UNIVERSIDADE ESTADUAL DE CAMPINAS
INSTITUTO DE BIOLOGIA - IB

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CAMUFLAGEM SOB DIFERENTES CONTEXTOS E PERSPECTIVAS: PADRÕES GERAIS, MECANISMOS E EFEITOS DO SUBSTRATO UTILIZADO

CAMPINAS
2023

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Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Ecologia.

Orientador: Dr. Gustavo Quevedo Romero

ESTE TRABALHO CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELO ALUNO JOÃO VITOR DE ALCANTARA VIANA E ORIENTADA PELO PROF. DR. GUSTAVO QUEVEDO ROMERO.

CAMPINAS

2023

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca do Instituto de Biologia
Mara Janaina de Oliveira - CRB 8/6972

D342c
de Alcantara Viana, João Vitor, 1992-
Camuflagem sob diferentes contextos e perspectivas : padrões gerais, mecanismos e efeitos do substrato utilizado / João Vitor de Alcantara Viana. – Campinas, SP : [s.n.], 2023.

Orientador: Gustavo Quevedo Romero.
Tese (doutorado) – Universidade Estadual de Campinas, Instituto de Biologia.

1. Animais - Comportamento. 2. Predador-presa. 3. Ecologia sensorial. 4. Cor - Percepção. 5. Camuflagem (Biologia). I. Romero, Gustavo Quevedo, 1974-. II. Universidade Estadual de Campinas. Instituto de Biologia. III. Título.

Informações Complementares

Título em outro idioma: Camouflage under different contexts and perspectives : general patterns, mechanisms and effects of the used background
Palavras-chave em inglês:
Animal behavior
Predator-prey
Sensory ecology
Color vision
Camouflage (Biology)
Área de concentração: Ecologia
Titulação: Doutor em Ecologia
Banca examinadora:
Gustavo Quevedo Romero [Orientador]
João Vasconcellos Neto
Yuri Fanchini Messas
Vinícius Lourenço Garcia de Brito
Marcelo de Oliveira Gonzaga
Data de defesa: 01-09-2023
Programa de Pós-Graduação: Ecologia

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0002-5249-5362
- Currículo Lattes do autor: http://lattes.cnpq.br/2274001910884190

Campinas, 01 de Setembro de 2023

COMISSÃO EXAMINADORA

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Os membros da Comissão Examinadora acima assinaram a Ata de defesa, que se encontra no processo de vida acadêmica do aluno.

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa de Pós-Graduação em Ecologia do Instituto de Biologia

Com orgulho de quem alcança um objetivo importante, dedico essa tese à minha família, que é a minha base e que me deu toda a estrutura para perseguir e alcançar meus sonhos.

AGRADECIMENTOS

Gostaria de agradecer ao **Prof. Dr. Gustavo Quevedo Romero**, por ter me aceitado como aluno, mesmo que meu tema de estudo não seja o mesmo da linha de pesquisa principal e atual do laboratório. Agradeço pela confiança, paciência e por toda a motivação que me deu. Agradeço pela oportunidade de participar de outros projetos não relacionados a minha tese, em especial pelas expedições de campos em que cruzamos o Brasil para trabalhar na Amazônia. Um bioma lindo que tive a oportunidade de conhecer. Obrigado por manter as portas do seu laboratório para mim, pelos conselhos acadêmicos e pessoais, em que aprendi de perto tantas coisas importantes que farão de mim melhor como pessoa e como profissional.

Agradeço aos meus principais colaboradores e membros do comitê de acompanhamento, à **Dra. Camila Vieira** e ao **Dr. Rafael Campos Duarte**, que muito me ajudaram desde o início do doutorado. Agradeço pela oportunidade de conhecer pessoas incríveis como vocês, que me deram suporte científico e emocional nessa caminhada acadêmica. Obrigado por confiarem em mim e por entrarem a fundo nas mais variadas ideias que tivemos juntos. Ainda temos muito a fazer em colaboração. Desejo todo o sucesso do mundo a vocês!

Agradeço à minha esposa **Carolina Lambertini de Alcantara**, a qual, dentre os tantos desencontros e probabilidades da vida, tive a sorte de encontrar durante o meu doutorado e de construir uma família com laços fortes para uma vida toda. Agradeço por me fazer uma pessoa melhor a cada dia, uma habilidade de transformação que poucos corações possuem. Agradeço por ser extremamente parceira, cuidadosa, paciente e pelos vários ensinamentos e ajudas no mundo acadêmico, incluindo tudo que passamos nos EUA. Por acreditar em mim mesmo quando eu mesmo não acredito. Agradeço ao universo pelo nosso amor e por nossa família.

Agradeço ao meu amigo **Wagner Luiz dos Santos**. Aprendi muito com você, em especial a não desanimar quando as coisas não dão certo. Criamos uma amizade com laços fortes e irmandade, baseada em muita parceira e ajuda mútua. Desejo que sua caminhada seja suave, pois merece o que o mundo tem de melhora a oferecer.

Aos meus amigos **Bruna** (Cine) e **Mateus**. Obrigado pelos aprendizados durante nossas discussões sobre estatística na pandemia. Todo esse tempo juntos nos trouxe estórias memoráveis, divertidas e alegres. Sempre recordarei o tempo que passamos juntos.

Ao meu amigo e físico **Lucas Pollyceno**. Obrigado pela amizade e parceria nos tempos de república. Sua amizade será sempre muito importante para mim. Agradeço também ao **Herivelton**, foi bom compartilhar com você duas repúblicas diferentes. Agradeço à **Marina Neves** pelos aprendizados, espero que tenha uma vida repleta de muitas alegrias e realizações.

Ao **Du "01", Mari**, **Pri** e **Migo** pela amizade e momentos compartilhados na república. Obrigado por abrirem as portas para mim e pelo acolhimento. À **Cris**, **Rose** e **Bruno** pela amizade e parceria. Ao **Trovão**, meu parceiro fiel de quatro patas.

Ao **Felipe Coelho**, **Anna Martins** e **Júlia Chechinnato** pelas ajudas de campo nos Cerrados de Uberlândia. Essa ajuda foi imprescindível para o desenvolvimento dos capítulos da minha tese. Agradeço também ao **Prof. Vinícius Brito**, pelos transportes e ajuda com os campos. Aos meus amigos de Uberlândia **Lino**, **Adilson**, **Vitor e João**. Obrigado por me hospedarem, por me ajudarem com o material de campo e por todas as risadas. Ao **Rogério** e **Paola** e **Jaqueline** pela amizade e parceria nos desafios do campo com as pererecas.

Ao **Pablo** por todos os aprendizados no campo da Amazônia e por compartilhar comigo momentos felizes que levarei para vida. À **Paula Omena**, **Thiago** e **Micael** por me receberem na república no início do doutorado. Aos amigos do Laboratório de Interações Multitróficas e Biodiversidade e por todos os momentos e aprendizados juntos, à **Izadora**, **Dai**, **Paula**, **Felipe**, **Rúbia**, **Thales, Lucas e Zé.**

Aos meus amigos e familiares de Botucatu, Uberlândia e Campos Novos Paulista, por todo apoio e por tantos momentos bons compartilhados juntos.

Agradeço ao **Nuno** e **Marlene Lambertini**, à **Fernanda** e **Bruno** por me aceitarem como família, por torcerem para mim e por me tratarem tão bem.

Agradeço as minhas irmãs **Camila** e **Isabella**. Vocês possuem um grande papel na minha formação como um todo, da graduação ao Doutorado, de financeiro a emocional. Obrigado por toda confiança, amor e carinho. Espero retribuir tudo o que fizeram por mim. Aos meus pais **Nilva** e **Vicente**, obrigado pela confiança e torcida durante todo esse processo. Obrigado pela criação que me deram, baseada em muito respeito com pessoas e tudo o que é vivo. Obrigado por todo amor que me deram. Realizo aqui um sonho pessoal, mas não é uma vitória só minha, pois o compartilho com minha família e com os que torcem por mim.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001

RESUMO

O sucesso da camuflagem depende de vários fatores, como variações na coloração do animal e do substrato, iluminação, observador e comportamento. O objetivo desta tese foi investigar padrões, mecanismos e processos em estratégias de camuflagem em diferentes grupos animais. No Capítulo 1, realizamos uma meta-análise para examinar como diferentes estratégias de camuflagem impactam o tempo de procura e a taxa de ataque dos predadores às presas. Constatamos que o mascaramento foi a estratégia mais eficaz no aumento do tempo de procura. A camuflagem por movimento mostrou eficácia apenas em relação à taxa de ataque, enquanto os ocelos não foram eficazes. No Capítulo 2, investigamos os efeitos das queimadas na camuflagem de artrópodes polimórficos no Cerrado. Calculamos o contraste de cor para aves predadoras em artrópodes melânicos e marrons (*Ronderosia bergii* e *Eumiopteryx laticollis*) e em uma espécie de aranha melânica (*Syntrechalea brasilia*), associando com o tempo de permanência em arenas experimentais. Os artrópodes melânicos apresentaram menores valores de contraste acromático em troncos queimados, e apenas a aranha mostrou menor contraste cromático e acromático em troncos queimados, escolhendo-os como substrato de camuflagem. Ao criar presas teóricas com diferentes estratégias de camuflagem, testamos o tempo de procura e a distância de encontro com participantes humanos como predadores, em áreas queimadas e não queimadas. Em áreas queimadas, apenas as presas melânicas se camuflaram efetivamente, enquanto, em áreas não queimadas, a coloração disruptiva foi mais efetiva que as presas marrons, destacando-se também na eficácia em relação à distância. No Capítulo 3, examinamos como as queimadas afetam as estratégias de camuflagem em comunidades. Coletamos artrópodes em troncos queimados e não queimados, verificando os contrastes de cores e níveis de coloração disruptiva para aves predadoras. Quantificamos os indivíduos categorizados em relação às estratégias de camuflagem, relacionando o tamanho com contraste e coloração disruptiva. Os contrastes nos troncos queimados foram menores, ao contrário dos animais encontrados nos troncos não queimados. A coloração disruptiva não diferiu entre os tipos de troncos, e não encontramos relação entre nenhuma variável e o tamanho. No Capítulo 4, analisamos a contribuição da correspondência de pano de fundo e coloração disruptiva na efetividade de camuflagem contra aves predadoras de morfoespécies de louva-deus simpátricas na Amazônia. Coletamos três morfotipos em troncos esbranquiçados e marrom-esverdeados. Morfoespécies branca e cinza correspondiam melhor a troncos esbranquiçados, enquanto a espécie verde apresentava menores contrastes em troncos esverdeados, sendo mais disruptiva. Experimentos de predação revelaram que morfoespécies brancas foram favorecidas em troncos esbranquiçados, enquanto morfoespécies verdes foram favorecidas em troncos marrons-esverdeados. No Capítulo 5, verificamos se a mudança de cor fisiológica em uma perereca noturna do Cerrado favorece sua camuflagem sob grama e serapilheira, respectivamente, contra corujas predadoras. As pererecas foram capazes de sucessivas mudanças de coloração, diminuindo seu contraste cromático conforme o substrato. Esta tese preenche lacunas na ecologia comportamental e sensorial, discutindo padrões nas estratégias de camuflagem, mecanismos e processos que as tornam amplamente utilizadas na natureza. Este trabalho abre novas possibilidades de estudos sobre camuflagem pouco explorados na região mais biodiversa do planeta, os neotrópicos.

ABSTRACT

The success of camouflage depends on various factors, such as variations in the animal and substrate coloration, lighting, observer characteristics, and behavior. The aim of this thesis was to investigate patterns, mechanisms, and processes in camouflage strategies across different animal groups. In Chapter 1, a meta-analysis was conducted to examine how different camouflage strategies impact the search time and predator attack rates on prey. It was found that masking was the most effective strategy in increasing search time. Camouflage through movement was effective only in relation to the attack rate, while ocelli were not effective. In Chapter 2, the effects of wildfires on the camouflage of polymorphic arthropods in the Cerrado were investigated. Color contrast for predatory birds was calculated for melanic and brown arthropods (*Ronderosia bergii* and *Eumiopteryx laticollis*) and a melanic spider species (*Syntrechalea brasilia*), associated with the time spent in experimental arenas. Melanic arthropods showed lower achromatic contrast on burned trunks, and only the spider exhibited lower chromatic and achromatic contrast on burned trunks, selecting them as camouflage substrates. The creation of theoretical prey with different camouflage strategies tested search time and encounter distance with human participants as predators in burned and unburned areas. In burned areas, only melanic prey effectively camouflaged themselves, while in unburned areas, disruptive coloration was more effective than brown prey, standing out in effectiveness even in terms of distance. In Chapter 3, the impact of Cerrado wildfires on camouflage strategies at the community level was examined. Arthropods were collected from burned and unburned tree trunks, and color contrasts and levels of disruptive coloration for predatory birds were assessed. Individuals were quantified in relation to their camouflage strategies, relating size to contrast and disruptive coloration. Contrasts on burned trunks were lower, unlike animals found on unburned trunks. Disruptive coloration did not differ between trunk types, and no relationship between any variable and size was found. Chapter 4 analyzed the contribution of background matching and disruptive coloration in the effectiveness of camouflage against avian predators for sympatric morphospecies of mantises in the Amazon. Three morphotypes occurring on whitish and brownish-greenish trunks were collected. White and gray morphospecies matched better on whitish trunks, while the green morphospecies showed lower contrasts on greenish-brown trunks and higher levels of disruption. Predation experiments revealed that white morphospecies were favored on whitish trunks, while green morphospecies were favored on brownish-greenish trunks.In Chapter 5, physiological color changes in a nocturnal Cerrado tree frog were investigated for their camouflage effectiveness under grass and leaf litter against predation by owls. The frogs were capable of successive color changes, reducing chromatic contrast depending on their substrate. This thesis fills gaps in behavioral and sensory ecology, discussing patterns in camouflage strategies, mechanisms, and processes that make them widely utilized in nature. This work opens new possibilities for studying camouflage that are still underexplored in the most biodiverse region on the planet, the neotropics.

SUMÁRIO

INTRODUÇÃO GERAL

O estudo da coloração animal como ciência teve sua fundação no século XIX por pioneiros naturalistas, que contribuíram para um variado e crescente campo de disciplinas na biologia (Caro et al., 2017). Dentre grandes nomes, podemos citar Erasmus Darwin (1794), com seu relato: "as cores de muitos animais parecem adaptadas aos seus propósitos de se esconderem, seja para evitar o perigo, seja para atacar suas presas". Wallace (1807), por sua vez, descreve que observações de insetos miméticos e crípticos o ajudaram a se convencer do poder da seleção natural. Posteriormente, Poulton (1890), em seu livro "The colours of animals", introduziu os temas camuflagem e aposematismo, assim como a ideia de predação dependente de densidade, na qual predadores selecionam presas com fenótipos de cor mais abundantes, o que mantem o polimorfismo nas populações (Vasconcelos-Neto & Gonzaga, 2000). O campo de estudo da coloração defensiva teve colaborações importantíssimas que introduziram vários termos atuais feitas por profissionais de áreas diversas, como o artista Thayer (1909) e o zoólogo Abbot Cott (1940). Essas contribuições, publicadas nos livros "Concealing-coloration in the animal kingdom" (Thayer,1909) e "Adaptive Coloration in Animals" (Cott, 1940), respectivamente, possuem ilustrações extremamente persuasivas, com associações entre coloração animal e o meio, que definiram as bases do que hoje chamamos correspondência de pano de fundo e coloração disruptiva, além de diversas outras estratégias defensivas (Cuthill, 2019). Muitas dessas primeiras observações foram aplicadas em conflitos mundiais, incluindo instruções de camuflagem feitas pelo próprio Cott para os Britânicos na Segunda Guerra Mundial (Caro et al., 2017). Nesses conflitos, a camuflagem foi utilizada desde pinturas (exemplo: manchas e listras) a arranjos de objetos (exemplo: folhas e galhos.) em uniformes, veículos, navios e aviões, que tinham o objetivo de desviar ou dificultar a identificação do alvo pelo inimigo e que são usadas e melhoradas até hoje nas forças armadas de todo o mundo (Talas et al., 2017).

Dentre as estratégias anti-predatórias registradas na natureza, talvez a camuflagem seja a mais amplamente empregada e distribuída (Stevens & Merilaita, 2011). Considerada um tipo de defesa anti-predatória primária, pois funciona passivamente protegendo a presa (Owen, 1980), é composta por diferentes tipos de adaptações, em especial relacionadas a coloração e morfologia do organismo, que reduzem a probabilidade de detecção ou reconhecimento deste pelos observadores (Merilaita et al., 2017). É descrita em animais e plantas, sendo descrita em fósseis de dinossauros a diversos táxons atuais (Vinther et al., 2016; Brown et al., 2017; Niu et al., 2021; Nokelainen et al., 2022). Também pode ser encontrada nos nos mais variados ambientes terrestres, desde desertos até florestas, e aquáticos, de águas rasas aos oceanos abertos e profundos (Cronin, 2016; Matchette et al. 2018; Nokelainen et al., 2020). Endler (1978) define três funções para a coloração animal: termorregulação, comunicação interespecífica e estratégias anti-predatórias. No entanto, apesar de muits estudos focarem no papel protetivo da camuflagem, vale ressaltar que essas estratégias de ocultação são também altamente empregadas por predadores, já que aumentam o sucesso de captura das presas por mecanismos similares que operam também na proteção (Vieira et al., 2015; Smith & Ruxton, 2020).

São descritas mais de 16 estratégias de camuflagem, cada uma agindo especificamente no mecanismo sensorial e cognitivo do observador (Merilaita et al., 2017). Nas rotas sensoriais visuais, há uma relação de sinal e ruído das informações obtidas, em que o organismo é mais facilmente detectado quando há um desbalanço para mais sinal e menos ruído. Os padrões de coloração dos variados tipos de camuflagem exploram as reduções massivas de processamento de informação de sinais físicos pelo cérebro, durante a criação de atalhos de percepção (Troscianko et al., 2009; Merilaita et al., 2017). Tendo isso em vista, estratégias como coloração de correspondência de pano de fundo, diminuem a informação de sinal por meio da semelhança em coloração e luminância (brilho) a uma amostra do seu substrato utilizado.A coloração disruptiva, por sua vez, é definida por padrões de coloração altamente contrastantes que quebram o contorno do animal e geram ruídos que dificultam detecção e reconhecimento (Merilaita et al., 2017; Cuthill, 2019).

