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A forma e o contexto: testes experimentais da camuflagem de ninhos em duas espécies de aves da Mata Atlântica

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Dedico este trabalho a todas as pessoas que, de forma direta ou indireta, contribuíram para que ele se tornasse possível. Com ajuda, paciência e compreensão, cada um de vocês fizeram parte desta jornada de quatro anos de muito empenho e aprendizado. Este trabalho carrega um pouco de cada um, e a todos vocês, compartilho com gratidão cada resultado aqui alcançado.

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Centro de Ciências Biológicas e da Saúde
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SUMÁRIO

RESUMO

A Mata Atlântica é um dos ecossistemas mais diversos e ameaçados do planeta, que abriga uma avifauna rica e de grande relevância ecológica. Apesar do expressivo número de espécies, ainda há lacunas significativas no conhecimento sobre aspectos reprodutivos e comportamentais das aves neotropicais, especialmente em relação às estratégias de defesa contra predadores. A predação de ninhos representa a principal causa de falha reprodutiva, constituindo uma das pressões seletivas mais intensas sobre a biologia das aves. Essa pressão influencia tanto a escolha de locais de nidificação quanto o *design* e a arquitetura dos ninhos, moldando, ao longo da evolução, um conjunto de comportamentos e estruturas voltados à redução da detecção por predadores. Entre as estratégias antipredatórias, a camuflagem destaca-se como um mecanismo central para diminuir a conspicuidade de ninhos e ovos, sobretudo diante de predadores orientados visualmente. Embora a maioria dos estudos sobre o tema se concentre em aves que depositam seus ovos diretamente sobre o solo, a função da camuflagem de ninhos estruturais e suspensos, comuns em passeriformes tropicais, permanece pouco explorada. Além disso, as distintas modalidades de detecção, como visual e olfativa, sugerem que diferentes tipos de predadores impõem pressões seletivas específicas sobre forma, os materiais e a localização dos ninhos. Neste contexto, apresentamos dois capítulos independentes, ambos voltados a testar, por meio de experimentos de campo, a função da camuflagem de ninhos de duas espécies de aves da Mata Atlântica. O primeiro capítulo investigou se a estrutura de “cauda” dos ninhos do tangará (*Chiroxiphia caudata*) atua como camuflagem disruptiva, reduzindo a detecção por predadores visuais. Os resultados demonstraram que ninhos sem cauda foram predados cerca de dez vezes mais do que os com cauda, e todos os eventos registrados envolveram predadores de busca visual, evidenciando o papel da “cauda” como uma estrutura funcional na redução da detectabilidade do ninho. O segundo capítulo avaliou se a cauda e ornamentação dos galhos laterais nos ninhos do patinho (*Platyrinchus mystaceus*) funcionam como um fenótipo estendido com função anti-predatória. Embora não tenha sido observada diferença significativa nas taxas de predação

entre ninhos ornamentados e não ornamentados, o padrão consistente de adição de materiais e a semelhança com o substrato indicam uma potencial função adaptativa. Em conjunto, os resultados apresentados reforçam a importância da camuflagem como uma força evolutiva atuante sobre o design e o posicionamento dos ninhos em aves tropicais. Além disso, destacam que estruturas aparentemente ornamentais, como a “cauda” dos ninhos de *Chiroxiphia caudata* e a decoração ao redor dos ninhos de *Platyrinchus mystaceus*, podem representar componentes funcionais da arquitetura do ninho, resultantes da seleção natural mediada pela predação visual.

ABSTRACT

The Atlantic Forest is one of the most diverse and threatened ecosystems on earth, harboring a rich avifauna of great ecological relevance. Despite the remarkable bird diversity, substantial knowledge gaps on their reproductive aspects still persist, particularly on the antipredatory strategies. Nest predation is the main cause of avian reproductive failure, representing one of the strongest selective pressures molding their biology. Such pressure influences both nest-site selection and nest design, reducing the probability of detection by predators. Among antipredator strategies, camouflage stands out as a central mechanism to decrease the conspicuousness of nests and eggs, especially against visually oriented predators. Although most studies have focused on species that lay their eggs directly on the ground, the camouflage function of nests built on the vegetation, a common pattern among passerines, remain poorly explored. Moreover, the distinct modes of nest detection, i.e. visual or olfactory, suggest that different predator types impose specific selective pressures on nest form, material, and placement. In this context, this thesis is composed of two independent chapters, each designed to test, through field experiments, the camouflaging function of nests from two bird species of the Atlantic Forest. In the first chapter we investigated whether the “tail” of the blue manakin (*Chiroxiphia caudata*) nests acts as disruptive camouflage, reducing detection by visually oriented predators. The results showed that tailless nests were preyed upon about ten times more frequently than those with tails, and all recorded predation events were caused by visual predators, highlighting the functional role of the “tail” in decreasing nest detectability. In the second chapter, we evaluated whether the ornamentation of lateral branches and tail in nests of the white-throated spadebill (*Platyrinchus mystaceus*) function as an extended phenotype with a camouflaging role. Although no significant difference in predation rates was found between ornamented and non-ornamented nests, the consistent pattern of material addition and the resemblance to the surrounding substrate suggest a potential adaptive function of camouflage. Together, these results reinforce the importance of camouflage as an evolutionary force shaping the design and placement of tropical bird nests. They also highlight that ornamental feature, such as the “tail” of *Chiroxiphia caudata* nests and the decoration around *Platyrinchus mystaceus* nests may represent

functional components of nest architecture, resulting from natural selection mediated by visual predation.

1. INTRODUÇÃO GERAL

Avifauna diversa e estudos escassos

A Mata Atlântica destaca-se entre as cinco ecorregiões mais megadiversas e ameaçadas do planeta, sendo reconhecida como um dos 25 principais *hotspots* de biodiversidade mundial (Myers *et al*., 2000; Galindo-Leal; Câmara, 2003). No passado, sua cobertura original se estendia por uma área de aproximadamente 1 a 1,5 milhão de km², abrangendo o sul, sudeste e nordeste do Brasil, o leste do Paraguai e o nordeste da Argentina. Hoje, porém, restam apenas fragmentos de sua cobertura original, estimados entre 7 e 12% no Brasil e no Paraguai, e cerca de 50% na Argentina (Galindo-Leal; Câmara, 2003; Fragano *et al*., 2003). Essa perda expressiva de habitat é consequência de um processo histórico de desmatamento e fragmentação, impulsionado principalmente pela expansão agrícola, pela urbanização e pela exploração madeireira (Ribeiro *et al*., 2009; Rezende *et al*., 2018).

A elevada heterogeneidade ambiental da Mata Atlântica, composta por florestas ombrófilas densas, florestas estacionais e ecossistemas associados como restingas e manguezais sustenta uma das maiores diversidades biológicas do planeta (Myers *et al*., 2000). Essa variação de habitats reflete-se na composição e na dinâmica das comunidades de aves, que exercem funções ecológicas cruciais, como a polinização, dispersão de sementes e controle de invertebrados (Brooks *et al*., 1999). Além disso, muitas espécies atuam como bioindicadoras sensíveis à degradação ambiental, sendo fundamentais para o monitoramento da integridade florestal (Ribeiro *et al*., 2009; Jenkins *et al*., 2013).

No entanto, apesar do número expressivo de estudos sobre a diversidade e a conservação da avifauna neotropical, ainda persistem lacunas significativas no conhecimento sobre aspectos da biologia reprodutiva, ecologia comportamental e estratégias antipredatórias das espécies. Pesquisas clássicas conduzidas principalmente na Amazônia já haviam revelado que, para muitas espécies tropicais, permanecem desconhecidos detalhes básicos de sua reprodução, como o tipo e a localização dos ninhos, o número de ovos, o período de incubação e permanência dos filhotes e o comportamento parental (Oniki; Willis, 1982; Greeney *et al*., 2019). Esse padrão se repete na Mata Atlântica, onde poucos trabalhos se dedicaram a investigar de forma sistemática a biologia reprodutiva, envolvendo a construção, arquitetura e

seleção de sítios de nidificação (Roper; Goldstein, 1997; Cockle *et al*., 2019).

Ninhos, predadores e suas interações

O propósito principal dos ninhos das aves é comumente definido como uma estrutura que oferece proteção aos ovos e filhotes, de forma que proporcione um desenvolvimento seguro, garantindo o sucesso reprodutivo da espécie (Skutch, 1961; Hansell, 2002, Healy *et al*., 2008; Mainwaring *et al*., 2014). Para além dessa função, a estrutura do ninho serve como um termorregulador, que permite a incubação mais eficaz e mantém a temperatura dos filhotes controlada, próxima aos 40°C (Deeming at al., 2020; Gray; Deeming, 2017).

A predação de ninhos representa a principal causa da falha reprodutiva nas aves, sendo um desafio constante para a maioria das espécies. Por essa razão, acredita-se que elas estejam sob fortes pressões seletivas para desenvolver comportamentos e características que reduzam as chances de seus ninhos serem detectados e atacados por predadores (Caro, 2005; Ibáñez-Álamo *et al*., 2015; Menezes; Marini, 2017). A seleção natural atua, portanto, não apenas sobre o *design* e a estrutura dos ninhos, mas também sobre o comportamento das aves durante o período de construção, especialmente nas fases de coleta e transporte de materiais até o local de nidificação (Lima, 2009).

Quando se trata de predação de ninhos, há uma escassez de conhecimentos sobre os hábitos de forrageamento de predadores, os mecanismos de localização de ninhos, interações entre predadores e habitats e a importância do conteúdo do ninho como alimento para predadores individuais (mas veja Schmidt *et al*., 2001; Schmidt; Schauer, 2007). Os predadores de ninhos mais frequentes são outras aves, como gaviões e corvos, além de mamíferos e serpentes (Reidy; Thompson II, 2012; Ribeiro-Silva *et al.,* 2018), que são funcionalmente distintos no que se refere à forma de forrageio e à capacidade de deslocamento até os ninhos (Perrella *et al.,* 2020). As aves que predam ninhos são em sua maioria guiadas pela visão e audição e têm maiores possibilidades de acesso aos locais dos ninhos devido à capacidade de voo, enquanto os mamíferos e serpentes são guiados principalmente pelo olfato e podem ter o acesso limitado a certos locais (Collias, 1997; Ibáñez-Álamo *et al.,* 2015).

