010).

Rejeitamos a atribuição do CERVUS e atribuímos a paternidade a um macho de classificação inferior sob estas circunstâncias: 1) se os machos tivessem pontuações LOD semelhantes, mas os machos de classificação inferior tivessem menos incompatibilidades entre alelos; 2) se os machos tivessem uma única incompatibilidade entre alelos, mas a incompatibilidade do macho de classificação inferior indicasse a presença de um alelo nulo; 3) se os machos tivessem pontuações LOD semelhantes e o mesmo número de incompatibilidades baixas (zero ou um), mas outras evidências sugeriam que o macho com classificação inferior era o pai mais provável. Nesse último caso, consideramos se o macho era o dono do território mais próximo do ninho ou se a incompatibilidade dos machos foi possivelmente causada por um erro de pontuação (por exemplo, alelos incompatíveis diferiram por apenas uma unidade de repetição). Finalmente, quando o CERVUS foi incapaz de atribuir parentesco com ambos os níveis de confiança (ou seja, as pontuações LOD do trio foram negativas para todos os pais candidatos para uma prole) deduzimos a paternidade para o indivíduo com a maior pontuação LOD de par e menores incompatibilidades de loci. Nossas simulações preliminares usaram 10.000 ciclos e 0,02% de erro de genotipagem por locus. O CERVUS mediu o verdadeiro erro de digitação como a porcentagem de incompatibilidades entre mães e filhos identificados.

RESULTADOS

Os 12 microssatélites apresentaram alta variabilidade, com uma média de 8 alelos por loco e elevados valores de heterozigosidade, indicando que os loci são informativos (Tabela 1). Todos os filhotes e mães conhecidas foram genotipados em seis ou mais loci. Houve apenas três casos de incompatibilidades e apenas um caso em vários loci (i.e., 2 loci). Nossos dados indicam que a maioria ou todas as incompatibilidades não foram causadas por erro na atribuição da maternidade, que seria causada pela incompatibilidade entre a presumida mãe e filhote em vários loci.

Tabela 1 Características dos loci de 12 microssatélites utilizados nas análises de parentesco de 77 amostras de *Antilophia galeata*. O número de alelos (k), a heterozigosidade observada (Ho), heterozigosidade esperada (He), probabilidade de não-exclusão para um dos pais candidatos (NE-1P) e para um pai candidato dado que o genótipo da mãe é conhecido (NE-2P), a significância de desvio do equilíbrio de Hardy-Weinberg (H-W) e frequência de alelos nulos (Fnull) são fornecidos.

Locus	**k**	**Ho**	**He**	**NE-1P**	**NE-2P**	**H-W**	**Fnull**
Chir1-6	3	0.44	0.41	0.917	0.832	NS	-0.034
Chir1-16	9	0.88	0.837	0.507	0.336	NS	-0.030
Chiro5	7	0.8	0.746	0.656	0.478	NS	-0.042
Chiro8	11	0.792	0.886	0.42	0.264	ND	0.046
Chiro10	9	0.863	0.831	0.524	0.351	NS	-0.023
Chiro12	15	0.895	0.876	0.408	0.255	NS	-0.019
Abom6	7	0.789	0.737	0.67	0.493	NS	-0.037
Abom7	11	0.862	0.853	0.475	0.308	NS	-0.013
Abom11	9	0.773	0.729	0.677	0.503	NS	-0.035
Abom12	3	0.294	0.369	0.933	0.839	NS	0.127
Xenat01	6	0.434	0.539	0.845	0.686	NS	0.128
Xenat02	5	0.44	0.543	0.844	0.685	NS	0.124

* NS = não significativo; ND = não determinado

O programa CERVUS atribuiu um pai a 18 dos 22 ninhegos (82%): três foram atribuídos com 95% de confiança e 15 com 50% de confiança. Utilizando as regras para aceitar ou rejeitar atribuições do CERVUS (consulte a seção Métodos), aceitamos todas as atribuições de alta e baixa confiança. Os quatro ninhegos que não tiveram paternidade atribuída pelo CERVUS foram manualmente analisados utilizando a abordagem de “evidência total” (detalhes na seção Métodos). Dos 45 machos genotipados, apenas 13 machos (29%) tiveram a paternidade atribuída a pelo menos um ninhego, e a maioria deles exibia a plumagem adulta (N = 10; 77%). O restante estava na plumagem verde (N = 1) e pré-definitiva (N = 2).

Identificamos os pais de 22 ninhegos pertencentes a 11 ninhadas completas; dessas ninhadas, mais da metade (55%, N = 6) possuiu diferentes pais. Dessas ninhadas com paternidade múltipla, a maioria dos ninhegos (82%, N = 18) foi gerada por machos na plumagem adulta, e os outros quatro a machos na plumagem verde ou pré-definitiva. A maioria dos machos (62%, N = 8) teve paternidade atribuída a metade de uma ou duas ninhadas, e cinco machos foram os pais de ninhadas inteiras. Dos machos que tiveram sucesso reprodutivo, a maioria obteve a paternidade de dois ninhegos (54%, N = 7) (Fig. 1) e de apenas um ninho (39%, N = 5), e alguns (N = 4) tiveram a paternidade atribuída a duas ninhadas de fêmeas distintas. Duas fêmeas nidificaram mais de uma vez na estação reprodutiva, mas a maioria (78%, N = 7) nidificou apenas uma vez.

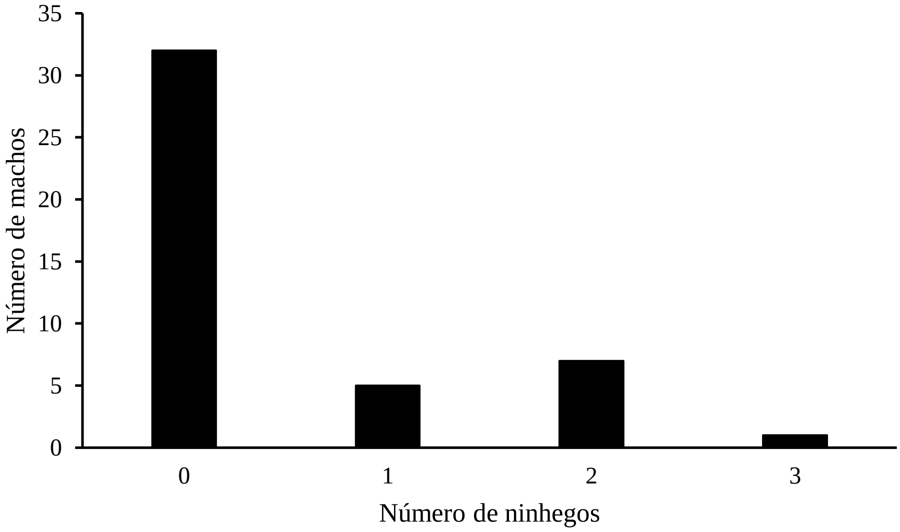

Figura 1 A distribuição do sucesso reprodutivo de 45 machos de *Antilophia galeata*.

Entre os treze machos que tiveram a paternidade biológica confirmada para pelo menos um ninhego, cinco deles possuíam territórios que foram confirmados pelos experimentos de territorialidade. Os territórios tiveram em média 1,16 ± 0.99 ha, e a maior parte deles estava totalmente inserido na área de vida (8.45 ± 13.49 ha) (Tabela 2). Nenhum dos ninhos atribuídos a esses machos estavam localizados dentro dos limites de seus territórios (Fig. 2). Em realidade, a maioria dos ninhegos estava em ninhos extremamente distantes dos territórios de seus pais (média ± desvio padrão: 1.752 ± 1.117m, 276 – 3.075m, N = 7 ninhos) (Tabela 2).

Tabela 2 Estimativas dos territórios de *Antilophia galeata* calculadas pela Estimativa de Densidade de Kernel. O número de localidades (n) utilizadas nas estimativas de kernel e a distância dos ninhos aos territórios dos pais são mostrados.

Território	***n***	**Território (ha)**	**Distância do ninho (m)**
1	27	0.22	2.563 e 3.075
2	5	0.12	276
3	24	1.20	900
4	10	2.29	624
5	8	1.99	2.525 e 2.303

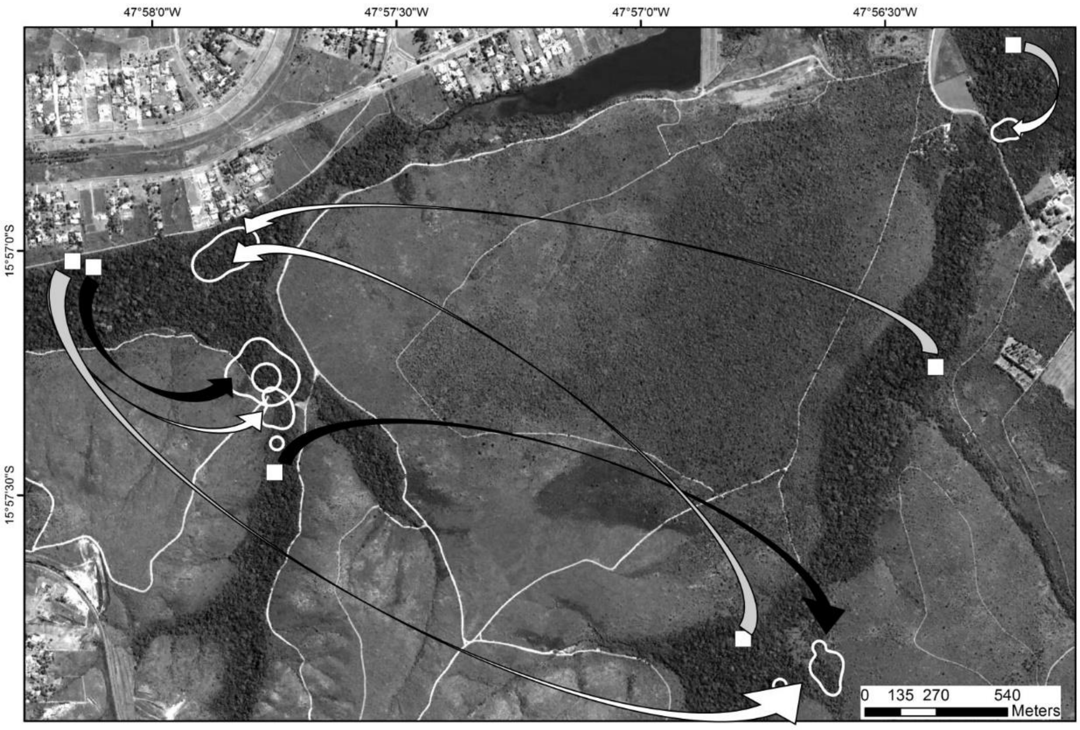

Figura 2 Configuração dos territórios de machos que obtiveram a paternidade de pelo menos um ninhego. Os territórios de cinco machos estão delimitados em branco. Esses machos adquiriram paternidade de pelo menos um ninhego dos ninhos (quadrados), e essa relação está indicada pela direção da seta. Setas pretas indicam que a ninhada inteira teve a paternidade atribuída a um único macho.

DISCUSSÃO

Os resultados encontrados nesse estudo a partir da análise genética de paternidade sugerem que a população de *A. galeata* investigada apresenta elevado desvio reprodutivo entre machos, além de evidências de poliginia e poliandria. Ademais, machos em plumagem adulta obtiveram paternidade da maioria dos ninhegos. Nenhum dos filhotes se encontrava em ninhos localizados dentro dos territórios identificados como pertencentes aos seus pais; em realidade, grande parte dos ninhegos estavam em ninhos bastante distantes dos territórios dos pais.

Em animais que se reproduzem sexuadamente, há um desequilíbrio de interesses entre o pais resultante dos custos na geração de descendentes (ARNQVIST e ROWE, 2005). O conflito sexual se intensifica quando a aptidão de um dos pais é aumentada se este investe menos na prole e o outro investe mais (TRIVERS, 1972). O resultado dos diferentes comportamentos, estratégias e táticas adotados por machos e fêmeas para aumentar sua aptidão resultam nos diferentes sistemas de acasalamento. No caso das espécies poligínicas em *lek*, o investimento parental de machos no cuidado da prole é inexistente e a escolha das fêmeas exerce intensa pressão de seleção sexual sobre os machos (HÖGLUND e ALATALO, 2014). Nessas espécies, o conflito sexual ocorre sobre a escolha da fêmea quanto à cópula (HARTLEY e ROYLE, 2007): machos buscam copular com o máximo possível de fêmeas, enquanto fêmeas aumentariam sua aptidão copulando com o macho de melhor qualidade genética ou fenotípica.

A espécie *A. galeata* pertente à família Pipridae, notavelmente conhecida pelos elaborados *displays* de corte realizados pelos machos submetidos a intensa seleção sexual exercida pela escolha da fêmea; nessa família, o sistema de acasalamento poligínico em *lek* prevalece. Contudo, a proposta inicial foi de que *A. galeata* possivelmente apresentasse um sistema de acasalamento social monogâmico. Tal proposta baseou-se em observações comportamentais de pareamento entre dois machos e duas fêmeas, territorialidade em quatro machos, e um ninho dentro de um desses territórios (MARINI, 1992; MARINI e CAVALCANTI, 1992). Na época o acesso a ferramentas moleculares para atribuição de paternidade genética era limitado. Tal hipótese recentemente foi contestada com evidências comportamentais sugerindo que a espécie, em realidade, possui um sistema de acasalamento poligâmico (MARÇAL e LOPES, 2019a; b; Capítulo 1 e 2). Entretanto, evidências comportamentais, como a ausência de cuidado biparental, apenas fornecem indícios sobre o sistema de acasalamento e devem ser avaliados com cautela. O cuidado biparental é associado a 90% das aves socialmente monogâmicas (ALCOCK, 2016), contudo tal associação se torna bem menos explícita em certas condições. Em espécies com sistemas de acasalamento mistos, como *Vanellus vanellus* (PARISH e COULSON, 1998) e *Prunella modularis* (DAVIES e LUNDBERG, 1984; SANTOS e NAKAGAWA, 2013), indivíduos na mesma população podem ser monogâmicos, poligínicos ou poliândricos, e os machos exercem diferentes níveis de cuidado parental. Assim, o uso de técnicas moleculares para confirmar as relações genéticas de parentesco é fundamental para a determinação do sistema de acasalamento.

Além da ausência de cuidado parental por parte dos machos, outra evidência comportamental que sugere uma semelhança no sistema de acasalamento de *A. galeata* com os outros piprídeos é a presença de um *display* de corte. Recentemente foi proposto que os voos circulares no qual machos se perseguem violentamente e que são realizados pelas duas espécies do gênero *Antilophia* (MARINI e CAVALCANTI, 1992; SICK, 1997; GAIOTTI, 2016; MARÇAL, 2017; MARÇAL e LOPES, 2019b; GAIOTTI et al., 2020) correspondem ao *display* em *lek* de *A. galeata* (MARÇAL e LOPES, 2019b). Esse comportamento também foi observado no presente estudo durante os experimentos de territorialidade, contudo as perseguições não eram consistentes durante o dia e nunca foram observadas fêmeas próximas (Capítulo 2). As observações dos machos monitorados neste estudo sugerem que *A. galeata* não perdeu completamente o comportamento de *lek*, mas realiza um *display* na forma de defesa de território, frequentes vocalizações no seu entorno ao longo do dia e algumas posturas pouco elaboradas (detalhes do *display* se encontram no Capítulo 2). *Display* similar foi observado em algumas outras espécies de Pipridae, como *Heterocercus flavivertex* (PRUM et al., 1996).

Em sistemas de acasalamento poligínico, a competição entre machos para adquirir cópulas é alta e resulta em elevados níveis de desvio reprodutivo entre eles (HÖGLUND e ALATALO, 2014). Os resultados das análises moleculares de parentesco sugerem que o sucesso reprodutivo é desigual (Fig. 1) e deve haver elevada competição por cópulas entre machos na população de *A. galeata* estudada. Ademais, 31% dos machos com sucesso reprodutivo obtiveram a paternidade de ninhadas pertencentes a fêmeas diferentes, fornecendo evidências de que machos são poligínicos. Apesar do tamanho amostral ser baixo, é possível que a análise da paternidade de mais ninhadas revelasse maiores níveis de poliginia. Ainda assim, esse padrão em que a maioria das ninhadas possuiu pais monogâmicos é comum em várias espécies de aves que fazem *lek* (HÖGLUND e ALATALO, 2014), como em *Lepidothrix coronata* (DURAES *et al.*, 2009), *Centrocercus urophasianus* (BIRD *et al.*, 2012), e *Tetrao tetrix* (LEBIGRE *et al.*, 2007).