Existem estratégias de camuflagem que não necessariamente reduzem as chances de detecção, mas de reconhecimento, tal qual o mascaramento, em que organismos se assemelham a elementos ou objetos inanimados do ambiente, como pedras, folhas ou gravetos (Skelhorn et al., 2010). A camuflagem por decoração se assemelha ao mascaramento, dificultando o reconhecimento de animais que fixam em seus corpos objetos inanimados (e.g., pedras ou grãos de areia) ou até mesmo outros organismos, como plantas, algas e esponjas (Allgaier, 2007; Yanes et al., 2009). A camuflagem por meio do movimento é alcançada por meio de listras ou padrões de cores que se alinham e se correspondem com a cor do fundo utilizado, sendo específicamente associada a comportamentos que minimizam ou perturbam o sinal de movimento, ou que mimetizam o fluxo óptico no sistema sensorial do observador (Stevens & Merilaita, 2011). Esse tipo de camuflagem ainda é questionado quanto sua eficiência, dado que muitos predadores são extremamente sensíveis ao movimento (Mizutani et al., 2003). Contudo, existem evidências que suportam que esses padrões de cores agem dificultando a identificação e prejudicando o ataque, ou mesmo a discriminação de trajetória da presa pelo predador (Stevens et al., 2011). Evidências robustas sobre a efetividade de algumas estratégias de camuflagem ainda são escassas, como o papel efetivo das marcas de distração, que são pequenas manchas nas extremidades do animal que distraem o predador de detalhes corporais que revelam a presença da presa (Stevens et al., 2013). Já os ocelos ou “manchas oculares” operam, potencialmente, defletindo o ataque do predador ou intimidando-o (Mukherjee & Kodandaramaiah, 2015; Prudic et al., 2015).

A efetividade da camuflagem depende de múltiplos fatores, de ambientais a intrínsecos ao organismo (de Alcantara Viana et al.,2022 a). Ela pode ser mais ou menos protetiva devido a variações de agentes físicos sazonais, estocásticos ou antrópicos, que alteram a coloração e textura dos substratos, ou que promovem alterações na iluminação ambiental (Walton & Stevens, 2018; Carter et al., 2020). Um caso clássico de como fatores antrópicos alteram as pressões de predação em fenótipos distintos de uma espécie (polimorfismo) é o da mariposa *Biston betullaria*, durante a revolução industrial na Inglaterra. Em um período de expansão da industrialização, muitos poluentes liberados no ar alteraram a cor dos substratos usados pelas mariposas ao escurecer os troncos e matar líquens que os cobriam. Esse escurecimento favoreceu o fenótipo escuro da mariposa (*f. carbonaria*), que era menos comum que o claro (*f. typica*), e que foi menos predado por aves insetívoras, que identificavam facilmente as mariposas claras sobre os troncos escuros. Esse padrão de predação e de expressão fenotípica na população foi posteriormente revertido com políticas de diminuição de poluentes. Algo semelhante pode ser observado em ambientes propensos a fogo, que favorecem o estabelecimento de polimorfismos (melanismo), como observado nas em florestas, savanas africanas e Cerrado (Owen, 1980; Forsman et al., 2011; de Alcantara Viana et al., 2022 b). Em relação aos aspectos intrínsecos aos organismos, além da própria morfologia, como formato corporal e coloração, o comportamento também tem um papel importante que pode promover o aumento do potencial de ocultação da presa (Stevens & Ruxton, 2018). Existem descrições de comportamentos de seleção ativa de hábitats que promovem ajustes em correspondência de fundo em táxons diversos, de gafanhotos que são capazes de selecionar micro-habitat, a mariposas que se orientam adequadamente com a textura troncos ao repousar sobre eles (Kang et al., 2012, Camacho et al., 2020). Em casos específicos, a seleção natural pode favorecer mecanismos de mudança de cor morfológicos ou fisiológicos que promovem correspondência de pano de fundo e até mesmo coloração disruptiva em múltiplos substratos (Kang et al., 2016).

Tendo em vista que a camuflagem é mplamente distribuída na natureza, a presente tese buscou averiguar mecanismos e processos por perspectivas distintas que se relacionam a efetividade de proteção para variados táxons de presas frente a predadores visualmente orientados. Deste modo, o trabalho possui cinco capítulos, em formato de artigos científicos, que estão detalhados a seguir. No capítulo 1) buscamos averiguar, através de uma meta-análise, como diferentes estratégias de camuflagem concedem proteção à presa em relação ao tempo de procura dos predadores e a taxa de ataque nas presas. No capítulo 2) averiguamos os efeitos das queimadas nas estratégias de camuflagem adotadas por espécies polimórficas de artrópodes que usam troncos para se camuflarem no Cerrado. Adicionamente, verificamos a correspondência de pano de fundo em três espécies de artrópodes, sendo duas polimórficas com colorações melânica e marrom (*Ronderosia bergii* e *Eumiopteryx laticollis*) e uma espécie de aranha monomórfica – melânica (*Syntrechalea brasilia*). Tambem buscamos testar a efetividade de diferentes estratégias de camuflagem frente a um cenário de queimada estocástico do Cerrado. No capítulo 3) averiguamos como eventos estocásticos de queimadas no Cerrado afetam as estratégias de camuflagem em comunidades de artrópodes que utilizam troncos paras se camuflarem de aves predadores. O capítulo 4) teve como objetivo averiguar a contribuição da correspondência de pano de fundo e coloração disruptiva na efetividade de camuflagem de morfoespécies de louva-deus simpátricas da Amazônia. Finalmente, no capítulo 5) verificamos se a adaptação de mudança de cor fisiológica de verde para marrom em uma espécie de perereca (*Pithecopus hypochondrialis*) noturna do Cerrado favorece sua camuflagem quando sob grama e serapilheira, respectivamente, frente a potencial predação por corujas. O presente trabalho traz informações inéditas sobre padrões gerais na ocorrência e efetividade de estratégias de camuflagem. Aqui, mostramos mecanismos e processos que contribuem para a ocultação de diversos táxons de presas sob diferentes condições de substratos, frente a predadores visualmente guiados.

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CAPÍTULO 1

Predator responses to prey camouflage strategies: a meta-analysis

Foto: Felipe Capoccia Coelho

Manuscrito publicado no perióidco “Proceedings of Royal Society B”

(https://doi.org/10.1098/rspb.2022.0980)

Predator responses to prey camouflage strategies: a meta-analysis

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Abstract

Although numerous studies about camouflage have been conducted in the last few decades, there is still a significant gap in our knowledge about the magnitude of protective value of different camouflage strategies in prey detection and survival. Furthermore, the functional significance of several camouflage strategies remains controversial. Here we carried out a comprehensive meta-analysis including comparisons of different camouflage strategies as well as predator and prey types, considering two response variables: mean predator search time (63 studies) and predator attack rate of camouflaged prey (28 studies). Overall, camouflage increased the predator search time by 62.56% and decreased the attack rate of prey by 27.34%. Masquerade was the camouflage strategy that most increased predator search time (295.43%). Background matching and disruptive coloration did not differ from each other. Motion camouflage did not increase search time but decreases attack rate on prey. We found no evidence that eyespot increases search time and decreases attack rate by predators. The different types of predators did not differ from each other, but caterpillars were the type of prey that most influenced the magnitude of camouflage's effect. We highlight the potential evolutionary mechanisms that led camouflage to be a highly effective anti-predatory adaptation, as well as potential discrepancies or redundancies among strategies, predator, and prey types.

Keywords: antipredator behaviour, background matching, concealment, disruptive coloration, masquerade

1. Introduction

One of the most widespread defensive strategies to avoid predation in nature is camouflage [1], which is defined as the use of colour patterns and other morphological adaptations by an organism to reduce the probability of being detected or recognized by an observer [2]. This anti-predatory strategy is found in many taxa with reports including from dinosaurs to plants and used both from prey and predators [2–6]. Even studied over a century, including observations and seminal studies made by Wallace and Poulton [7,8], camouflage is frequently defined as the simple association between the colour patterns of the organisms and their backgrounds [9]. However, currently, 16 types of camouflage strategies are described in the literature, each with its distinct sensory and cognitive mechanisms [2].

The different camouflage strategies can be classified according to the underlying mechanisms that minimise the signal-to-noise ratio regarding the type of signal or noise information [2]. These strategies can also be classified if they act in the recognition (i.e., masquerade) or detection of the target (i.e., crypsis), as well as if they lead to a reduction in the accuracy or a deflection in predator's strikes (i.e., motion camouflage and eyespot) [2,9]. For example, background matching, one of the best-known strategies of camouflage, occurs when the animal appearance contains random samples of colours and/or patterns of the occupied background, and works by impeding an observer to distinguish the animal surfaces from the background, therefore minimising signal information [10–12]. On the other hand, disruptive coloration creates contrasting edges in the animal's silhouette that intersect with the real body margins, which makes detection or recognition difficult, as this strategy minimises signals but creates noise [10,13,14]. Masquerade differs from the other strategies since while allowing detection, it works by reducing the probability of prey recognition by predators by increasing false signals, since the animal resembles an inanimate and not interesting object from its surrounding, such as rocks, bark, twigs, leaves or even bird droppings [15]. In addition, other strategies that are primarily involved in reducing strike accuracy, intimidation and deflection of attacks made by predators instead of minimizing recognition, such as distractive marks, eyespots and motion camouflage, can either increase noise or decrease signals [2], being employed by many animal species from different taxa [2, 16–19]. However, despite being well-documented, the functional significance of several camouflage strategies remains controversial in the literature [20,21].

Although numerous studies about camouflage have been done in the last few decades, there is still a lack of experimental tests on the efficiency of the different camouflage strategies in relation to prey concealment and survival [21]. In addition, only a few studies seek to contrast the effectiveness of camouflage strategies, being those almost restricted to background matching and disruptive coloration comparisons [22–24]. Besides these comparative assessments, as far as we know, no study has tested the efficiency of several camouflage strategies under a comparative approach. In this study, we use a meta-analysis to address, for the first time, the efficacy of the different camouflage strategies in the survival and detection/recognition of prey by predators. Our analysis focuses on the two most common predator responses found in experimental studies about camouflage, namely the mean time to find and attack prey (search time – ST) and the mean percentage of prey consumption (attack rate - AR). Therefore, we seek to understand if (i) camouflage (i.e., the overall outcome of all camouflage strategies) is effective in promoting protection to prey; (ii) there is a difference in the degree of prey protection among the different camouflage strategies; (iii) the two response variables differ concerning the different types of predators or prey.

We predict that (i) camouflaged prey will be more difficult to be found and attacked by predators than non-camouflaged (i.e., control) prey models. Since there exist reported differences in the effectiveness of camouflage strategies in the literature, we believe that (ii) camouflage strategies will promote different degrees of protection for prey. Specifically, based on previous studies showing that disruptive coloration is less dependent on the type of background than background matching, and that this could translate into increased survival [21,22], we predict that disruptive coloration will promote a greater degree of protection than background matching. Additionally, we predict that strategies primarily focused on prey concealment will strengthen prey survival by promoting increase in search time and decrease in attack rate compared to strategies primarily focused on promoting prey escape, such as those involved in intimidation, deflection, distraction, or reduction of strike accuracy by predators (e.g., eyespots and motion camouflage). We expect that (iii) there will be no differences in the response variables according to the predator type, given that some studies show similar patterns of search image formation during foraging of humans and birds, which are the main predators used in the experiments [25,26]. Since camouflage is a widespread anti-predatory strategy in several taxa, we expect (iv) no differences in terms of protective effectiveness among prey types.

2. Material and methods

(a) Literature search and database

To understand the effects of the different camouflage strategies on prey protection, we extensively search for articles in the Web of Science (all databases) published from 1900 to July 2022. The Google Scholar online database was used as a secondary and complementary source of record. Our search in the Web of Science allows the insertion of logical arguments (AND/OR) and asterisk (*), which expand the searching process to consider both American and British spelling, as well as word variations (e.g., colo* could indicate “color”, “colour”, “coloration” or “colouration”). Therefore, differences in word spelling and use of word variations of similar terms do not influence the searching accuracy of our study. Based on that, we used the following search string in our searching process: [(camouflage* OR cryp*) AND background matching* OR countershad* OR dazzle colo* OR distractive marking* OR disruptive colo* OR eyespot* OR masquerade* OR motion camouflage*]. We refined the search into the following Web of Science categories: behavioural sciences, biology, ecology, environmental sciences, evolutionary biology, entomology, and zoology. We also looked at cited references in research articles and reviews to identify additional papers. Our initial search resulted in 1,188 records following the removal of duplicates according to PRISMA statement 2020 [27] (see electronic supplementary material – ESM l - figure S1 for PRISMA flow diagram).

After the initial searching, we used a set of different criteria for the inclusion of the articles in our analysis (see ESM 2 - table S1A, S1B). To be included in our final list, the study should have (i) experimentally tested at least one camouflage strategy; (ii) reported the time spent by the predator to find the prey (in seconds or another transformable data) or (iii) presented the percentage of attacked camouflaged prey (or another transformable data) by predators; (iv) contrasted the outcomes of the camouflage strategies with experimental controls (i.e., non-camouflaged prey model); and finally (v) presented the mean, standard deviation (or any transformable measure of variation) and sample size of the measured variables.

Several studies about camouflage showed more than one outcome per camouflage strategy. For example, Fraser and colleagues [28], in a study about the role of disruptive coloration in reducing detectability, used two different variations of disruptive and background pattern models and contrasted them with an average colour control model. Therefore, to deal with studies that used different experiments to test the efficacy of a specific camouflage strategy, we first calculated the mean value for each experiment and then derived a single effect size estimate per experiment and camouflage strategy, allowing us to control for pseudoreplication. We obtained mean and standard deviation values as well as sample sizes from texts, tables, and graphics, digitized using Web Plot Digitizer v.4.4 software (https://automeris.io/WebPlotDigitizer/) to extract the aforementioned statistics.

(b) Sources of variation and meta-analysis

To test the overall effect of camouflage in both ST and AR, we used all studies that have tested at least one camouflage strategy. Although these variables could be potentially sequential in predator foraging activity in nature, as reported in studies that had measured both in similar experimental conditions [28-30] (also, see details of AR-ST Pearson correlation in Results section), we used the two variables in our meta-analysis to broaden the scope and evaluate the distinct ways to test for camouflage effectiveness. While ST may determine the effectiveness of a strategy when the prey is within the field of vision of their predator, AR denotes the mean percentage of individuals that was attacked after a certain period. The two variables were considered for all camouflage strategies that we found in the literature, both those that prevent detection or recognition and those that primarily deflect the attack or cause confusion effects on predator's strikes

We considered in the specific analysis of moderators categories that have been tested in at least four articles since this is an adequate sample size to perform a robust meta-analysis [31] (see ESM 1, 2 and 3 for additional details). We used the following moderators in our analysis: "camouflage strategy", "predator type", and "prey type". The categories for "camouflage strategy" were 'background matching', 'disruptive coloration', 'eyespot', 'masquerade', and 'motion camouflage'. The categories for the "predator type" moderator were 'avian', 'fish', and 'human'. Finally, for "prey type", we considered 'theoretical prey models' (i.e., prey that do not resemble a specific taxon, theoretically representing one type of camouflage strategy), 'Lepidoptera' (considering only adult stage), and 'caterpillar'. Although the 'caterpillar' category included studies about Lepidoptera, we preferred to define it as a distinct level, since the shape and colour of many Lepidoptera species change dramatically along ontogeny [32]. We decided to not include background type as a moderator because this feature was very variable among the experiments, and consequently difficult to quantify the match of the prey model to the background and to control for the comparisons of our response variables for all selected studies [2].

To test the magnitude of camouflage in prey protection, we converted the data to log response ratio (ln*R*) and used it as a metric of effect size [33,34]. In this metric, positive or negative values indicate that predators increase or decrease both the search time and the percentage of attacked camouflaged models, respectively. To ease interpretation, we back transformed ln*R* to percentage $[(\exp \ln R - 1) \times 100\%]$ to obtain the differences between treatment and control. We used a mixed effects model for moderator analysis using maximum likelihood (ML) as the estimator parameter (throughout the *rma* function in R) [34], which assumes that variation among studies within a group are random and among groups are fixed [35]. We removed the intercept from the model throughout the "mods-1" argument [33], and then all moderators' subgroups were included in the model [33].

The Wald-type chi-square test for heterogeneity (*Qt*) was used to determine if the amount of heterogeneity in effect sizes could be due to sampling error alone. I^2 statistic was used to access the percentage of heterogeneity between studies, in which larger values suggest that studies did not belong to the same population and therefore subgroup analysis of moderators could be performed [35]. The Cochran's Q test (*Qm*) was used to access the significance of moderators [35]. We also conducted pairwise contrasts between moderator levels using the Tukey significant difference test with Bonferroni adjustment for multiple comparisons in the "multcomp" package [36]. The meta-analysis was carried out using the "metafor" package available in R statistical software and language (version 4.1.2) [34,37].

(c) Publication bias

We tested the robustness of our results to publication bias using funnel plots. For that, we plotted the effect size values against their respective sample sizes and verified if plots were asymmetrical by calculating the rank correlations (z) between the ln*R* effect size and the standard error [34]. Rosenthal's fail-safe number was used to test how many unpublished studies with non-significant results had to be added to the analysis to change the observed significant effect sizes to non-significant [38]. Based on this metric, the results of the meta-analysis are considered robust against publication bias when the number of observations included in the study (n) is greater than $5n + 10$ [38].

3. Results

(a) Meta-analysis

Our literature search resulted in 84 articles that met our inclusion criteria, with most experiments performed in the northern hemisphere (figure 1, ESM 2 and 3 for additional details). We found 63 studies that compared the search time (ST) of camouflaged prey by predators, from which we obtained 335 mean outcomes values that were averaged into 98 mean values of effect sizes from the different types of camouflage strategies (ESM 4). Additionally, we selected 28 studies that compared the attack rate (AR) of camouflaged models with different types of camouflage strategies in relation to their control groups, from which we found 94 mean outcomes values that resulted in 38 mean values of effect sizes (ESM 1 - figure S2 and ESM 5).

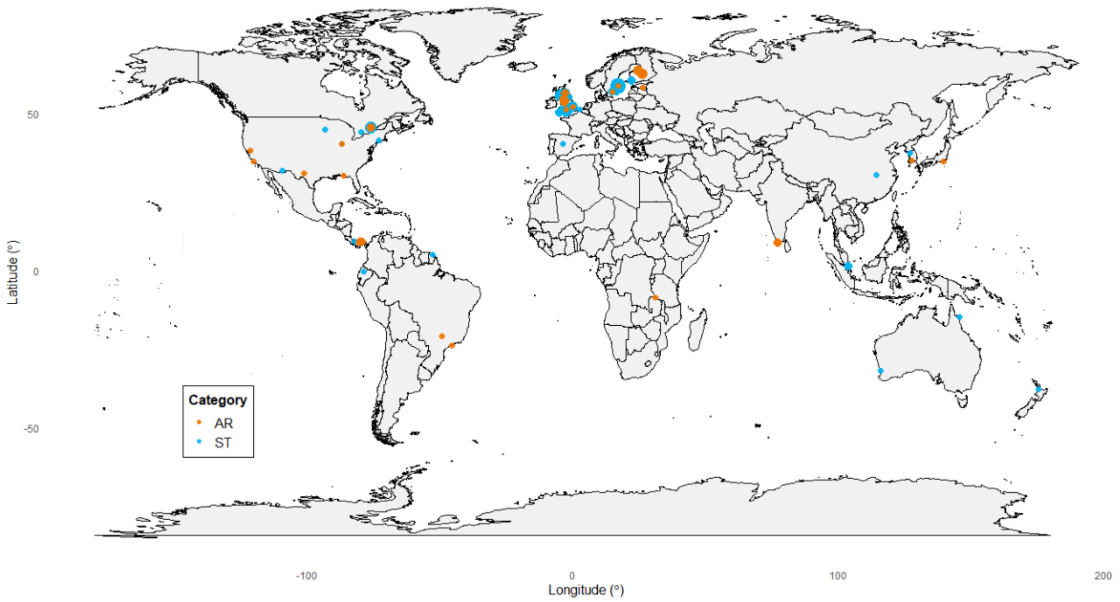

Figure 1. The map shows the locations where the original studies used in the meta-analysis were conducted. The map shows 63 locations for the search time variable (ST) and 28 locations for the attack rate variable (AR). The size of the points is proportional to the number of studies conducted in each location.