Os predadores podem ter diferentes preferências ou habilidades para lidar

com o conteúdo do ninho, devido a diversidade de tipos de ninhos e de nichos de nidificação, e dessa forma, estabelecer pressões de seleção diferentes (Bayne; Hobson, 1997). Três tipos básicos de ninhos são descritos entre os passeriformes: ninhos em cavidades, ninhos abertos e ninhos fechados (Collias, 1997), além disso, apresentam diferentes tamanhos, formatos e meios de fixação (*nest attachment*) (Hansell, 2000). Cada tipo básico de ninho concede vantagens e desvantagens ecológicas, dependendo do local em que se encontra em relação aos predadores, clima e concorrentes (Collias, 1997).

Ninhos suspensos por galhos finos e periféricos, ao que tudo indica, fornecem menos acessibilidade aos predadores arbóreos e devido a sua localização periférica, geralmente apresentam uma cobertura para proteger os ovos da exposição a fatores climáticos (Collias, 1997). Ninhos construídos em cavidades ficam mais visíveis, além disso, alguns predadores retornam ao local ano após ano (Brightsmith, 2005). Já os ninhos abertos deixam os ovos e filhotes completamente expostos quando o indivíduo adulto não está presente, diferente dos fechados, que geralmente apresentam apenas uma pequena entrada (Collias; Collias, 1984; Collias, 1997).

Além do tipo do ninho, a facilidade de acesso dos predadores, bem como a altura em que ele é construído podem ser considerados fatores determinantes para as taxas de predação e tipos de predadores (Mason *et al*., 2005; Takahashi *et al*., 2013). Ninhos da mesma espécie de ave podem apresentar taxas de predação diferentes de acordo com as características do habitat em que está inserido (Weidinger; Kocˇvara, 2010). Um local de ninho pode ser altamente suscetível à predação por um tipo de predador (por exemplo, aves), mas demonstra baixa predação de outra classe de predador (por exemplo, serpentes) (Kleindorfer *et al*., 2003). Dito isto, a importância da ocultação do ninho para a predação está relacionada ao tipo de predador e à conspicuidade do ninho (Burhans; Thompson III, 1998).

Embora Collias e Collias (1984) e Hansell (2000) forneçam descrições gerais da construção de ninhos de aves, e apesar da convicção do efeito da predação de ninhos na reprodução das aves, sabemos relativamente poucos detalhes sobre os ninhos para espécies particulares (Healy *et al*., 2015) e pouco sobre as identidades dos predadores nos diferentes tipos de habitats, bem como suas interações com diferentes tipos de ninhos (Weidinger, 2009).

Ninhos camuflados: uma estratégia pouco estudada

A camuflagem é uma estratégia evolutiva através da qual os organismos evitam ser detectados e/ou identificados por suas presas ou predadores, presente em muitos sistemas predador-presa (Merilaita *et al*., 2017; Stevens; Ruxton, 2018). Os tipos de camuflagem podem ser agrupados em cripsis, mascaramento (*masquerade*), desorientação por movimento (*motion dazzle*) e camuflagem por movimento (*motion camouflage*) (Merilaita *et al*., 2017; Stevens; Merilaita, 2009). A cripsis envolve uma variedade de estratégias desenvolvidas principalmente para dificultar a detecção, incluindo mimetismo de fundo (*background matching*; um objeto torna-se inconspícuo porque combina com a aparência do ambiente de fundo), camuflagem disruptiva (*disruptive camouflage*; marcas ou estruturas conferem a um objeto limites falsos ou um formato irreal, evitando sua detecção) (Stevens; Merilaita, 2009), marcas distrativas (*distractive markings*; marcas conspícuas direcionam a atenção de um observador para evitar revelar as principais partes do corpo) (Merilaita *et al*., 2017), contrassombreamento (*countershading*; organismos apresentam uma superfície mais escura nas partes do corpo que recebem luz solar, por exemplo no dorso, o que pode evitar a criação de sombras ou distorcer a forma tridimensional) (Merilaita *et al*., 2017) e a fusão de cintilação (*flicker-fusion*; marcas como listras que se tornam borradas para evitar a detecção durante o movimento) (Merilaita *et al*., 2017). O mascaramento, por outro lado, está relacionado à redução no reconhecimento quando um objeto se assemelha a estruturas presentes no ambiente que não são de interesse (Skelhorn *et al*., 2010; Skelhorn *et al*., 2011; Nafus *et al*., 2015). Outros tipos de camuflagem são a desorientação por movimento (marcas que dificultam estimar direção e velocidade) ou a camuflagem por movimento (movimentos inconspícuos que tornam a percepção do movimento improvável) (Stevens; Merilaita, 2009). Devido às altas taxas de predação, acredita-se que algumas espécies de aves possam construir ninhos camuflados (Hansell, 1996; Hansell, 2000), partindo da premissa de que o design de ninhos construídos também influencia o risco de predação (Caro, 2005). Uma série de estudos foram realizados com aves que nidificam no chão, como bacuraus e aves marinhas, para as quais foi verificado que, de uma maneira geral, ninhadas cujos ovos, filhotes e indivíduos parentais se assemelham mais ao substrato têm menores chances de serem predados por predadores visuais (Gómez *et al*., 2018; Stevens *et*

al., 2017). Além disso, em alguns trabalhos foi demonstrado que estas aves podem escolher locais para nidificar que maximizem estas semelhanças (Gómez *et al.,* 2018; Stevens *et al*., 2017).

No entanto, estas aves colocam os ovos diretamente no chão, não construindo ninhos propriamente ditos, de maneira que estudos empíricos sobre o papel da camuflagem dos ninhos estruturais permanecem escassos (Mulder *et al*., 2021). Os estudos que avaliaram o efeito da camuflagem em ninhos "construídos" são relativamente poucos. Entre eles, alguns abordaram a camuflagem disruptiva (Hansell, 1996; Mulder *et al*., 2021; Master; Allen, 2012), o *background matching* (Bailey *et al*., 2015; Hansell, 1996) e o mascaramento (*masquerade*) (Master; Allen, 2012; Zima *et al*., 2022). Apesar desses avanços, nenhum dos estudos considerou explicitamente os diferentes tipos de predadores envolvidos, como os de busca visual e os de busca olfativa.

Pouco se sabe sobre o gasto energético envolvido na construção de ninhos, embora se presuma que seja uma atividade energeticamente custosa. A necessidade de minimizar o risco de predação influencia fortemente tanto a escolha do local quanto o design dos ninhos (Mainwaring *et al*., 2014). Apesar das evidências de que as taxas de predação moldam o projeto dos ninhos em escalas evolutivas, ainda são escassos os estudos que investigam variações adaptativas no design dos ninhos ao longo da vida de uma espécie (Lima, 2009). O campo de pesquisa sobre ninhos e sua construção é promissor, com uma ampla variedade de questões a serem exploradas sob diferentes perspectivas: evolutiva, funcional, de desenvolvimento ou mecanicista (Healy *et al*., 2023).

Diante dessas lacunas, esta tese foi estruturada em dois capítulos independentes, porém complementares, que abordam experimentalmente o papel da forma e do contexto na camuflagem de ninhos em duas espécies de aves da Mata Atlântica. O primeiro capítulo investiga a função da estrutura de "cauda" nos ninhos de *Chiroxiphia caudata* (Pipridae), testando a hipótese de que essa extensão atua como um elemento de camuflagem disruptiva capaz de reduzir a detecção por predadores visuais. O segundo capítulo avalia, em *Platyrinchus mystaceus* (Platyrinchidae), se a ornamentação dos galhos de sustentação do ninho contribui para sua integração visual com o ambiente, explorando a possibilidade de um fenótipo estendido decorrente do comportamento construtivo da espécie. Em conjunto, os dois

estudos buscam compreender de que forma a arquitetura e o posicionamento dos ninhos refletem adaptações a pressões seletivas distintas, contribuindo para ampliar o entendimento sobre os mecanismos evolutivos que moldam as estratégias de camuflagem e o sucesso reprodutivo das aves tropicais.

2. CAPÍTULOS

2.1. Why do birds construct nest tails? A test of disruptive camouflage in the blue manakin

Artigo publicado em acesso aberto e irrestrito no periódico *Biology Letters*.

INTRODUCTION

Camouflage has evolved in many animals to avoid detection or identification by visually-oriented predators or preys (Merilaita *et al*., 2017; Nokelainen; Stevens, 2016; Stevens; Ruxton, 2018). Although most studies on camouflage are concerned with body color or shape and how they interact with the surrounding context (Stevens; Ruxton, 2018; Stevens; Merilaita, 2009), the principles of camouflage also apply to extended phenotypes (Hansell, 2000). Nests are motionless structures that hold eggs, nestlings and parents that are vulnerable to detection by predators or brood parasites (Hansell, 2000), and a recent study provided support to the nest-crypsis hypothesis (Skutch, 1976), i.e., nests can be recognizable by visually oriented predators independently of their contents (eggs or nestlings) (Zima *et al*., 2021). While the construction of nests in hidden or in inaccessible sites is commonly observed as a first line of defense (Chalfoun; Schmidt, 2012; Perrella *et al*., 2021), the evolution of camouflage is also expected.

The camouflage strategies so far hypothesized for bird nests are background matching (an object become inconspicuous because it matches the appearance of the background environment), disruptive camouflage (markings or structures give an object false boundaries or shape, avoiding detection), and masquerading (an object resembles structures present in the environment that are not of interest, reducing recognition) (Nafus *et al*., 2015; Skelhorn *et al*., 2010; 2011). A number of studies has revealed the antipredatory function of background matching for eggs and parental individuals of species which nests are simple scratches on the ground, such as nightjars and marine birds (Gómez *et al*., 2018; 2019; Solís; de Lope, 1995; Stevens at al., 2017; Troscianko *et al*., 2016), and some of these birds also seek for suitable microhabitats to optimize camouflage (Gómez *et al*., 2018; Stevens *et al*., 2017;

Troscianko *et al*., 2016).

While these studies did not involve the camouflage of nests per se, the first evidence for nest background matching was derived from an experiment in captivity in which zebra finches *Taeniopygia guttata* selected nest materials that matched the color of their cage walls (Bailey *et al*., 2015). The idea of nest background matching was rejected for the long-tailed tit *Aegithalos caudatus* and the blue-gray gnatcatcher *Polyoptila caerulea* because their nests, that are densely covered with lichen flakes, were more often constructed on branches that were not covered by these materials. Then, because in some nests the lichens were replaced by artificial materials or spider cocoons, the alternative hypothesis of disruptive camouflage was suggested, i.e., the light reflection of these materials against an illuminated background could create the effect of light passing through a hollow object (Hansell, 1996), but nest survival was not addressed. The predation rates of natural nests of passerines did not differ between those adorned with pieces of paper and chalk markings and control nests, but the support for disruptive camouflage came from an experiment that demonstrated that the adorned nests were less visible to human eyes (Mulder *et al*., 2021).