Além disso, nenhuma das ninhadas que tiveram paternidade atribuída a um macho territorial estavam localizadas nos territórios desses machos. Em realidade, as ninhadas estavam localizadas bastante distantes desses territórios, muitas vezes em seções da mata de galeria separadas por área de savana (Fig. 2), sugerindo que fêmeas de *A. galeata* têm elevada mobilidade e possivelmente extensas áreas de vida na área de estudo. Nossos registros de recapturas e reavistamentos de fêmeas anilhadas na área de estudo revelam que a movimentação de fêmeas pelos corredores de mata de galeria é ampla: uma fêmea capturada em uma parte do fragmento (Córrego da Onça) após 4 meses foi recapturada em outro extremo (Córrego do Capetinga), percorrendo ca. 2,3 km e, provavelmente, atravessando a savana que separa as duas partes do fragmento. Movimentação parecida foi registrada para outra fêmea que percorreu 2,8 km, a distância máxima registrada entre local de anilhamento e recaptura/reavistamento para fêmeas nesse estudo. Em um estudo recente com a espécie, a área de vida de quatro fêmeas correspondeu em média a ca. 20 ha, e os autores consideraram que esse valor poderia estar subestimado (MARÇAL e LOPES, 2019b). Assim, a espécie parece fazer amplo uso de manchas florestais minimamente conectadas (ANDRADE e MARINI, 2001). Essa capacidade de dispersão é observada em outras espécies da família. Em *Chiroxiphia linearis*, uma fêmea foi observada visitando um *lek* distante pelo menos 2 km da área onde havia sido anilhada quatro dias antes (MCDONALD, 2010). Em outro estudo investigando a movimentação de passeriformes em uma paisagem fragmentada, um indivíduo de *C. caudata* foi recapturado a uma distância mínima de 2.100 m do local de anilhamento e percorreu pelo menos 650 m pela matriz de vegetação não-florestal que separava os dois pontos (MARINI, 2010).

Os padrões de deslocamento de fêmeas, incluindo a dispersão, são tipicamente associados aos custos relativos da endogamia, que são maiores para as fêmeas em espécies poligínicas (GREENWOOD, 1980); mas outros fatores, como níveis de competição intrassexual, sistemas de acasalamento e territorialidade têm sido considerados (PUSEY, 1987; WOLFF e PLISSNER, 1998). Essas hipóteses não podem ser descartadas para os deslocamentos de fêmeas em Pipridae, mas nesse caso, possivelmente fêmeas que possuem grandes áreas de vida têm a oportunidade de visitar vários *leks* e avaliar melhor os machos (MCDONALD, 2010). Em *A. galeata*, o comportamento de deslocamento das fêmeas e as evidências que encontramos de paternidade múltipla parecem sugerir que fêmeas conseguem visitar mais territórios e avaliar melhor os machos.

No presente estudo mais da metade das ninhadas tiveram a paternidade distribuída entre diferentes pais. Supõe-se que a frequência de ocorrência de paternidades múltiplas em espécies que fazem *lek* seja baixa, pois fêmeas têm a oportunidade de avaliar vários machos e escolher o macho com melhor qualidade (BRADBURY e GIBSON, 1983). Porém, dados que evidenciam a paternidade múltipla dentro da mesma ninhada têm se acumulado para muitas espécies de aves que fazem *lek*, como *T. tetrix* (4%; LEBIGRE *et al.*, 2007), *Rupicola rupicola* (25%; TRAIL, 1985), *C. urophasianus* (7,9%; BIRD *et al.*, 2012) e *Philomachus pugnax* (50%; LANK *et al.*, 2002). A proporção de paternidade múltipla encontrada para *A. galeata* é bastante elevada (55% das ninhadas amostradas) comparando-se com os valores encontrados para outras espécies de piprídeos, como *Lepidothrix coronata* (5%; DURAES *et al.*, 2009), *Pipra filicauda* (18%; RYDER *et al.*, 2009), *Chiroxiphia lanceolata* (14%; RIVERS e DUVAL, 2020), sendo apenas menor do que a proporção encontrada em *A. bokermanni*, na qual 81% das ninhadas apresentaram paternidade múltipla (GAIOTTI *et al.*, 2020). Três hipóteses principais foram propostas para explicar por que fêmeas realizam múltiplas cópulas: 1) é uma maneira de assegurar a fertilidade, caso o parceiro inicial seja infértil (BIRKHEAD *et al.*, 1987); 2) para criar oportunidades de escolha pós-copula, quando os sinais dos machos não são honestos (PARKER, 1970); e 3) para aumentar a chance de diversificar a variabilidade genética da ninhada (WATSON, 1991). As três hipóteses não são excludentes, e poderiam explicar as elevadas taxas de paternidade múltipla no gênero *Antilophia*.

Além disso, nossos resultados indicam que fêmeas não escolheram copular apenas com os machos que sinalizariam melhor qualidade: das seis ninhadas no qual foi encontrada paternidade múltipla, em duas delas machos adultos (em plumagem definitiva) compartilharam a paternidade da ninhada com um macho jovem (em plumagem verde) e em outras duas ninhadas, compartilharam com dois machos em plumagem pré-definitiva. Várias espécies na família Pipridae passam por diversos estágios de plumagens intermediárias até atingirem a plumagem adulta (KIRWAN e GREEN, 2012; SCHAEDLER *et al.*, 2021), muitas vezes atingindo esse último estágio apenas quatro anos depois (*Chiroxiphia linearis*; LUKIANCHUK e DOUCET, 2014; *Chiroxiphia caudata*: MALLET-RODRIGUES e DUTRA, 2012). Esse padrão de ciclos prolongados de muda também é constatado nos machos de *A. galeata*, no qual machos podem levar pelo menos três anos para atingirem a plumagem definitiva (KAJIKI e MACEDO dados não publicados). Apesar de machos em plumagem verde ou pré-definitiva serem usualmente denominados de ‘machos imaturos’, esses machos estão aptos à reprodução (FOSTER, 1987; MARINI, 1989; MARINI, 1992; GAIOTTI *et al.*, 2020). Os resultados apresentados aqui, apesar de limitados, sugerem que machos jovens podem apresentar maturidade sexual e conseguir cópulas que resultam em fertilizações. Ademais, na espécie-irmã *A. bokermanni*, a proporção de sucesso reprodutivo adquirido por machos jovens foi elevada e eles adquiriram paternidade de 48% dos ninhegos (GAIOTTI *et al.*, 2020).

Existem três principais hipóteses para a função do atraso na muda e potenciais benefícios adquiridos por machos jovens em plumagens não definitivas: a de cripsis, no qual esses machos seriam menos detectados por predadores; a de mimetismo, no qual esses machos seriam confundidos com fêmeas e assim conseguiriam entrar nos territórios de adversários; e a de sinalização de status, no qual a plumagem sinaliza explicitamente o menor status social e reprodutivo, reduzindo a agressão por machos dominantes (HAWKINS *et al*. 2012). No caso dos piprídeos, a última hipótese foi sugerida como mais plausível (SCHAEDLER *et al.*, 2021), e pode se estender a *A. galeata*. Observações de machos em plumagem verde e pré-definitiva com machos adultos já foram reportadas (MARINI, 1992; MARINI e CAVALCANTI, 1992; MARÇAL e LOPES, 2019b), e durante os experimentos de territorialidade também avistamos essa dinâmica (Capítulo 2). Em uma ocasião, realizamos esse mesmo experimento de territorialidade, porém apresentamos um modelo taxidermizado na plumagem verde acompanhado de *playback* com as vocalizações típicas de machos e de fêmeas. Não houve resposta ou aproximação de outros machos; ao invés disso, uma fêmea anilhada que nidificou em anos consecutivos próximo do local onde conduzimos o experimento se aproximou gradualmente do modelo até ficar a menos de 2m de distância. A fêmea não emitiu nenhum som, e também não atacou o modelo, porém ficou observando-o por ca. 3 min. Em outra ocasião, próximo a um ninho ativo onde se encontrava uma fêmea incubando, realizamos o mesmo tipo de experimento, introduzindo um modelo em plumagem verde. Tanto a fêmea anilhada quanto um macho em plumagem pré-definitiva se aproximaram da área, mas não demonstraram interesse no modelo. Apesar de serem observações anedóticas, a ausência de respostas do macho adulto territorial pode indicar maior tolerância a indivíduos na plumagem verde, que poderiam estar sinalizando status de subordinado. Acreditamos ser improvável que machos jovens na plumagem verde consigam cópulas por mimetizarem a plumagem de fêmeas, pois fêmeas parecem ser intolerantes a outras fêmeas, pelo menos nas áreas onde nidificam durante o período reprodutivo. Em uma ocasião, observamos um indivíduo na plumagem verde (sem anilhas) atacar uma fêmea que estava incubando, e ambos indivíduos saíram em uma rápida perseguição. Poucos minutos depois, a fêmea anilhada retornou a incubar o ninho, e o outro indivíduo não foi avistado novamente nos 30 min seguintes. Fêmeas em geral se mostraram bastante agressivas, inclusive, com indivíduos de outras espécies de aves que se aproximaram dos ninhos (Capítulo 1).

Por fim, a ausência de cuidado biparental (Capítulo 1), os indicativos de execução de *display* solitário com evidências de territorialidade (Capítulo 2), o desvio reprodutivo entre machos (Capítulo 3) e as evidências de poliginia e de paternidades múltiplas nas ninhadas (Capítulo 3) sugerem que *A. galeata* não possui um sistema de acasalamento monogâmico como inicialmente proposto. No entanto, os dados do nosso trabalho não permitem uma classificação inequívoca de *A. galeata* em uma categoria específica de sistema de acasalamento. Uma possibilidade seria a classificação do sistema como de *lek* solitário poligínico. Esse tipo de *lek* possivelmente derivou do *lek* tradicional, no qual machos se distribuem em múltiplas áreas de *display* ou cortejo (também chamadas de zonas de empoleiramento ou arenas) agregadas; no *lek* do tipo solitário, as arenas são isoladas e fora do alcance visual ou auditivo. Contudo, no presente estudo machos territoriais possivelmente conseguem escutar outros machos com territórios, pois muitas vezes a distância entre eles é de algumas centenas de metros (Capítulo 2). Essa configuração espacial possivelmente resulta da restrição de hábitat, limitada na população estudada às estreitas matas de galeria que percorrem cursos d’água. Outro desvio dessa possível classificação do sistema de acasalamento em *lek* é a ausência de *displays* elaborados, restando apenas resquícios de comportamentos possivelmente mais complexos.

Os resultados desse estudo nos possibilitaram responder algumas questões básicas sobre o sistema de acasalamento da espécie, porém outras questões surgiram ou se mostraram bastante complexas que requerem aprofundamento em estudos futuros. O padrão de distribuição espacial bastante disperso dos machos e suas ninhadas, por exemplo, compelem ao questionamento do porquê fêmeas estão se deslocando tanto para encontrar parceiros. Seria importante que os próximos estudos priorizem avaliar a distribuição dos recursos alimentares de *A. galeata* e como isso poderia afetar a capacidade de dispersão dos adultos – por exemplo, as fêmeas talvez necessitem buscar alimento em áreas distantes e, eventualmente, também encontrem parceiros. A análise da compatibilidade genética (e.g., heterozigosidade) entre machos e fêmeas pareados também é interessante para esclarecer a hipótese dos custos da endogamia. Além disso, em função da heterogeneidade do habitat de *A. galeata* ao longo de sua distribuição no Cerrado, populações possivelmente diferem nas proporções e intensidades de poliginia e poliandria. Assim, estudos que investigassem as relações de parentesco em outras populações situadas em fragmentos que estejam sob diferentes níveis de limitação de habitat forneceriam evidências para a discussão do efeito do ambiente no comportamento da espécie e as consequentes implicações evolutivas no sistema de acasalamento.

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CAPÍTULO 4

Less is more: female choice for discrete and simple ornaments in helmeted manakins (*Antilophia galeata*) (AVES: Pipridae)

Uma versão deste capítulo será submetida para publicação no periódico *Animal Behaviour* e está redigido de acordo com as normas de formatação da revista.

Less is more: female choice for discrete and simple ornaments in helmeted manakins (*Antilophia galeata*) (AVES: Pipridae)

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Abstract

The manakins (family Pipridae) are neotropical birds known for their striking sexual dichromatism and elaborate courtship displays performed in lekking arenas. The helmeted manakin was considered the only case of monogamy in the family, but recent findings suggest males are polygynous and defend individual territories where they display solitarily. They do not perform elaborate courtship exhibitions, but their plumage is highly contrasting and conspicuous, and the advertising song is loud and different from other manakins. Thus, we tested the hypotheses that ornaments function as indicators of males' quality and have evolved via female choice. We investigated the interrelationship between plumage coloration, song, and putative indicators of male quality. We further assessed if the secondary sexual traits are associated with female choice using molecular parentage analysis. We captured individuals and obtained plumage coloration data using reflectance spectrometry and avian visual models; song acoustic parameters were extracted from recordings of the adult males. A scaled mass index and the leucocyte profile were used as indicators of male quality, and we developed a set of generalized linear models and regressions to test our hypotheses. We found that plumage coloration and song are correlated with indicators of health and body condition. Furthermore, females preferred males with shorter songs, duller mantle feathers, and reduced achromatic contrast between mantle and breast regions. Our results indicate that the secondary sexual traits of the helmeted manakin appear to be under sexual selection via female choice but potentially provide different information depending on whether the targeted viewers are females or competing males.

Keywords: Cerrado, condition-dependent, endemic, mate choice, multiple ornaments

Introduction

Birds are well-known for exhibiting multiple types of sexual secondary traits, from extremely loud songs to mesmerizing courtship displays. In general, ornaments are often designated as secondary sexual traits. Some of them reach such high levels of exaggeration that it becomes an arduous task to explain their evolution apart from sexual selection theory (Darwin 1871). In many species, these secondary sexual traits encompass distinct signals such as song, plumage, and display behaviour (e.g., courtship dances), and generally they may convey information on the owner's quality (Zahavi 1977; Andersson 1994). It has been proposed that mate choice should favour a single most-revealing signal at the expense of others (Johnstone 1996). Alternatively, it has been suggested that multiple quality-revealing sexual ornaments could evolve under specific circumstances (Johnstone 1995).

Several hypotheses attempt to explain the evolution of multiple ornaments. However, it can be preferentially attributed to the Fisherian runaway selection hypothesis and handicap (Zahavi 1975, 1977) or index processes (Maynard-Smith and Harper 2003). In the first, exaggerated traits evolve as the selection on the preference increases (Fisher 1915; Fisher 1958), and in the second, ornaments may reach more elaborate forms due to intrinsic costs (i.e., handicaps: Zahavi 1975, 1977) or constraints (Podos 2021) that favour those males that can sustain such detriments. Then, selective females could rely on the expression of these costly-ornaments to assess male quality (Andersson 1994). Secondary sexual traits may also contain information on genetic quality (e.g., disease resistance) and could be selected through mate choice (good genes hypothesis: Weatherhead and Robertson 1979; Hamilton and Zuk 1982; Ryan 2018). Finally, secondary sexual characters may be expressed as multiple ornaments. Three main hypotheses suggest plausible explanations for how such ornaments could have evolved: 1) the signal amplification hypothesis, in which amplifying traits that are not the immediate object of choice increase mating success of the higher quality males (Hasson 1989); 2) the enhanced signal detection hypothesis, in which multi-components of a sexual display enhance signal reception in receivers (Rowe 1999); and 3) the multiple receiver hypothesis, in which ornaments convey information that is differentially assessed depending on the receiver's sex (Andersson *et al.* 2002).

The helmeted manakin is a sexually dimorphic and large (~ 21 g) piprid that belongs to the neotropical manakin clade (family Pipridae). Most species in this avian family have highly sexually dichromatic plumage, and males perform elaborate courtship displays in communal arenas known as leks (Kirwan and Green 2012; Höglund and Alatalo 2014). Like other lekking birds, males in this family do not provide parental care, and male mating success is highly skewed, characterizing a polygynous lekking mating system (Höglund and Alatalo 2014). The evolution of exaggerated secondary sexual traits in manakins is thought to be chiefly driven by sexual selection via female choice (Darwin 1871; Andersson 1994; Prum 1998; McDonald *et al*. 2001). However, courtship displays have so far been the central multi-component ornament investigated and viewed as resulting from female choice (Théry 1992; McDonald and Potts 1994; Duval 2007; Anciães and Prum 2008; Lukianchuk and Doucet 2014; but see Trainer and McDonald 1995, McDonald *et al.* 2001, Stein and Uy 2006, Ryder *et al.* 2010 and Schaedler *et al.* 2019 for other components analysed).