There were only three studies that have experimentally tested both ST and AR of different camouflaged models (i.e., varying in the level of concealment) together (i.e., using the same experimental protocol). After pooling the data of these studies, we found no correlation between the two response variables (r = 0.26, t_{11} = 0.90, p = 0.38; ESM 5), indicating that searching times are not associated to consumption rates of prey models by predators. The two most common strategies were background matching and disruptive coloration, corresponding together for 51% of the studies for the variable ST (background matching = 23 articles and disruptive coloration = 20 articles) and 67% for the variable AR (background matching = 13 articles and disruptive coloration = 6 articles). In relation to the types of predators and prey used in the experiments, we found avian (ST: 28 articles; AR: 10 articles) and humans (ST: 28 articles; AR: 5 articles) as the most common predators, while adult Lepidoptera (ST: 22 articles; AR: 5 articles) and theoretical prey models (ST: 15 articles) were the most commonly type of prey used. In addition, we found that for ST, the most common model of prey used in the predation experiments were artificial models placed in the field or in laboratory conditions (23 articles), followed by computer-generated prey models (18 articles), live animals (12 articles), and animal photographs used in computer citizen science games (11 articles). The same pattern was observed in AR experiments, with artificial models being the most used (13 articles), followed by computer-generated prey (6 articles), live animals (6 articles), and animal photographs (1 article).

The overall main effect of camouflage was positive for the search time of camouflaged prey by predators ($\ln R = 0.48$, CI = 0.34 to 0.63, N = 98; figure 2). Predators spent 62.57% longer to find and attack the camouflaged models than the non-camouflaged control models. On the other hand, the attack rate on camouflaged prey decreased by 27.34% compared to controls ($\ln R = -0.32$, CI = -0.46 to -0.18, N = 38; figure 3). The total heterogeneity of our model was significant for both measured variables (ST: $Qt = 4091.81$, $df = 97$, $p < 0.01$; AR: $Qt = 1114.05$, $df = 37$, $p < 0.01$) and their total variance was due to the differences among articles (ST: $I^2 = 98.63\%$; AR: $I^2 = 98.10\%$), which allow us to achieve the condition to continue with moderator's analysis (ESM 6 - tables S1 and S2).

The search time of camouflaged prey by predators differed among the camouflage strategies ($Qm = 89.90$, $df = 5$, $p < 0.01$). Masquerade was the strategy that most increased (295.43%) the predators' search time compared to control groups ($\ln R = 1.39$, CI = 1.03 to 1.74, N = 12) and significantly differed from all other strategies (figure 2, ESM 6 - table S1). Camouflage by background matching and disruptive coloration increased predators' search time by 56.63% ($\ln R = 0.44$, CI = 0.29 to 0.65, N = 23) and 52.75% ($\ln R = 0.42$, CI = 0.16 to 0.64, N = 20), respectively. In contrast, eyespot and motion strategies had no effect on predator search time (eyespot: $\ln R = 0.11$, CI= -0.13 to 0.40, N = 11, $p = 0.21$; motion: $\ln R = 0.04$, CI = -0.26 to 0.36, N = 10, $p = 0.51$). Finally, the type of predators ($Qm = 30.23$, $df = 3$, $p < 0.01$) and prey ($Qm = 74.17$, $df = 3$, $p < 0.01$) used in the experiments had a significant effect in predator search time (figure 2). Although predators spent more time searching for camouflaged than non-camouflaged prey, we did not find contrasting differences among the predator types used in the experiments (figure 2, ESM 6 - table S3). However, for prey types, caterpillars differed from all other prey categories (figure 2, ESM 6 - table S3), increasing the search time of predators by 279.49% when compared to control conditions ($\ln R = 1.32$, CI = 0.80 to 1.86, N = 11).

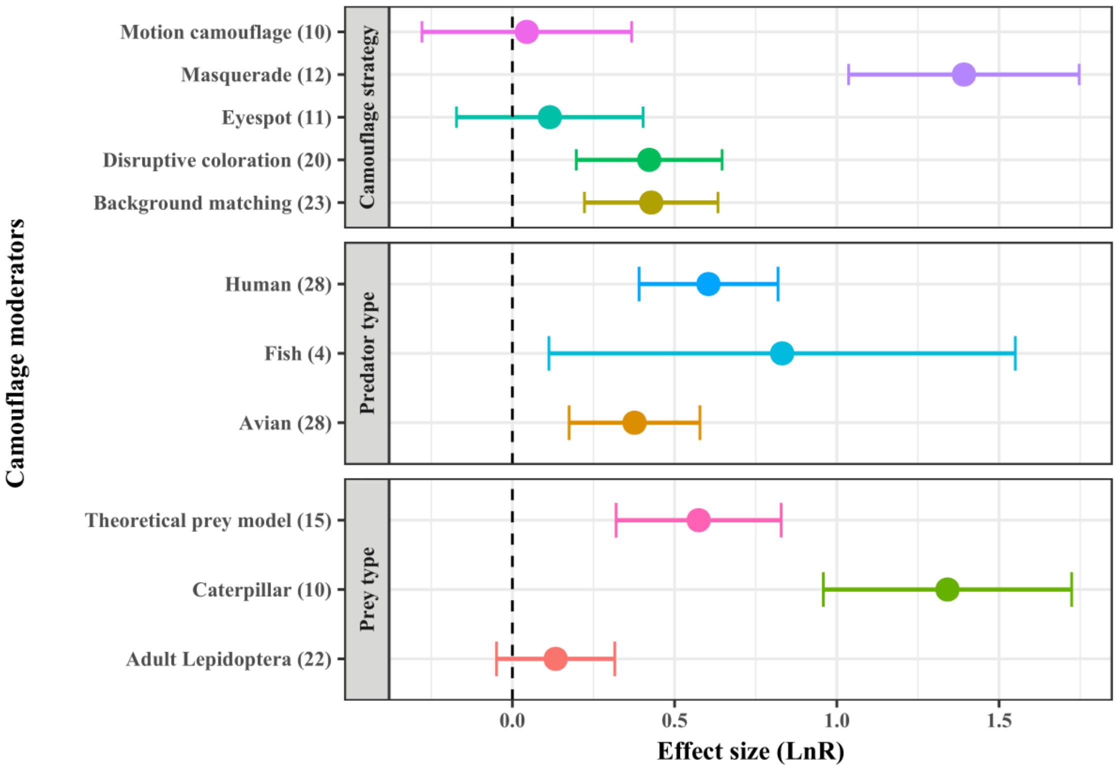

Figure 2. Differences in the search time of camouflaged prey by predators according to different moderators, including camouflage strategies, predator and prey types. The dots represent the mean effect sizes and the bars 95% confidence intervals (CI). Positive effects indicate an increase in the time spent by the predator to find the camouflaged prey. Effects are considered significant if 95% CI does not include 0. The numbers following the moderator levels indicate the respective number of articles from which the effect sizes were extracted.

The attack rate of predators on camouflaged models were also affected by camouflage (*Qm* = 24.47, *df* = 3, *p* < 0.01; figure 3). Background matching decreased the mean percentage of attacks by 22.69% (ln*R* = -0.30, CI = -0.48 to -0.13, N = 15), while disruptive coloration decreased by 18.73% (ln*R* = -0.28, CI = -0.54 to -0.02, N = 7), and motion camouflage by 29.99% (ln*R* = -0.37, CI = -0.63 to -0.11) (ESM 6 - table S2). However, we found no differences between the protective values of these camouflage strategies (ESM 6 - table S4). In general, both the type of predators (*Qm* = 14.81, *df* = 2, *p* < 0.00) and prey (*Qm* = 8.66, *df* = 2, *p* < 0.01) used in the experiments significantly affected the mean attack rate on camouflaged models. While caterpillar prey increased protection in 30.80% (ln*R* = -0.41, CI = -0.73 to -0.10, N = 8), for adult Lepidoptera there was no difference between the attack rate on camouflaged prey compared to non-camouflaged control models (ln*R* = -0.15, CI = -0.37 to

0.06, N=10, *p* = 0.16). In addition, we found no differences between predator and prey categories in pairwise contrast comparisons (figure 3, ESM 6 - table S4).

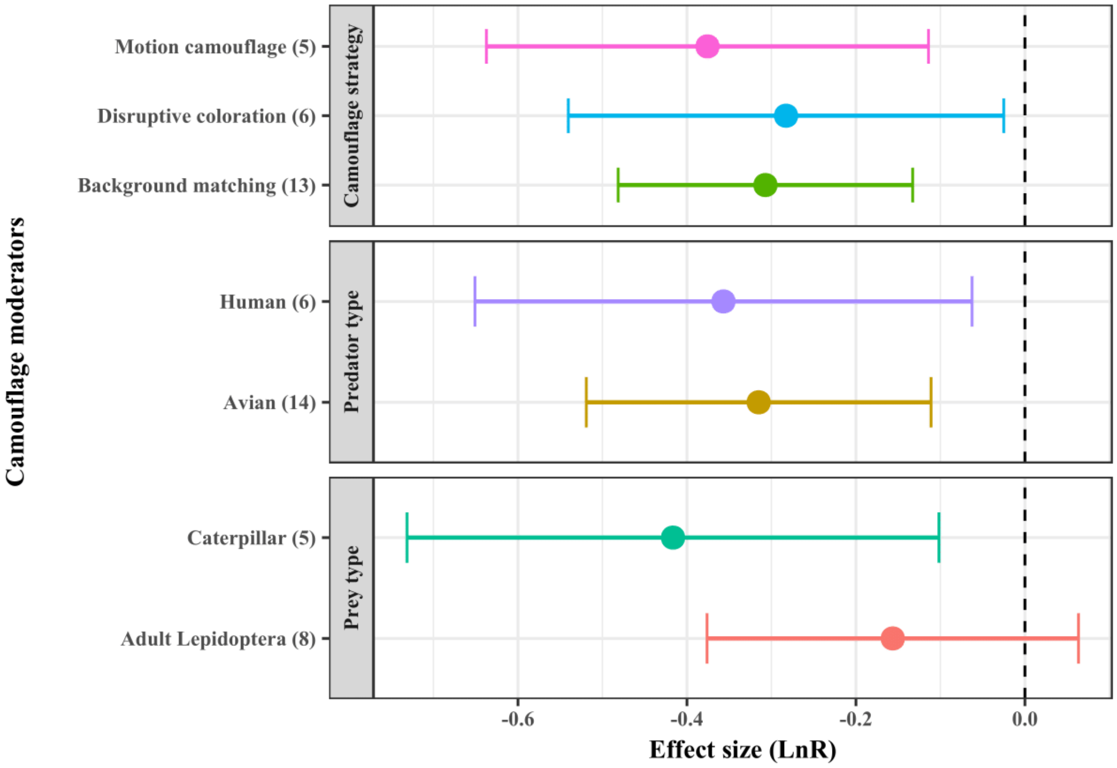

Figure 3. Differences in the attack rate of camouflaged models by predators according to different moderators, including camouflage strategies, predator and prey types. The dots represent the mean effect sizes and the bars 95% confidence intervals (CI). Negative effects indicate a decrease in the percentage of camouflaged prey being attacked by predators. Effects are considered significant if 95% CI does not include 0. The numbers following the moderator levels indicate the respective number of articles from which the effect sizes were extracted.

(b) Publication bias

The results of the regression tests we applied to assess funnel plot asymmetry were not significant for overall comparisons considering both search time by predators (*z* = 1.90, *p* = 0.06, ESM 1 - figure S3) and for the attack rate of camouflaged prey models (*z* = -1.27, *p* = 0.20, ESM1 - figure S4). The calculated Rosenthal's fail-safe numbers were higher than the cut-off for both variables (ST: 10286 fail-safe numbers, which is higher than the 485 cut-offs;

AR: 5381 fail-safe numbers, which is higher than the 200 cut-off). Results of these two procedures indicated that our meta-analysis were robust against potential publication bias.

4. Discussion

Our meta-analysis indicated that camouflage is a highly efficient anti-predatory adaptation, overall increasing the predator’s search time to find camouflaged prey and decreasing their attack rate to camouflaged models. However, the several camouflage strategies may provide different levels of protection for prey types, ranging from less protective for prey that use strategies aiming to deflect and reduce the accuracy of predator attacks (e.g., motion and eyespot strategies, mainly in adult lepidopteran prey) to highly protective for strategies working to improve prey concealment or to prevent prey recognition (e.g., masquerade, background matching and disruptive coloration, mainly in caterpillar prey). In conjunction, our findings suggest that the type of strategy animals adopt, the appearance and identity of prey, as well as the cognitive mechanism and behaviour of predators are important evolutionary pressures shaping camouflage in nature [39].

Since new descriptions and definitions about the different types of camouflage have emerged in the literature, questions about the variation in their effectiveness were also raised [9]. Our study shows that masquerade was the camouflage strategy that most influenced prey protection during predator search time. This probably occurs because masquerade is a highly specialized strategy in which the animal resembles inanimate and uninteresting objects from the surroundings [6,40], acting specifically to alter cognitive and perception mechanisms and benefit from the predator’s misidentification of the masquerading organism [41]. Regardless of the high similarity of the animal to the inanimate object, the lack of the predator's experience in interacting with the mimicked object can make it even more difficult for the predator to identify the prey [15]. The most accepted hypothesis to date is that masquerade evolved from cryptic ancestors [21,41]. However, unlike cryptic coloration, there are behavioural and environmental limiting factors required for the evolution of masquerade organisms, such as the low mobility of the species [21]. Additionally, masquerade is more likely to evolve when masquerading organisms exhibit similar size to the mimic object [42,43], as well as when they occupy areas with similar light conditions to those in which the mimic inanimate objects is found [21].

Our results point out that both background matching and disruptive coloration increase predators’ search time and reduce prey consumption rates, but they do not differ in terms of the magnitude of prey protection. These findings contrast with previous empirical studies [22–24], which suggested disruptive coloration to be more effective than background matching in increasing prey protection. A classical study that also found no differences between the two strategies argued that they are not mutually exclusive, but indeed that disruptive prey can also exhibit some degree of background matching traits [43]. However, recent studies have shown that the detection of cryptic and disruptive prey by predators does not depend exclusively on the appearance of background and prey colour, but also on background complexity and animal size [44,45]. Thus, future studies are needed to better elucidate the relationship between animal coloration and morphological traits, such as body size, as well as according to the type and visual complexity of substrates that animals occupy [44].

There is an intense debate about the protective function of eyespots and motion camouflage, and our meta-analysis highlight that such mechanisms are not effective to increase searching time by predators [18,46–49]. However, this does not mean that these camouflage strategies are not adaptive. In the case of eyespots, for example, some studies have shown that this strategy works not only by diffusing the attack, but also by directing the predator to an area of the prey that is not lethal, increasing the chances of prey escaping [50]. Concerning the search time, our study supports the hypothesis that concealment is very difficult to achieve during movement when camouflage is broken, probably because predators are highly sensitive to motion information [51]. However, our results support the protective value of motion camouflage in decreasing the attack rate by predators. One of the most raised hypotheses about the protective value of motion camouflage is that motion strategies may interact with confounding effects when used in groups by some animals [52,53]. Therefore, new comparative studies are necessary to better elucidate the evolutionary benefits of eyespots and motion camouflage strategies, which operate differently than concealment strategies, such as background matching, to prevent predation.

As expected, the type of predators used in the experiments did not affect the magnitude of the measured effect sizes. Since most of the studies used humans and birds as predators, our findings reveal that even with existing taxon-specific sensory and cognitive abilities, there is no change in the efficiency of prey detection and attack rate between predator types [25,26]. Therefore, our study reinforces that experiments using humans as predators represent a reliable proxy of predation and can provide interesting insights about the effectiveness of camouflage strategies [25,26]

Contrary to our expectations, the type of prey used in the experiments affected the predators’ search time. This was particularly evident for caterpillar, which was the prey type that elicited longer predator responses probably because it is the most used model for studies on masquerade [40-41,54]. In addition, most masquerade studies have used live caterpillar and not computer models or artificial prey, which can make them difficult for predators to find, as realism is increased in the experimental setting (see ESM 2). Moreover, considering that immature forms of insects are less mobile and more vulnerable to predation than adults [32], it makes sense to find more effective protection in terms of concealment in caterpillar than adults. Although many studies have provided evidence that prey shape is important for detection, to date few studies have compared the detection of prey taxa according to their underlying camouflage strategy [29,45,54,55]. Future experimental studies should independently test the function of animal silhouette and camouflage strategy on prey detection to tease apart these effects.

In this study, we use a reductionist approach to test the protective value of different camouflage strategies independently. However, it is important to clarify that many of the strategies in nature occur simultaneously, which gives different levels of protection to the prey that are still poorly understood [21]. For example, many prey species can benefit from both disruptive coloration and background matching when selecting a tree trunk, where they can also mimic the substrate shape and texture, which also may favour masquerade. Additionally, many species can switch between different types of strategy along ontogeny [21,56], benefiting from the protection of each of them during a specific life-stage [31,57–59]. Isolating the effects of each strategy is challenging and opens a valuable topic for research in future studies. Furthermore, something still unexplored is the trade-offs inherent to each strategy, especially those related to the behavioural repertoire that many animals use to choose substrates. Since the different camouflage strategies have different levels of specialization regarding the requirements of the habitat, the choice for a specific substrate must not relate only to individual survival, but also to other components of the organisms' life history [43].