Another potential type of disruptive camouflage is the addition of hung materials in nest underparts, forming a “nest tail” that could disrupt the traditional cup outline (Master; Allen, 2012). Although nest tails are constructed by species from different bird families (e.g., Tyrannidae, Thamnophilidae, Tityridae, Trochilidae, etc.), their adaptive functions have been poorly explored. For the acadian flycatcher *Empidonax virescens*, which cup nests measure 7.3 cm in outer diameter and have nest tails averaging 21 cm, an index that considered tail length and material abundance was not correlated with nest survival probability, rejecting the idea of disruptive camouflage. However, nest sites in southeastern Pennsylvania had more debris materials similar to those used for nest tail construction than random sites (Master; Allen, 2012) which could suggest a type of masquerading.

The blue manakin *Chiroxiphia caudata* is a forest understory passerine endemic to the Atlantic Forest from southeastern Brazil and eastern Paraguay (Ridgely; Tudor, 1994). This species selects the edges of small forest streams to construct their nests (Perrela *et al*., 2021; Zima *et al*., 2017), that are shallow cups measuring 7.6 cm in outer diameter and 4.6 cm in height, attached at the rim to horizontal forks of shrubs or saplings, with a little more than 50% of the nests constructed over forest streams

(Zima *et al*., 2017). The nests have an elongated tail composed of moss and other plant fibers attached to the underparts (Zima *et al*., 2017; 2022), varying from 8 cm to 1.25 m in length (average = 57 cm). Although hung materials can be observed at the bottom of the nests of some bird species just because the materials used in nest wall construction are loosely attached, the blue manakin indeed construct the nest tails, and the main evidence is that the materials used for tail construction differ in length and in type when compared with those used in the construction of nest walls (Zima *et al*., 2022). In this species the density of tufts of moss and debris resembling nest tails was significantly higher in nest sites than at random sites, but nest survival was negatively correlated with the abundance of these materials near the nests, which was interpreted as a mixed support for the masquerading strategy (Zima *et al*., 2022). Then, the knowledge on avian nest camouflage is limited not only by the reduced number of studies, but also because most studies have not controlled to the potential effects of olfactory predators, which could produce misleading results (but see Zima *et al*., 2022; Rangen *et al*., 2000).

Here, we experimentally tested the hypothesis of nest tail serving as disruptive camouflage for nests of the blue manakin in a well-preserved Brazilian Atlantic Forest area. Natural nests were collected and relocated in the study area to sites with similar characteristics, and predation of plasticine eggs was compared between nests with and without tails. Nests were full-time monitored with infra-red camera traps for predators' identification and we predicted that the nest tail disruptive camouflage hypothesis would receive support if: i) tail presence is positively correlated with nest survival, controlled to other variables capable of affecting nest success such as vegetation cover, height above ground and distance from water and ii) nest predators are visually oriented.

METHODS

(a) Study Area

We conducted the experiment at Carlos Botelho State Park-PECB (24°06'55" to 24°14'41" S, 47°47'18" to 48 07'17" W), São Paulo state, southeastern Brazil. This conservation unit has 37,644 hectares of well- preserved Atlantic Forest and it is part of the largest remaining continuum of this biome, which exceeds 1.1 million

hectares (Perrella *et al*., 2021; Mattoso *et al*., 2008). In this area, carnivores (jaguars, cougars, ocelots) and large herbivores (tapirs and peccaries) are still present and are indicators that the whole community of potential nest predators also may be preserved. In the areas we conducted our study (figure 1) the altitude varied from 714 to 837 m and the vegetation is classified as submontane rainforest (Oliveira-Filho; Fontes, 2000). Average annual temperature ranges from 18 ° to 20 °C, and the annual precipitation is 1,500–2,200 mm (Ferraz; Varjabedian, 1999) with a humid subtropical climate (Cfb according to the Köppen classification) (Alvares *et al*., 2013; Peel *et al*., 2007).

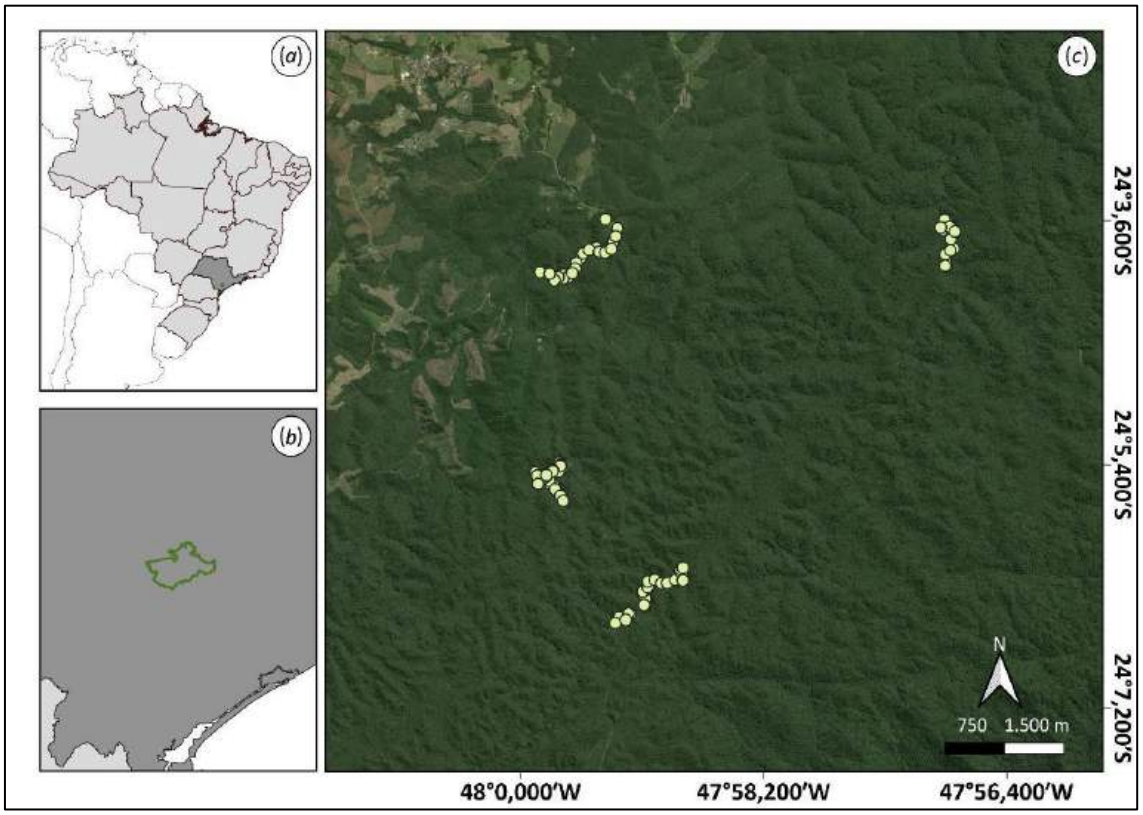

Figure 1. Study sites at Carlos Botelho State Park (PECB). (*a*) São Paulo state, in southeastern Brazil; (*b*) PECB location in São Paulo state (dark green polygon); (*c*) sites to where the blue manakin nests were relocated (circles).

(b) Experimental design

In our study area, active nests of the blue manakin (those containing eggs or nestlings) can be found from October to February and our experiment was conducted within this period. We searched for active and inactive nests in good conditions (i.e., nests with intact shape and not dispersed nest materials) by thoroughly inspecting the vegetation across the edges of four forest streams from the PECB during two breeding

seasons, 2023/2024 and 2024/2025, with active nests being used only after fledging or predation (figure 1). For each nest we obtained a set of concurrent variables, i.e., nest height above ground or water, vegetation cover, and for this specific case also distance to the nearest forest stream (Perrella *et al*., 2021).This is because increased vegetation cover, for instance, could interact with nest tail to difficult detection by visually oriented nest predators; proximity to water could increase nest detection by visually oriented animals due to the open spaces above streams, and nests constructed higher above ground also could be more exposed to forest canopy birds that can depredate nests in the study area, such as toucans (see Results). Then, we predicted that these variables should be modelled in our nest camouflage study.

For vegetation cover, nests were observed by a single person (DLB) at the four cardinal directions at a 1m radius with the eyes of the observer positioned at the same height of the nest. Then, for each direction, we adopted five visual categories of vegetation cover that were based on the percentage of the nest body that could be visualized without the interference of vegetation, i.e. 0 (absolutely no vegetation blocking the nest view); 25 (up to 25% of the nest view obstructed by vegetation); 50 (26 to 50% of the nest view obstructed by the vegetation; 75 (51 to 75% of the nest view obstructed by vegetation), and 100 (more than 75% of the nest view obstructed by vegetation). Then, we averaged the values to obtain the final vegetation cover estimate. We also used the vegetation cover above nest as a distinct variable, which was estimated by taking a picture with a smartphone positioned 1 m above the center of the nest, and the same categorization of nest cover used above was adopted based on the picture (0, 25, 50, 75, 100).

Nest height above ground or water was obtained from the base of the nest, without considering the tail, with a measuring tape accurate to 1 cm. When nests were above water, we never obtained this measure in rainy days to avoid potential variations in water level. For the distance of nest to water, we also used a measuring tape to obtain the distance from the nest border to nearest stream edge, with nests above ground receiving positive values and nests constructed over the water receiving negative values (see also Perrella *et al*., 2021).

After the above parameters were measured, we relocated each nest by assorting between up and downstream directions by tossing a coin, and then we walked along the stream to a site located 30 m from the original nest site. When in this location,

we searched for a horizontal fork in bushes and saplings of the forest understory for which vegetation density, height above ground or water and distance to stream edge was as similar as possible to the original nest site. Furthermore, because the hypothesis of nest masquerading has received partial support for the blue manakin, i.e., parental birds strongly selected nest sites with higher densities of tufts of moss or debris that closely resembled their own nests (Zima *et al*., 2022), we only chose sites in which tufts of moss or debris were absent in a radius of 5 m to avoid the potential masquerading effect of the tail. Then, the nest was tied up to the fork by its rim using a thin black sewing thread of 0.18 mm that was virtually invisible amid the nest material. When a nest was in the new site, the same habitat variables mentioned above were estimated for the validation of the experiment (see below). In each nest we placed two eggs of non-toxic plasticine with the same average measures and color pattern of the blue manakin eggs (17.2 ± 1.3 in width and 24.8 ± 1.3 mm in length) (figure 2), and we removed the tail of every other nest using scissors.