Historically, the helmeted manakin was hypothesized as a unique case of monogamy in the family (Marini and Cavalcanti 1992), but recent investigations have questioned this assumption (Marçal and Lopes 2019) (Chapter 2). The sole sister species, Araripe manakin (*Antilophia bokermanni*), is polygynous (Gaiotti *et al.* 2020), and molecular parentage analysis has confirmed that the helmeted manakin is polygynous as well, with high levels of female polyandry and high reproductive skew among males (Chapter 3). Males appear to display in 'solitary' leks (sensu Prum 1994), but contrary to most lekking manakins, the helmeted manakin defends individual territories and has a simple male display in the form of frequent advertising vocalizations around its territory associated with a few postures (Chapter 2). Nonetheless, male plumage is highly contrasting, with most of the body covered by black plumage contrasting a bright crimson frontal crest and mantle. Additionally, males sing throughout the year (Marini 1992) a rollicking (Ridgely and Tudor 2009), 'noisy, sonorous, and varied song, rather different from other manakins' (Sick 1967).

Body and health (i.e., immunological) conditions are widely used to assess individual quality in birds (Davis *et al.* 2008; Labocha and Hayes 2012). Body condition has been related to birds' survival, reproduction, and behaviour and is often correlated with fat content in birds and used as a measure of energy reserves (reviewed by Labocha and Hayes 2012). Among the many different parameters that may be used to assess an individual's health condition, leucocyte profiles are particularly useful because they may be altered by stress and related to stress hormone levels (Davis et al. 2008). For example, in the helmeted manakin, lymphocytes were positively correlated with tick infestation (Ribeiro *et al*. 2021). Notably, the heterophil to lymphocyte ratio (H/L ratio) is considered a robust measure of physiological stress in vertebrates, and it has been reported to increase in response to parasitic infection (Davis *et al.* 2004; Lobato *et al.* 2005; Minias 2019) and with susceptibility to infection (Al-Murrani *et al.* 2002). High H/L ratios were associated with reduced growth in pied flycatcher (*Ficedula hypoleuca*) nestlings, indicating a high level of stress and worse health condition that affected growth (Moreno *et al*. 2002).

Previous studies found that colour properties of the crimson-red plumage in helmeted manakin males were associated with the individual condition, and males with brighter feathers exhibited a better body condition (Silva *et al.* 2017). The red colour in animals is mainly derived from carotenoid pigments obtained from the diet (Latscha 1990), thus imposing a cost to birds with carotenoid-based plumage that will spend more time and energy to acquire food with those pigments when resources are limited. Carotenoids are known to be immune boosters (Chew and Park 2004), and it has been hypothesized that there is a trade-off in carotenoid allocation between immune functions and ornament exhibition (Lozano 1994; Mougeot *et al.* 2007). Thus, variations in the colour parameters of carotenoid-based feathers potentially indicate overall male quality (Hill *et al.* 2002; Weaver *et al.* 2018).

Songs may also reliably convey information on individual quality because their expression is limited by the properties of vocal mechanisms (Podos *et al.* 2004) and affected by immune activation (Saino *et al.* 1997; Owen-Ashley *et al.* 2006; Munoz *et al.* 2010). Manakins have a wide array of vocalizations (Kirwan and Green 2012), and song performance and coordination in leks may function as an important cue that females can assess (Trainer and McDonald 1995; Duval 2007; Schaedler *et al.* 2019). The helmeted manakin does not perform duets or any coordinated songs; thus, other acoustic parameters, such as song duration, might reveal male quality and be favoured by females. For example, in white-crowned sparrows (*Zonotrichia leucophrys*), females prefer males with longer songs (Wasserman and Cigliano 1991).

In this study, we hypothesized that ornaments and songs might function as indicators of male quality in helmeted manakins, and males bearing and performing these exaggerated traits would signal their condition to females and have higher mating success. We predicted that males in better conditions would exhibit exaggerated ornaments (i.e., brighter, more intense hue of red plumage and greater contrast among plumage patches) and sing higher performance songs, and thus sire more nestlings. To test this hypothesis, we investigated how two types of sexual signals (plumage colour and song) of male helmeted manakins can potentially convey information about male quality. To this end, we evaluated the relationship between male plumage coloration and song with two putative indicators of male condition (i.e., immunological and body condition, Fig. 1). We then investigated whether these traits are under sexual selection and are potentially selected via female choice.

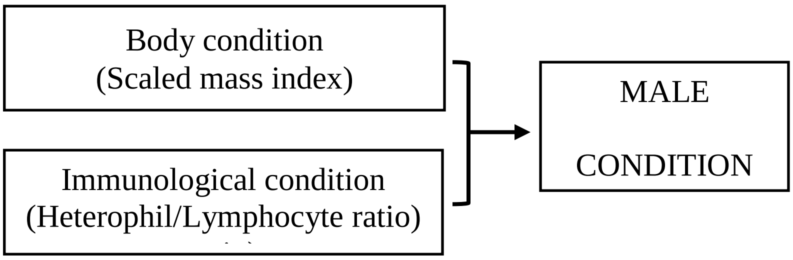

Figure 1 A scheme summarizing the two parameters we used to assess male condition.

Methods

General field methods and study animal

Fieldwork was conducted from 2017 to 2020 at the “Fazenda Água Limpa”, an experimental farm that belongs to the University of Brasília. The farm is located near the city of Brasília (15° 56’ 56.1” S, 47° 56’ 02.6” W), in central Brazil, and is characterized by 4,500 ha of Cerrado savanna traversed by strips of humid gallery forest. The local climate has two well-defined seasons, one that is hot and rainy (October – April) and another that is cold and dry (May – September) (Almeida *et al.* 2014). Our study area encompassed some 120 ha of gallery forests bordering three main rivers within the farm.

Helmeted manakins are neotropical birds mainly distributed within the Cerrado region in South America, ranging from central Brazil to north-eastern Bolivia and Paraguay (Snow and de Juana 2020). This species belongs to the Pipridae family, birds known for their striking plumage dimorphism and complex courtship displays. Helmeted manakins (~21 g) are sexually dichromatic, with adult males characterized by a bright crimson frontal crest and mantle, contrasting with the uniform black body plumage. Males sing throughout the year, with peaks in vocal activity before and during the breeding season, from August to December (Marini 1992). The main song is characterized by seven to eight short and fast ascending notes, resulting in a distinctive and rollicking song, as Ridgely and Tudor (2009) described.

We captured individuals with mist-nets and banded them with numbered aluminium bands from the National Center of Research and Bird Conservation (CEMAVE, ICMBio, Brazil) and with a unique combination of three coloured plastic bands (research permits SISBIO 58094-3 and CEMAVE 4221) to allow identification and monitoring of individuals in the field. Nesting females were captured with mist-nets only during the nestling stage or when eggs were in the final stages of development.

We searched for nests between August and January of three breeding seasons (2017 – 2020). We located nests by following birds carrying food or nest material and by searching vegetation within areas where males were continuously observed and along the length of rivers. Females often re-nest in a particular area (LNK, pers. obs.); thus, we searched more intensely in a 25 m radius from the nest in the following months and breeding seasons when a nest was found.

Male quality (body and immunological conditions)

We used morphological parameters and immunological condition to assess individual condition, used as a proxy for male quality. Here, body condition is represented by an index of body mass, and immunological condition by the heterophils/leucocyte (H/L) ratio, described below. For all the 48 sampled males in adult plumage, we measured beak length, depth and width, wing chord (unflattened wing length), tarsus length, and body length (from the tip of the beak to the end of the tail) with a caliper, and body mass with a 30 g (accuracy 0.25 g) spring scale. Because morphometrics and body mass often present a linear relationship, we adopted the scaled mass index (SMI herein) to estimate the total energy reserves following Peig and Green's (2009) recommendations. The SMI was computed with the equation:

$$\widehat{M_\iota} = M_i \left[\frac{L_0}{L_i}\right]^{b_{SMA}}$$

Where M_i and L_i are the body mass and the linear body measurement of individual i, respectively; L_0 is the arithmetic mean value for the study population; b_{SMA} is the scaling exponent estimated by the SMA regression of M on L, and $\widehat{M_\iota}$ is the predicted body mass when the linear body measure is standardized to L_0.

To derive the SMI, we first analyzed Spearman's correlation coefficients among all morphometric variables in a log-log scale and selected the one with the highest correlation with body mass. None of the morphometric measurements were highly correlated with body mass ($r_S \leq 0.50$, Supplementary Information Table S1), but the highest correlation was with beak height ($r_S = 0.32$). Therefore, we derived the SMI from beak height.

We placed single drops of blood on slides and produced smears that were allowed to air dry, then fixed with 100% methanol and stained with the Instant-Prov kit

(NewProv®). Differential leucocyte counts were carried out with a light microscope (1000x magnification with oil immersion) at the Laboratório Veterinário Santé in Brasília, Brazil. In each smear, a total of 100 leucocytes were counted; therefore, heterophils, lymphocytes, eosinophils, and monocytes were calculated as percentages. Across the three breeding seasons, we obtained and analyzed blood smears from 37 adult males (2017: 14 individuals; 2018: 12 individuals; 2019: 11 individuals). Due to the absence or reduced quantities of monocytes and eosinophils in most blood smears, only heterophil/lymphocyte (H/L) ratios were included in statistical analyses. All birds were sampled within 30 minutes of capture to ensure that H/L ratios were not affected by routine handling (Davis 2005).

Colour measurements and visual modelling

We plucked ten feathers from the crest, mantle, and breast regions of all the 50 adult males caught, stored in small plastic bags covered with aluminium foil, and kept in the dark at room temperature. After that, reflectance spectra were obtained with the spectrometer Ocean Optics USB4000 with a PX-2 pulsed xenon light source (Ocean Optics, Dunedin, Florida). We calibrated the spectrometer with a white reflectance standard of Barium Sulphate ($BaSO_4$). The fibre was positioned over a surface covered with black tape in darkroom conditions to obtain the black reflectance standard. We performed this calibration procedure at the beginning of every reading session. The ten feathers were stacked in the same orientation to simulate their natural configuration over the bird’s body and mounted over the same black surface used for calibration. The bifurcated fibre-optic cable was fitted at the end with a cylindrical probe to standardize the measuring distance from the feather and block ambient light. The probe was positioned at a 45° angle to the feather surface. We took three readings for each feather assemblage (crest, mantle, and breast) in randomly chosen points about 2 mm apart, lifting the probe between each measurement. We used the software OceanView (Ocean Optics, Dunedin, Florida) to analyse readings between 300 and 700 nm, as most birds are sensitive to ultraviolet (UV) wavelengths (300-400 nm; Cuthill *et al.* (2000), and 700 nm is probably the upper limit of the vertebrate visual spectrum (Jacobs 1981).

We used the package "pavo" (Maia *et al.* 2019) in RStudio 1.4.1103 (RStudio, Inc., Boston, MA, USA) running the R version 3.6.3 to import, process, and extract plumage reflectance from spectra data. We calculated the mean relative reflectance brightness for each plumage area. We obtained colour variables of hue ('theta' and 'phi') and chroma (r achieved or r_a) (see Stoddard and Prum 2008 for details) considering an avian visual model to ensure that our spectra analyses did not overlook variances perceived by birds but not by humans. This is a flexible way to represent stimuli, in which photon catches are expressed in relative values. Here, a tetrahedron encompasses all colours perceived by birds, and each of the vertices represents the maximum stimulation of a particular cone type. The values for hue (theta and phi) are both angles measured from the achromatic origin. Theta is the angular displacement around a circle parallel to the tetrahedron base, where perceived red-greens are close to 0°, reds and purples are negative, and greens and blues are positive. Phi is the vertical angle and represents the UV/Violet (UV/V) contribution to perceived colour, with more positive values indicating more UV/V perceived. R achieved is an estimate of the ratio of r to the maximum possible chroma along the hue vector (Dakin and Montgomerie 2013). Four types of single cones that are sensitive to very short (VS), short (S), medium (M), and long (L) wavelengths mediate the perception of colour in birds (Hart 2001b). Therefore, we computed quantum catches (*Qi*) for each cone type under a blue tit (*Cyanistes caeruleus*) visual system, considering forest shade as an illuminant and the gallery forest as background. The receptor quantum catches were calculated with the equation:

$$Q\iota = \int_{\lambda} R\iota(\lambda)S(\lambda)I(\lambda)d\lambda$$

Where λ represents the wavelength, $R\iota(\lambda)$ the spectral sensitivity of the receptor ι, $S(\lambda)$ the reflectance spectrum of the colour, and $I(\lambda)$ the illuminant spectrum.

We calculated chromatic and achromatic contrasts across the three body regions (crest, mantle, and breast) of helmeted manakin males in adult plumage (N = 50) by estimating colour distances. The procedure weighs the Euclidian distances of photoreceptor quantum catches by the Weber fraction of the cones under the receptor-noise limited (RNL) model (Vorobyev *et al.* 1998; Vorobyev *et al.* 2001). These distances are referred to as delta S (ΔS) for chromatic distances and delta L (ΔL) for achromatic (luminance) distances and are expressed in Just Noticeable Distances (JND) (Vorobyev *et al*. 2001). Values lower than 1 are predicted to be indistinguishable, and distinctiveness increases with distance to 1. We assumed a Weber fraction of 0.1 for the long-wavelength sensitive photoreceptor (Hart *et al.* 2000), and relative cone proportions for the blue tit (wavelengths: UV = 1, short = 1.92, medium = 2.68, long = 2.7; Hart (2001a)). Chromatic and achromatic contrasts indicate how two different spectra are perceived given the visual space of the receiver, and values > 1 are considered discernible by birds (Vorobyev *et al.* 1998; Endler and Mielke 2005).

Song measurements

During the monitoring of adults, we conducted recording sessions whenever a banded adult male was found actively singing. Recording sessions were initiated after the bird was identified and continued for at least five minutes and a maximum of 15 minutes. We recorded uninterruptedly until the bird flew away or moved to another perch. The sessions occurred from 9h00 to 16h00, when adult males were most active. We used a Zoom H5n recorder with Sennheiser ME66 unidirectional microphone.

Our protocol focused on sampling the maximum number of males per day as possible; therefore, the combined sample from a particular male was relatively short. This sampling protocol did not allow accurate quantification of performance and song complexity variables, such as total time spent singing and the number of songs performed. Instead, we focused on variables that could be assessed over short listening periods (see below).

We only used recordings of the main song of 15 adult males, which consists of three syllables (Fig. 2). The first syllable contains three to four fast, low-pitched, ascending whistles, and the second and third syllables contain a lower-pitched whistle followed by a higher-pitched ascending whistle with greater frequency bandwidth, which starts at ~ 2Hz and quickly reaches ~ 4Hz. This is the main song of helmeted manakin adult males, potentially used during their display to advertise and acoustically defend a territory (Chapter 2).

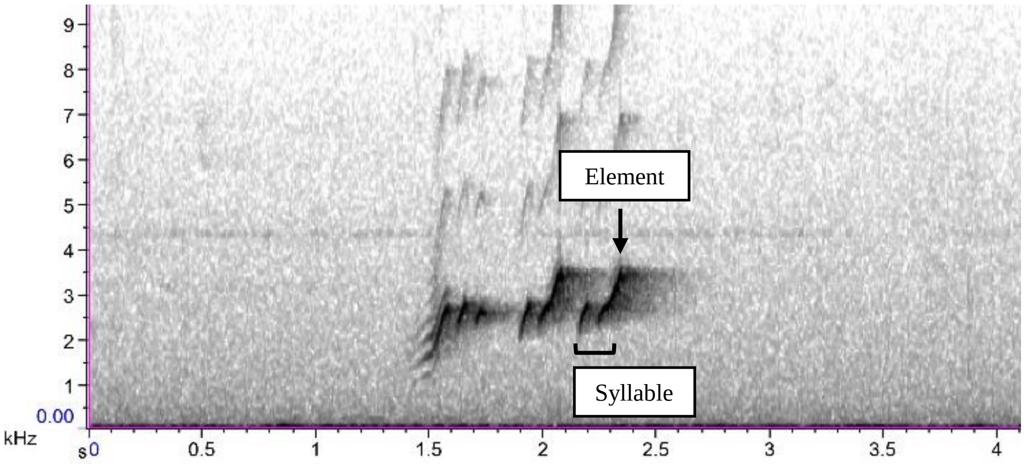

Figure 2 The distinctive rollicking song of helmeted manakin represented as a sonogram.