Our study also points to the commonality in the use of artificial prey models and computer-generated shapes in studies about camouflage. Although such studies are important and meritorious, having provided the basis of camouflage theory in the last years, they are probably under- or overestimating the protective function of the different camouflage strategies, since they did not consider the behavioural interactions between predators and prey under natural conditions [57,60]. Therefore, there is an urgent need for studies testing the protective function of the different camouflage strategies using real prey and predators under natural conditions. There is also a need to standardize the methods to quantify camouflage when testing prey protection. Powerful metrics such as JNDs (i.e., just noticeable differences), pattern and edge disruption analysis (e.g., GabRat) [61,62] are now available and are being used in different studies to obtain more robust and controlled results to better elucidate the role of animal and background coloration in the effectiveness of such anti-predatory strategies. We also claim for more studies be carried out in the tropics, since it is the region of the globe most neglected in camouflage studies (figure 1). In addition, the tropics are also the region where prey and predators interact most strongly, which can bring us new insights into the efficiency of the most diverse protective colorations for different type of predators [63-64].

5. Conclusion

Here, we show that there are relevant differences in the degree of prey protection offered by each type of camouflage strategy and that such protection differs according to the type of prey. Finally, we suggest that strategies preventing the recognition of prey models (i.e., masquerade) are more effective in increasing predator search time than those that avoid reduction of signals or even detection. This pattern may be related to the high degree of specialization in which this type of strategy has evolved. We conclude that, in general, camouflage is an efficient anti-predatory strategy, either by increasing the search time for predators, which also increases the probability of prey escape, or by decreasing the prey predation rate.

Acknowledgements

We would like to thank CAPES, PPG-ECO and IB-Unicamp for the financial support and library access for searching articles during the research activities. We would like to thank the associated editor and three anonymous reviewers for their substantial and valuable comments on our article.

Data accessibility

All data underlying our analyses are available as supplementary material.

Funding

JVAV is funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES#88887.338483/2019-00) through a doctoral fellowship. CV received a fellowship from FAPESP (2010/51523-5) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES#88882.314749/2019-01). RCD is funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP #2019/01934-3, #2022/00946-0) through a postdoctoral fellowship. GQR acknowledges financial support for research provided by FAPESP (grants 2018/12225- 0 and 2019/08474- 8), CNPq-Brazil productivity grant, and funding from the Royal Society, Newton Advanced Fellowship (grant no. NAF/R2/180791).

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CAPÍTULO 2

Differential survival and background selection in arthropod camouflage strategies in fire-prone environments

Manuscrito sob revisão no periódico “The American Naturalist”

Differential survival and background selection in arthropod camouflage strategies in fire-prone environments

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Abstract

Fire events change background colour impairing camouflage strategies. However, selection for polymorphic populations may balance the effects of camouflage by reducing predation risks. We conducted experiments addressing background selection and predation pressures on the effectiveness of arthropod camouflage against predation in burned and unburned trunks. We tested colour contrasts and trunk preferences in a colour polymorphic grasshopper and a praying mantis species with melanic and brown morphotypes, and in a spider species with a single dark colour. We also performed predation experiments using theoretical prey exhibiting different camouflage strategies and humans as a proxy of "predators" to understand which strategy is most effective against both trunk conditions. Melanic morphs had lower achromatic contrast in burned backgrounds, suggesting that melanism promotes advantages against predation over long distances. However, only spiders actively selected the low contrasting burned trunks, indicating habitat specialization. The predation experiments showed that only black models have benefited from camouflage in burned trunks. In the unburned area, background matching and disruptive models did not differ concerning search time, but the last was found at short distances, suggesting that disruption impairs recognition over long distances. We suggest that post-fire effects can enhance colour contrasts and increase predation over colour-mismatching individuals, also favouring selection of colour polymorphism and matching background choices.

Keywords: crypsis; habitat selection; savanna; fire; predation.

1. Introduction

Anti-predatory defences are related to the cognitive ability of prey to recognize features of predation risk (Ruxton et al., 2004). The behavioural responses of prey may vary according to both predator identity and hunting strategy, ranging from an increase in surveillance to the adoption of evasive behaviours, as well as through the selection of specific backgrounds to improve individual concealment (Nafus et al., 2015; Stevens & Ruxton, 2019). Background choices can result from three main mechanisms: (i) species-level preferences, where all individuals within a species share fixed choices; (ii) context-dependent choices, such as the preferences of all individuals when better backgrounds are available (microhabitat preferences); and (iii) individual-level choices, such as white morphs selecting light backgrounds and black morphs choosing dark ones, which can improve individual concealment (Stenves & Ruxton, 2019). Therefore, when considering crypsis as a mechanism of anti-predatory strategy, the type of background choice behavior plays a significant role in individual survival

Camouflage is one of the most widespread adaptations that reduce the chances of predation in nature (Cott, 1940; Cuthill, 2019; Stevens & Merilaita, 2011; Thayer, 1909). This anti-predatory defence is defined by an organism exhibiting body colour patterns and morphological traits that interact with background features and decrease the probability of individuals being detected or recognized by an observer (Merilaita et al., 2017). Although camouflage has been used as a broad ecological term in the literature, it comprises several strategies that act in a variety of ways by exploiting prey ability to disrupt the predator's perception and provide different degrees of protection (de Alcantara Viana, Vieira, et al., 2022; Stevens & Merilaita, 2016). There are types of camouflage that hinder the observers to detect animal's body, such as background matching, in which individuals match the colour, brightness and/or pattern of a background sample (Cuthill, 2019; Merilaita et al., 2017). Other strategies, such as disruptive coloration, are less dependent on the background and function by increasing visual confusion through the creation of false contrasting body edges in animals, which can be more effective in certain scenarios compared to background matching (Cuthill et al., 2005; Price et al., 2019; Stevens & Merilaita, 2009).

A straight route to achieve camouflage is habitat selection, in which the individual actively chooses the backgrounds that it resembles mostly (i.e., matching habitat choice) (Camacho et al., 2020; Heinze et al., 2022; Ruxton et al., 2004). For that, the organism must be capable of recognizing environmental cues such as the colour and pattern of backgrounds to choose the most concealing and protective habitat (Baños-Villalba et al., 2018; Camacho et al., 2020). In different background conditions, animals can either be well-hidden or easily noticeable. To achieve effective crypsis, the chosen substrate should closely match in coloration and brightness or enable the use of other camouflage strategies that minimize the likelihood of detection by predators. (Bond & Kamil, 2002; Stevens et al., 2017; Stevens & Ruxton, 2019).

Beyond the selection of of suitable backgrounds, many animals have also to deal with environmental changes that can drastically modify the coloration of backgrounds and impair concealment (Ahnesjö & Forsman, 2006; Caro et al., 2016; Cook et al., 2002; Forsman et al., 2011). In this scenario, natural selection may either favour adaptations of animals to change colour over different time scales to chromatically adapt to background changes (Duarte et al., 2017; Zimova et al., 2018) or promote the coexistence of two or more discrete colour morphotypes (= morphs) within the population (e.g., colour polymorphism) (Karpestam et al., 2016). Colour polymorphic populations are expected to be more stable and persistent than monomorphic populations, since the predation risks over the whole population are reduced due to the existence of alternative morphotypes that impair search image formation by predators (Forsman et al., 2011; Karpestam et al., 2014; Troscianko et al., 2021; White & Kemp, 2016).

The Neotropical Cerrado savanna is the most biodiverse savanna in the world, considered a hotspot for biodiversity and conservation, which contain several fire-prone ecosystems (He et al., 2019; Oliveira & Marquis, 2002). Many plant species in the Cerrado are resistant to fire, as they have high amounts of suberin on their trunk that protect against burning (Scariot et al., 2005). However, despite the vegetation remaining alive after these stochastic and severe events, the colour of the landscape (i.e., tree trunks) is changed significantly from a heterogeneous green and brown to a more homogeneous black background (He et al., 2019). Landscapes with high environmental heterogeneity favour the selection of colour polymorphism in cryptic animals (Bond, 2007; Forsman et al., 2011; Karpestam et al., 2016). Previous studies with the community of arthropods occupying trunks in burned and non-burned environments in both tropical and temperate woodlands found that animals often occupy backgrounds where they achieve lower colour/brightness contrast to the view of avian predators (de Alcantara Viana, Brito et al., 2022; Forsman et al., 2011; Karpestam et al., 2012). The occurrence of brown and melanic morphs in polymorphic populations was observed for species of different taxa occupying fire-prone environments, from herbivores (grasshoppers - Acrididae) to primary predatory insects (praying mantis - Thespidae) (de Alcantara Viana, Brito, et al., 2022; Forsman et al., 2011; Karpestam et al., 2012). In addition, these areas are also suitable for monomorphic trunk-specialized spiders (Trechaleidae) (de Alcantara Viana, Brito, et al., 2022). However, there is still little information on how the fire-mediated changes in the colour of backgrounds in Cerrado savannas affect the effectiveness of the different camouflage strategies used by trunk-dwelling arthropods.

Considering that the fire modifies the colour heterogeneity of landscapes generating burned and unburned areas, we aim to understand whether these effects can: (i) mediate morph-specific patterns of background selection for camouflage in colour monomorphic and polymorphic species, and (ii) affect the survival of trunk-dwelling arthropods that use different camouflage strategies to hide from predators on trunks. Here, we first performed a background-choice experiment to test the preference of both melanic and brown morphs of the grasshopper *Ronderosia bergii* (Acrididae) and the praying mantis *Eumiopteryx laticollis* (Thespidae) for burned and unburned trunks. This choice experiment also included the spider *Syntrechalea brasilia* (Trechaleidae), which exhibits a dark, sooty general colour pattern and a lifestyle highly associated with tree trunks in the study area (de Alcantara Viana, Brito, et al., 2022). In order to understand whether the choices made by the arthropods are related to the colour of backgrounds and could be a mechanism for animals to improve camouflage, we calculated the colour contrasts of the different morphs and species against burned and unburned trunks to the view of avian predators.

On a broader and theoretical scope, our second aim was to understand how post-fire effects affect the survival of trunk-dwelling arthropods that use different camouflage strategies to camouflage themselves from their predators. One of the current and most used ways to test the survival of camouflaged organisms is to use citizen science methods that consider humans as "predators" in both online and in-person approaches. Data obtained from human predators can be extrapolated to understand how predator-prey interactions occur in nature, including those with predatory birds and arthropod prey (de Alcantara Viana, Vieira, et al., 2022; Karpestam et al., 2012; Michalis et al., 2017). Therefore, we tested the effectiveness of different camouflage strategies employed by a theoretical prey considering both the searching time (ST) that humans spend to find prey and the encounter distance (ED) at which detection occurs. For that, we created theoretical moth-shaped prey models matching the colour and/or pattern of burned and unburned Cerrado trunks as well as one model exhibiting disruptive coloration, with models being placed on natural trunks in the field.

Our first hypothesis is that the brown and melanic/dark colour morphs would exhibit lower colour contrasts on unburned and burned trunks, respectively. There are few studies about what ecological and evolutionary processes drive matching background choice patterns in nature. However, evidence suggests that these processes are widespread among taxa (Kang et al., 2012; Troscianko et al., 2016; Green et al., 2019; Boyle & Start, 2020; Camacho & Hendry, 2020). Therefore, we expect that lower contrasts between morphs and the different backgrounds (e.g., melanic/dark on burned and brown on unburned trunks) will translate into selection by individuals for more concealing backgrounds (Owen, 1982). Our second hypothesis is that theoretical prey models exhibiting contrasting coloration against trunks (e.g., brown models on burned trunks) will be found faster and at greater distances when compared to camouflaged models (e.g., brown models on unburned trunks). We also predict that models exhibiting disruptive coloration will exhibit higher fitness (i.e., will take longer to find, and detection will occur at shorter distances).

2. Materials and Methods

2.1 Study site and field sampling

The present study was conducted at the Panga Ecological Reserve (REP), which covers 409.05 hectares of the natural Cerrado domain, located in the municipality of Uberlândia, Southeast Brazil (19º11'40" S, 48º19'06" W). The REP is the largest continuous area of native vegetation in the region and is home to several vegetation types of Cerrado (Vasconcelos & Araujo, 2014). For this study, we chose the dense Cerrado, which is a vegetation type characterized by a reduced density of shrubs and herbaceous plants, and mainly composed of trees from up to 8 meters high (Gonçalves et al., 2021). At this location, natural fire events occurred both in the years 2017 and 2021, which therefore provided an ideal environment timing to conduct our study.

We sampled three representative arthropod species that were recorded resting on tree trunks in four adjacent burned and unburned plots (approximately 29.86 $m^2$of unburned plots; 29.55 m^2 of burned plots) (Figure 1 - A and B) of the study area, two represented by two distinct, discrete colour morphs: the grasshopper *Ronderosia bergii* (Acrididae) (Figure 1- C and D), from which we collected 15 melanic (burned: *n* =15; unburned: *n* =0) and 15 brown individuals (burned: *n* = 7; unburned: *n* = 8), the praying mantis *Eumiopteryx laticollis* (Thespidae) (Figure 1 - E and F) which we collected 21 melanic (burned: *n* =21; unburned: *n* =0) and 10 brown individuals (burned: *n* =3; unburned: *n* =7). We also sampled the single dark morph of the spider *Syntrechalea brasilia* (Trechaleidae) (Figure 1 - G) in a total of 17 individuals (burned: *n* =17; unburned: *n* =0). *Ronderosia bergii* is a widely distributed grasshopper species with variable colour patterns along its distribution range (Cigliano, 1999). In the study area, we recorded *R. bergii* resting on tree trunks, as well on litter and grass. *Eumiopteryx laticollis* belongs to the Thespidae family, which is the most diverse Mantodea group in Neotropical regions. Individuals of this species exhibits preferences for vertical substrates, mostly on tree trunks, but it was also recorded on large twigs and shrubs, and exhibit remarkable cryptic adaptations (pers. obs, de Alcantara Viana, Brito et al. 2022; Rivieira and Svenson, 2016). *Syntrechalea brasilia* belongs to the genus *Syntrechalea*, named Neotropical arboreal spiders, and besides already being found on soil, our observations and field collections indicate that it is highly associated with arboreal lifestyle (Carico, 2008; de Alcantara Viana, Brito, et al., 2022). We carried out four sampling campaigns between 2019 and 2022 (November 2019, January -

February 2020, July 2021, and February 2022), with collections during the morning, summing up 740 h in the field divided between plots with burned (370 h) and plots with unburned (370 h) trees. We conducted active searches (N = 4 well-trained researchers) for arthropods in trunks of *Qualea grandiflora* (Vochysiaceae) from burned and unburned plots. This fire-resistant tree species is highly widespread in the Cerrado domain and is used as the resting site by many arthropod species (de Alcantara Viana, Brito et al., 2022).

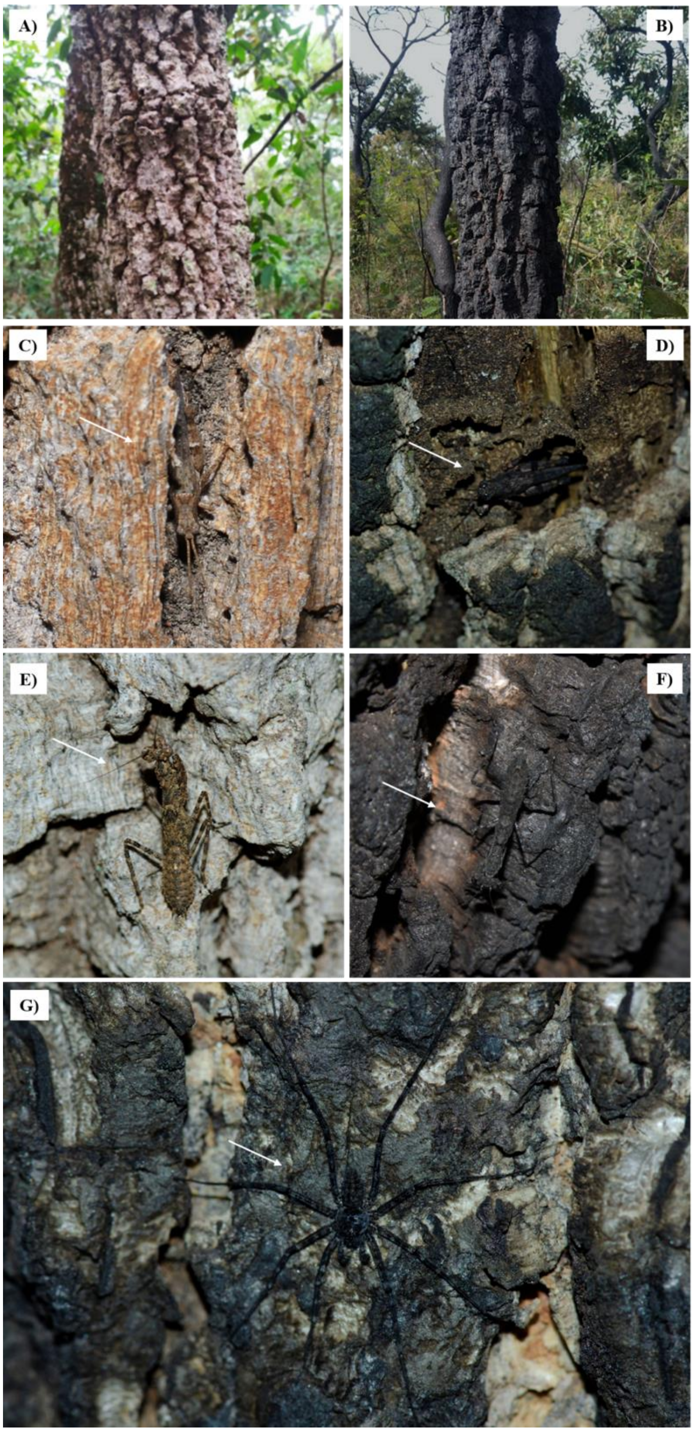

Figure 1. Colour polymorphism of different arthropods occupying tree trunks in Cerrado savannas. (A) Unburned and (B) burned trunks of *Qualea grandiflora*. (C) Brown and (D) black morphs of the grasshopper *Ronderosia bergii* on unburned and burned trunks, respectively. (E) Brown and (F) black morphs of the praying mantis *Eumiopteryx laticollis* on unburned and burned trunks, respectively. (G) *Syntrechalea brasilia*, a monochromatic (dark), sooty spider on a burned trunk.

2.2 Measuring arthropod coloration and camouflage

We used a spectrophotometer (Jaz; Ocean Optics Inc., Dunedin, FL, USA) to measure the spectral reflectance from 300 to 700 nm of the arthropods. We positioned the probe of the reflectance spectrophotometer at an angle of 45° and at approximately 2 mm of distance from the targets. Each read was a result of a reflectance spot of around 1 mm in diameter, to record the spectral reflectance of the head, thorax, and abdomen of both *R. bergii* and *E. laticollis*, and the cephalothorax and abdomen of *S. brasilia*. We then averaged the spectral measurements taken on the same individual for further analyses. We also randomly selected 60 *Q. grandiflora* trunks (30 homogenous burned pattern trunks and 30 homogenous unburned pattern trunk) at the sampling sites and measured their spectral reflectance as above to obtain mean reflectance values for each trunk condition. The calibration process was performed always before a measurement session and consisted of removing the spectrophotometer fibre and blocking the light to standardize the black reference and measuring the reflectance of a circularly arranged and homogenized pellet composed of barium sulphate to standardize the white reference. We corrected the noise of the reflectance curves by employing a local regression through a smoothing function (span = 0.5) using the “PAVO” package (Maia et al., 2019; R Development Core Team, 2022).