Figure 2. Nesting sites and nests of the blue manakin *Chiroxiphia caudata* in a well- preserved Brazilian Atlantic Forest area. (*a*) overview of the blue manakin nesting habitat; (*b*) details of a nest with a tail of decaying materials; (*c*) a relocated nest which tail was experimentally removed; (*d*) an active nest containing real eggs; (*e*) a relocated nest containing plasticine eggs

Because average incubation period of this species is 18 days (Zima *et al*., 2017; Ribeiro-Silva *et al*. 2018), we exposed each nest for a similar period (20 days) and monitored with an infrared camera trap (Bushnell TrophyCam, model 119437C, Bushnell Outdoor Products, Kansas City, USA) for nest predator identification. We programmed the cameras to record 30-second videos with a 3- second interval between triggers, containing date and hour. The cameras were tied to available branches at distances varying from one to three meters from the nests, following the optimization procedures suggested in (Ribeiro-Silva *et al*., 2018). We considered as depredated nests in which at least one egg was removed during the 20 days exposure.

(c) Experiment validation and statistical approaches

We considered that nest relocation was important because potential predators could have learned that the nests in their original sites were empty, reducing their interest for checking them (Latif *et al*., 2011). However, nest relocation requires the validation of the experiment ensuring that nest site characteristics capable of affecting predation were not changed by relocation. Here, we addressed the experiment validation in two different ways. First, we compared nest site variables between the "original" and "relocated" nest sites. To achieve this purpose, we used a generalized linear modeling (GLM) with binomial distribution and logit link function, with "original" and "relocated" nest sites coded as 0 and 1, respectively, for the response variable, and vegetation cover in the four cardinal directions, vegetation cover above nest, distance to water, and nest height above ground or water as explanatory variables. Because some nests were relocated more than once to increase sample size, the number of original nest sites was smaller than that of relocated sites.

For addressing the influence of each parameter in the modeling, we estimated parameter values, their standard errors (SE), and the significance of each variable was tested using Z- tests, which addresses whether each variable has differed significantly from zero. All of the explanatory variables were Z-transformed before the analysis. Second, to address whether relocations could have led to distortions in the nest site parameters between nests with and without tails, we used the same variables and statistical approach described above to compare the sites of relocated nests with and without tails.

To address the influence of nest tail in nest survival, we used a mixed effect

generalized linear model (GLMM) also with binomial distribution and logit link function, with the R package lme4 (Bates *et al*., 2015). Successful nests were coded as 0 and depredated nests were coded as 1 for the response variable, and nest tail was used as a categorical fixed-effect explanatory variable (Yes/No). To account for concurrent variables that can affect nest predation, we also used the same Z-transformed nest site variables described above as fixed-effect explanatory variables, including the interaction between tail and vegetation density. Because some nests were used more than once, and our experiment involved four different regions of the study area (Figure 1), we used Nest ID and site (categorical variable) as random effect variables

RESULTS

We used a total of 50 nests to generate 108 experimental replicates, being 54 replicates with, and 54 without tail. In the experiment validation analyses, vegetation cover in the four cardinal directions was significantly greater in the original nest sites when compared to the sites to where the nests were relocated (p = 0.012), and vegetation cover was also lower in the sites containing nests without tails when compared to sites that received the nests with tails (p = 0.025).

Four nests were visited by nocturnal, olfactory animals: The southeastern four-eyed opossum *Philander frenatus* (n = 2), the brazilian gracile opossum *Gracilinanus microtarsus* (n = 1), and an unidentified arboreal rat (n = 1). However, because they have just checked the nests without depredating the eggs, these records were not considered as predation and these nests were maintained in the experiment because they remained available for visual predators. Of the 54 nests with tail, only one was depredated (1.9%), while 11 of the 54 nests without tail were depredated (20.4%) (figure 2). Predators were recorded by the camera traps in six (all in nests without tail) of the 12 predation events, and they were all visually-oriented (other birds): The rufous-capped motmot *Baryphthengus ruficapillus* (n = 2), the red-breasted toucan *Ramphastos dicolorus* (n = 1), the giant antshrike *Batara cinerea* (n = 1), the red-crowned ant- tanager *Habia rubica* (n = 1) and the white-necked thrush *Turdus albicollis* (n = 1) (Figure 3).

The GLMM indicated tail presence/absence as the only variable significantly associated with nest predation (p = 0.032) (figure 4; table 1), in addition to an only

marginal trend for increased predation with increasing distance to water (p = 0.077) (table 1).

Figure 3. Examples of visually oriented predators recorded by camera traps during nest predation events: (a) rufous-capped motmot (*Baryphthengus ruficapillus*), (b) red- breasted toucan (*Ramphastos dicolorus*), (c) giant antshrike (*Batara cinerea*), (d) red- crowned ant-tanager (*Habia rubica*), and (e) white-necked thrush (*Turdus albicollis*).

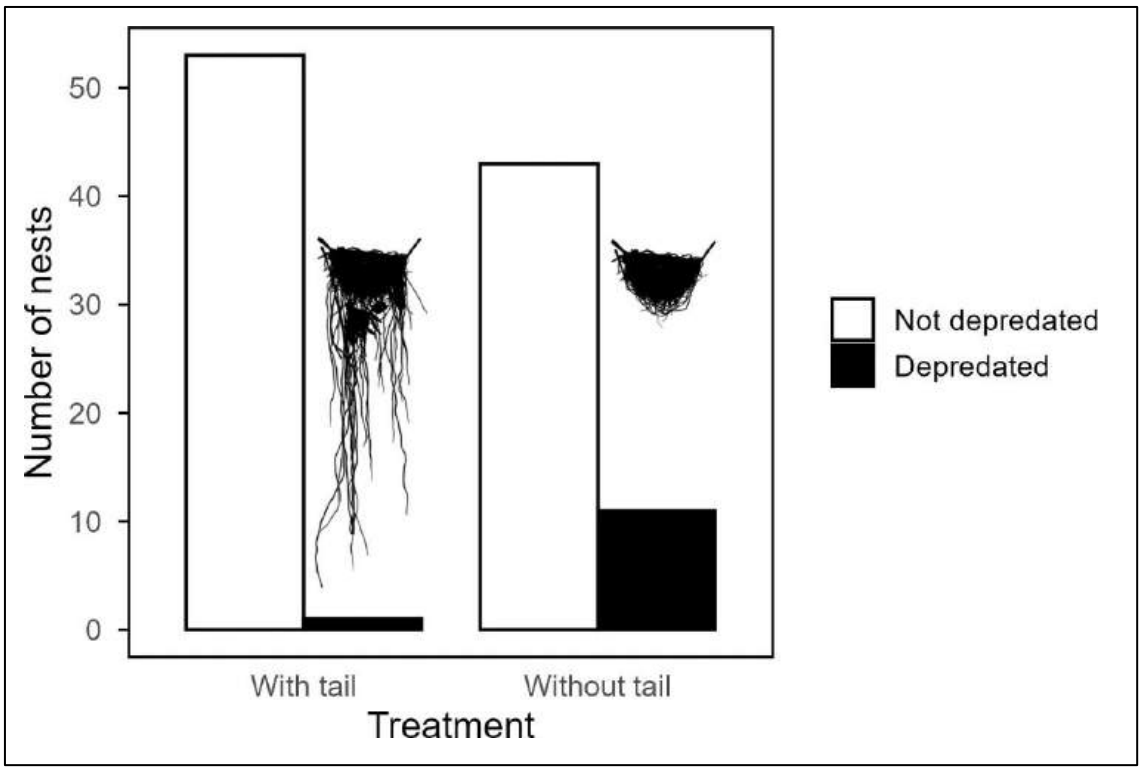

Figure 4. Comparative numbers of predations in nests of the blue manakin with and without tail, providing support to the hypothesis of nest tail as a type of disruptive camouflage.

Table 1. Results of a binomial GLMM modeling used to address predation of relocated nests of the blue manakin *Chiroxiphia caudata.* Explanatory variables are the presence/absence of nest tail (Tail); nest height above water or ground (Height); nest distance to stream edge (Distance); vegetation cover across the four cardinal directions (Vegetation cover), and vegetation cover above nests (Vegetation above). Nest ID and sampling areas were used as random effect variables. The values estimated for each model parameter (Estimate), standard error (SE), value of the Z-statistic (Z), and the level of significance of the Z-test (*p*) are provided.

Parameter	Estimate	SE	Z	*P*
(Intercept)	-2.088	0.908	-2.299	0.021*
Tail	-2.818	1.317	-2.140	0.032*
Height	0.101	0.477	0.212	0.832
Distance	1.191	0.673	1.769	0.077 ·
Vegetation cover	0.767	0.589	1.304	0.192
Vegetation above	-0.879	0.617	-1.425	0.154
Tail*Vegetation cover	-0.445	1.125	-0.396	0.692

DISCUSSION

Our main finding was the first empirical support for the adaptive function of avian nest tail, which was also the first statistically significant evidence for bird nest disruptive camouflage obtained in the field. We consider that our experimental approach using natural nests and realistic artificial eggs was ideal because we eliminated the interference of parental birds and nestlings. In the same study area, nest predation rate in active nests of the blue manakin was much higher (66%), likely because of parental activities attracting visually oriented predators and begging calls attracting olfactorily oriented animals (Zima *et al*., 2017). In the other study that to our knowledge has investigated nest tail as disruptive camouflage, only tail length variations were addressed in active nests of the acadian flycatcher, and predator types were not controlled, which may have contributed with the non-significance of the tests (Master; Allen, 2012). Although it is not expected that camera traps can record all nest predation events, they are valuable tools for assessing the main types of predators (Master; Allen, 2012; Ribeiro-Silva *et al*., 2018). The mammals recorded here on the videos commonly depredate natural eggs in the study area (Ribeiro-Silva *et al*., 2018), but in our experiment they just checked the nests, without handling or consuming the eggs, likely

because of their unnatural smell. Together with the observation that all of the egg removals captured on cameras were carried out by birds, it suggests that egg removal was caused predominantly by birds, i.e., visually oriented predators. In addition to birds, the only other visually oriented nest predator documented at the PECB is a monkey, the black capuchin *Sapajus nigritus*, which however, was not detected here and it is among the least frequent nest predators present in the area (Ribeiro-Silva *et al*., 2018). Then, it is unlikely that this species could have contributed with the predations not recorded by the cameras.