We extracted 20 song metrics, following Odom *et al.* (2021), and assessed if any variables were redundant with a Spearman correlation. This left us with seven uncorrelated variables ($r_S < 0.70$), which include mean song duration 90%, frequency bandwidth 95%, frequency bandwidth 5%, maximum frequency 25%, frequency excursion index, number of elements, and maximum number of inflections (see Supplementary Information Table S2 for a detailed description). Selections of sounds were first conducted in Raven Pro 1.6 (Cornell Laboratory of Ornithology, Ithaca, NY; www.ravensoundsoftware.com), and acoustic parameters were obtained with the R package "warbleR" (Araya-Salas and Smith-Vidaurre 2017).

Molecular methods

Blood samples (~ 50 µl) were collected through brachial venipuncture and stored in 99% ethanol at - 4° C. We extracted DNA from blood samples following the phenol:chloroform and precipitation with ethanol protocol (Sambrook *et al.* 1989), and sexed adults and nestlings with P2/P8 primers (Griffiths *et al.* 1998; Çakmak *et al.* 2017). We genotyped 12 polymorphic microsatellite markers (see Supplementary Information Table S3) and assigned parentage to 22 nestlings from complete clutches found in 11 nests of the 2019/2020 breeding season. We genotyped nine females observed attending these nests and 45 males captured in the surrounding areas (ca. 250m from the nest) from 2017 – 2020.

PCR amplifications were performed in 11.5 µl reaction volumes using 50 ng genomic DNA, 1 pmol of unlabelled forward primer with the M13 sequence (5'- 3': CAC GAC GTT GTA AAA CGA), 3 pmoles of unlabelled reverse primer, 2 pmoles of M13 primer labelled with 6-FAM, VIC or NED (Applied Biosystems) dye, 1.2 µl PCR Buffer 10x (Invitrogen), 0.4 µl dNTPs (25 mM), 0.4 µl MgCl2 (25mM), and 0.5 U Taq polymerase (5 U/µl, Invitrogen). General conditions of the PCR were as follows: 94°C (7 min), 35 cycles at 94°C (30 s) / annealing temperature (Supplementary Information Table S3; 30 s) / 72°C (30 s), followed by an extension at 72°C for 10 min. PCR amplicons from one to three loci labelled with different dyes were combined in appropriate dilution ratios with sterile water, formamide, and a size standard (ROX 500; Perkin-Elmer) to run on an ABI 3730 automated capillary sequencer.

Parentage analysis

Allele sizes were scored manually using the software GeneMarker 2.6.3. We used CERVUS 3.0.0 (Kalinowski *et al.* 2007) to assign parentage of nestlings. We calculated the allelic diversity, observed (Ho) and expected heterozygosity (He), exclusion probability for the first (NE-1P) and second candidate parent (NE-2P), Hardy-Weinberg equilibrium (H-W), and frequency of null alleles (Fnull). We obtained allelic frequencies from 77 individuals, and typing error was low (~ 7.8%) for most of the markers used (except for loci Abom12). We assumed females attending nests were the biological mothers and that the mother-offspring relationship was thus known for all nestling samples analysed. We assumed all males could be candidate fathers and thus included all individuals captured with mature male plumage or sexed as males in the paternity analysis.

We genotyped 45 candidate males (ca. 34% of males sampled), nearly completely typed at the 12 loci (70%). We assigned parentage using relaxed (50%) and strict (95%) confidence levels by calculating the likelihood ratio scores (hereafter LOD) as well as using a “total evidence” approach. This assignment approach uses simulations to determine differences between the most likely candidate parents and produce confidence levels in assignments (Jones *et al.* 2010). First, we selected the male from the population with the highest trio LOD score as the most likely sire. The trio LOD score is the log-likelihood ratio for a parent-offspring relationship between the candidate parent and the offspring given the known parent. Second, we used a “total evidence” approach for each paternity assignment to determine if the CERVUS assignment was reasonable (Webster *et al.* 2004; Ryder *et al.* 2010).

We rejected the CERVUS assignment and assigned paternity to a lower-ranked male under these circumstances: 1) if males had similar LOD scores, but the lower-ranked males had fewer mismatches; 2) if males had a single mismatch, but the lower-ranked male’s mismatch indicated the presence of a null allele; 3) if males had similar LOD scores and the same number of low mismatches (zero or one), but other evidence (as follows) suggested that the lower-ranked male was a more likely sire. In the latter situation, we considered whether either male was the territory owner closest to the nest or male’s mismatch was caused by a scoring error (e.g., alleles that mismatched differed by only one repeat unit). Finally, when CERVUS was unable to assign parentage with both confidence levels (i.e., the trio LOD scores were negative for all candidate fathers for an offspring), we assigned paternity for the individual with the highest pair LOD score and minimum loci mismatches. Our preliminary simulations used 10,000 cycles and 0.02% as per locus genotyping error. CERVUS measured true typing error as the percentage of mismatches between identified mothers and offspring.

Statistical analyses

We first predicted that ornaments and songs might signal male quality. In order to test it, we performed a series of models and regressions for each set of variables. First, we fitted generalized linear models (GLM) to check whether colour parameters (predictor variables) predicted health condition (H/L) and body condition (SMI) in helmeted manakin adult males. We used Gaussian family and identity link to develop models with SMI as the response variable and Gamma family and log link to develop models with H/L as the response variable. Second, due to the low number of males that yielded good quality recordings, we conducted separate linear regressions between the song parameters and H/L ratio and SMI.

Next, in our second hypothesis, we asked whether individual coloration and acoustic song parameters predicted a higher probability of paternity among males. We fitted a set of candidate binomial GLMs with song and coloration parameters as potential predictors and the paternity of nestlings as a binary response variable. Males were assigned 1 when they sired at least one nestling and 0 when they did not sire any nestlings. We generated distinct models for each type of variable (song and plumage) and separate models for the body regions when analysing plumage. Multicollinearity can be a problem when using several independent variables; therefore, we used variance inflation factors (VIFs) higher than ten as a sign of severe collinearity (Thompson *et al.* 2017). We removed independent variables with high VIFs and included only those with low VIF (< 4). The most parsimonious models were chosen by a stepwise-backward procedure using AICc scores, and models with $\Delta AIC_c < 4$ were considered to have support (Burnham *et al*. 2011). Finally, a one-way ANOVA was performed to evaluate whether chromatic and achromatic contrast between colour patches was associated with the probability of siring a nestling (breeding success).

All statistical analyses were performed on RStudio 1.4.1103 (RStudio, Inc., Boston, MA, USA) running R version 3.6.3. Sample sizes differed among statistical tests because not all birds captured and assessed for ornamentation and morphological measures were genotyped for parentage assignment, and not all sexual secondary characteristics were measured in some individuals.

Results

Immunological and body conditions

We captured, banded, and took morphological measurements of 48 males in adult plumage (Fig.3b) and nine females (see Supplementary Information Table S4). Three females molecularly sexed had a few reddish feathers on the mantle, the crest, or both regions. Blood smears and morphometric measurements of 38 adult males were selected to evaluate the immunological condition (through calculation of the heterophil/lymphocyte [H/L] ratio) and body condition (by deriving the scaled mass index [SMI]). We obtained good quality recordings and extracted feathers from eight and 48 individuals, respectively. The mean SMI was 20.56 ± 1.9 (range = 14.16 – 23.9) and mean H/L ratio was 0.27 ± 0.19 (range = 0.02 – 0.92). Body condition was not correlated with immunological condition of birds ($r_S = 0.017$, $P = 0.92$).

Secondary sexual traits and male quality

Correlation between plumage colour and male quality

We obtained the reflectance spectra (Supplementary Information Fig. S1) and the tetrahedral colourspace model for helmeted manakin adult males' crests, mantles, and breast regions (Fig. 3, a summary of values for theta, phi, and r_a are available in Supplementary Information Table S5). Generally, more positive phi values indicate stronger stimulation of UV/Violet cones. In this study, more negative values of theta indicate a slightly weaker stimulation of long-wave sensitive cones and slightly stronger stimulation of short wave-sensitive cones (i.e., more purple hue was perceived) (see Methods). Chromatic contrast between patches (crest-breast and mantle-breast) was high (Supplementary Information Fig. S2), but overall achromatic contrast was not noticeable, except for the mantle-breast contrast (Supplementary Information Fig. S2).

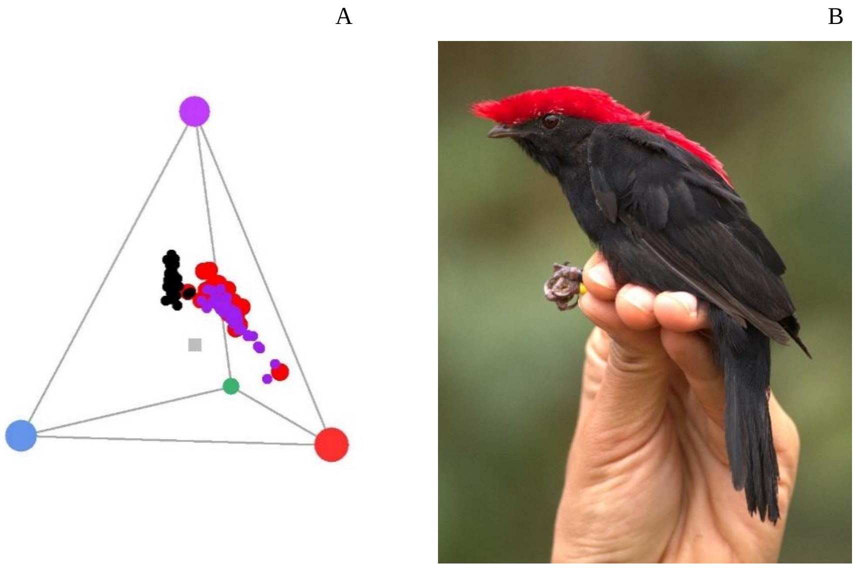

Figure 3 The tetrahedral colourspace model (a) for helmeted manakin in adult plumage (b). Samples of the crest (red dots), mantle (purple dots), and breast (black dots) of 48 helmeted manakin males were obtained. The achromatic point is at the origin, where there is equivalent stimulation of all channels (ultraviolet or violet, short, medium, and long wavelengths represented as the purple, blue, green, and red vertices, respectively).

For all plumage regions, the model with the best fit included the intercept only, but the other models have support ($\Delta AIC_c < 4$) (Table 1). The second most parsimonious model that assessed the relationship between colour parameters and the H/L ratio included theta for crest feathers, and it carried 35% of the explanation found with all models. Theta had a weak negative correlation (GLM; β estimate = -2.00, SE = 1.17, t = -1.72, P = 0.09) with the H/L ratio (Fig. 4). For mantle feathers, theta and phi were selected in the second model with the best fit, and the model carried 33% of the total explanation found in the full set of models. We found that the H/L ratio had a strong positive correlation with theta (GLM; β estimate = 6.41, SE = 2.91, t = 2.20, *P* = 0.04; Fig. 4) and a marginal correlation with phi (GLM; β estimate = 1.85, SE = 0.98, t = 1.88, *P* = 0.07). For breast feathers, the second model with the best fit included brightness, and it carried 30% of the model weight but did not indicate as correlating with the H/L ratio (P = 0.27) (Table 1). Thus, males with a purer red hue and less UV/V perceived in the mantle feathers were in better health condition (less stressed). Healthier males possibly exhibited crest feathers with a less pure red hue (i.e., more purple perceived).

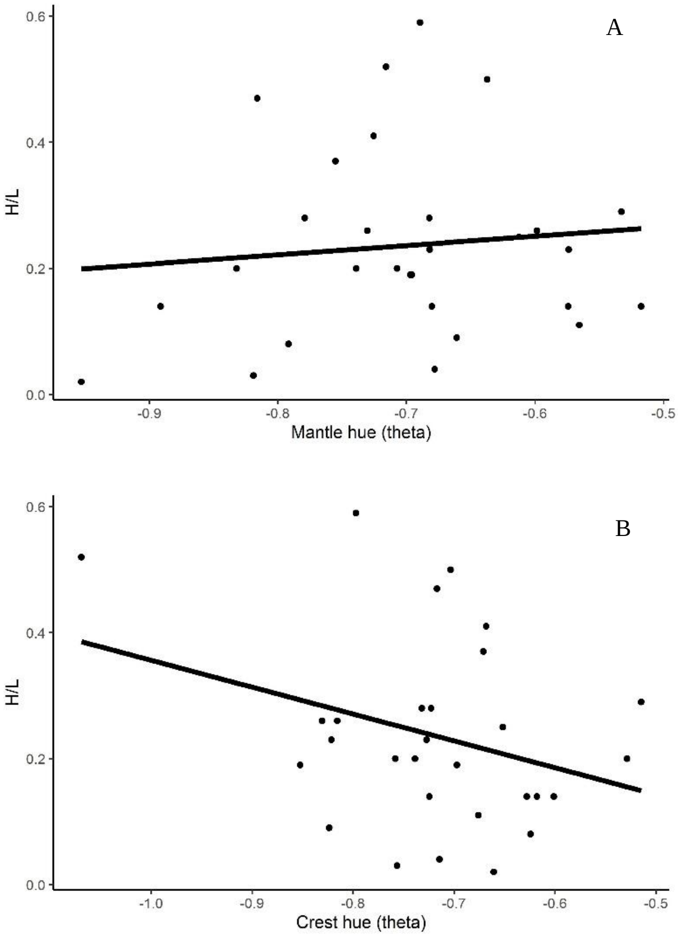

Figure 4 Correlation between hue (theta) from the mantle feathers (A) and from the crest (B) with H/L ratio.

Table 1 Generalized linear models between plumage parameters and H/L ratio (N = 30).

Variables	**Log Likelihood**	**Delta AICc**	**AICc weight**	**Residual deviance**
Crest				
Intercept	16.79	0	0.49	
Theta	17.71	0.66	0.35	13.70
Theta(.) + r.achieved	18.31	1.49	0.17	13.17
Mantle				
Intercept	16.79		0.57	
Theta* + phi(.)	18.84	1.11	0.33	12.74
Theta(.) + phi(.) + r.achieved	19.05	3.61	0.09	12.56
Breast				
Intercept	16.79	0	0.61	
B2	17.30	1.47	0.30	14.06
B2 + r.achieved	17.47	3.83	0.09	13.90

(.) $p < 0.1$; * $p < 0.05$

Only brightness in the mantle feathers had a strong correlation to males' SMI (GLM; β estimate = 0.18, SE = 0.07, *t* = 2.37, *P* = 0.03) (Fig. 5), and the most parsimonious model carried 46% of the model weight. Thus, males with brighter red feathers in the mantle have greater energetic reserves and body condition.

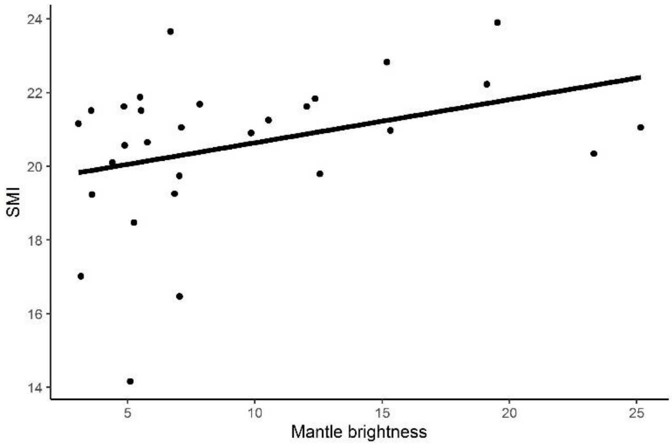

Figure 5 Relationship between the mantle brightness on the scaled mass index (SMI) of helmeted manakin males.

Song and male quality

We analysed seven acoustic parameters of helmeted manakin males' songs (Supplementary Information Table S6). We found that none of the song parameters significantly covaried with the H/L ratio or SMI (Supplementary Information Table S7). However, there was a marginal positive correlation of song mean duration 90% (ANOVA; *P* = 0.06, t = 2.42, *F* = 5.86) with the H/L ratio. This result suggests that males with shorter songs had reduced H/L ratios, thus were less stressed and in better health conditions.