In order to obtain colour contrasts, we analysed the reflectance data for both arthropods and trunks through the Receptor Noise-Limited Model (RNL) (Vorobyev & Osorio, 1998) considering the Eurasian blue tit (*Cyanestes caraleus*) as a potential avian predator. Although this bird species does not occur in the Brazilian Cerrado, birds are highly conserved in their visual pigment characteristics (Hart, 2001), with blue tits being widely used in camouflage studies as the representative visual model for all Passeriformes birds, which are natural predators of arthropods worldwide (Owen, 1982). For that, we calculated chromatic (= colour) and achromatic (= luminance) contrasts in the form of JNDs (just noticeable differences) to understand how avian predators discriminate individuals of the different species and colour morphs against burned and unburned trunks. Since the RNL model was initially designed to deal with chromatic signals (Vorobyev & Osorio, 1998), using the model for achromatic comparisons needs caution because there is still no formal testing about the adjustability for this type of signal (Olsson et al., 2018). However, although birds are known to use dedicated single cones located at their retina to discriminate colour signals and create high-resolution images, double cones are important for luminance vision, which is especially important under low-light conditions (Mitkus et al., 2017; Kelber, 2019). Therefore, following the previous recommendation (Olsson et al., 2018), we chose to calculate both colour and luminance contrasts in our study and interpret their results independently.

For both chromatic and achromatic contrasts, values below 1 JND indicate that predators cannot distinguish prey from their background. Therefore, the higher the contrast value, the easier it will be for the predator to discriminate the arthropod from the tree trunk (Siddiqi, 2004; Maia et al., 2019). In addition, we considered the D65 standard irradiance spectrum as a measure of incident illumination in the model (Wyszecki & Stiles, 1982) since this is the most comparable spectrum to the natural lighting of Cerrado savannas. All analyses were performed in the R software using the "PAVO" 2.40 package (Maia et al., 2019; R Development Core Team, 2022).

2.3 Background selection experiment

Experiments were conducted in circular transparent arenas (27 cm in diameter and 20 cm in height) containing holes for air entry and two pieces of both burned and unburned branches of *Qualea grandiflora* (≅ 3.5 cm in diameter and 11 cm in height) as well as natural soil with local site leaf litter covering the bottom (Supporting Information 1- Figure S1). Arenas remained under constant and non-direct natural light (D65) throughout the entire experiment, with cloudy days being avoided. Before starting the experiment, all sampled individuals of the three arthropod species (*R. bergii, E. laticollis,* and *S. brasilia*) were acclimated in pots with moistened cotton wool for 30 minutes. After that, animals were carefully placed at the centre of the arena to prevent any possible interference in their behaviour and were recorded with a Sony - HD Video Recording Handycam for 6 hours (as in Vieira et al., 2015). Two trials were run at the same time and new arenas and backgrounds were used for subsequent trials to prevent the influence of any cue released by a previous animal on the behaviour of the next individual tested in the experiment. Given that the burned and unburned plots of the reserve are adjacent and that adult mobile arthropods can cross them, we cannot guarantee that the individuals we collected in the unburned area never occupied burned trunks and *vice versa*. In this way, our data and analysis focused only on the individuals, colour morphs, and their time of permanence (in minutes) in both types of trunks of the experimental arena, not relating to the occupation before the experiments.

2.4 Predation experiment

Our experiment using human predators followed previous protocols described elsewhere (Cuthill et al., 2005; Xiao & Cuthill, 2016; Kjernsmo et al., 2020). We preferred to run the experiment directly in the field instead of through online approaches (i.e., games) because by simulating predation in the field we believe that the realism of the predator-prey interaction is improved (Xiao & Cuthill, 2016; Kjernsmo et al., 2020). We asked 23 participants to walk along Cerrado savanna trails to look for artificial moth-shaped prey models attached to burned and unburned *Qualea grandiflora* trunks. Four different types of artificial models were used: (i) a brown model matching the colour of unburned trunks (brown colour matching - Br), (ii) a black melanic model (black - Bl) matching the colour of burned trunks, (iii) a brown model containing variable pattern markings matching the colour and pattern shape of unburned trunks (Bpm), and (iv) a brown model exhibiting disruptive markings of variable coloration around the target edges (Dis) (Supporting information - Figure S2).

The colour of all models was based on photographs obtained from unburned (*n* = 50, for the Br, Bpm, and Dis models) and burned (*n* = 50, for the Bl model) trunks of *Q. grandiflora* in the REP. Burned trunks resulted from a burning event that occurred at the reserve in September 2021. Trunks were photographed using a Nikon D7000 digital camera coupled with a Nikkor 105 mm macro lens mounted on a tripod and a diffuser umbrella. Images were taken in RAW format, with manual white balancing and fixed aperture (f8) settings to avoid overexposure (Stevens et al., 2007) at a distance of one meter and included a standard pellet of Barium sulphate (99% reflectance) to control for changes in lighting conditions, following current standard protocols (Troscianko & Stevens, 2015). After being equalized using the white standard, the image channels were scaled to reflectance values, where an image value of 255 on an eight-bit scale equals 100% reflectance (Stevens et al., 2007). Finally, we converted camera-based reflectance values to human cone catches using models and functions implemented within the MicaToolbox in ImageJ (Troscianko & Stevens, 2015). The cone-catches values for the three human colour channels, which are analogous to the RGB system, were averaged for both burned and unburned trunks and used to represent the black and brown coloration of our artificial prey models, respectively. The pattern markings included inside (for the Bpm model) or at the border (for the Dis model) of models were based on the spatial variation of real patterns observed in unburned *Q. grandiflora* trunks. We used ImageJ to convert the trunk images to greyscale and applied a 50% threshold to binary (black/white) images (Supporting Information - Figure S2); with the generated patterns being manually applied to the artificial models using Adobe Photoshop (version 2.2). This procedure resulted in 10 different versions of Bpm and Dis models (as in Cuthill et al., 2005), which were used for both burned and unburned areas (see details below). The printed colour patches were then photographed and their RGB values were measured and compared to the reflectance values of real trunks, modelled for human vision, with the most similar values being selected for both black and brown models. Finally, prey models (3.6 cm in width and 2 cm in height) were printed on a waterproof paper (A4 F22) with a Colour LaserJet printer at 600 dpi resolution.

We randomly chose 20 burned and 20 unburned *Q. grandiflora* trees separated by at least 15 m within the REP. Em cada tronco foram colocados aleatoriamente dois tipos de alvos a aproximadamente 1,5 metros de altura, incluindo sempre um modelo Br e um Bl, ou um modelo Bpm e um modelo Dis no mesmo nível do plano de visualização. All the models were kept at the same location for all participants. Volunteers aged 18 to 48 of both genders ($n_{\text{female}} = 12$; $n_{\text{male}} = 11$) and with normal or corrected-to-normal vision participated in the experiment. We asked participants to follow the instructors (J.V.A.V, C.L., F.C.C., and A.L.O.M.) with their faces down so they were not able to see any of the models before starting the trial. Upon reaching the initial distance of 7.5 m from the first experimental tree, participants were warned that prey models were located on a tree ahead of them. We advised that participants could walk and approach the trunks as much as necessary until they spot the target and indicate its location with a laser pointer. After finding the first target, participants remained still and looked down to prevent from seeing the other target on the trunk. Instructors then collected the time (ST) and the distance (ED) in which each participant found the first target. After that, participants were free to search for the second target on the trunk. This procedure was performed for all subjects in 20 unburned and 20 burned trees, with an average duration of 2.5 hours per participant. Although the two variables considered in this experiment can be correlated, we preferred to use them as different metrics given that participants may use different search tactics to find the models (e.g., some would prefer to get close to the tree to identify the targets, while others would favour searching models from a distance before approaching to the tree).

2.5 Statistical analyses

All statistical analyses were undertaken using the software R v.4.2.2 (R Core Team, 2022). We used linear mixed-effects models to test for differences in both colour and luminance contrasts (JNDs) of the colour morphs of the three arthropod species between burned and unburned trunks. Models were fitted separately for each species, considering JNDs as the response variable, morph (only for *R. bergii* and *E. laticollis*) and trunk condition (burned and unburned) as fixed between-subject factors, and arthropod identity as a random factor to control for repeated measurements on the same individual (Zuur et al., 2009). A different version of the model (i.e., with only trunk condition as the fixed factor) was used for the *S. brasilia*. The same model was used to test for differences in the preference (i.e., the occupation time on trunks, in minutes) of the different colour morphs of the arthropod species between burned and unburned trunks. For this analysis, we disregarded the time that animals remained on the horizontal substrate (leaf litter), considering only the vertical structures (trunks). For all models, we visually inspected the normality of residuals through q-q plots and test the homogeneity of variances by the Fligner-Killeen test, for which the occupation time of *S. brasilia* required log transformation to meet model assumptions. Finally, in the case of significant effects, we applied Tukey's post-hoc tests to test for differences between factor levels using the ‘emmeans’ package in R (Lenth, 2016).

For the predation experiment, we calculated the mean and standard deviation for both ST and ED of each participant separately for unburned and burned trunks. We then converted these values into log response ratios (lnR) which was used as a metric of effect size. In this metric, positive or negative values indicate that participants increase or decrease the search time and the encounter distance of models in burned trunks in comparison with unburned substrates. For better interpretation, we back-transformed lnR to percentage [(exp lnR −1) × 100%] to obtain differences between burned and unburned conditions for the different prey types. We used mixed-effects models to test for differences in the effect sizes calculated for the two variables between prey types on burned and unburned areas using maximum likelihood (throughout the ‘rma’ function). We removed the intercept from the models throughout the ‘mods-1’ argument, and then all subgroups (i.e., prey models) were included in the model (Hedges et al., 1999). All analyses of the predation experiment were conducted using the “METAFOR” package (Viechtbauer, 2010).

3. Results

3.1 Camouflage of arthropods on trunks

Colour and luminance contrasts of the grasshopper *R. bergii* significantly differed between trunk conditions, but differences depended on the colour morphs (Table 1). While black individuals showed similar colour JNDs between burned and unburned trunks, brown grasshoppers had lower contrasts against unburned trunks. On the other hand, black individuals had lower luminance JNDs in burned than in unburned trunks, but brown grasshoppers exhibited similar contrasts on both trunk types (Figure 2A). Comparable results were found for the praying mantis *E. laticollis*, with both colour and luminance contrasts differing between trunk types but depending on the colour morph (Table 1). Colour and luminance JNDs of black mantis were lower on burned than on unburned trunks. In the case of brown mantis, while colour JNDs were similar between trunk conditions, luminance JNDs were lower against unburned trunks (Table 1; Figure 2B). Finally, individuals of the spider *S. brasilia* showed significantly lower colour and luminance contrasts against burned trunks compared to unburned substrates (Table 1; Figure 2C).

Table 1. Arthropod camouflage against different trunk conditions in Brazilian Cerrado based on the blue tit bird vision. Results of the analysis of variance (ANOVA) applied to different linear mixed-effects models (lmer) testing differences in just-noticeable-differences (JNDs) for colour and luminance measurements of the black and brown "morphs" of the grasshopper *Ronderosia bergii* and the praying mantis *Eumiopteryx laticollis*, as well as individuals of the spider *Syntrechalea brasilia*, against burned and unburned trunks ("trunk condition"). Model intercept includes arthropod identity as a random factor to control for repeated measurements in the same individual. Significant values are shown in bold.

	Colour JNDs				Luminance JNDs			
	df	*MS*	*F*	*p*	*df*	*MS*	*F*	*p*
Ronderosia bergii								
Trunk condition	1	39.31	20.08	**< 0.001**	1	123.46	12.13	**< 0.001**
Morph	1	7.26	3.71	0.064	1	58.49	5.74	**0.020**
Trunk * Morph	1	16.81	8.59	**< 0.001**	1	263.26	25.86	**< 0.001**
Residuals	28	1.96			56	10.18		
Eumiopteryx laticollis								
Trunk condition	1	8.82	6.01	**0.017**	1	146.37	18.34	**< 0.001**
Morph	1	0.70	0.48	0.490	1	63.66	7.98	**0.007**
Trunk * Morph	1	38.49	26.20	**< 0.001**	1	516.41	64.73	**< 0.001**
Residuals	60	1.45			29	7.72		

Syntrechalea brasilia								
Trunk condition	1	104.8	52.86	**< 0.001**	1	675.20	82.36	**< 0.001**
Residuals	32	1.98			16	8.20		

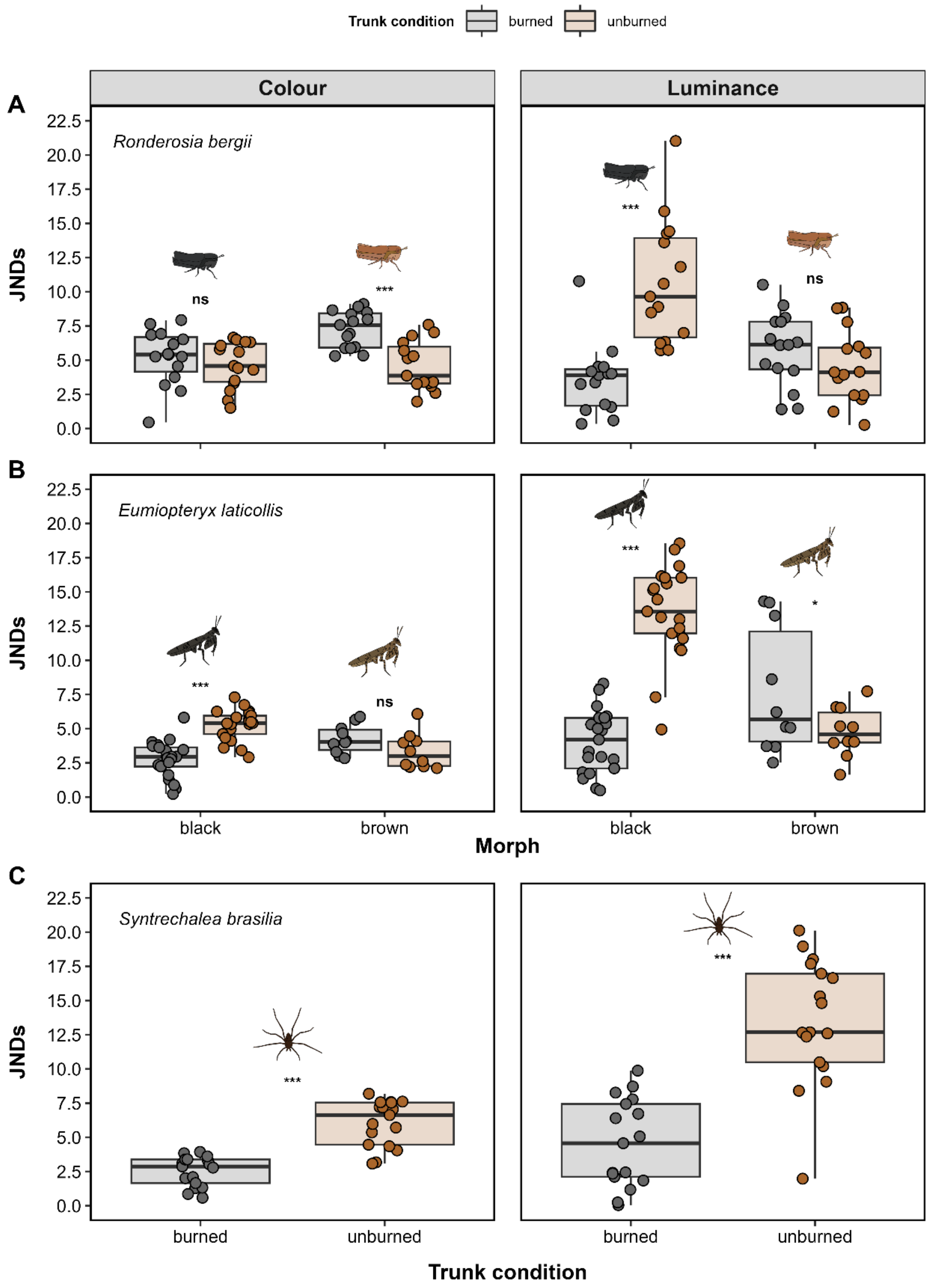

Figure 2. Colour and luminance contrasts of arthropods against Cerrado savanna trunks. Blue tit bird vision discrimination (as just noticeable differences; JNDs) of black and brown morphs

of the (A) grasshopper *Ronderosia bergii* and the (B) praying mantis *Eumiopteryx laticollis*, as well as of the (C) spider *Syntrechalea brasilia* against burned and unburned trunks of *Qualea grandiflora* in Cerrado savanna. Boxes show medians and interquartile ranges (IQR), whiskers represent the lowest and highest values within 1.5∗IQRs, and filled circles represent raw data. *ns*: not significant; **p* < 0.05; ****p* < 0.001.

3.2. Arthropod background selection experiment

Regardless of colour morph, we did not find evidence that *R. bergii* prefer trunks based on their conditions (Table 2). The individuals spent 137.81 ± 19.90 (mean ± standard error) min in unburned and 100.70 ± 17.93 min in burned substrates (Figure 3A). In contrast, the praying mantis *E. laticollis*, regardless of its colour morphs, showed individual preferences to settle longer on unburned (173.20 ± 22.47 min) than burned (114.01 ± 19.39 min) trunks (Table 2; Figure 3B). On the other hand, individuals of the monochromatic dark, sooty spider *S. brasilia* spent approximately 126% longer on burned (102.91 ± 25.86 min) than unburned (45.42 ± 25.14 min) substrates (Table 2; Figure 3C).

Table 2. Arthropod background selection experiment. Results of the analysis of variance (ANOVA) applied to different linear mixed-effects models (lmer) testing differences in trunk condition selection preferences of the black and brown “morphs” of the grasshopper *Ronderosia bergii* and the praying mantis *Eumiopteryx laticollis,* as well as individuals of the spider *Syntrechalea Brasilia*. Model intercept includes arthropod identity as a random factor to control for repeated measurements in the same individual. Significant values are shown in bold.