Nest relocation is a fundamental step of experimental approaches because of the potential saturation of predator's visits to nests that remained empty for certain amounts of time (Latif *et al*., 2011). However, relocated nests can only be useful if they can mirror the original nest site characteristics. Although we have done our best to choose relocation sites that were as similar as possible to the original nest sites, the new sites had lower vegetation cover, suggesting that replicating all of the original parameters is not straightforward. Importantly, among the relocated nests, those without tail had significantly lower vegetation cover, which in addition to the lack of tail also could increase detection by visually oriented predators, obscuring the tail effect. However, the lack of association between vegetation cover, as well as of the interaction between vegetation cover and tail with nest predation probability observed in the GLMM suggested that this flaw may not have weakened our finding of nest tail being the main parameter driving nest predation. A number of study systems have suggested that masquerading relies on the proximity to models (sticks, rocks, etc.) to operate (Nafus *et al*., 2015), but this idea is still controversial (Skelhorn *et al*., 2010; 2011). Here, we cannot exclude the possibility that a blue manakin nest could resemble a tuft of moss or debris, avoiding recognition by visually oriented predators, even when far from these models. Although we have relocated the nests to places without potential masquerading models, we cannot promptly discard the masquerading hypothesis, and our support for disruptive camouflage is based on the assumption that masquerading would have a reduced effect in sites where the models are absent.

Although a detailed quantification is still unavailable, a variety of birds present potential nest camouflage adaptations. Nesting stages are the steps of avian life cycle in which they experience the highest mortality rates, and most of the losses are caused by eggs and nestlings' predation (Matysioková; Remeš, 2022; Ricklefs, 1969),

meaning that the architectural aspects of the nests can be under intense selective pressures (Fang *et al*., 2018; Martin *et al*.; 2017). Then, it is surprising that so few studies have addressed nest camouflage strategies. Here we add knowledge to this field of investigation by providing robust evidence for the adaptive function of nest camouflage, increasing the theoretical background for future works on this overlooked aspect of avian evolution.

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2.2. A test for adornments as disruptive camouflage and records of active background modification in nests of the white-throated spadebill *Platyrinchus mystaceus* (Aves: Platyrinchidae)

Artigo a ser submetido para publicação em periódico científico.

INTRODUCTION

Nest predation is the main cause of avian offspring mortality (Martin, 1993; Ricklefs, 1969) and it occurs because eggs and nestlings remain for long periods of time at the same place, which increases the chances of encounter by a variety of visually or olfactorily-oriented animals (Collias, 1997; Ibáñez-Álamo *et al*., 2015; Martin, 1993; Ricklefs, 1969). Then, strategies that reduce nest detectability and access by predators are expected to evolve (Caro 2005; Ibáñez-Álamo *et al*., 2015; Solis; de Lope, 1995), and for many species it can include camouflage mechanisms (Mulder *et al*., 2021; Zima *et al*., 2023).

For bird nests, camouflage involve modifications in shape, color, texture, or in the materials used for nest construction as ways of minimizing visual detection or perception by predators (Hansell, 2000; Martin, 1993; Skutch, 1976). Types of nest camouflage so far hypothesized and tested are background matching, in which incubating individuals, eggs or nestlings become inconspicuous because they are similar in color to the surrounding substrate (Alothyqi *et al*., 2024; Gómez *et al*., 2018, 2019; Solis; de Lope, 1995; Troscianko *et al*., 2016); disruptive camouflage, when markings or structures avoid nest detection by producing false shapes (Hansell, 1996; Mulder *et al*., 2021), and masquerading, when nest shape, material or appendices make nests similar to structures present in the environment that are not of interest to predators, such as tufts of moss or debris deposits (Galligan; Kleindorfer, 2008; Nafus *et al*., 2015; Skelhorn *et al*., 2010; Zima *et al*., 2023). However, nest camouflage has been an overlooked aspect of avian evolution (Zima *et al*., 2023; Martins *et al*., *in prep;*), and works have found only mixed support for nest crypsis as antipredatory strategies (Hansell 1996; Mulder *et al*., 2021, Zima *et al*., 2023).

The white-throated spadebill, *Platyrinchus mystaceus* (Platyrinchidae) is a small passerine (12 g, 10 cm) that occurs in the understory of lowland and montane humid forests, tall secondary forests, and gallery forests from northeastern to southern Brazil,

Paraguay, Argentina, Bolivia, and parts of the Amazon (del Hoyo *et al*., 2020; Remsen *et al*., 1991). It builds conical nests attached to vertical forks of small shrubs or saplings, near the ground (del Hoyo *et al*., 2020). Nest outer walls are adorned with elongated stripes of leaves that are firmly adhered, forming a homogeneous surface, and most nests have a tail underneath (del Hoyo *et al*., 2020). For other birds, nest tails have been hypothesized to be forms of disruptive camouflage, i.e. appendices providing nests with unreal shapes to confound visual predators, an idea that received support from field experiments with nests of the Blue Manakin *Chiroxiphia caudata* (Martins *et al*., *in prep*), but not for nests of the Acadian flycatcher *Empidonax virescens* (Master; Allen, 2012).

In addition of being another suitable species for testing the adaptive function of nest tail, we observed that some of the reproductive white-throated spadebills used an uncommon strategy for tail construction. In birds of various families (e.g. Trochilidae, Pipridae, Tyrannidae, Thraupidae) nest tails are formed of loose materials adhered to nest underparts (del Hoyo *et al*., 2020). Although tails in some white-throated spadebill's nests also had hung materials, they were partially or totally formed of leaves adhered to the supporting branch below the nest. In most nests, the branches supporting the nest walls laterally were also adorned, and in a few nesting sites, stripes of the same nest material used to adorn nest walls were adhered to branchlets surrounding the nest, suggesting that at least to some level, active background modification (Stevens; Ruxton, 2018) can occur in this species. It is well known that birds can search for nesting microhabitats where the substrate matches better the color or shape of eggs, nestlings or incubating individuals (Mulder *et al*., 2021; Stevens; Ruxton, 2018; Troscianko *et al*., 2016), and that they can actively select nest materials that increase background matching (Bailey *et al*., 2015). Based on these findings, it also would be expected that birds could modify the surrounding environment to improve crypsis of nests or their contents (Stevens; Ruxton, 2018), but evidence for this idea is limited only to the egg covering behavior of plovers, which cover the eggs with materials that match egg color when nests remain unattended (Stevens, Ruxton 2018; Troscianko *et al*., 2016). To our knowledge, the potential of birds to modify the environment beyond their own nests (active background modification), theoretically for camouflage purposes, is a poorly addressed issue (see Stevens; Ruxton, 2018).

Here, we conducted an experiment to test the hypothesis that nest ornaments,

i.e. nest tail, together with the covering of branches touching the nests laterally with the same nest materials used in nest adornment can provide disruptive camouflage for nests of the white-throated spadebill. Specifically, we constructed artificial nests mimicking real nests, and we placed them in a well-preserved Atlantic Forest area following nest site and nest placement parameters obtained from real nests. Then, we compared predation rates of plasticine eggs between nests with and without these ornaments, controlled to a set variables capable of affecting nest predation (vegetation density, nest height above ground and proximity to forest streams) (Perrella *et al*., 2021). Because disruptive camouflage is a strategy evolved to confound visually oriented organisms, we monitored the nests with infra-red camera traps to confirm if nest predators were visually oriented. We predict that disruptive camouflage would receive support if nest predation is higher in nests without ornaments, and nest predators are visually oriented. Because adhering nest materials beyond nest body was a strategy used for tail construction in a number of nests, we also describe in details and discuss the idea of active background modification (Stevens; Ruxton, 2018) occurring in this species.

METHODS

(a) Study area

The study was conducted in two Atlantic Forest fragments (10 and 38 ha) located at the buffer zone of Parque Estadual Carlos Botelho (PECB), state of São Paulo, southeastern Brazil. The PECB has approximately 37.000 ha and it is part of the largest remaining Atlantic Forest continuum, with more than 1 million ha (Mattoso *et al*., 2008). The fragments belong to Fazenda Elguero – RPPN Trápaga and are characterized by primary submontane Atlantic Forest. They are 150 m far from each other and both are only 100 m from the forest continuum of the PECB, isolated by a matrix of *Pinus* spp. silviculture (Figure S1). This work was part of a major project in which nests were searched at the fragments and at the forest continuum, and this experiment was conducted at the fragments because we found much more white-throated spadebill nests at the fragments (n = 09) than at the continuous forests (n = 02). Notably, we found that communities of the main nest predators and nest predation rates in two model bird species did not differ between these fragments and the forest

continuum (Bruno *et al*., *in prep*). The local climate is classified as Cfb according to Köppen (humid subtropical climate, with temperate summer and no dry season) (Alvares *et al*., 2013; Peel *et al*., 2007).

(b) Nest searches and experimental design

During two breeding seasons (2023/2024 and 2024/2025), we searched for nests through *ad libitum* walks at least twice a week, from October to February. Nests were found by thoroughly inspecting the vegetation or when individuals were flushed from their nests due to observers' approach. For each active nest (those for which eggs or nestlings were observed), we collected a set of variables potentially related with nest survival, i.e. height above the ground, vegetation cover around and above the nest, and distance to the nearest watercourse (Perrella *et al*., 2021). To avoid nest disturbances, we measured these variables only after nest predation of fledging.

Vegetation cover was estimated by a single observer (DLB) at the four cardinal directions at distances of 1 m from the nest. In each direction, vegetation cover was visually assessed and classified into five categories based on the approximate percentage of the nest that could be visible: 0 (no obstruction), 25 (up to 25% obstruction), 50 (26–50% obstruction), 75 (51–75% obstruction), and 100 (more than 75% obstruction) (Martins *et al*., *in prep*). The final estimate for each nest was obtained by calculating the arithmetic mean of the estimates obtained in the four directions. Vegetation cover above nest was assessed separately. We positioned a smartphone 1 m above the center of the nest to take a photograph, and the same cover scale (0, 25, 50, 75, 100) was applied. We measured nest height above ground from nest bottom, disregarding nest tail, using a measuring tape with 1 cm precision. The distance to the nearest watercourse was measured with a tape, considering the shortest distance from the nest to the stream edge.