Molecular paternity analysis

The 12 microsatellites were highly variable, with an average of 8 alleles per locus and a high heterozygosity value (Table 2). All offspring and known mothers were genotyped at six or more loci. Our data indicate that most or all of the mismatches were not caused by misassigned maternity, which would be caused by the presumed mother and nestling mismatching at several loci. There were only three cases of mismatches and only one case at multiple loci (i.e., 2 loci).

Table 2 Characteristics of 12 microsatellite loci used in parentage analysis for the helmeted manakin based on 77 samples. Provided in the table are data on number of alleles (k), observed heterozygosity (Ho), expected heterozygosity (He), non-exclusion probability for one candidate parent (NE-1P) and for one candidate father given the known genotype of the mother (NE-2P), departure from Hardy-Weinberg equilibrium (H-W) and frequency of null alleles (Fnull).

Locus	**k**	**Ho**	**He**	**NE-1P**	**NE-2P**	**H-W**	**Fnull**
Chir1-6	3	0.44	0.41	0.917	0.832	NS	-0.034
Chir1-16	9	0.88	0.837	0.507	0.336	NS	-0.030
Chiro5	7	0.8	0.746	0.656	0.478	NS	-0.042
Chiro8	11	0.792	0.886	0.42	0.264	ND	0.046
Chiro10	9	0.863	0.831	0.524	0.351	NS	-0.023
Chiro12	15	0.895	0.876	0.408	0.255	NS	-0.019
Abom6	7	0.789	0.737	0.67	0.493	NS	-0.037
Abom7	11	0.862	0.853	0.475	0.308	NS	-0.013
Abom11	9	0.773	0.729	0.677	0.503	NS	-0.035
Abom12	3	0.294	0.369	0.933	0.839	NS	0.127
Xenat01	6	0.434	0.539	0.845	0.686	NS	0.128
Xenat02	5	0.44	0.543	0.844	0.685	NS	0.124

NS: not significant; ND: not determined

CERVUS assigned a sire to 18 of the 22 nestlings (82%): three were assigned with 95% confidence (strict) and 15 with 50% confidence (relaxed). We accepted all of the high and low confidence assignments using the rules for accepting or rejecting CERVUS assignments (see Methods). From the 46 males genotyped, 13 males (28%) sired at least one nestling, and the majority of these were in adult plumage (*N* = 11 of 13 males). The two juvenile males that sired a nestling had the paternity assigned with relaxed and strict confidence, but both had positive LOD scores of 2.89 and 5.26, respectively.

Effects of male sexual secondary traits on breeding success

Our results from analyses associating probability of paternity and song acoustic parameters suggested that among the seven acoustic variables measured, only mean song duration 90% had a weak correlation with breeding success (binomial GLM; β estimate = -12.45, SE = 7.36, z = -1.69, *P* = 0.09, *N* = 15) (Fig. 6) (Table 3). Males with shorter songs had a higher probability of siring nestlings.

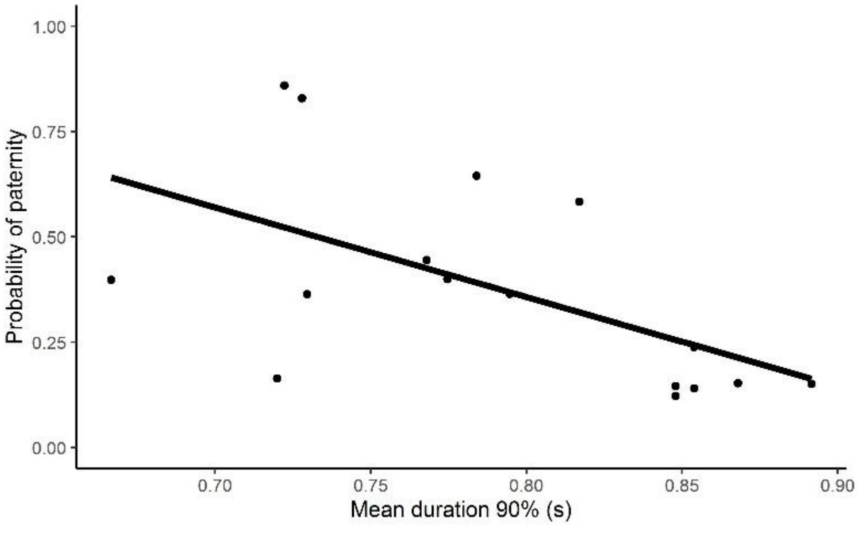

Figure 6 Relationship between probability of a male gaining paternity and mean song duration.

Table 3 Model selection assessing the effects of song and plumage coloration parameters on breeding success.

Variables	**Log Likelihood**	**Delta AICc**	**AICc weight**	**Residual deviance**
Song acoustic parameters (*N* = 15)				
Intercept	-10.59	0.00	0.65	
Mean.duration90(.) + mean.freq.band5	-8.57	1.69	0.28	17.15
Mean.duration90(.) + mean.freq.band5 + max.inflections	-8.08	4.35	0.07	16.17
Colour parameters (crest) (*N* = 18)				
Intercept	-11.46	0.00	0.61	
B2	-10.93	1.49	0.29	21.86
Theta + B2	-10.74	4.03	0.08	21.49
Theta + r.achieved + B2	-10.74	7.40	0.02	21.48
Colour parameters (mantle) (*N* = 18)				
B2(.)	-6.94	0	0.69	13.89
Theta + B2(.)	-6.37	1.78	0.28	12.75
Intercept	-11.46	6.48	0.03	
Colour parameters (breast) (*N* = 18)				
Theta(.)	-8.86	0.00	0.28	17.73
Theta(.) + B2	-7.44	0.07	0.27	14.89
Phi(.) + r achieved*	-7.78	0.74	0.20	15.55
Theta(.) + r achieved	-7.93	1.05	0.17	15.86
Intercept	-11.46	2.64	0.08	

(.) $p < 0.1$; * $p < 0.05$

Our analyses with plumage colour parameters indicated that the probability of siring a nestling was marginally related to mantle brightness (binomial GLM; β estimate = -0.94, SE = 0.5, z = -1.88, *P* = 0.06, *N* = 18) (Fig. 7, Table 3). Furthermore, the most parsimonious model included brightness, and it carried 69% of the model weight. The chromatic (hue-theta, binomial GLM; β estimate = -20.55, SE = 11.46, z = -1.79, *P*= 0.07, *N* = 18) (Fig. 7) component of breast plumage colour was also marginally correlated with breeding success (Table 3), however, the model contained < 30% of the total explanation. Thus, males with duller mantles had a higher probability of siring nestlings.

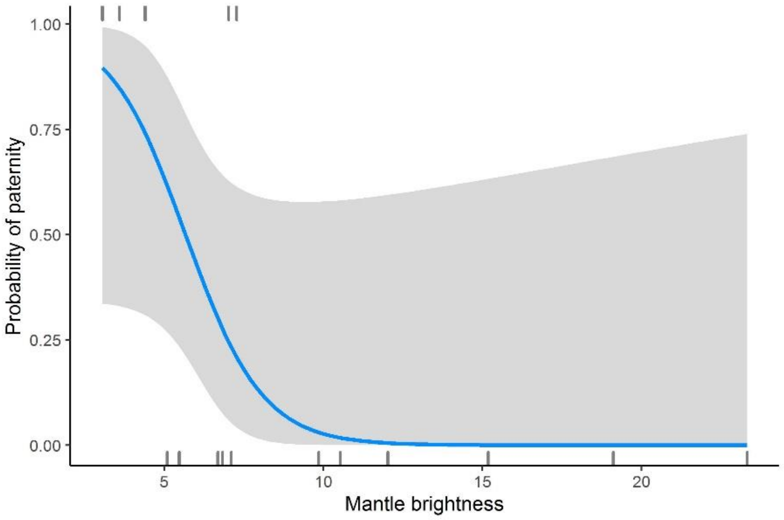

Figure 7 The relationship between brightness from mantle feathers and the probability of males gaining paternity.

We found a statistically significant difference in mean mantle-breast ΔL distances (achromatic contrast) between males that gained and those that did not gain paternity (ANOVA; *P* = 0.05, *F* = 4.54). This suggests that males with lower achromatic contrast between mantle and breast patches had higher breeding success than males with greater contrast (Fig. 8). The colorimetric parameters from the crest feathers did not have correlation with the probability of a male gaining paternity.

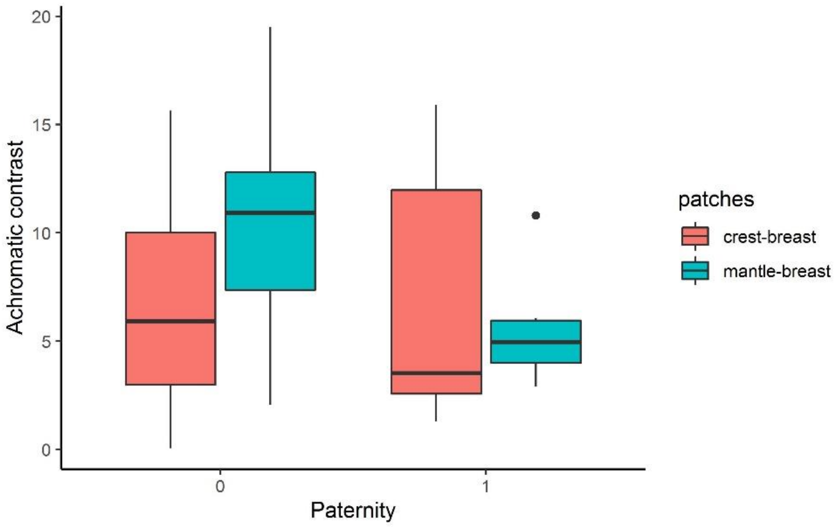

Figure 8 Differences in achromatic contrast between plumage patches and breeding success (0: no paternity and 1: with paternity). Males with less achromatic contrast between mantle and breast patches were more successful (ANOVA; P = 0.05, F = 4.54).

Discussion

This study examined whether sexual secondary traits exhibited by helmeted manakin males possibly reflect their quality and could be selected via female choice. Our results supported our initial prediction that some sexual secondary traits reflect male quality and partially supported our prediction that females prefer males bearing costly secondary sexual traits. First, we found that health condition was negatively correlated with the hue of the red crest and positively correlated with the hue of mantle feathers and song duration. Body condition was only positively associated with feather brightness from the mantle. Second, we found that reproductive success was predicted by the brightness of mantle plumage but not by colour parameters from the red crest feathers. Thus, males with less bright plumage on the mantle had a higher probability of siring a nestling. Finally, our results indicated that females prefer males with reduced achromatic contrast between mantle and breast feathers and males with shorter songs.

In species under strong sexual selection, secondary sexual characters that function as reliable signals of an individual's quality (phenotypic and genotypic), such as plumage coloration, could explain males' high reproductive skew via female choice (Darwin 1871; Fisher 1915; Andersson 1982; Andersson 1986). Plumage colour is a primary sexual ornament among birds, and variations in the components of the colour are usually investigated under the hypothesis that sexual ornaments are condition-dependent due to physical, developmental, or physiological constraints (Hamilton and Zuk 1982). This assumption suggests that colour components can reveal competitive ability or individual quality (Fisher 1915; Zahavi 1977; Andersson 1994).

In the neotropical manakins (family Pipridae), ornamental expression varies significantly among species, and some studies have found a correlation between plumage brightness and male condition. A study with the golden-collared manakin (*Manacus vitellinus*), for instance, demonstrated that the yellow collar brightness was related to the male condition, and females copulated more with brighter males (Stein and Uy 2006). Similarly, high values of brightness and saturation, but not hue, found in red crest feathers of helmeted manakin males have also been associated with greater energetic reserves (Silva *et al.* 2017). We found that males with brighter feathers in the mantle had better body condition (Fig. 4). However, we found no significant correlation between the other colour parameters of helmeted manakin male plumage and energetic reserves. Avian plumage coloration results from melanins, carotenoids, tissue structure, or a mix of these components. Carotenoid ornaments (e.g., coloured bright yellows, oranges, and reds) are solely exhibited by birds if the pigment is included in their diets (Latscha 1990). Therefore, limitations in its acquisition and expression may have significant implications for mate choice (Hill 1991; Hill *et al.* 2002). Carotenoids may be related to different aspects of individual quality, such as body and immune conditions (Hõrak *et al.* 2001; Fitze and

Richner 2002; Beltrão *et al.* 2021). Several experiments that manipulated diet have found an overall effect of nutritional deficiency on carotenoid-based plumage (reviewed in Griffith *et al.* 2006). Thus, we hypothesize that bright plumage may indicate male condition in the helmeted manakin.

Individual quality may be assessed through many parameters. However, leucocyte profiles can be particularly suitable for measuring an individual's quality because they are generally modified by stress and directly associated with stress hormone levels (Davis *et al.* 2008). We found that the H/L ratio was positively correlated with the hue of the red mantle plumage and had a weak negative correlation with the hue of the red crest feathers. These results may indicate that healthier males had a purer hue of red and less UV/V in the mantle feathers and possibly exhibited crest feathers with a less pure hue of red (i.e., more purple perceived). Previous studies have observed a similar pattern in both structural colours (Doucet and Montgomerie 2003; Costa and Macedo 2005; Macedo *et al.* 2012) and pigment colours in plumage (Minias *et al.* 2019). Carotenoids are widely recognized as immune boosters (Chew and Park 2004); thus, less infected helmeted manakin males may be able to allocate more carotenoids to feathers.

However, the opposing effects of health condition on two patches with reduced chromatic contrast suggest that carotenoids may be preferentially allocated to a particular patch to the detriment of the other. Increasing carotenoid content has been related to a decrease in theta values of house finch *Carpodacus mexicanus* feathers, which in this species indicate a shift towards increased stimulation of long versus medium wavelength sensitivity, representing a red-shifted spectrum (Butler et al. 2011). The same pattern is possibly observed in the helmeted manakin; thus, it appears that healthier helmeted manakin males preferentially allocate more carotenoids to the mantle feathers, and males in worse health conditions allocate more carotenoids to the crest feathers. We hypothesize that males adopt this strategy to maximize the display of the carotenoid pigment and reduce the costs associated with using it. Previous studies have found that birds may concentrate pigments into a smaller patch preferentially assessed by females (Hill 1993; Hill 1994) or allocate pigments to certain feathers (Surmacki et al. 2016) to minimize the amount of costly carotenoids used and keep the effectiveness of colour signalling. The deposition and exhibition of carotenoid pigments in large patches, such as the crest and mantle plumage together, is probably more costly than pigment concentration in a single patch (Hill 1993; Hill 1994).

The cost of exhibiting carotenoid pigment is also associated with the specificity of the carotenoid pigment used (Hill 1994). Rhodoxanthin is the primary carotenoid pigment deposited in the red feathers of helmeted manakins and is thought to be converted from two common dietary carotenoids (zeaxanthin and lutein) (Hudon *et al.* 2012). The metabolic pathways conjectured for this conversion suggests that the production of rhodoxanthin requires four extra independent enzymatic reactions followed by a retro configuration (i.e., rearrangement of the molecules), which is rare in animals (Hudon *et al.* 2012; Prum *et al.* 2012) and that could add physiological costs for individuals. Thus, the red mantle of males may convey information on their health condition and thus communicate individual quality.

Efforts in signalling quality are not limited to producing and exhibiting conspicuous colours or patterns in birds' plumage but extend to the rich variation of parameters related to song production (Searcy and Andersson 1986; Kroodsma and Byers 1991; Hutchinson *et al.* 1993; Gil and Gahr 2002; Odom *et al*. 2021). Previous evidence has demonstrated an association between the immunological condition and song expression, indicating that song may also function as an honest signal of health condition (Saino *et al.* 1997; Møller *et al.* 2000; Munoz *et al.* 2010) and could be negatively affected by immune activation (Owen-Ashley *et al.* 2006; Munoz *et al.* 2010). Our data shows a marginal positive correlation between the H/L ratio and song duration, suggesting that males with higher stress levels (worse health condition) performed longer songs. The helmeted manakin song comprises seven to eight ascending whistles divided by very short silent gaps. This song is very stereotyped (but minor variations exist in the number of elements, for example) and is thought to be mainly performed for territorial defence and courtship display (Chapter 2). Possibly, the production of shorter songs may be more challenging because it could require a superior modulation ability to be delivered in a shorter duration (Podos and Nowicki 2004). Furthermore, helmeted manakin song may be subject to performance constraints because inter-syllable frequency shift (i.e., the end of one syllable is produced at a different frequency than the start of the following syllable) is significant (Podos *et al*. 2009). Thus, only individuals in better condition might be able to produce the entire stereotyped song of the species within shorter intervals.