	Arthropods background selection			
	df	*MS*	*F*	*p*
Ronderosia bergii				
Trunk condition	1	16.95	1.65	0.200
Morph	1	42	0.004	0.940
Trunk * Morph	1	33.41	8.59	0.540
Residuals	46	1.02		
Eumiopteryx laticollis				
Trunk condition	1	60.27	4.48	**0.038**
Morph	1	11.73	0.87	0.3539

Trunk * Morph	1	19.19	1.42	0.2329
Residuals	58	1.45		
Syntrechalea brasilia				
Trunk condition	1	33.74	9.97	**< 0.001**
Residuals	32	1.83		

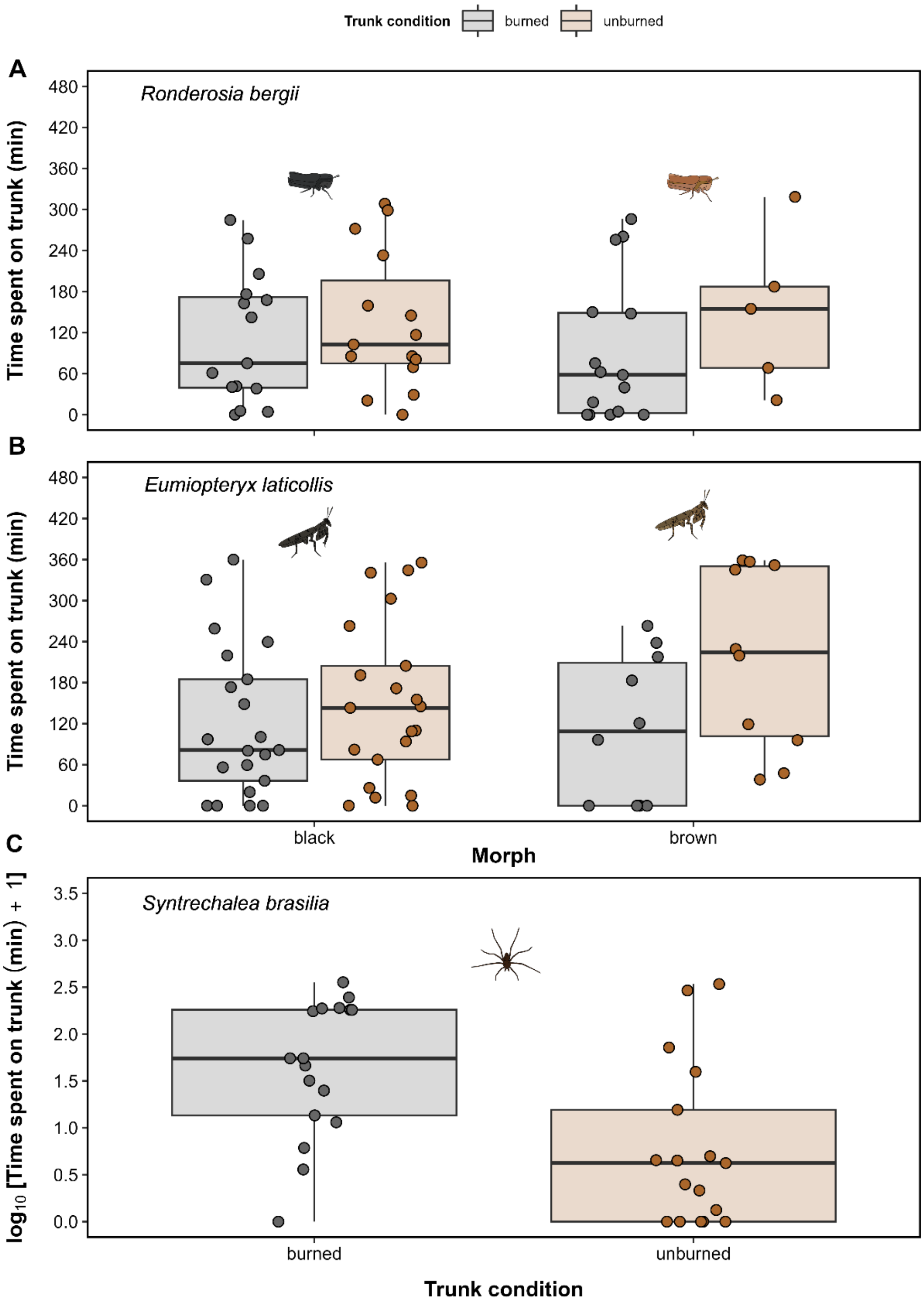

Figure 3. Background selection experiment of arthropods for different trunk conditions in Cerrado savanna. Time spent on burned and unburned trunks (in minutes) of *Qualea grandiflora* in Brazilian Cerrado of black and brown morphs of the (A) grasshopper *Ronderosia bergii*; the (B) praying mantis *Eumiopteryx laticollis* as well as the black individuals of the (C) spider *Syntrechalea brasilia*. Boxes show medians and interquartile ranges (IQR), whiskers represent the lowest and highest values within 1.5∗IQRs, and filled circles represent raw data. Data for *S. brasilia* were log-transformed to attend to the model assumptions (see the main text for more details).

3.3 Human predation experiment

Overall, the time that human predators spent to find the prey models differed between trunk conditions (*Qt* = 1398.44; *df* = 91; *p* < 0.001), with the searching time being 52% shorter in burned than in unburned trunks. The effect sizes calculated for predator searching time significantly differed between prey models (*Q*m = 1070.87; *df* = 4; *p* < 0.001), being positive for the black ("Bl") (i.e., models took longer to be detected on burned than unburned trunks) and negative for the other model types (i.e., models were detected faster on burned than unburned trunks) (Figure 4A). Although disruptive models ("Dis") were found faster than the brown ("Br") type on burned trunks (and consequently slower on unburned trunks), they showed similar detection time to the background pattern matching models ("Bpm"), which were also similar to brown models (Figure 4A). Similarly, the distance that human predators detected the targets also differed between trunk conditions (*Q*t = 1267.18; *df* = 91; *p* < 0.001), with the encounter distance of models decreasing by 61% in burned trunks. The effect sizes calculated for the predator encounter distance significantly differed between prey models (*Q*m = 403.64; *df* = 4; *p* < 0.001), being, however, negative for the black ("Bl") model (i.e., models were detected within a shorter distance on burned than unburned trunks) and positive for the other types (i.e., models were detected within a larger distance on burned than unburned trunks) (Figure 4B). The "Dis" models were detected at a larger distance than both "Br" and "Bpm" targets on burned trunks (and consequently at a shorter distance on unburned trunks), with the last two models exhibiting similar values between them (Figure 4B).

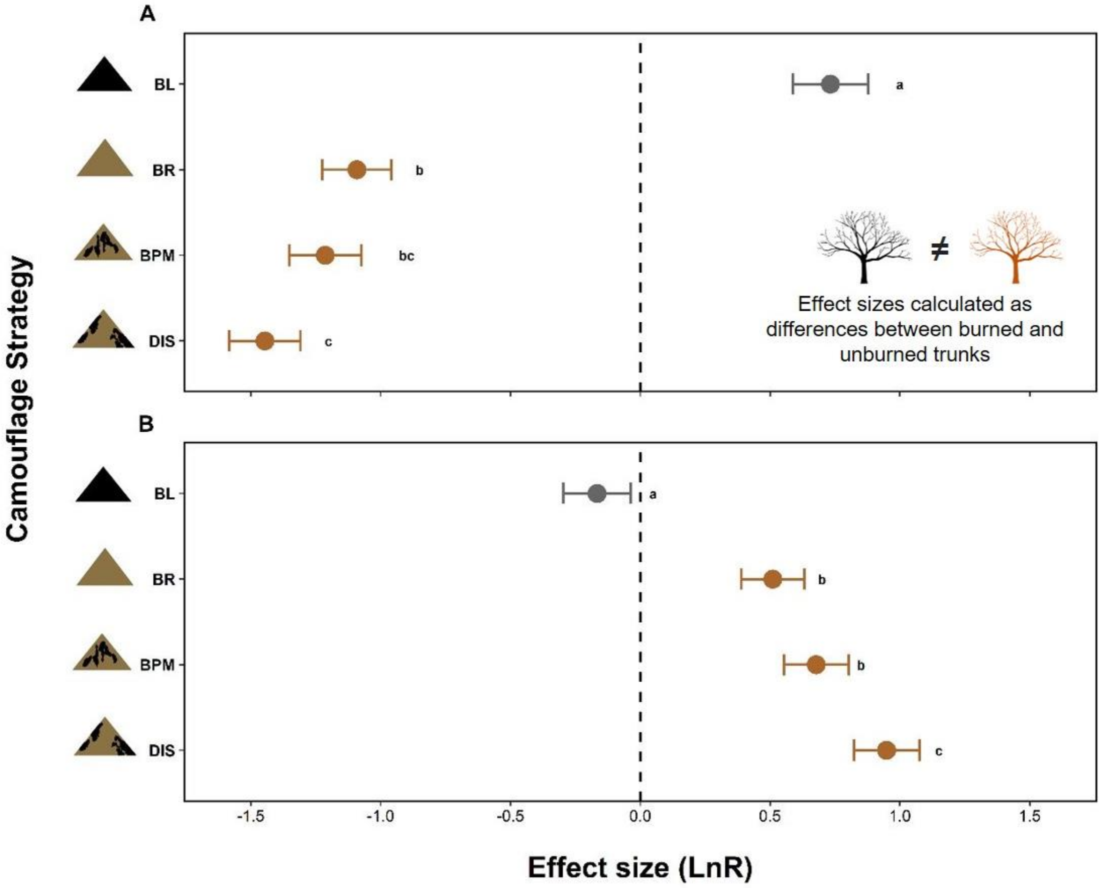

Figure 4. Predation experiment using humans as predators to test the effectiveness of different camouflage strategies on burned and unburned trunks in Brazilian Cerrado. Differences in (A) the searching time and (B) the encounter distance of artificial prey models exhibiting different camouflage strategies (BL – black; BR – brown; BPM – background pattern matching; DIS – disruptive) on burned and unburned trunks. In A, positive and negative values indicate an increase or decrease in the time spent by the predator to find the prey, respectively. In B, positive and negative values indicate an increase or decrease in the distance at which predators encounter the models, respectively. Effects are considered significant if the 95% CI does not include 0. Different letters indicate significant differences between factor levels.

4. Discussion

Together, our results highlight the importance of different conditions that influence the success of camouflage in unpredictable habitats subject to dramatic alterations of background coloration. We show the role of behaviour in choosing a suitable substrate that matches the coloration and brightness as well as the differences between the effectiveness of camouflage strategies adopted and used against predators in different background conditions. Arthropods living in fire-prone environments in the Brazilian Cerrado savanna exhibit effective morphological and behavioural strategies to obtain camouflage against tree trunks of different burning conditions. However, the adoption of these strategies is species-specific and depends primarily on the behaviour and life history traits of the organism. For almost all arthropod species studied, the melanic (= black) individuals exhibited lower luminance and colour contrasts in burned trunks. On the other hand, the contrast of brown individuals against trunks did not follow a general pattern, with some individuals exhibiting lower colour and luminance contrasts against unburned trunks, as expected, but others showing similar concealment against backgrounds. In addition, only the monochromatic dark spider *S. brasilia* was able to behaviourally optimize its crypsis at burned trunks by spending more time on this background, since grasshoppers (*R. bergii*) have no preferences and praying mantis (*E. laticollis*) preferred to settle longer on unburned trunks regardless of their colour. The preference for non-matching substrates of the black individuals of *E. laticollis* that are better concealed at burned trunks may increase their risk of being preyed upon on preferred unburned backgrounds. This is supported by the results of our predation experiment using humans as predators, in which black models were found faster and at a larger distance in unburned trunks, showing higher survival probability at burned substrates. Altogether our results suggest that achromatic (i.e., brightness) information in burned savannas could be an important visual signal used by predators at long and medium distances to identify prey in a more open vegetation landscape with heterogeneous substrates (de Alcantara Viana, Brito, et al., 2022; Hart, 2001). Individuals of species that do not show specific background matching behaviour possibly may reduce predation risks by achieving camouflage against concealing substrates when these are highly available within their habitat or by adopting alternative behavioural strategies that reduce predation against non-concealing substrates.

As expected, the spider *S. brasilia*, which is the most arboreal specialized arthropod species from our study system, showed the best matching against burned trunks for both colour contrasts in comparison to the melanic morphs of the other two polymorphic species. Spider camouflage is optimized through a behavioural preference for burned trunks, which is supported by the high specialization of this species for vertical substrates, including body adaptations such as slender legs and low profile that can increase concealment (Carico, 2008). Once the spiders of the *Syntrechalea* genus (Trechaleidae) are predators of landing arthropods on trunks (Carico, 2008; de Alcantara, Brito, et al., 2022), exhibiting effective camouflage is, thus, essential for individuals to improve both the capture of prey and to avoid detection by their natural enemies, as reported for crab spiders upon flowers (Théry & Casas, 2002). Furthermore, the spiders' habitat selection behaviour can be partially explained by imprinting, as they were especially found in burned trunks in our study area, which can also indicate a juvenile phase of the learning process for burned trunks (Davis & Stamps, 2004). On the other hand, the preference of black and brown morphs of the praying mantis *E. laticollis* for unburned trunks could be explained by the less arboreal fidelity of these species when compared with *S. brasilia* and other trunk-specialized praying mantis, such as the bark mantis of the Liturgusidae family (de Alcantara Viana, Brito, et al. 2022; Svenson, 2014). The preference for brown backgrounds may have evolved in *E. laticollis* because this specie also occupies other types of brownish vertical habitats, such as twigs and shrubs (de Alcantara Viana, Brito, 2022; pers. obs.). During the dry season and in the absence of fire events, such habitats form a homogeneous brown landscape in Cerrado savannas (pers. obs.), which could optimize the camouflage of grasshoppers and praying mantises at these alternative habitats. The preference of *E. laticollis* praying mantis for brown substrates may have evolved faster than the unpredictability of the fires in Cerrado landscapes. In this way, the differential predation over colour-mismatching individuals is probably maintaining the stable colour polymorphism with melanic and brown morphs in the population of these species (Bond, 2007; Karpestam, et al. 2012). Considering that predation risks will be much higher for the melanic morphs at the preferred unburned trunks, it can be expected that these individuals would be strongly consumed by predators and will be likely to become locally extinct over time, as reported by brown morphs of pygmy grasshoppers in fire-prone woodlands of Sweden (Forsman et al., 2011). However, this relationship may be different depending on the availability of different background types in the habitat. In some Cerrado areas, the cover of burned areas can be exceedingly higher than unburned ones, and therefore even showing preferences for unburned backgrounds, the melanic individuals of both *R. bergii* and *E. laticollis* would benefit from their cryptic appearance and be less predated at these places due the low availability of unburned trunks.

It is also possible that for some morph - background combinations, individuals of *R. bergii* and *E. laticollis* would benefit from a generalist (= compromise) camouflage strategy, in which coloration matches many backgrounds to some extent but none closely (Forsman et al., 2011; Hughes et al., 2019). Black and brown *R. bergii* grasshoppers exhibit comparably high and similar colour and luminance contrasts, respectively, against burned and unburned trunks. The same is observed for the colour contrasts of brown *E. laticollis* against trunk types. Therefore, in specific situations, predators would be able to detect both colour morphs equally, regardless of the trunk conditions, which would contribute to balancing the frequency of melanic and brown morphs in the population (Hughes et al., 2019). Moreover, some grasshoppers and praying mantis can change their body colour over different timescales, mainly for thermoregulation or to obtain camouflage (Battiston & Fontana, 2010; Edelaar et al., 2017; Valverde & Schielzeth 2015). Thus, it could be possible that the melanic and non-melanic morphs of *R. bergii* and *E. laticollis* in the Brazilian Cerrado result from phenotypic plasticity (Duarte et al., 2017; Umbers et al., 2014). Non-reversible colour changes were registered for the praying mantis *Galepsus toganus* and *Pyrgomantis pallida* from African savannas, in which colour plasticity takes place when grass backgrounds are affected by burning (Edmunds, 1976; Owen, 1982). However, there is no information about the capacity of colour change of any polymorphic arthropod species from fire-prone savannas in Brazil, which opens an important line of research for future investigation.

Predation experiments using humans have been increasingly used to understand the evolution and the survival benefits of different camouflage strategies in nature, with the results frequently matching those generated by experiments using real predators (Karpestam et al., 2013; Xiao & Cuthill, 2016). In our experiment, we used theoretical prey models to generalize the melanic and brown morphs that are observed in a range of arthropod species in the Brazilian Cerrado and test how their differential camouflage against burned and unburned trunks translates to survival in a natural setting. The melanic (= black) models had increased fitness at burned trunks, with predators spending more time and needing to get closer to detect black targets in comparison to the non-melanic brown models. Therefore, in substrate-darkening scenarios, melanism will be favoured quickly and will be important to reduce the risks of population extinction due to strong predation pressures after fire events (Karpestam et al., 2016; Vignieri et al., 2010). This result is comparable to the findings by Kettlewell (1955; 1956), when studying the effects of industrial pollution on the differential survival of the melanic and non-melanic morphs of the peppered moth (*Biston betularia*) in England woodlands. Although this classic experiment, which is considered as one of the most important examples about evolutionary processes in action in nature (Cook, 2012), has received several criticisms and being questioned by possible fraud (Hooper, 2002), recent evidence showed that the pale (*typica*) morph of the peppered moth exhibited lower colour contrasts against trees covered by lichen in unpolluted areas, where they also had higher survival when compared to the melanic (*carbonaria*) morph (Walton & Stevens, 2018). In our study, besides testing differences in the survival of homogeneously black and brown prey types, we also added to our experiment models containing internal pattern markings closely corresponding to a background pattern matching (i.e., markings are distributed randomly inside the model) and a disruptive (i.e., markings are distributed internally close to the border of the model) strategy. As expected, regardless of having internal markings or not, all brown models performed worst in burned trunks and conversely had higher fitness against unburned backgrounds. However, compared to homogeneously coloured brown models, disruptive targets were the hardest to find against unburned trunks, with predators spending more time and needing to get closer to capture prey models. On the other hand, on this background, predators had similar detection times between pattern matching and disruptive models. These results are similar to the observed in a recent meta-analysis that compares the effectiveness of different camouflage strategies (de Alcantara Viana, Vieira, et al., 2022), as well as a classic study of birds predating computer-based theoretical prey (Merilaita & Lind, 2005).

Altogether, our results show that in burned trunks, the simple melanic prey models had the highest survival probability, indicating that in less complex textured habitats, a strategy promoting effective colour background matching is favoured. On the other hand, in more complex habitats, such as the unburned trunks, a strategy enhancing edge disruption would be more successful (Cuthill et al., 2005; Webster et al., 2013) since disruptive coloration can impair object identification, especially over long distances (Adams et al., 2019; Barnett et al., 2014; Barnett et al., 2018).

5. Conclusion

We provide novel experimental evidence about ecological and behavioural factors controlling the colour polymorphism of different arthropod species in fire-prone environments. Colour morphs may benefit from differential concealment against burned and unburned trunks and in some situations would be able to select substrates where they are best concealed to improve crypsis. In addition, our predation experiment showed that human predators increase searching time and decrease the distance to find melanic prey in burned areas, with individuals being quickly predated in unburned backgrounds. Although there were no differences in predator searching time between pattern matching and disruptive models, predators needed to get closer to find disruptive targets against unburned trunks. Therefore, the possible effect of obscuring outlines and creating false boundaries due to the presence of pattern markings in disruptive models would be more effective against complex unburned trunks than the other strategies, especially over long distances. Finally, we recommend the inclusion of the effects caused by fire on predator-prey interactions in future fire management policies, since burning cannot only lead to direct animal mortality caused by fire but also can indirectly modify the survival chances of several organisms through disruption of visual and behavioural interactions in natural environments.