Because not many nests of the white-throated spadebill were found during the two years of searches (see Results), we hand-crafted artificial nests using air-hardening modeling clay (DAS ®), with outer walls covered with dry grass leaves (*Brachiaria spp*.) glued with liquid silicon, and the incubatory chamber filled with dark-colored fungal hyphae collected in the study area to mimic natural nests (Figure S2). To respect the dimensions of real nests, we used the average measures obtained from

active nests (55.9 mm in external height and 53,4 mm in external width) and we attached them to the chosen forks (see below) using cream-colored waxed wire, which color was similar to that of the external nest materials to make it as inconspicuous as possible (Figure 1, S2). Although we searched for nests by walking through all parts of the fragments, nests of the white-throated spadebill were always found from 0.15 to 15 m from two small forest streams (one stream in each fragment). Then, for distributing the artificial nests in the field, we used the two streams as transects. Every 30 m we searched for the first vertical fork of sapling or bush that was within the range of variations of vegetation density, heigh above ground and distance from water observed for the natural nests, in such a way that these artificial nests were at least 30 m far from each other and from any fragment border.

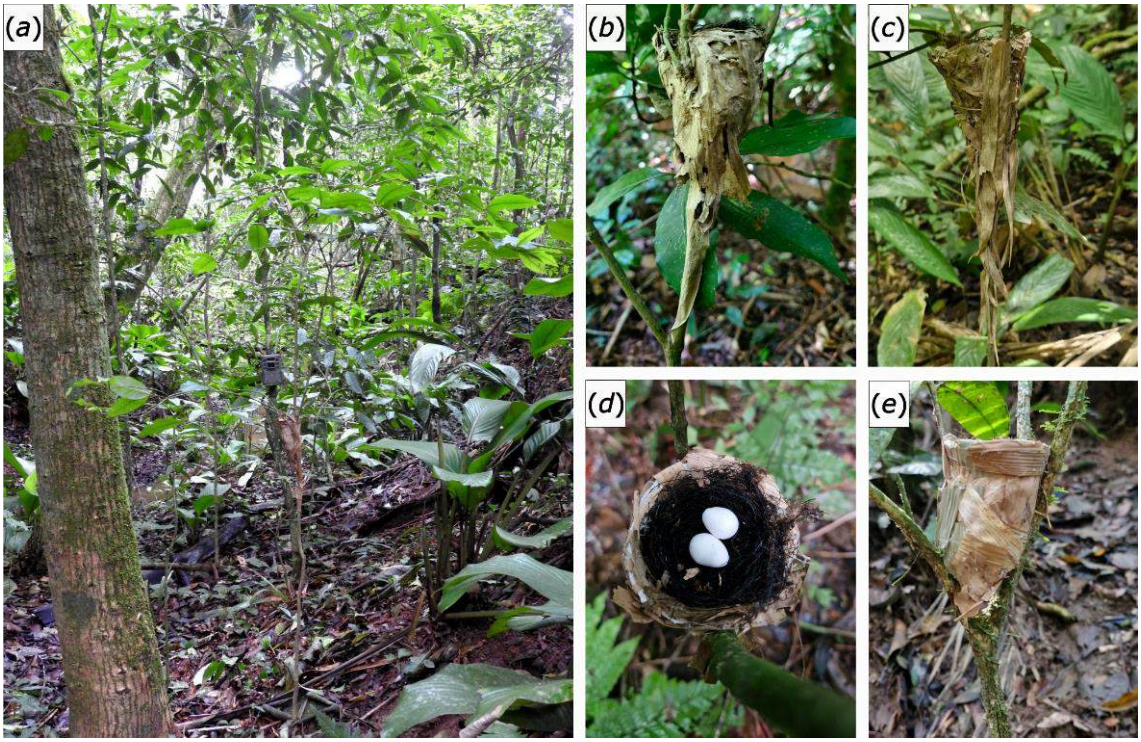

Figure 1. Environmental context and nests of the white-throated spadebill Platyrinchus mystaceus. (a) General view of the nesting habitat and of a camera trap used for nest predator identification; (b) A natural nest of the white-throated spadebill; (c) An artificial nest adorned with tail and covering of the lateral branches; (d) Artificial clay eggs; (e) Artificial nest without adornments.

For the artificial nests we also measured the same habitat variables previously described for active nests (see also Latif *et al*., 2011), and each received two non-toxic plasticine eggs with dimensions and color patterns matching that of real white-throated

spadebill's eggs (17.0 ± 0.9 mm length; 13.6 ± 0.4 mm width, Figure 1). To address the hypothesis of disruptive camouflage, alternate nests had the lateral branches and the basal fork branch, 10 cm below the nest, covered with dry grass leaves (n = 30 replicates), using liquid silicone, while in 30 replicates only nest walls were adorned, totaling 60 replicates.

From March to May 2025 we exposed each nest for 20 days, which is the approximate incubation period of the species in the study area (Bruno et al., *in prep*), and we monitored them with infrared camera traps (Bushnell TrophyCam, model 119437C, Bushnell Outdoor Products, Kansas City, USA) for predators' identification (Figure 1). We programmed the cameras to record 30-second videos, with a 3-second interval between triggers, recording date and time. We installed them on available branches at distances ranging from 1 to 3 m from the nests, following the optimization recommendations of Ribeiro Silva *et al*., (2018). We considered predation when the egg manipulation was recorded by the cameras, or when at least one egg disappeared during the exposure period.

(c) Statistical analyses

We considered that for our experiment to be valid, the nesting microhabitats of real nests should be mirrored in the artificial nests (see also Latif *et al*., 2011, Martins *et al*., *in prep*). Then, we used a PERMANOVA to compare Z-transformed vegetation density, height above ground and distance to water between real and artificial nests. To achieve this purpose, we used an Euclidean distance matrix implemented in the software PAST (Hammer *et al*., 2001), with 9,999 permutations. To address the antipredatory function of nest ornaments, we constructed a binomial GLM modeling for artificial nests, with nest fate as the response variable (depredated = 1; not depredated = 0) and ornament presence as categorical explanatory variable (yes or not), controlled to the above, Z-transformed environmental variables. The contribution of each variable for the model was evaluated by estimating the coefficient values, their standard errors (SE), and the significance of each parameter was tested using *Z*-test, with P value determining whether each parameter value differed significantly from zero.

RESULTS

From October 2023 to January 2025, we found nine active nests of the white-throated spadebill, and we conducted our experiment from March to May 2025 (Figure 1). Nest height above ground varied from 38 to 70 cm (53.2 ±14.86); distance from water varied from 0.15 m to 15 m (5.75 ± 6.18); vegetation density in the four cardinal directions varied from 0% to 100% (32.2% ± 17.61) and vegetation density above nest varied from 0% to 100% (55.5 ± 30.05). Of the nine active natural nests we found, seven had tail. Although all tails had hung loose materials (like other birds), in six of these nests stripes of the same material used for nest outer wall adornment were adhered to the basal branch of the supporting fork (Figure 2), and all nests also had the lateral forks adorned where touching the nest walls (Figure 2). Furthermore, in one nest the breeding individuals adhered nest materials throughout branchlets surrounding the nests (Figure 2).

Our PERMANOVA revealed no significant differences between the variables measured for natural nests and the experimental replicas, ensuring that environmental characteristics were not potential flaws in our experiment. Of the 60 replicates, 11 were depredated (19.33%) (seven nests with ornaments and four nests without ornaments), and the recorded predator species were the white-lipped peccarie *Tayassu pecari* (Tayassuidae; n = 1), the southeastern four-eyed opossum *Philander frenatus* (Didelphidae; n = 2) and the big-eared opossum *Didelphis aurita* (Didelphidae; n = 2) (Figure 3). None of the analyzed variables were significantly associated with nest predation probability (Table 1).

Figure 2. Natural nests of the white-throated spadebill Platyrinchus mystaceus. (a – d) Arrows depicting nest materials adhered to the supporting branches below the nests; (b and d) Arrows indicating the covering of the lateral branches with the same nest materials; (e) The adherence of nest materials at branchlets surrounding a nest, evidencing the occurrence of active background modification.

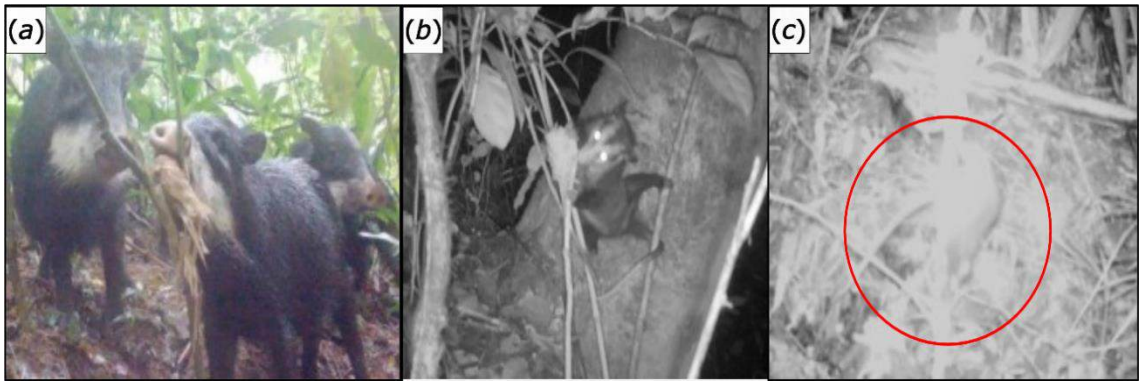

Figure 3. Olfactory-oriented predators recorded during the experiment: (a) white-lipped peccary (*Tayassu pecari*), (b) big-eared opossum (*Didelphis aurita*), and (c) southeastern four-eyed opossum (*Philander frenatus*).

Table 1. Results of the binomial GLM using predation/survival (0 or 1) of artificial nests of the white-throated spadebill as response variable. Explanatory variables are the presence or absence of nest adornment (Adornment), nest height above the ground (Height), distance from the nearest stream (Distance), vegetation cover in the four cardinal directions (Vegetation cover), and overhead vegetation cover (Vegetation above). For each parameter, we report the estimated coefficient (Estimate), its standard error (SE), the Z-statistic (Z), and the corresponding *P*-value.