Bird song is widely recognized as a sexually selected trait (Searcy and Andersson 1986; Buchanan and Catchpole 1997; Byers and Kroodsma 2009), but evidence for this is unusually scarce in manakins (but see McDonald 1989 and Duval 2007). For this lekking polygynous group of birds, acoustic signals reach farther than visual elements and are likely initial and long-distance cues that attract females to the lekking arenas (McDonald 1989; McDonald 2010). Our results, however, demonstrate that song may have a more critical role than serving as an attractor to leks. Helmeted manakin males that performed shorter songs had higher breeding success when compared with those that sang longer songs (Fig. 5). Adding to the evidence that song may function as a condition-dependent trait in this species, our results suggest it is also under sexual selection. Helmeted manakins sing the main song throughout the day, possibly for territorial defence (Chapter 2) and attracting females. It is possible that males singing shorter songs are closer to their performance limits and signal their singing ability to rivals (Geberzahn and Aubin 2014) and choosy females. We postulate that this sexual trait may be selected via handicap processes (sensu Podos 2021) and could be explained by females choosing males with genes related to quality and disease resistance (good genes hypothesis) expressed in their singing ability.

In addition to shorter songs being preferred, we found a correlation between males' breeding success and mantle feather colour parameters, in which females preferred males with duller mantle plumage (Fig. 7). In contrast with our initial prediction that helmeted manakin males bearing exaggerated ornaments (i.e., the red crest and mantle) would be preferred by females, no other chromatic plumage parameters were significantly correlated with the probability of siring a nestling. Helmeted manakin males defend their territories from other adult males (Chapter 2), thus a possibility is that these ornaments may primarily function to indicate quality for potential rivals. At the same time, the colour properties of the red crest may have been initially targeted by females to assess male quality. However, once the trait spread among males, females opted to 'raise the bar', and the mantle plumage became a higher stimulus for mating response than the crest plumage (chase-away sexual selection process; Holland and Rice 1998). Thus, the information conveyed by the crest and mantle plumage may be differently evaluated by males and females (multiple receiver hypothesis). Another possibility is that we overlooked the combination of multiple plumage ornaments for enhancing signal design.

Many other species of manakins exhibit a similar visual contrasting pattern of colours as the helmeted manakin (i.e., a bright, colourful patch on the crest and mantle and an overall black body). The high chromatic and achromatic contrast within plumage patches in this family suggests that multiple and contrasting plumage patches may be sexually selected (Doucet *et al.* 2007). We found that the helmeted manakin plumage is also highly contrasting (Supplementary Information Fig. S2); nonetheless, contrary to our predictions, our findings suggest that females favoured males with less achromatic contrasting patches (Fig. 7). In an experiment testing for achromatic and chromatic vision tuning in budgerigars (*Melopsittacus undulatus*), gratings with low achromatic and high chromatic contrast were detected over broader acuity ranges than when contrasts were isolated (Lind and Kelber 2011). Thus, we hypothesize that the combination of the overall high chromatic contrast among patches combined with low achromatic contrast would enhance the signal perception by females. Hence, the preference for this trait could be explained by the signal amplification hypothesis (Hasson 1989) and the enhanced signal detection hypothesis (Rowe 1999).

Despite uncertainties related to the position of the helmeted manakin in the phylogenies suggested so far, the species is closely related to the blue manakin *Chiroxiphia caudata* (Tello *et al.* 2009, Ohlson *et al*. 2013, Silva *et al*. 2018), and it has recently been suggested to be nested within the *Chiroxiphia* genus (Leite *et al.* 2021). Although markedly different in several behavioural aspects, one could imagine that helmeted manakins integrate a set of unusual behaviours that possibly represent an evolutionary transition scenario from its putative ancestor, the blue manakin. The continuous reduction in habitat availability that confined the species to the narrow gallery forests in the Cerrado biome could have affected its behaviour and mating system. The resulting lack of complex courtship displays and clumped aggregations of males that, instead, defend individual territories (Chapter 2), added to the overall lack of female preference for conspicuous ornaments found in this study, appear to indicate a potential relaxation of sexual selection on males. Despite the intense sexual plumage dichromatism found in the species, bird plumage colour is a highly labile trait (Price and Birch 1996). Thus, a loss of male elaboration associated with weakened sexual selection, such as in the green manakin *Xenopipo holochlora* (Ribeiro *et al.* 2015), could potentially be an ongoing process in the helmeted manakin.

In conclusion, we found that specific elements of the helmeted manakin male song and plumage convey aspects of individual quality, indicating that both traits may signal information important in female choice. We also found evidence for differential allocation of carotenoid pigments among plumage patches, which may serve as a strategy to minimize costs and enhance signal efficacy. However, contrary to our initial predictions, the chromatic features of colour did not appear to be directly favoured by females, as they preferred males expressing the trait in moderate levels. Instead, females selected males with decreased brightness in their mantle plumage and lower achromatic contrast between colour patches, which may be enhancing the signal design of colours. Together, these results show that helmeted manakins males have multiple quality-revealing ornaments but are not associated with an intense sexual selection via female choice. Future studies should extract and investigate other metrics of song performance and ornamentation complexity, in addition to evaluating traits that were not analysed here and could be potentially under sexual selection, such as age, territory size, and personality. Further investigation on male-male competition and potential attributes associated with social dominance should also provide new perspectives on the selective forces operating in the species.

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Supplementary Information

Table S1 Spearman correlation among the acoustic song measurements.

	Beak_l	**Beak_h**	**Beak_w**	**Body length**	**Wing chord**	**Tarsus length**
Beak_h	-0.22					
Beak_w	0.36*	0.3*				
TL	0.07	0.07	0.16			
Wing	-0.14	0.25	-0.05	0.16		
Tarsus	0.11	-0.16	-0.31*	0.01	0.18	
Body mass	-0.14	0.32*	0.28	0.27	-0.03	-0.12

*** $P < 0.05$**

Beak_l: beak length; beak_h: beak height; beak_w: beak width

Table S2 Detailed description of acoustic song measurements

Acoustic variables	**Description**
Duration 90%	The duration of the unit section containing 90% of spectral energy. It is the difference between 5% and 95% time, which consists of the point in time that divides the acoustic unit in two time intervals, containing 5% and 95%of the energy.
Frequency bandwidth 95%	The average frequency bandwidth containing 95% of the energy in the selection
Frequency bandwidth 5%	The average frequency bandwidth containing 5% of the energy in the selection
Max frequency bandwidth 25%	Maximum frequency bandwidth containing 25% of the energy in the selection
Frequency excursion index	The sum of frequency modulations both within and between notes on a per-time basis. First, peak frequencies are calculated in successive time bins for each vocal segment, then the spectrogram distances between successive frequency points and time are summed across the sample, including inter-note and inter-syllable intervals (details on Podos *et al*. 2016).
Number of elements	The average number of elements in each acoustic unit
Max inflections	The maximum number of inflections per acoustic unit. A frequency inflection is either a change from increasing to decreasing frequency and vice-versa, or a break in a continuous signal where the sound following the break is at a different frequency.

Table S3 Description of 12 microsatellite loci without the M13 sequence used for genotyping helmeted manakin samples. The dyes, annealing temperatures (Ta) and the name of the species for which the primers were described are provided.

Locus	**Primer sequence (5' - 3')**	**Dye**	**Ta (°C)**	**Source species (reference)**
Chir1-6	F: ATACTAAATGCGTTACCAAGC	6-FAM	58	*Chiroxiphia caudata*
	R: ATCTTGGGTTAACATAACCTG			(Francisco *et al.* 2004)
Chir1-16	F: AGAGCCACCTAGTTAAACTGC	6-FAM	51	*Chiroxiphia caudata*
	R: TTAGATCACCTGGTGTATGTTC			(Francisco *et al.* 2004)
Chiro5	F: TGAGAGGCAGAAAATATTCCAG	6-FAM	57	*Chiroxiphia lanceolata*
	R: GATTAGATCTTCATCCTGAGAGT			(DuVal *et al.* 2007)
Chiro8	F: TTGTACAGACAATCACATCTACCTC	VIC	55	*Chiroxiphia lanceolata*
	R: TGACAATGTGGGTGTATGCAG			(DuVal *et al.* 2007)
Chiro10	F: GGAGGGATAGTGGGCAAGAT	VIC	56	*Chiroxiphia lanceolata*
	R: CTTTCTGGAGTTCTTTCCATGC			(DuVal *et al.* 2007)
Chiro12	F: TGTCCCTTATTTGCCTTAGTGC	NED	58	*Chiroxiphia lanceolata*
	R: GCTTCACTTGCAATACATGTCTATCT			(DuVal *et al.* 2007)
Abom6	F: CTGCCGTATGTGACAATTTGAA	6-FAM	57	*Antilophia bokermmani*
	R: AAAAGTGATACCCACCACATCC			(Souza *et al.* 2019)
Abom7	F: CACCCATTGCTATTGGGCAAC	6-FAM	53	*Antilophia bokermmani*
	R: CAGGACTGCCTTTTAGATGAGG			(Souza *et al.* 2019)
Abom11	F: CGGGACAAAAGGCAATGGTTA	NED	57	*Antilophia bokermmani*
	R: AGATTGCTCAGGGCTGTGTT			(Souza *et al.* 2019)
Abom12	F: CTGCAAGGATGGACTGACTCTT	VIC	55	*Antilophia bokermmani*
	R: CACTTTTTCAAGGGTTTCAAAT			(Souza *et al.* 2019)
Xenat01	F: GGAATGGATGCTAACTCTGGC	6-FAM	60	*Xenopipo atronitens*
	R: AAGGGAAAGGTCTGGAATGC			(Gubili *et al.* 2016)
Xenat02	F: AGGGAAAGGTCTGGAATGCA	6-FAM	59	*Xenopipo atronitens*
	R: TGGATGCTAACTCTGGCTATGT			(Gubili *et al.* 2016)

Table S4 Morphological measurements of males (N = 48) and females (N = 9).

	Males			**Females**		
	mean ± SE	min	max	mean ± SE	min	max
Beak						
Length (mm)	6.93 ± 0.64	5.1	8.3	6.95 ± 0.52	6.35	7.7
Height (mm)	4.03 ± 0.24	3.6	5	3.93 ± 0.15	3.7	4.1
Width (mm)	4.63 ± 0.36	3.65	5.6	4.79 ± 0.29	4.3	5.1
Wing chord (mm)	79.2 ± 2.1	72	83	76.33 ± 2.45	72	80
Tarsus length (mm)	19.04 ± 0.8	17.4	21.2	18.26 ± 1.16	16.5	19.8
Body length (mm)	162.9 ± 4.34	150	170	162.9 ± 4.34	150	170
Mass (g)	20 ± 1.44	17.5	23	21 ± 1.06	19.2	22.35

Table S5 Summary of values of theta, phi and r achieved obtained for each region of the body of 50 male helmeted manakins in adult plumage. Values represent stimuli in an avian colourspace.

	Crest		**Mantle**		**Breast**	
	min	max	min	max	min	mx
Theta	-2.514	-0.463	-0.953	0.260	-2.726	-2.435
Phi	-0.094	1.406	-0.158	1.403	0.900	1.459
R achieved	0.253	0.783	0.275	0.775	0.274	0.6

Table S6 Summary of acoustic measurements extracted from songs of 15 helmeted manakin males.

	mean ± SE	**min**	**max**
Mean duration 90% (s)	0.77 ± 0.16	0.21	0.89
Mean frequency bandwidth 95% (Hz)	3575 ± 163	3129	3766
Mean frequency bandwidth 5% (Hz)	2409 ±103	2203	2625
Maximum frequency bandwidth 25% (Hz)	2683 ± 125	2535	2960
Mean excursion index (cum. Δ Hz/s)	1615 ± 474	706	2245
Average number of elements	7 ± 1	3	8
Maximum number of inflections	14 ± 2	9	17

Table S7 Linear regressions between song parameters and indicators of individual quality (N = 15).

	H/L					**SMI**				
	Estimate	**SE**	***t***	**R^2**	***P***	**Estimate**	**SE**	***t***	**R^2**	***P***
mean.duration.90 (.)	1.70	0.70	2.42	0.54	**0.06**	6.02	3.80	1.59	0.30	0.16
mean.freq.band.95	0.00	0.00	0.22	0.01	0.83	0.00	0.01	0.29	0.01	0.78
mean.freq.band.5	0.00	0.00	-0.12	0.00	0.91	0.00	0.01	-0.10	0.00	0.92
max.freq.band.Q1	0.00	0.00	0.35	0.02	0.74	-0.01	0.00	-2.00	0.40	0.09
frequency excursion index	0.00	0.00	-0.93	0.15	0.40	0.00	0.00	2.04	0.41	0.09
mean number of elements	0.04	0.04	1.01	0.14	0.35	0.59	0.56	1.06	0.16	0.33
maximum number of inflections	0.04	0.03	1.58	0.29	0.18	0.01	0.38	0.02	0.00	0.99

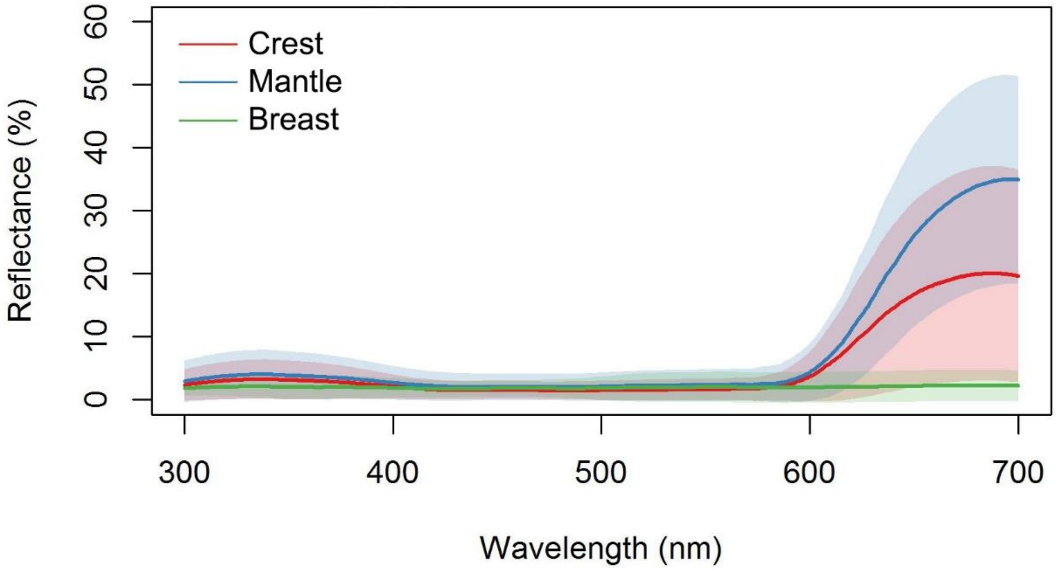

Figure S1 Average reflectance spectra (line) and corresponding standard deviation (shaded area) of the plumage of 50 adult male helmeted manakins.

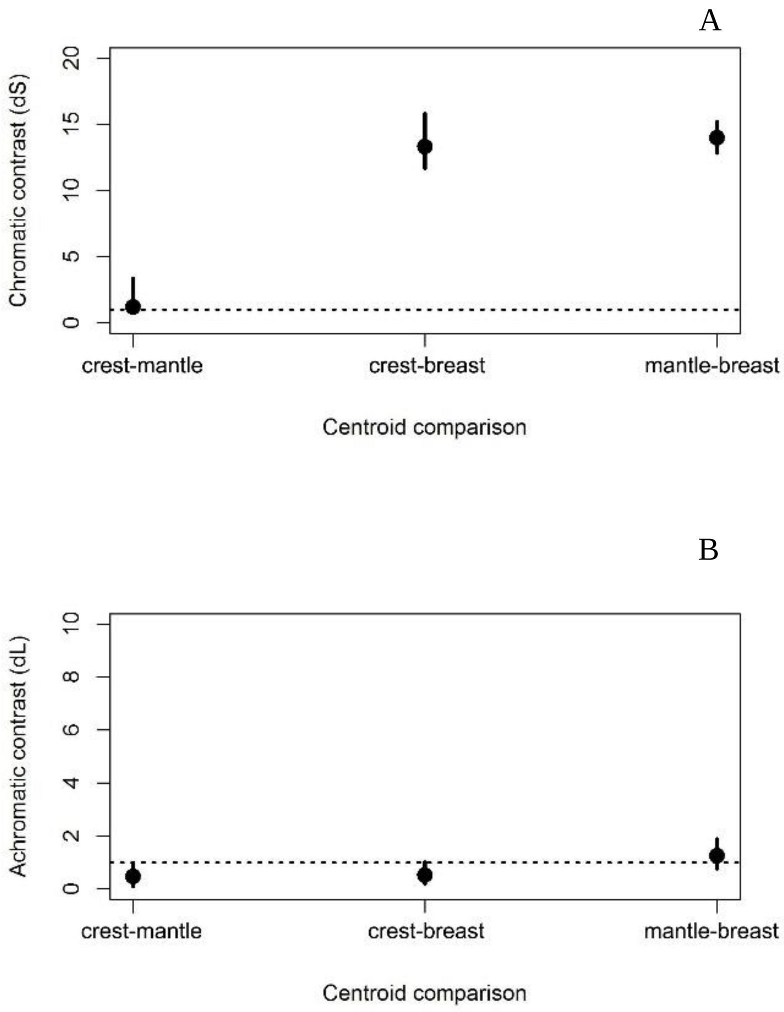

Figure S2 Chromatic (A) and achromatic (B) contrasts as perceived by birds in Just Noticeable Distances. Contrasts with JND > 1 are perceived by birds.