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CAPÍTULO 3

Post-fire effects on the camouflage strategies of Cerrado arthropods

Post-fire effects on the camouflage strategies of Cerrado arthropods

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Abstract

Camouflage encompasses more than a dozen defensive coloration strategies, each one exploring a different sensory route to avoid detection and recognition by predators. To date, it is still unclear how color changes in the background and animal prey size are related to the effectiveness and occurrence of different camouflage strategies. In this study, we investigated the relationship between background color changes mediated by fire and the occurrence of camouflage strategies in trunk-dwelling arthropod communities of a Brazilian savanna. First, we recorded the number of individuals of morphospecies in each trunk condition (burned or unburned) and quantified the dissimilarity between morphospecies composition as well as account for the occurrence of one or more camouflage strategies in each individual. Second, we measured the degree of background matching on occupied and non-occupied (control) backgrounds. Third, we examined disruptive coloration (GabRat) between trunk conditions. Finally, we explored the relationship between both camouflage metrics, arthropod taxonomic Order, and prey size in both background conditions. We sampled 639 individuals, with 69.3% in burned and 30.7% in unburned areas. Trunk conditions contributed to 45% of morphospecies composition dissimilarity but did not influence the frequency occurrence of the varying camouflage strategies on them. Background matching (37.5% of sampled individuals) was the most abundant strategy observed on both trunk conditions, followed by disruptive coloration (37.1% of sampled individuals). The arthropods found on the burned occupied trunks had lower color and luminance contrast when compared to the unburned non-occupied trunks. Conversely, arthropods on the unburned occupied trunks had lower color and contrast values compared to the non-occupied burned trunks. Disruptive coloration in arthropods did not differ between trunk conditions. We did not find an overall relationship between arthropod size and camouflage metrics. However, we found interactions between taxonomic Order, size, and camouflage metrics. Overall, our results provide strong evidence that, even in a fire-mediated background color change scenario, background matching is the main anti-predatory camouflage strategy adopted by prey, followed by disruptive coloration. These results suggest that predation pressures on arthropods occupying trunks of burned and unburned areas adjust communities to show low color and luminance contrasts and intermediate levels of GabRat against predatory birds.

Keywords: background matching; camouflage; disruptive coloration; fire; savanna.

1. Introduction

Predation exerts one of the strongest evolutionary pressures on organisms, contributing to the structure and complexity of ecological communities (Glasser, 1979; Ruxton et al., 2004). Camouflage is, perhaps, the most widespread anti-predatory strategy used by prey to avoid predation (Cott, 1940; Troscianko et al., 2016). As a primary defense, it is defined as morphological traits or color patterns that organisms possess for concealment, thereby preventing detection and/or recognition (Stevens and Merilaita, 2011). Camouflage is a comprehensive ecological concept that encompasses distinct types of strategies employed single or together by prey and applied in several cognitive routes to distort the perception of the viewer (Merilaita et al., 2017). Surprisingly, the mechanisms of camouflage and how different strategies work in concert are still poorly understood because researchers have historically underestimated the topic as something already well-known (Stevens and Merilaita, 2011; Merilaita et al., 2017).

Camouflage operates by exploiting vulnerabilities in the sensory and cognitive perception mechanisms of predators, where the detection and recognition of prey result from the balance between target signals and background noise information (Cuthill, 2019). Background matching occurs when the animal matches the color, brightness, and/or pattern with its surrounding background, making it challenging for predators to distinguish the prey by blending it with its background (Stevens and Merilaita, 2011). Disruptive coloration is defined as the color patterns consisting of contrasting coloration that creates the illusion of false boundaries and body edges, thereby increasing visual noise and impairing detection (Merilaita et al., 2017). Masquerade, however, operates by diminishing the probability of recognition and not necessarily the detection, as the organisms can be conspicuous but misinterpreted by the predator as an inanimate object from the environment, such as a rock, twig, leaf, or even a bird dropping (Skelhorn et al., 2010).

Animal color patterns per se are not the only factors in camouflage. The size of the animal itself, as well as changes in the color patterns of the background, also play a significant impact in the selection and effectiveness of camouflage strategies (Murali and Kodandaramaiah, 2018; Smith and Ruxton, 2021). Concerning the size, Smith and Ruxton (2021) found that increasing the size of disruptive prey also increased their susceptibility to predation compared to uniformly colored prey. Taking into account the changes in the background, a classic example illustrating the relationship of background color change on phenotype selection due to predation pressures is the industrial melanism in *Biston betularia*

(Walton and Stevens, 2018). However, how the animal size and background color change patterns are related to different camouflage strategies is still under discussion.

Brazil is home to a unique neotropical savanna known as the Cerrado, which has evolved under strong stochastic fire selection pressures (Oliveira and Marquis, 2002). The trees in this region have developed fire-resistant adaptations. As fire result, the landscape undergoes a significant color transformation to black, becoming completely different compared to its previous brown and green state. This phenomenon creates a scenario similar to industrial melanism, particularly for the associated fauna, including the arthropod community that relies on tree trunks to avoid predation by visually guided predators. The altered landscape resulting from fires can lead to shifts in predation pressures, potentially resulting in changes in the phenotypic frequencies of animals in that specific location (Price et al., 2019; de Alcantara Viana et al., unpublished data).

Recently, de Alcantara Viana et al. (2022) found that the arthropod community of Neotropical Savanna (Cerrado) exhibited lower values of achromatic contrasts to potential avian predators on both burned and unburned trunks. However, the degree of disruptive coloration, as well as the occurrence of camouflage strategies in the arthropod community in burned and unburned landscapes, remain largely unknown. Furthermore, natural experiments, such as the Cerrado stochastic fires, provide an ideal setting to test hypotheses that have not yet been applied to real prey, such as the relationship between animal size, color contrasts, and disruptive coloration. Here, we aim to investigate the relationship of post-fire effects on i) the occurrence of morphospecies and their composition dissimilarity, ii) the camouflage strategies that operate solely or in concert; iii) the degree of color and luminance contrasts, as well as iv) the disruptive coloration of the arthropod community that rests on burned and unburned tree trunks to avoid bird predation on Cerrado savanna. Additionally, we aim to understand v) the relationship between background matching and disruptive coloration strategies with prey size, concerning both trunk conditions for the overall arthropod community as well for the taxonomic Orders.

We expected that i) a larger number of individuals collected on both burned and unburned trunks would exhibit predominantly Bm and Dis strategies compared to other strategies, either as sole or in combination (*e.g.*, Bm+Dis). We predicted that ii) the color contrasts of arthropods resting on burned or unburned trunks would be lower compared with potentially and non-occupied unburned or burned trunks, respectively. Studies have shown that disruptive coloration can function independently of background matching, being more effective in heterogenous habitats (Shaefer and Stobbbe, 2006; Stevens et al., 2006). Thus, we expected iii) differences in the degree of disruptive coloration between burned and unburned trunks. As severe fires leads to homogenization of backgrounds, we expected that unburned trunk, which is more heterogeneous in coloration, will lead to higher levels of disruptive coloration to the arthropod community than homogeneous burned trunks. Increases in body size also increase the probability of detection by predators. Thus, we predicted that iv) larger-sized arthropods will show overall lower values of color contrasts and higher levels of disruptive coloration.

2. Methods

2.1. Study site and arthropod collections

The study was conducted in the Panga Ecological Reserve (PER), located in Uberlândia City, Brazil (19º11' S and 48º19' W). PER holds several types of Cerrado vegetation (Vasconcelos and Araujo, 2014). The location was chosen because in 2017 and 2021 the PER suffer severe fire events, in which ~75% of the park was burned, providing an ideal natural experimental area for the study. The collections were performed in four burned and four unburned plots of dense Cerrado (approximately 29.86 $m^2$of unburned areas; 29.55 m^2 of burned areas), which is a vegetation type mainly composed of trees up to eight meters high (Gonçalves et al., 2021). We carried out six monthly sampling campaigns from 2019 to 2022, as follows: one campaign in 2019, two in 2020, two in 2021, and one in 2022 (Supplementary Material 1). Samplings consisted of active searches by four observers for arthropods in trunks of *Qualea grandiflora* in burned and unburned plots areas, observing each type of trunk for 3 minutes, totaling 740 hours in the field divided between areas. The collections were made from 0 m to 3m in height on the trunk. For additional details, please refer to the study conducted by de Alcantara et al. (2022), as it contains the same sampling protocol.

Each arthropod found was collected using plastic pots or falcon tubes. After collection, the arthropods were placed in the freezer to reduce their metabolism and prevent movement during subsequent photographing and reflectance procedures. Following the reflectance measurements and photography (as described in sections 2.3 and 2.4), the arthropods were sacrificed by immersing them in 70% ethanol for further identification and size measurements. The identification of the collected animals was carried out with the aid of stereomicroscopes, consulting dichotomous keys, as well as taxonomist researchers for finer identification at the possible taxonomic level (Supplementary Material 1). We also separated and recorded the number of different morphospecies present in each of the families, and all collected individuals were used in subsequent analyses. The total length of each arthropod was measured in centimeters (cm) from the photographs using the Image J measurement tool. This measurement was used as a proxy for size. The length was determined from the head to the abdomen of each individual, with reference to a scale provided in the photographs. The study has authorization for collection, which was granted by Brazilian environmental legislation (SISBIO application number: 66836).

2.2. Categorization of camouflage strategies in sampled individuals

Different individuals can exhibit more than one camouflage strategy, and it is common to observe natural variations in phenotypes within species across different taxa (Caro and Koneru, 2020). Based on these assumptions, in our study, we classified each collected individual based on the camouflage strategies they exhibited, using established definitions from the literature (Stevens and Merilaita, 2009; Merilaita et al., 2017; Caro and Koneru, 2020; Pembury Smith and Ruxton, 2020). The classification process involved visual inspections and categorical variables, which were independent of the background matching (color contrasts) and disruptive coloration analyses (GabRat). For example, an individual could be classified as exhibiting masquerade (Masq) or disruptive coloration (Dis), or even multiple strategies simultaneously, such as masquerade and background matching (Bm + Masq). Further details can be found in Supplementary Material 1.

2.3. Spectral reflectance and image analysis

For spectral reflectance measurements of the arthropod community, we used a spectrophotometer (Jaz; Ocean Optics Inc., Dunedin, FL, USA) and collected the spectral reflectance from 300 to 700 nm of each collected animal. The reflectance was measured at approximately a 2 mm distance from the targets and at an angle of 45° from the head, thorax, and abdomen for the insects, and cephalothorax and abdomen for the spiders. Each individual in the further color analysis contained a unique averaged measurement of the reflectance of the three body regions, as in the previous study (de Alcantara et al. 2022). We always calibrated the spectrophotometer before each measurement session. To this, we removed the spectrophotometer fiber and block light to standardize the black, as well we measured the reflectance of Spectraloon 99% (Labsphere ®) to standardize the white. We corrected the reflectance noises by employing a local regression through a smoothing function (span = 0.5) using the “PAVO” package (Maia et al., 2013; R Development Core Team, 2022).

Background and arthropod photograph methods followed standard protocols (Troscianko et al., 2016; Wilson-Aggarwal et al., 2016; Price et al., 2019). We used a full-spectrum Nikon D7001 digital camera with a 105mm Nikkor macro lens fitted on a tripod. The RGB photographs were taken through an visible-spectrum-pass Kolari Vision UV / IR Cut filter (transmitting between 400 and 700 nm) and UV photographs were taken with a UV-pass filter (Optic Makario) (transmitting between 300 and 400 nm). We calibrated the ambient lightness by photographing a 99% wavelength pellet of barium sulfate placed in each image. We used diffusers to avoid shadows and the photographs were taken at 100 mm from the trunks and 100 mm height. The photos were taken on sunny days with a fixed aperture of F5 (ISO 100) in raw image format and with a bar scale for subsequent measurement analysis (Troscianko and Stevens, 2015).

We used Multispectral Image Calibration Toolbox (MICA -v2.2.2), an ImageJ plug-in, to transform photos into multispectral images for further analysis. First, we aligned the UV and RBG channels, as well as the white standards to linearize and standardize the pixels concerning light conditions (Troscianko and Stevens, 2015). We created regions of interest (ROIs) of the arthropod dorsal area and trunks (5 cm square) (Troscianko and Stevens, 2015). In this study, we chose to exclude appendage coloration since it is linked to motion camouflage or group behaviors in herds, aspects not investigated in this research (Negro et al., 2020), although it may impact the degree of disruption Then, we converted each ROI into cone catch images, taking into account predatory birds (*Cyanestes careleus*) for further analysis (Stevens and Troscianko, 2017).

2.4. Background matching analysis

We used the Receptor Noise Limited model taking blue tit (*Cyanestes careleus*) as potential predators (Vorobyev and Osorio, 1998). For each animal (n = 639), we measured its discrimination threshold concerning its substrate in chromatic (color) and achromatic (luminance) just-noticeable difference (JND) units. The chromatic contrast (=color) means how the color of the arthropod is different from the color properties of the background on which it is found from the point of view of a predator. The achromatic contrast (=luminance), on the other hand, indicates the brightness of visual information at medium and long distances (Hart, 2001; Siddiqi, 2004). It is important to point out that achromatic channels were never formally tested, but the best recommendation is to maintain both contrasts and draw conclusions based on both results (Olson et al., 2018). In this analysis, color and luminance contrast values lower than 1 to 3 JNDs indicate that the predator cannot or has difficulty distinguishing the prey from the substrate. Therefore, as the contrast value increases, it becomes easier to detect the animal in its background. For each individual collected, we compared the color contrasts (color and luminance) between the occupied background in which the arthropod was collected and the opposite trunk condition non-occupied (control) by the individual. The background had a medium spectral reflectance value of 50 trunks, as in a previous study (de Alcantara Viana et al., 2022).

2.5. Disruptive coloration analysis

We used the GabRat tool in micaTollbox to estimate the false ad coherent edges of the dorsal body area of each collected arthropod from campaigns 3 to 6, resulting in 484 animal disruptive coloration measurements (Troscianko and Stevens, 2015; Troscianko et al., 2017). GabRat is based on the Garbor band pass filter, which was an angle-sensitive filter applied to an algorithm that compares the ratio of the true outline of arthropods and compared with false edges (Troscianko et al., 2017). For GabRat analysis, we used bluet tit system vision (*Cyanistes caeruleus*) and corrected the acuity value for 6 units at 100 mm distance, as this value resembles the flight initiation distance of insectivore avian predators (Troscianko et al., 2017; Caves et al., 2018; van den Berg et al., 2020; Wuthrich et al., 2022). Following previous recommendations, we set the Garbor filter sigma value of 3 units (Troscianko et al., 2017). The analysis consisted of a customized JavaScript code in Image J that cropped each photographed arthropod's ROI, placed them randomly in 10 non-overlapping positions on occupied trunks, and calculated the false and coherent edges for blue tit vision in each position. This procedure resulted in 4.840 disruption measurements for all arthropod communities. For each individual, we calculated an average of GabRat for the 10 total positions of occupied backgrounds.

GabRat values can be interpreted as follows: higher rates of false edges to coherent edges mean increased disruption of body edges and makes the animal more difficult to be detected, while lower values suggest salient coherent edges, which turns easy for avian detection. GabRat values range between 0 and 1. Values below 0.20 are low disruptive, between 0.20 and 0.40 intermediate, and above 0.40 highly disruptive (Troscianko et al., 2017; Price et al., 2019).

2.6. Statistical analysis

To assess the compositional dissimilarity between morphospecies between trunk conditions, we performed the Bray–Curtis dissimilarity index (Bray and Curtis, 1957). Bray-Curtis is an index that varies between 0 and 1, where 0 means the two trunk conditions share the same morphospecies composition, and 1 means the two trunk conditions do not share any species.

We performed a generalized linear model (GLM) to understand whether each camouflage strategy operating solely or in concert is more frequent in individuals of the sampling community. We took the number of individuals and their respective camouflage strategies as the response variable, the trunk type as the interaction factor, and the camouflage strategy as the predictor variable, assuming a gaussian distribution.

To assess the effectiveness of background matching, we calculated the mean and standard deviation of each morphospecies separately for burned and unburned trunks. As control comparisons, we took the color and luminance contrasts of opposite trunks in which each arthropod where non-founded, for example, arthropods found on burned trunks were compared with non-recorded control unburned trunks and vice versa. First, we converted mean and standard deviation values into log response ratios (lnR), which were used as a metric of effect size. Positive or negative values indicate that color and luminance contrast increase or decrease in arthropods found in burned and unburned trunks in comparison with unburned and burned non-occupied trunks, respectively. We back-transformed lnR to percentage [(exp lnR −1) × 100%] to obtain differences between burned and unburned trunks for better interpretation of background matching effectiveness in each occupied trunk condition. We used Mixed-Effects Models to test for differences in the effect sizes calculated for color and luminance contrasts on burned and unburned trunks using maximum likelihood (throughout the ‘rma’ function in R). We removed the intercept from the models throughout the ‘mods-1’ argument to include subgroups in the model (Hedges et al., 1999). The analyses were conducted using the “METAFOR” package in R (Viechtbauer, 2010).

For the disruptive coloration analysis, we performed a Linear Mixed Effect Model (LME). We took GabRat values as the response variable and trunk conditions as the fixed factor, and morphospecies as the random effect. We also performed LME to understand the overall relationship of camouflage metrics – color and luminance JNDs as well as GabRat with arthropod size. After the overall comparisons, we performed LME to understand the relationship between size and trunk conditions on camouflage metrics by also considering the arthropod taxonomic Order. Then, we performed individual linear mixed models for the Orders that showed significant responses between prey size and camouflage metrics. We assumed the camouflage metrics as response variables, arthropod size as interaction argument, Order and trunk condition (burned or unburned) as fixed factors as well morphospecies as the random effect.

In all analyses, we checked the model residuals for assumptions of homogeneity and normality of variances, as well overdispersion. When was necessary, we log converted the data to correct for skewness, and homogeneity to best fit the i analysis assumptions (Zuur et al., 2009; Bates et al., 2015). LME and GLMs were performed using the lme4 package (Pinheiro and Bates, 2000). All analysis was conducted using the R statistical software and programming language (v.4.2.2).