Parameter	**Estimate**	**SE**	**Z**	***P***
(Intercept)	-2.652	0.848	-3.126	0.00177 **
Adornment	0.745	0.733	1.018	0.30881
Height	-0.633	0.373	-1.699	0.08930 .
Distance	-4.33	3.799	-1.140	0.25439
Vegetation cover	-0.05	0.490	-0.101	0.91993
Vegetation above	-0.198	0.484	-0.407	0.68396

DISCUSSION

Our main finding was the lack of support for nest tail as an antipredatory strategy (disruptive camouflage) in the studied population of the white-throated spadebill. A previous study on the acadian flycatcher also failed to find support for this hypothesis likely because active nests were used and parameters other than nest tail, e.g. parental movements, odor of nest contents and nestling begging calls could have affected the results. Furthermore, only tail volume variations were considered and predation by olfactively oriented animals, such as nocturnal mammals, were not controlled (Master;

Allen 2012). However, this hypothesis received support from an experiment using real nests of the Blue Manakin containing plasticine eggs, with nests that had their tails experimentally removed having a 10 times higher predation rate, caused mostly by other birds, as revealed by camera trap monitoring (Martins *et al*., *in prep*). Although here we also have eliminated the confounding effects of parental care and begging calls with the use of artificial nests and eggs, neither nest tail presence/absence, nor vegetation densities and distance to water explained nest losses. Because nest tail could theoretically confound nest recognition by visually oriented predators and the vegetation density variables we used are related with nest exposure also to visually oriented animals, the non-significant results we obtained could be at least partially explained by the reduced attraction of visually oriented predators to nests of both experimental conditions, i.e. visually oriented animals were not frequent neither in nest with, nor without ornaments.

In our study area, predators of real active nests of the white-throated spadebill were the green-billed toucan (*Ramphastos dicolorus*) (n = 3 camera trap records) and the White-necked Hawk (*Amadonastur lacernulatus*) (n = 1 record) (Ribeiro-Silva *et al*., 2018, Bruno *et al*., *in prep*), both diurnal and visually/audibly oriented birds, while two of the three animals that we recorded on camera traps, the *Tayassu pecari* and the *Didelphis aurita*, were never detected depredating arboreal nests across eight years of nest monitoring at the PECB. Then, despite our precautions to avoid human odor impregnation at nests and eggs, it is possible that these olfactorily oriented mammals could have been attracted by anthropogenic smell. Most of the nest predation events of our experiment were confirmed by egg disappearance, and the very low efficiency of camera traps to record these events (5 of 11 predations; 45,4%) was not consistent with previous works in which cameras with the same specifications (fabricant and model) recorded predator species from 50 to 57% of the predation events (Lobo-Araújo *et al*., 2024; Ribeiro-Silva *et al*., 2018). Although detection problems are difficult to explain, it is unlikely that the potential visually oriented predators of white-throated spadebill nests, i.e. green-billed toucan and white-necked Hawk, could have been missed due to camera detection failures, once they are big birds that have been frequently recorded depredating nests of Atlantic Forest passerine in general (Ribeiro-Silva *et al*., 2018, Lobo-Araújo *et al*., 2024). Then, it plausible to assume that visually oriented nest predators have played a reduced role

in the nest predations, while small rats, for instance, that are more difficult to detect, could have been potentially responsible for the unrecorded egg removals.

Then, we see two potential explanations for the lack of support to the disruptive camouflage hypothesis. First, nest placement below leaves near the ground (conditions replicated in our experiment), and small nest size may have been efficient to avoid attracting the main visually oriented nest predators (toucans and hawks) to the nests *per se*, with these predators attacking real nests likely because of adults or nestlings' behavioral cues (see also Zima *et al*., 2021). Second, the reduced probability of nest predation by visually oriented animals (other birds) could be a result of temporal nest survival effects, as predation rates and predators' willingness to consume nest contents can vary between years and throughout a reproductive season (Dinsmore *et al*., 2002, Rotella, 2018). Although our experiment did not coincide with the breeding season of *Platyrinchus mystaceus*, it was conducted within the broader reproductive period of several understory bird species in the PECB, when nest predators remain active and responsive to cues associated with nests. Nevertheless, our study covered only a single reproductive cycle, which may limit inferences about interannual variation in predation pressure. Although artificial nests have been used in many works (Gantz; Valdivia, 2021; Jokimäki; Huhta, 2000; Sloan; Holmes *et al*., 1998; Thompson *et al*., 2004), studies aiming to replicate real nests' appearance and placement of target species are uncommon (Wilson *et al*., 1998). The use of active nests (those containing real eggs or nestlings) for testing nest camouflage hypotheses can be problematic because of the confounding effects of parental and offspring movements, sounds, and smell (Master; Allen, 2012). A way of controlling to these concurrent variables is the use of real inactive nests with plasticine eggs (Mulder *et al*., 2021, Zima *et al*., 2021, 2023), and in these cases nest relocation to sites with similar microhabitat parameters is advised because predators could learn that old nests are empty, not visiting them (Martins *et al*., *in prep*). Although nests of some bird species permit manipulation (Mulder *et al*., 2021, Zima *et al*., 2021, 2023), others could fall apart when removed from the supporting branches, and it was the case of the white-throated spadebills' nests. They were so firmly attached to the supporting branches, that parts of the nests would remain attached to the forks with nest removal attempts. Furthermore, across the two years of nest searches only nine active nests of the white-throated spadebill were found, in such a way that the construction of artificial nests and the replication of

the microhabitat conditions of real nests permitted hypothesis testing with increased sample sizes. Here, the main evidence that the artificial nests were capable of mimicking real nests were 07 records of white-throated spadebills checking these nests. We obtained 07 video records from 04 nests in which white-throated spadebills (likely territorial individuals) not only perched at the artificial nests, but also perforated the plasticine eggs, a behavior that should be better investigated in this species.

It is important to highlight that our non-significant result does not invalidate the potential antipredatory function of white-throated spadebill nest tails and the disruptive camouflage hypothesis should be addressed in other contexts. The white-throated spadebill is a widely distributed species that occurs throughout many different types of habitats beyond the Atlantic Forest, including gallery forests and the Amazon (del Hoyo *et al*., 2020). Across these environments, not only vegetation structure can vary, but also the types of visually oriented predators, and we are unaware whether nest tails could be more correlated with nest survival in other populations.

Although we focused our tests in the potential antipredatory effect of the final product, i.e. nest ornaments, in most nests at least part of the tails were constructed by adhering stripes of leaves to the supporting branch, which is an example of active modification of the nest background environment (Stevens; Ruxton, 2018). It is also noticeable that in virtually all of the nests the branches touching nests laterally were also adorned and, in a few nests, even branches beyond the nest supporting fork were adorned with the same material used in nest walls, which can be intuitively interpreted as a nest camouflage strategy. Here we considered only the combined effect of nest tail and lateral fork adornment (nest ornaments) because the addition of nest materials to surrounding branches was rare, but it should be investigated whether this strategy can be more common in other populations, deserving experimental analyses. Independently of the frequency, this behavior is different from the egg covering behavior observed for plovers (Stevens; Ruxton, 2018; Troscianko *et al*., 2016).

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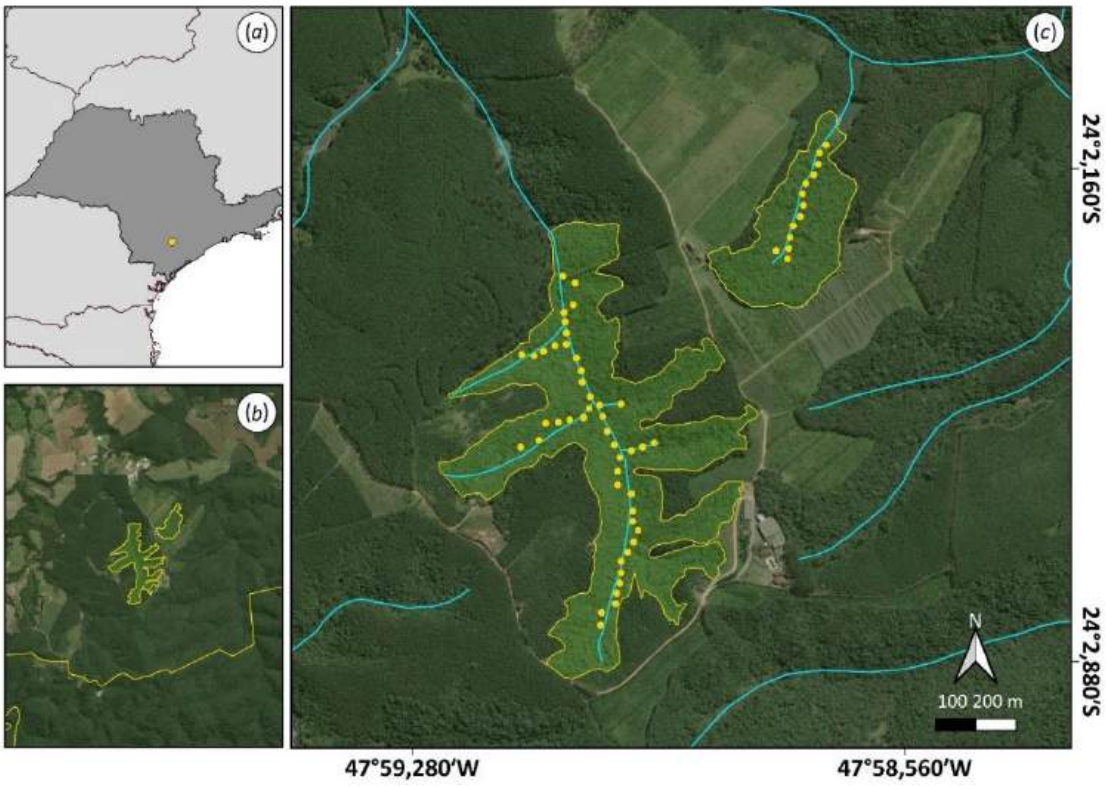

Figure S1. Study sites. (a) São Paulo state, southeastern Brazil; (b) forest fragments (polygons) and the delimitation of the limits of Parque Estadual Carlos Botelho - PECB (yellow line below the polygons); (c) locations where artificial nests of the white- white-throated spadebill Platyrinchus mystaceus were installed (yellow dots), with watercourses represented by blue lines.