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CONSIDERAÇÕES FINAIS

Nessa tese eu investiguei a biologia reprodutiva, o comportamento territorial de machos, as relações de parentesco e se há seleção sexual por determinados caracteres sexuais secundários de machos para embasar a discussão do sistema de acasalamento de *Antilophia galeata*. Essa espécie pertence à família Pipridae, conhecida pelo dimorfismo sexual acentuado, no qual machos possuem ornamentos extravagantes e elaborados *displays* de corte e a maioria das espécies apresenta um sistema de acasalamento poligínico em *lek*. O gênero *Antilophia* se destaca por ter sido considerado monogâmico, porém estudos recentes indicam que tal afirmação seria equivocada.

No primeiro capítulo descrevi aspectos básicos da biologia reprodutiva da espécie, identifiquei os predadores de ninhos de *A. galeata*, e avaliei os fatores que influenciam na sobrevivência de ninhos. Confirmei que apenas a fêmea exerce todas as atividades relativas ao cuidado parental na espécie, apresentei o primeiro registro documentado de predação de ninho de ave por macaco-prego (*Sapajus libidinosus*), e descobri que a taxa de predação dos ninhos de *A. galeata* é elevada: 59%, no qual foram registradas predação por outras aves, uma espécie de roedor e primata. Por fim, constatei que ninhos mais altos e mais próximos de corpos d’água tiveram maior sobrevivência.

No segundo capítulo, o objetivo foi averiguar se machos defendiam territórios individuais que potencialmente conteriam recursos importantes para as fêmeas, como sítios de nidificação. Conduzi experimentos de territorialidade utilizando um modelo taxidermizado e a técnica do *playback*. Nesse estudo confirmei que machos defendem territórios individuais que possuem pouca sobreposição, e que alguns desses territórios continham ninhos ativos. As evidências desses dois capítulos já sugeriam que o sistema de acasalamento da espécie não é monogâmico, porém isso apenas seria confirmado com análises moleculares de parentesco.

No terceiro capítulo analisei a paternidade de 22 filhotes cujas mães eram conhecidas. Encontrei elevado desvio reprodutivo entre machos, evidências de poliginia e alta proporção de paternidades múltiplas nas ninhadas. Assim, confirmei que o sistema de acasalamento de *A. galeata* é poligínico e poliândrico (poliginândrico), e os dados comportamentais sugerem que machos talvez apresentem *lek* solitário. Contudo, os resultados não permitiram uma classificação inequívoca do sistema de acasalamento. Além disso, encontrei que a maioria dos machos que adquiriram paternidade eram adultos, levantando a questão se tais machos foram escolhidos pelas fêmeas em função da exibição de caracteres sexuais secundários que sinalizaram sua condição.

Assim, no quarto capítulo investiguei se parâmetros acústicos do canto de machos e da cor da plumagem sinalizam sua qualidade (saúde e condição corporal) para fêmeas, e se essas características estão sob seleção sexual pela escolha da fêmea. Encontrei que a matiz do manto e da crista foram correlacionados com o nível de estresse fisiológico em machos, indicando que machos com a matiz mais pura no vermelho na crista, mais violeta e com menos UV no manto possuem melhor condição imunológica. Além disso, apenas o brilho da plumagem do manto é um indicativo de condição corporal. Dentre as características sexuais secundárias avaliadas, fêmeas preferiram machos com cantos mais curtos, menos brilho no manto e menor contraste acromático entre a plumagem do manto e ventre. Esses resultados indicam que as características sexuais secundárias de machos revelam diferentes aspectos da qualidade dos machos, mas contrário às predições, não estão associadas a intensa seleção sexual pela escolha da fêmea.

NOTA DE ESCLARECIMENTO

A coleta de dados dessa tese se iniciou em 2017 e foi concluída em 2020, ano no qual estava previsto um estágio no exterior, para que eu pudesse conduzir as análises de paternidade na Cornell University com financiamento de bolsa escolar do programa de internacionalização CAPES PrInt. A bolsa estava na fase final de implementação quando a pandemia pelo COVID-19 foi confirmada. Na esperança (e ingenuidade) de que a situação calamitosa não se estenderia por muito tempo, tentei retardar o início da bolsa para setembro de 2020. Logo a situação da pandemia piorou em todos os cantos do mundo, e realizar as análises moleculares fora do país se tornou inviável. A partir da metade do segundo semestre de 2020 iniciamos o processo de colaboração com o Laboratório de Genética e Evolução Molecular de Aves – USP, chefiado pela Dra. Cristina Y. Miyaki. Contudo, a situação da pandemia ainda era muito instável no Brasil no início de 2021, resultando em uma rotina laboratorial mais lenta em virtude das restrições de entrada e sistema de rodízio de alunos. Além disso, muitos reagentes utilizados nas análises moleculares foram importados e a logística de compra e chegada dos materiais provavelmente se tornou mais complicada em função da pandemia. Todos esses fatores contribuíram para um atraso significativo na realização das análises moleculares, e resultaram na impossibilidade de incluir todas as amostras coletadas durante os quatro anos de campo. Consideramos, no entanto, que a análise de uma amostra reduzida já era suficiente para gerar conclusões pertinentes para a tese, permitindo assim a finalização desta etapa de minha formação. Todavia, a análise das amostras restantes será conduzida nos próximos meses, permitindo subsidiar de forma mais robusta os resultados, que serão incluídos nos manuscritos que envolvem as análises moleculares.

ANEXOS

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 58094-1	**Data da Emissão: 18/05/2017 15:01**	**Data para Revalidação*: 17/06/2018**

* De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.

Dados do titular

Nome: LIA NAHOMI KAJIKI	CPF: 355.125.798-19
Título do Projeto: Re-evolução da monogamia: a seleção sexual sob pressões divergentes	
Nome da Instituição : FUNDAÇÃO UNIVERSIDADE DE BRASÍLIA	CNPJ: 00.038.174/0001-43

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	amostragem dos dados durante campanhas na época reprodutiva e não-reprodutiva	03/2017	03/2021

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1	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
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Outras ressalvas

1	Solicita-se que a pesquisadora visite o PNB para orientações, coleta de informações complementares ou referentes ao deslocamento na UC, antes do início da captura e repasse à Coordenação de Pesquisa a localização dos locais onde ocorrerão as capturas por contato com a analista Cibele Barreto.

Equipe

#	Nome	Função	CPF	Doc. Identidade	Nacionalidade
1	Carlos Humberto Biagolini Junior	Apoio na execução das atividades	373.864.488-13	378667919 SSP-SP	Brasileira
2	Pedro Zurvãino Palmeira Melo Rosa de Moraes	Apoio na execução das atividades	067.083.044-57	1597184 SSP-AL	Brasileira
3	RENATA DUARTE ALQUEZAR DE OLIVEIRA	Apoio na execução de atividades	019.550.731-20	2527814 SSP-DF	Brasileira
4	REGINA HELENA FERRAZ MACEDO	Supervisão	301.639.191-00	452409 SSP-DF	Brasileira

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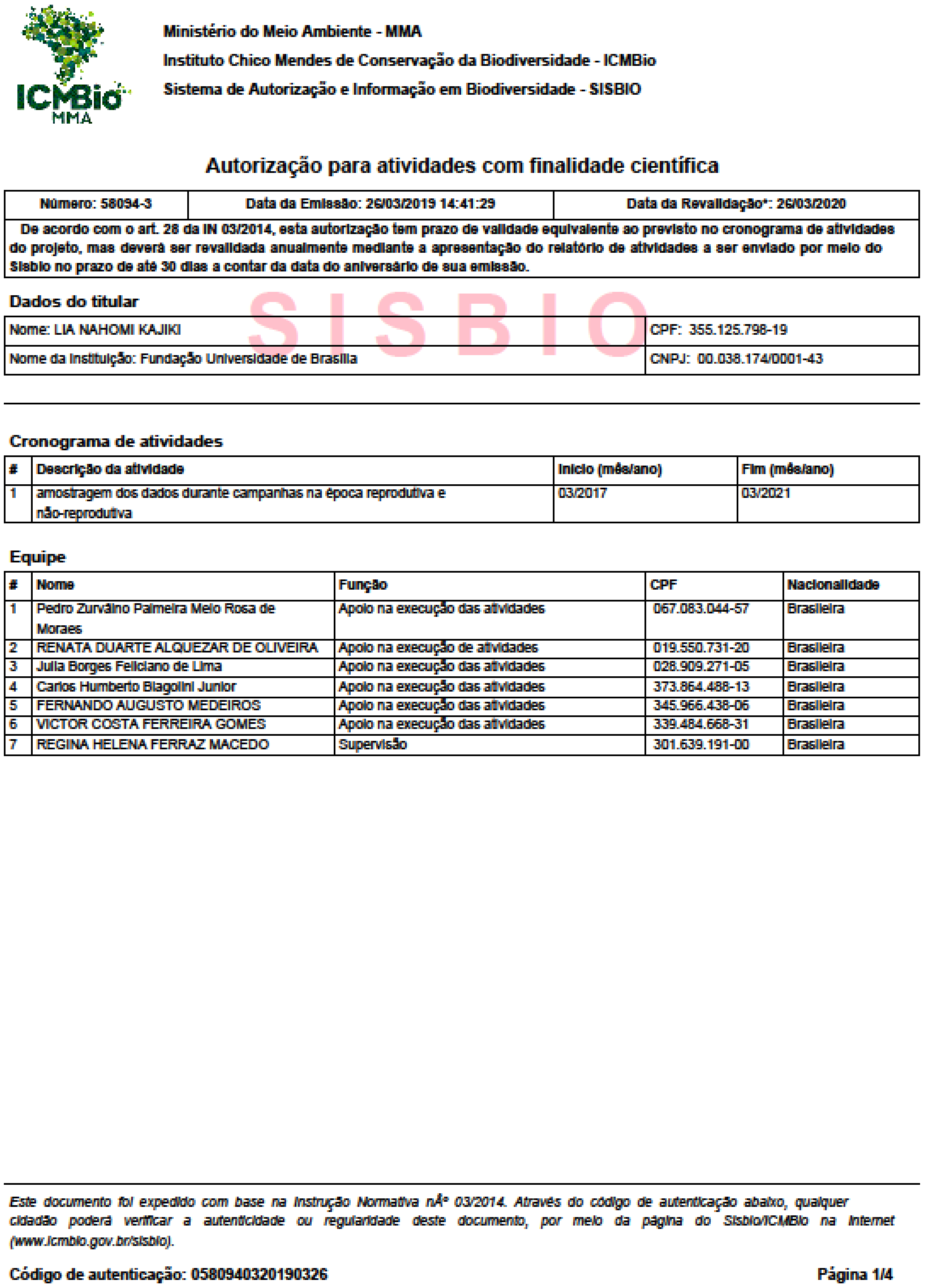

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Autorização para atividades com finalidade científica

Número: 58094-3	**Data da Emissão: 26/03/2019 14:41:29**	**Data da Revalidação*: 26/03/2020**

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Dados do titular

Nome: LIA NAHOMI KAJIKI	CPF: 355.125.798-19
Nome da Instituição: Fundação Universidade de Brasília	CNPJ: 00.038.174/0001-43

Cronograma de atividades

#	**Descrição da atividade**	**Início (mês/ano)**	**Fim (mês/ano)**
1	amostragem dos dados durante campanhas na época reprodutiva e não-reprodutiva	03/2017	03/2021

Equipe

#	**Nome**	**Função**	**CPF**	**Nacionalidade**
1	Pedro Zurvaino Palmeira Melo Rosa de Moraes	Apoio na execução das atividades	067.083.044-57	Brasileira
2	RENATA DUARTE ALQUEZAR DE OLIVEIRA	Apoio na execução de atividades	019.550.731-20	Brasileira
3	Julia Borges Feliciano de Lima	Apoio na execução das atividades	028.909.271-05	Brasileira
4	Carlos Humberto Biagolini Junior	Apoio na execução das atividades	373.864.488-13	Brasileira
5	FERNANDO AUGUSTO MEDEIROS	Apoio na execução das atividades	345.966.438-06	Brasileira
6	VICTOR COSTA FERREIRA GOMES	Apoio na execução das atividades	339.484.668-31	Brasileira
7	REGINA HELENA FERRAZ MACEDO	Supervisão	301.639.191-00	Brasileira

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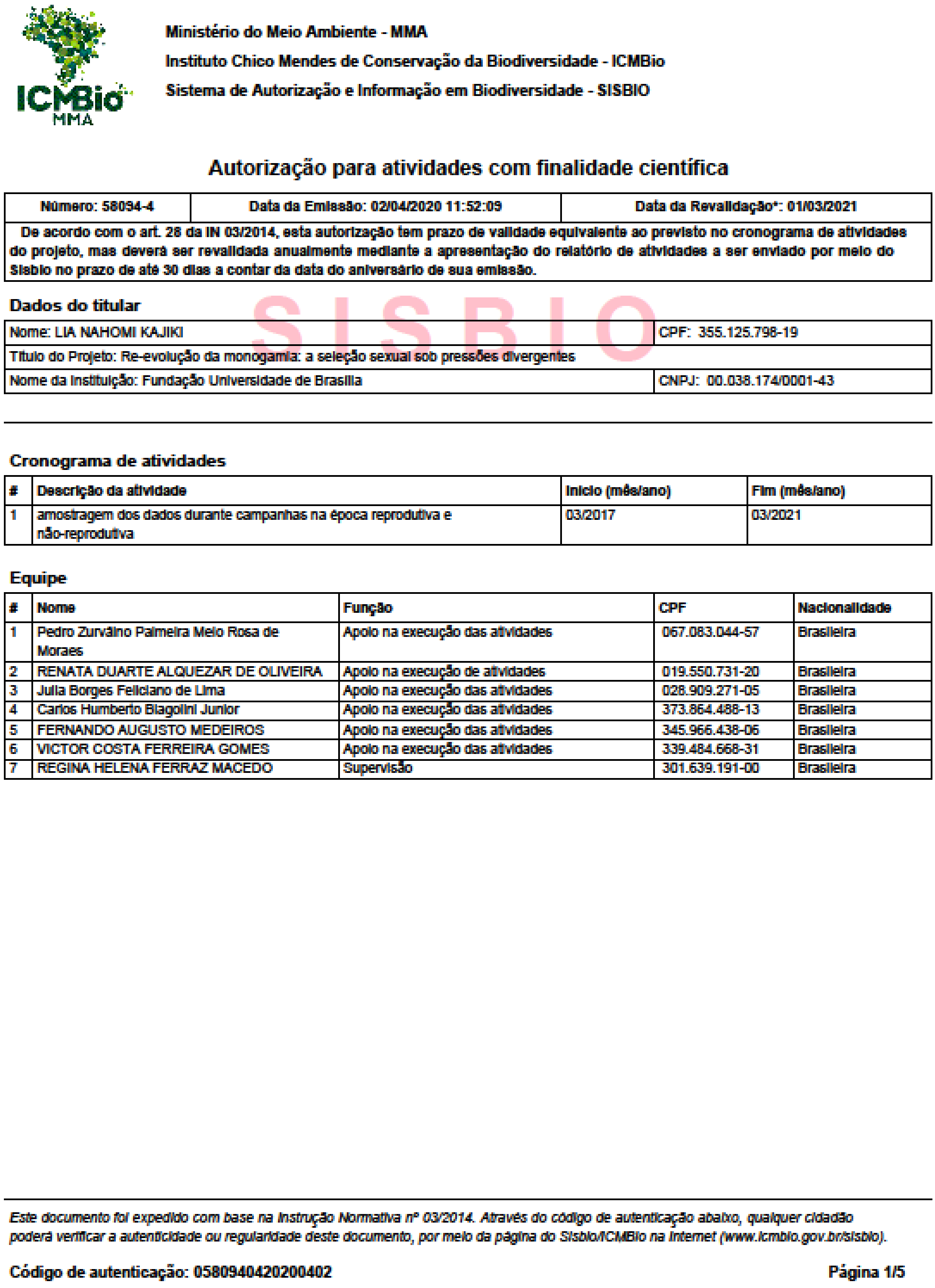