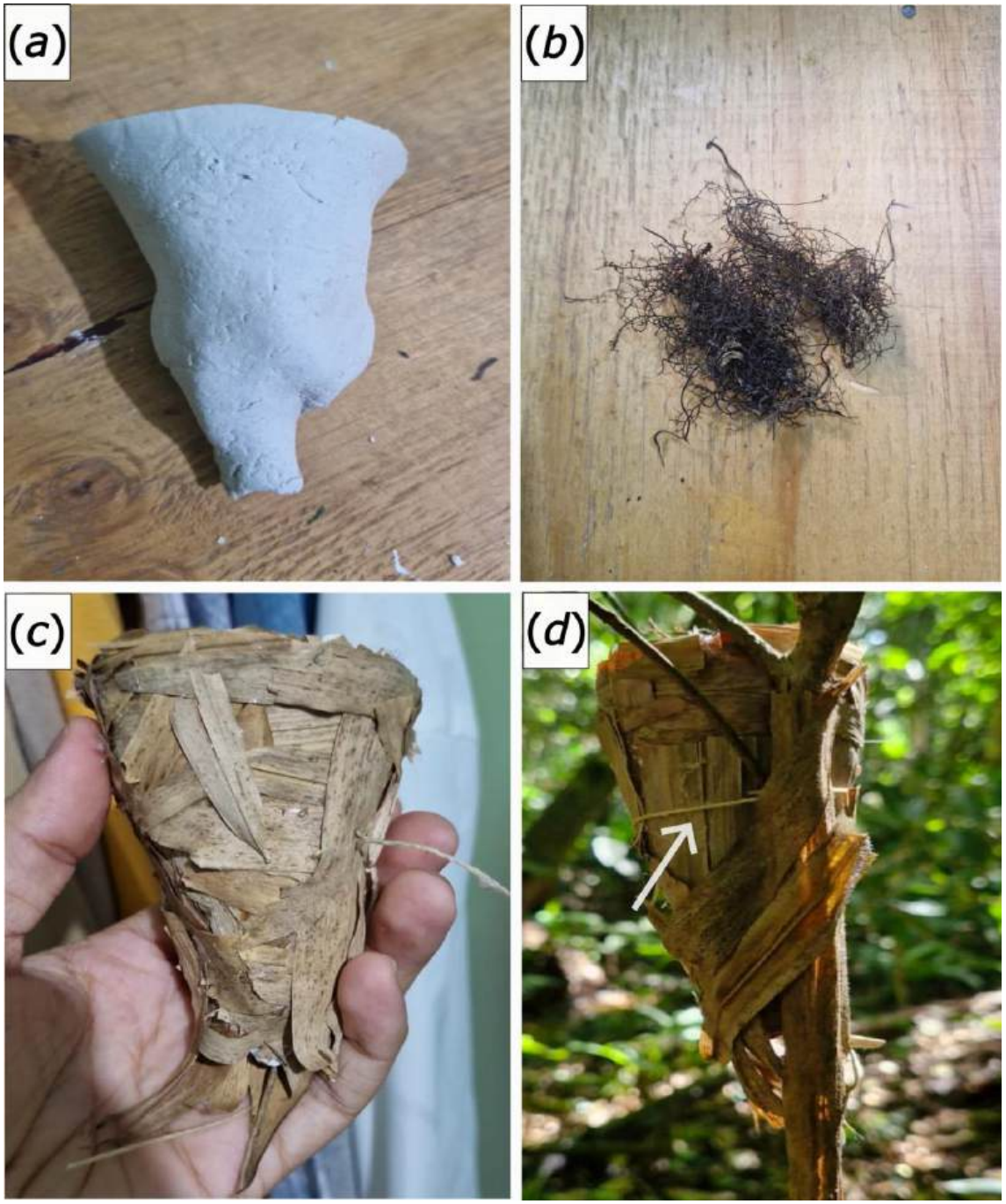

Figure S2. Steps of the construction of artificial nests of the white-throated spadebill Platyrinchus mystaceus. (a) Nest basis constructed of air-hardening modeling clay (DAS ®); (b) Tufts of black fungal hyphae used for the lining of the incubatory chamber; (c) Clay nest adorned with dry leaves of *Brachiaria* spp.; (c) Artificial nest placed in the field, with the white arrow indicating the wire used for nest attachment.

3. CONSIDERAÇÕES FINAIS

A abordagem experimental realizada neste estudo permitiu avaliar de maneira direta o papel de estruturas e ornamentações na redução da detectabilidade de ninhos por predadores visuais, oferecendo evidências inéditas sobre os mecanismos e a eficácia da camuflagem em espécies tropicais. Ao integrar dados de predação, monitoramento por câmeras e experimentos controlados, este trabalho contribui significativamente para preencher uma lacuna histórica na literatura sobre o tema; a ausência de testes empíricos robustos sobre o papel funcional da camuflagem em ninhos construídos por passeriformes neotropicais. Embora o tema da camuflagem seja amplamente abordado em grupos que nidificam no solo ou em ambientes abertos (Gómez *et al*., 2018; Stevens *et al*., 2017), os estudos voltados a espécies florestais, especialmente da Mata Atlântica, permanecem escassos e fragmentados (Cockle *et al*., 2019; Mulder *et al*., 2020; Roper; Goldstein, 1997). Até o presente, o número de trabalhos publicados que testaram diretamente hipóteses de camuflagem de ninhos estruturais ainda é reduzido, concentrando-se em poucos grupos e carecendo de replicações metodológicas em diferentes contextos ecológicos.

Os resultados obtidos para o tangará (*C. caudata*) representam um marco no avanço desse campo, pois constituem a primeira demonstração empírica da função adaptativa da “cauda” do ninho como camuflagem disruptiva. A diferença expressiva nas taxas de predação entre ninhos com e sem essa estrutura confirma que ela atua funcionalmente na distorção do contorno do ninho e consequentemente, na diminuição de sua conspicuidade no ambiente. Trata-se, portanto, da primeira evidência direta de que a “cauda”, uma característica frequentemente descrita apenas de forma descritiva em piprídeos, tem papel funcional claro na redução da detecção por predadores visuais. Esse resultado não apenas confirma a hipótese de camuflagem disruptiva, mas também estabelece *C. caudata* como uma espécie modelo excepcional para o estudo de estratégias antipredatórias associadas à arquitetura de ninhos, dada sua abundância relativa, acessibilidade dos ninhos e facilidade de observação em campo. Em contraste, para *Platyrinchus mystaceus*, revelou-se um sistema mais desafiador. A raridade de ninhos ativos e a dificuldade de localização no sub- bosque denso da Mata Atlântica impuseram limitações que dificultam experimentos com grandes amostras. Por outro lado, esse desafio metodológico fomentou uma inovação

importante deste trabalho: o uso de ninhos artificiais realistas. Essa estratégia, além de representar uma novidade metodológica no contexto de estudos tropicais, oferece uma solução plausível e replicável para contornar a escassez de ninhos naturais e controlar variáveis críticas, como formato, exposição e conteúdo. O uso de modelos artificiais validados por testes prévios aproxima o experimento das condições naturais, mantendo a padronização necessária para testar hipóteses funcionais com maior precisão. Essa abordagem, portanto, contribui não apenas para o avanço conceitual, mas também metodológico da área, propondo um caminho promissor para estudos futuros.

Os resultados obtidos para o patinho, ainda que não tenham revelado diferença significativa nas taxas de predação entre ninhos ornamentados e não ornamentados, indicam que o comportamento consistente de adicionar materiais aos galhos de sustentação demostra uma possível função complementar. Essa ornamentação pode estar ligada à integração visual do ninho com o substrato, no reforço estrutural ou regulação microclimática, hipóteses que ainda carecem de testes direcionados. O padrão observado reforça que as pressões seletivas sobre o design dos ninhos são multifatoriais, variando entre espécies e ambientes, e que diferentes modalidades de camuflagem, como *background matching*, *masquerade* e *camuflagem disruptiva* podem coexistir e atuar de forma complementar. Essa diversidade de mecanismos de camuflagem, amplamente reconhecida em outros grupos animais, ainda é pouco explorada em aves tropicais (Lima, 2009), o que evidencia o vasto potencial para novas investigações.

Outro aspecto relevante diz respeito à validação experimental dos métodos. Muitos trabalhos anteriores, como o estudo de Master e Allen (2012) com ninhos de *Empidonax* virescens em ambientes temperados, não controlaram adequadamente variáveis fundamentais, como o tipo de predador ou a distinção entre predação visual e olfativa. A ausência desses controles metodológicos dificultou interpretações conclusivas sobre os mecanismos de camuflagem. Ao empregar câmeras para identificar predadores e considerar apenas eventos de predação feitos por animais visualmente orientados, o presente estudo estabelece um padrão de validação e rigor experimental que deve ser seguido em trabalhos futuros. Essa padronização é essencial para garantir comparabilidade entre estudos e para compreender de forma mais precisa como as diferentes pressões seletivas moldam a evolução dos ninhos.

Em conjunto, os achados reforçam que a arquitetura dos ninhos é moldada por múltiplas forças seletivas, nas quais a camuflagem visual constitui uma força evolutiva relevante, porém variável entre espécies e contextos. Ao demonstrar que estruturas aparentemente ornamentais podem desempenhar funções adaptativas diretas, este trabalho amplia a compreensão dos ninhos como fenótipos estendidos sujeitos à seleção natural. Além disso, evidencia que a diversidade de formas e estratégias observadas entre espécies não é apenas resultado de circunstâncias ecológicas, mas também de trajetórias evolutivas distintas, nas quais a interação entre predadores e presas desempenha papel fundamental.

Por fim, este trabalho reforça a importância da Mata Atlântica como cenário privilegiado para estudos sobre evolução comportamental e morfológica em aves. Ambientes de alta complexidade estrutural e diversidade taxonômica, como esse bioma, oferecem oportunidades únicas para investigar como a forma e o contexto se combinam na evolução de estruturas adaptativas. O conjunto de resultados aqui apresentados consolida o *Chiroxiphia caudata* como uma espécie modelo para experimentos de camuflagem e propõe o *Platyrinchus mystaceus* como um novo sistema promissor, cuja abordagem experimental com ninhos artificiais poderá ser expandida e refinada em estudos futuros.

Assim, esta tese estabelece novos fundamentos empíricos e metodológicos para o estudo da camuflagem de ninhos tropicais, ressaltando a importância da experimentação validada, da diversidade de estratégias de camuflagem e da escolha de espécies-modelo adequadas. Esses avanços não apenas preenchem lacunas na literatura, mas também apontam caminhos para um meio de pesquisa mais integrado e comparativo sobre a ecologia e a evolução dos ninhos das aves.

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