Ministério do Meio Ambiente - MMA
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Autorização para atividades com finalidade científica

Número: 58094-4	**Data da Emissão: 02/04/2020 11:52:09**	**Data da Revalidação*: 01/03/2021**

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Dados do titular

Nome: LIA NAHOMI KAJIKI	CPF: 355.125.798-19
Título do Projeto: Re-evolução da monogamia: a seleção sexual sob pressões divergentes	
Nome da Instituição: Fundação Universidade de Brasília	CNPJ: 00.038.174/0001-43

Cronograma de atividades

#	**Descrição da atividade**	**Início (mês/ano)**	**Fim (mês/ano)**
1	amostragem dos dados durante campanhas na época reprodutiva e não-reprodutiva	03/2017	03/2021

Equipe

#	**Nome**	**Função**	**CPF**	**Nacionalidade**
1	Pedro Zurvaino Palmeira Melo Rosa de Moraes	Apoio na execução das atividades	067.083.044-57	Brasileira
2	RENATA DUARTE ALQUEZAR DE OLIVEIRA	Apoio na execução de atividades	019.550.731-20	Brasileira
3	Julia Borges Feliciano de Lima	Apoio na execução das atividades	028.909.271-05	Brasileira
4	Carlos Humberto Biagolini Junior	Apoio na execução das atividades	373.864.488-13	Brasileira
5	FERNANDO AUGUSTO MEDEIROS	Apoio na execução das atividades	345.966.438-06	Brasileira
6	VICTOR COSTA FERREIRA GOMES	Apoio na execução das atividades	339.484.668-31	Brasileira
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Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
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DADOS DO PROJETO E DO ANILHADOR:
Título do Projeto: Re-evolução da monogamia: a seleção sexual sob pressões divergentes
Nome do Anilhador Titular: LIA NAHOMI KAJIKI Nº do Registro: 5331333 CPF: 355.125.798-19

RELAÇÃO DOS ANILHADORES AUXILIARES

NOME	Nº REGISTRO	INCLUÍDO EM	CPF	CATEGORIA	SITUAÇÃO
PEDRO DINIZ ALVES	1635902	31/05/2017	02044033160	Senior	Autorizado
Carlos Humberto Biagolini Junior	5753756	31/05/2017	37386448813	Junior	Autorizado
Pedro Zurvâino Palmeira Melo Rosa de Moraes	5926354	31/05/2017	06708304457	Junior	Autorizado
VICTOR COSTA FERREIRA GOMES	6559029	31/05/2017	33948466831	Junior	Autorizado
Julia Borges Feliciano de Lima	6432482	31/05/2017	02890927105	Junior	Autorizado
RENATA DUARTE ALQUEZAR DE OLIVEIRA	5384464	31/05/2017	01955073120	Senior	Autorizado
Doiglas Phelipe Soares Rodrigues	6575043	31/05/2017	01472146107	Junior	Autorizado
REGINA HELENA FERRAZ MACEDO	324672	31/05/2017	30163919100	Senior	Autorizado
FERNANDO AUGUSTO MEDEIROS	4451004	31/05/2017	34596643806	Senior	Autorizado
RAVEL TIBURCIO MOREIRA TRINDADE	6894550	21/06/2017	13676354621	Junior	Autorizado

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ITENS AUTORIZADOS

ITEM	DESCRIÇÃO
LOCAIS DE ANILHAMENTO	APA Gama e Cabeça de Veado (BRASILIA-DF); Parque Nacional de Brasília (BRASILIA-DF)
INSTRUMENTOS DE CAPTURA	Rede Neblina (Qtde: 15)
MARCADORES	Anilhas coloridas; Anilhas de Alumínio (padrão CEMAVE)

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2. A coleta ou posse de ovos, peles, carcaças ou quaisquer outros produtos ou subprodutos da avifauna silvestre;
3. O anilhamento em unidades de conservação, devendo o interessado obter a licença junto ao órgão ambiental competente;
4. O anilhamento em propriedades privadas ou públicas sem a devida anuência de seu responsável ou proprietário legal.
5. O transporte, destinação ou manutenção de aves silvestres em cativeiro.

LISTA DOS TÁXONS AUTORIZADOS

NÍVEL	TÁXONS
CLASSE	AVES

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Centro Nacional de Pesquisa e Conservação de Aves Silvestres - CEMAVE
Sistema Nacional de Anilhamento de Aves Silvestres - SNA

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DADOS DO PROJETO E DO ANILHADOR:
Titulo do Projeto: Re-evolução da monogamia: a seleção sexual sob pressões divergentes
Nome do Anilhador Titular: LIA NAHOMI KAJIKI   Nº do Registro: 5331333   CPF: 355.125.798-19

RELAÇÃO DOS ANILHADORES AUXILIARES

NOME	Nº REGISTRO	INCLUÍDO EM	CPF	CATEGORIA	SITUAÇÃO
Lucas Peres Saavedra	7144496	26/08/2019	06581831174	Junior	Autorizado
Vinicius Rozendo Vianna	6873191	26/08/2019	03741011169	Junior	Autorizado
PEDRO DINIZ ALVES	1635902	31/05/2017	02044033160	Senior	Autorizado
CARLOS HUMBERTO BIAGOLINI JUNIOR	5753756	31/05/2017	37386448813	Senior	Autorizado
Pedro Zurvâino Palmeira Melo Rosa de Moraes	5926354	31/05/2017	06708304457	Junior	Autorizado
VICTOR COSTA FERREIRA GOMES	6559029	31/05/2017	33948466831	Senior	Autorizado
Julia Borges Feliciano de Lima	6432482	31/05/2017	02890927105	Junior	Autorizado
RENATA DUARTE ALQUEZAR DE OLIVEIRA	5384464	31/05/2017	01955073120	Senior	Autorizado
Felipe Brito Soares Rodrigues	6575043	31/05/2017	01472146107	Junior	Autorizado
MARIANA DE CARVALHO	5747583	26/08/2019	14101940789	Senior	Autorizado
REGINA HELENA FERRAZ MACEDO	324672	31/05/2017	30163919100	Senior	Autorizado
FERNANDO AUGUSTO MEDEIROS	4451004	31/05/2017	34596643806	Senior	Autorizado
RAVEL TIBURCIO MOREIRA TRINDADE	6894550	21/06/2017	13676354621	Junior	Autorizado

Esta Autorização concede ao(s) anilhador(es)acima identificado(s) o direito de proceder ao anilhamento de aves silvestres, de acordo com as condições abaixo descritas, podendo a referida autorização ser cancelada ou suspensa, quando constatado os descumprimento das normas previstas na legislação. O anilhador titular ou um dos membros da equipe de auxiliares deverá portar esta Autorização durante as atividades de anilhamento, devendo apresentá-la aos agentes públicos durante ações fiscalizatórias, devidamente acompanhada de um documento de identidade.

ITENS AUTORIZADOS

ITEM	DESCRIÇÃO
LOCAIS DE ANILHAMENTO	Córrego da Onça (FAL) (BRASILIA-DF); Córrego Capetinga (FAL) (BRASILIA-DF); Parque Nacional de Brasilia (BRASILIA-DF); APA Gama e Cabeça de Veado (BRASILIA-DF)
INSTRUMENTOS DE CAPTURA	Rede Neblina (Qtde: 15)
MARCADORES	Anilhas coloridas; Anilhas de Aluminio (padrão CEMAVE)

É proibida a utilização de artefato de marcação ou instrumento de captura não previstos nesta autorização, ou ainda, a utilização destes instrumentos em quantitativo superior ao autorizado.
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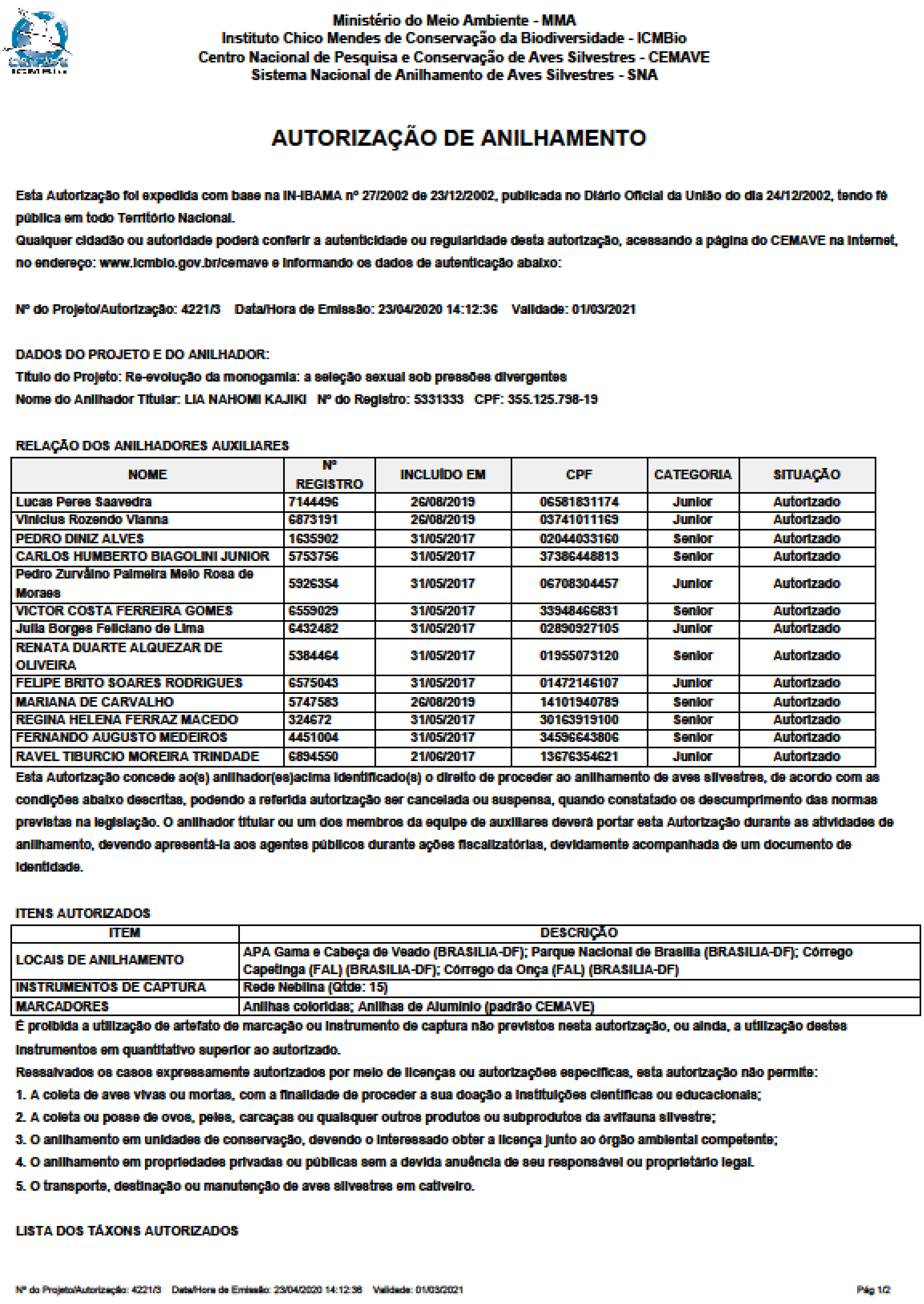
2. A coleta ou posse de ovos, peles, carcaças ou quaisquer outros produtos ou subprodutos da avifauna silvestre;
3. O anilhamento em unidades de conservação, devendo o interessado obter a licença junto ao órgão ambiental competente;
4. O anilhamento em propriedades privadas ou públicas sem a devida anuência de seu responsável ou proprietário legal.
5. O transporte, destinação ou manutenção de aves silvestres em cativeiro.

LISTA DOS TÁXONS AUTORIZADOS



Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Centro Nacional de Pesquisa e Conservação de Aves Silvestres - CEMAVE
Sistema Nacional de Anilhamento de Aves Silvestres - SNA

AUTORIZAÇÃO DE ANILHAMENTO

Esta Autorização foi expedida com base na IN-IBAMA nº 27/2002 de 23/12/2002, publicada no Diário Oficial da União do dia 24/12/2002, tendo fé pública em todo Território Nacional.
Qualquer cidadão ou autoridade poderá conferir a autenticidade ou regularidade desta autorização, acessando a página do CEMAVE na Internet, no endereço: www.icmbio.gov.br/cemave e informando os dados de autenticação abaixo:

Nº do Projeto/Autorização: 4221/3 Data/Hora de Emissão: 23/04/2020 14:12:36 Validade: 01/03/2021

DADOS DO PROJETO E DO ANILHADOR:
Título do Projeto: Re-evolução da monogamia: a seleção sexual sob pressões divergentes
Nome do Anilhador Titular: LIA NAHOMI KAJIKI Nº do Registro: 5331333 CPF: 355.125.798-19

RELAÇÃO DOS ANILHADORES AUXILIARES

NOME	Nº REGISTRO	INCLUÍDO EM	CPF	CATEGORIA	SITUAÇÃO
Lucas Peres Saavedra	7144496	26/08/2019	06581831174	Junior	Autorizado
Vinicius Rozendo Vianna	6873191	26/08/2019	03741011169	Junior	Autorizado
PEDRO DINIZ ALVES	1635902	31/05/2017	02044033160	Senior	Autorizado
CARLOS HUMBERTO BIAGOLINI JUNIOR	5753756	31/05/2017	37386448813	Senior	Autorizado
Pedro Zurvâino Palmeira Melo Rosa de Moraes	5926354	31/05/2017	06708304457	Junior	Autorizado
VICTOR COSTA FERREIRA GOMES	6559029	31/05/2017	33948466831	Senior	Autorizado
Julia Borges Feliciano de Lima	6432482	31/05/2017	02890927105	Junior	Autorizado
RENATA DUARTE ALQUEZAR DE OLIVEIRA	5384464	31/05/2017	01955073120	Senior	Autorizado
FELIPE BRITO SOARES RODRIGUES	6575043	31/05/2017	01472146107	Junior	Autorizado
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LISTA DOS TÁXONS AUTORIZADOS

Este documento não possui valor jurídico

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso
Cadastro nº A0ECFB3

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A0ECFB3**
Usuário:	**Lia Nahomi Kajiki**
CPF/CNPJ:	**355.125.798-19**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Antilophia galeata

Título da Atividade:	**Projeto de pesquisa de doutorado: Biologia reprodutiva, sistema de acasalamento e seleção sexual em Antilophia galeata (Aves: Pipridae)**

Equipe

Lia Nahomi Kajiki	**Universidade de Brasília**
Regina Helena Ferraz Macedo	**Universidade de Brasília**

Data do Cadastro:	**21/12/2021 21:35:26**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **21:35** de **21/12/2021**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN

Universidade de Brasília
Instituto de Ciências Biológicas
Comissão de Ética no Uso Animal

Brasília, 12 de dezembro de 2019.

DECLARAÇÃO

Declaramos que o projeto intitulado “**RE-EVOLUÇÃO DA MONOGAMIA: A SELEÇÃO SEXUAL SOB PRESSÕES DIVERGENTES”** Protocolo n.º 54/2019, sob responsabilidade da Professora Regina Helena Ferraz Macedo foi avaliado e aprovado pela Comissão de Ética no Uso Animal (CEUA) da Universidade de Brasília. Este projeto foi aprovado para utilização de: *Passeriformes (100 machos e 100 fêmeas).* A presente aprovação é válida pelo período de: *12/12/2019* a *04/02/2021*.

Dr. José Luiz Jivago de Paula Rôlo
Coordenador da CEUA – UnB

*Este documento se restringe à avaliação ética do projeto supracitado e não substitui outras licenças e permissões que porventura se façam necessárias.