3. Results

3.1. Arthropod collections and camouflage strategies

We collected 639 arthropod individuals, of which 445 were found in burned trunks (69.64%) and 194 in unburned trunks (30.35%) (Figure 1). The Bray-Curtis index revealed that 45% (0.45) of morphospecies are dissimilar on burned and unburned trunks. We identified 7 orders of insects and 1 arachnid (Araneae), comprising a total of 91 morphospecies. The insect sampling comprised 23 families, including 2 Coleoptera, 8 Hemiptera, 6 Lepidoptera, 3 Mantodea, 1 Neuroptera, 2 Orthoptera, and 1 Psocoptera. Concerning the spiders, we found 25 morphospecies belonging to 8 families, as follows: Anyphaenidae, Araneidae, Ctenidae, Hersiliidae, Salticidade, Selenopidae, Thomisidae, and Trechaleidae. Of the total arthropods collected, 2 families were found exclusively in the burned areas: Cerambycidae (Coleoptera) and Hersillidae (Araneae). In the unburned area, one insect family was exclusively found, the Liturgursidae (Mantodea). See online Supplementary Material 1 for additional details on the morphospecies list.

Figure 1. Arthropod community found on unburned (A-J) and burned (K-T) trunks of the Cerrado savanna. A-Selenopidae; B-Salticidae; C-Araneidae; D-Crysopidae (preying upon an ant); E-Acridiidae; F-Cicadellidae; G-Liturgusidae; H-Curculionidae; I-Psychidae; J-Fulgoridae; K-Trechaleidae; L-Salticidae; M-Araneidae; N-Cicadellidae; O-Orthoptera; P-Tineidae; Q- Thespidae, R-Achillidae, S-Tineidae and T-Noctuidae.

We recorded five camouflage strategies occurring without exclusivity on both trunk conditions: background matching, decoration, disruptive coloration, distractive markings, and masquerade (Figure 2). In addition, we found individuals showing aposematic signals as well as mimicry. Considering background matching and disruptive coloration, we found at least four taxa showing polymorphism patterns (Melanism), as follows: brown and black *Eustala* sp. (Araneidae), brown and black *Eumiopteryx laticollis* (Thespidae), gray and black *Issus* sp. (Issidae), and brown and black *Ronderosia bergii* (Orthoptera).

We found no difference between the individuals sampled and their camouflage strategies between trunk conditions ($F_{1,\ 13} = 1.73$; $p = 0.28$). However, we found overall differences in the camouflage strategies exhibited by individuals ($F_{1,13} = 4.09$, $p < 0.01$). Concerning camouflage strategies recorded alone on the individuals, we found that background matching was the most observed (37.55%), followed by disruptive coloration (7.12%), and masquerade (2.32%). We found that 52.2% of sampled individuals showed more than one camouflage strategy, and we recorded color pattern traits of up to three camouflage strategies occurring in the same individual. Regarding the occurrence of more than one camouflage strategy on the same animal, the most abundant combinations were: Bm+Dis (34.74%), followed by Bm+ Masq (5.32%) and Bm+Dis+Masq (1.25%) (Figure 2). Details of pairwise contrasts of least squares mean can be accessed in Supplementary Table 1.

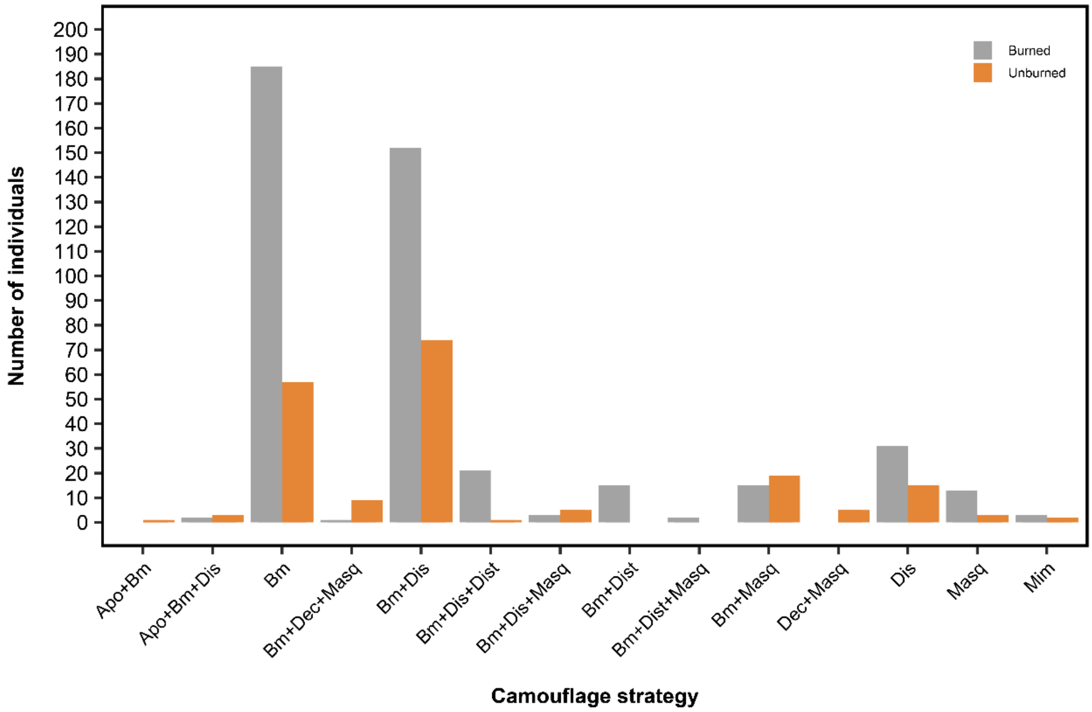

Figure 2. The bar plots indicate the number of individuals collected and their respective camouflage strategies in the burned (gray) and unburned (orange) trunks of Cerrado-savanna. The abbreviations for camouflage strategies correspond to Apo (aposematism); Bm (background matching); Dec (decoration); Dis (disruptive coloration); Dist (distractive markings); Masq (masquerade) and Mim (mimicry). Individual labels represent only one camouflage strategy operating on the individual, whereas joint labels (signal +) indicate the presence of multiple camouflage strategies at work on the individual.

3.2. Color contrasts of arthropod community

Overall, the color contrasts of the arthropod community on recorded trunks were 21% lower when compared with control comparisons (non-recorded trunks) (Qt = 302.44; df =

85; $p < 0.01$). The effect sizes calculated for color contrasts significantly differed between occupation status (*Q*m = 20.75; *df* = 2; $p < 0.01$), being negative for both trunk conditions (Figure 3A). Luminance contrasts were 27% lower on occupied when compared with non-recorded trunks (*Qt* = 314.23; *df* = 85; $p < 0.01$). In the same way, the effect sizes calculated for luminance differed between occupation status for both trunk conditions (*Q*m = 14.10; *df* = 2; $p < 0.01$) (Figure 3B). The results support the expectations that the arthropod community has a closer match on occupied backgrounds than non-occupied, regardless of trunk condition.

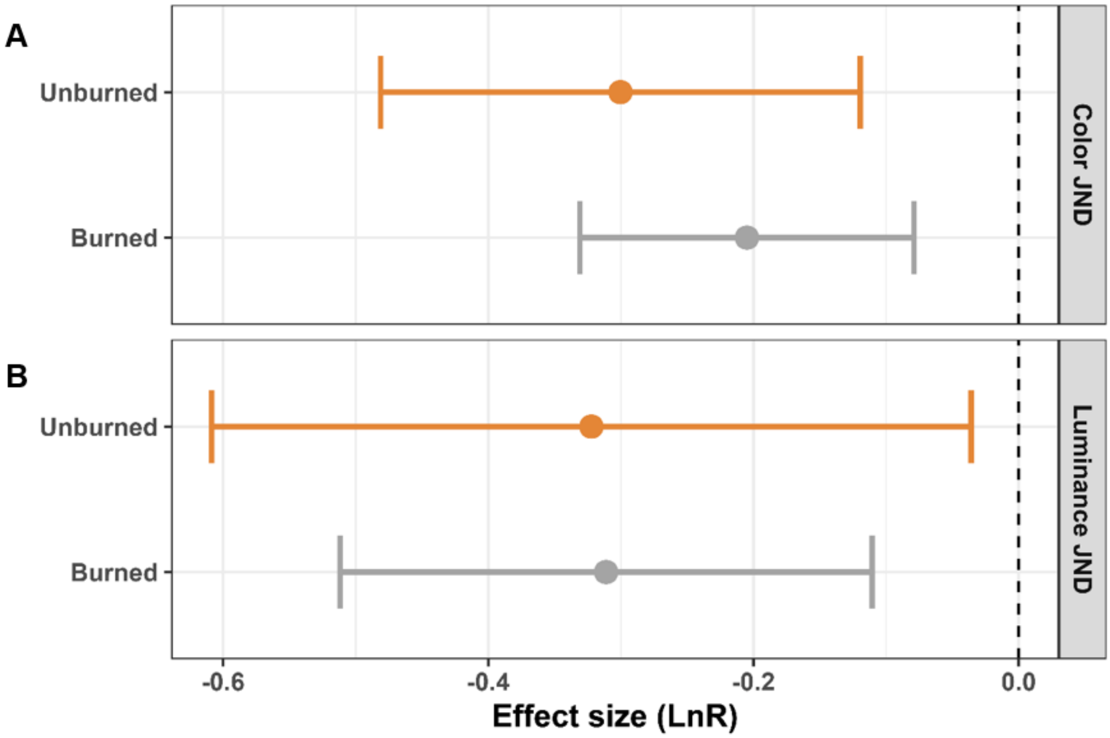

Figure 3. Effect sizes to test the effectiveness of background matching of the arthropod community in a Brazilian Cerrado Savanna. Differences in (A) the color and (B) luminance JND contrasts of arthropod community as perceived by avian predators on burned and unburned trunks. Negative values indicate a decrease in the color and luminance contrasts of occupied trunks when compared with non-occupied (control) trunks for both trunk conditions. Effects are considered significant if the 95% CI does not include 0.

3.3 Disruptive coloration of arthropod community

We did not find any significant difference in the GabRat values of the arthropod community between burned and unburned trunks ($F_{1, 467} = 2.93$, $p = 0.08$; Figure 4). Irrespective of the trunk condition, the arthropod community exhibited intermediate levels of disruptive coloration. Specifically, the average GabRat value for the burned trunks was 0.24 ± 0.09 standard error, while for the unburned trunks, it was 0.26 ± 0.10 standard error.

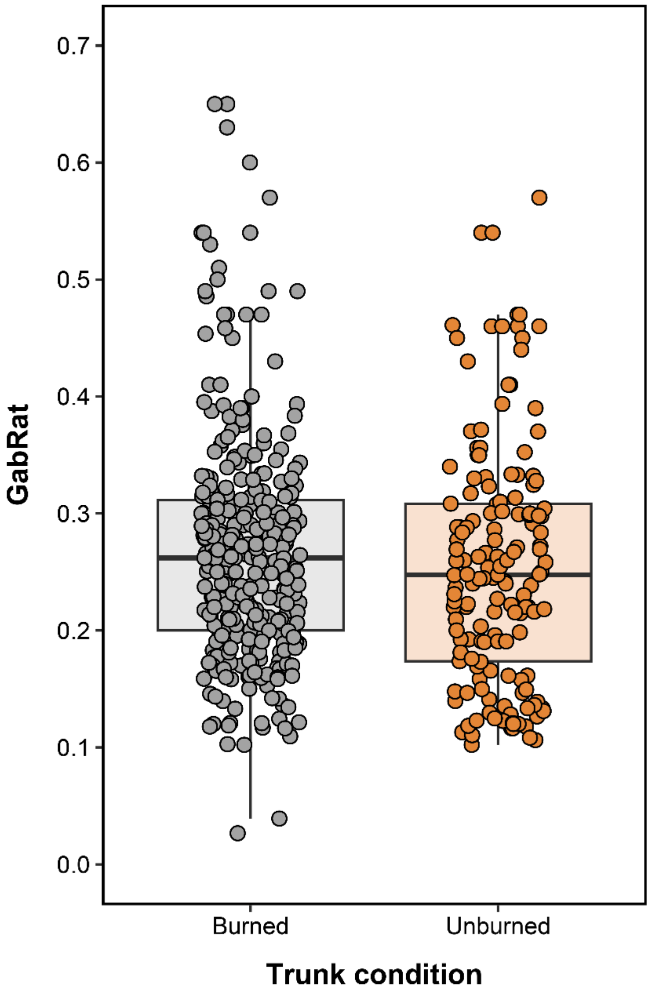

Figure 4. Disruptive coloration (GabRat) of the arthropod community when resting on burned (gray) and unburned trunks (orange) in the Brazilian Cerrado Savanna, as perceived by avian vision. Box plots show boxes with medians and interquartile ranges (IQRs), whiskers represent the lowest and highest values within 1.5 × IQRs and circles represent raw data.

3.4. Relationship between arthropods' size, trunk condition, and camouflage metrics

Regardless of trunk condition, we found no overall relationship between the color ($F_{1, 550}$ = 0.84, *p* = 0.35) and luminance contrasts ($F_{1, 550}$ =0.37, *p* = 0.53), as well the GabRat ($F_{1, 445}$ = 2.96, *p* = 0.08) values of arthropods community with animal size (cm) (Figure 5).When considering the taxonomic Order in the analysis, we observed an interaction effect between the arthropod Order and size on color contrasts ($F_{7, 341}$ = 2.16, *p* = 0.03). The linear mixed effect models individually tested for the Coleoptera revealed a significant relationship between size and color contrasts ($F_{1,14} = 8.94$, $p < 0.01$, Adjusted R squared = 0.34, Supplementary Figure 1). On the other hand, for luminance contrasts, only the arthropod Order was found to be associated ($F_{7,\ 343} = 2.65$, $p = 0.02$). The linear mixed models applied to the Lepidoptera Order revealed an effect of trunk condition on the relationship between size and luminance contrasts ($F_{1,82} = 6.66$, $p = 0.01$, Adjusted R squared = 0.09, Supplementary Figure 2). Regardless of the Order, we did not observe any effect of size on the GabRat of arthropods for both trunk conditions ($F_{1,459} = 2.31$, $p = 0.12$) (Table 1).

Table 2. Results of the analysis of variance applied to different linear mixed-effects models (lmer) testing relationships of just-noticeable-differences (JNDs) for color and luminance, as well GabRat with arthropod size and against burned and unburned trunks ("trunk condition"). Significant values are shown in bold.

	Color JNDs				Luminance JNDs				GabRat			
	df	*MS*	*F*	*p*	*df*	*MS*	*F*	*p*	*df*	*MS*	*F*	*p*
Size	1	0.10	0.02	0.90	1	53.68	1.43	0.23	1	0.00	0.02	0.88
Order	7	10.92	1.69	0.11	7	113.64	3.02	**0.00**	7	0.00	0.48	0.85
Trunk condition	1	7.57	1.17	0.28	1	17.59	0.47	0.49	1	0.03	3.18	0.08
Size * Order	7	14.00	2.17	**0.03**	7	46.53	1.24	0.28	7	0.00	0.46	0.86
Residuals	32	1.98										

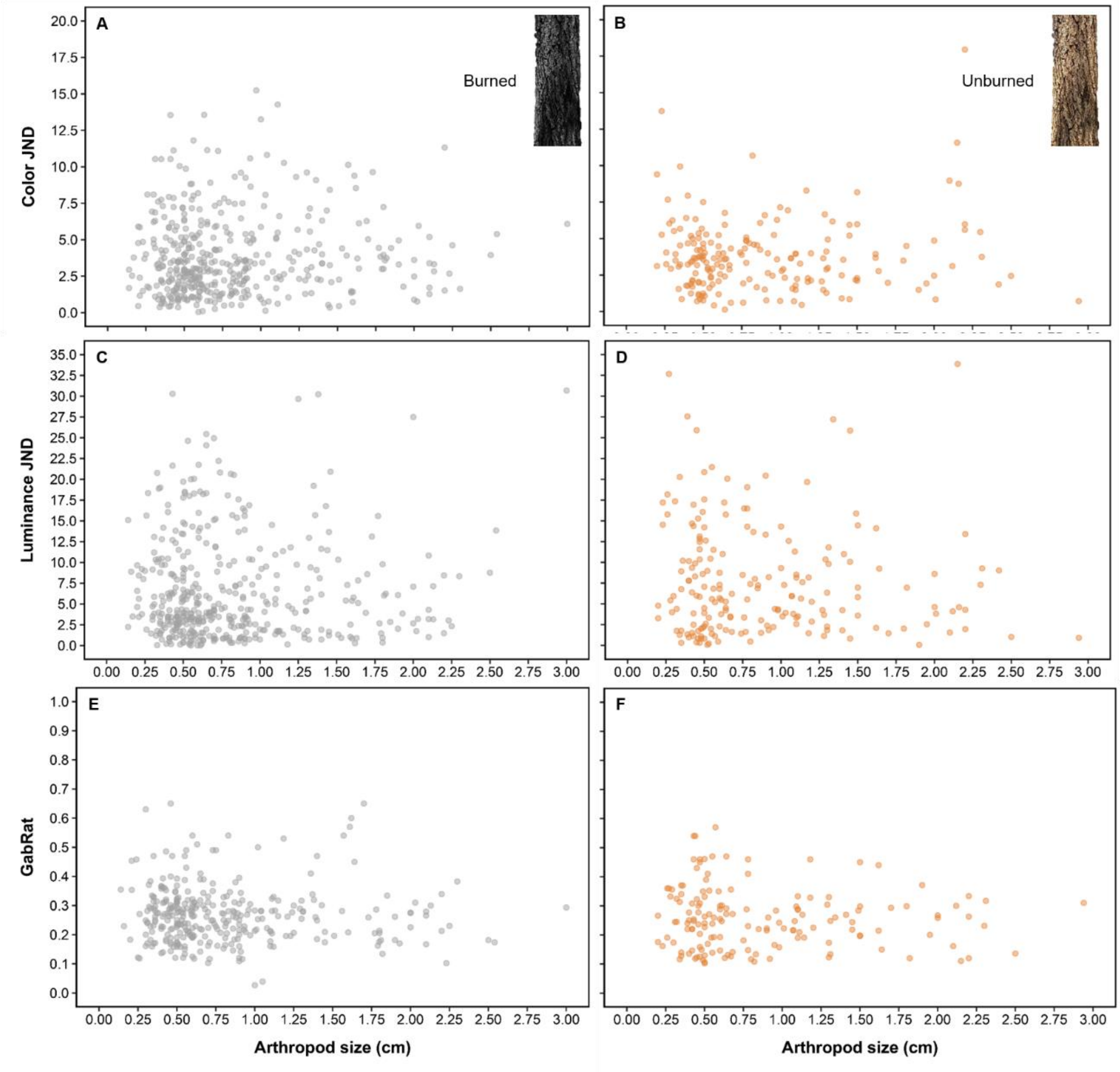

Figure 5. Overall relationships between camouflage metrics and arthropod size. Dot plots show the relationship between color, luminance, and GabRat of arthropod community with arthropod size (cm) in burned (gray panels: A, C, and E) as well in unburned trunks (orange panels: B, D, and F), respectively.

4. Discussion

We have demonstrated that the arthropod community in the Cerrado savanna consists of intermediate levels of species composition differences with individuals displaying similar camouflage strategies that inhabit both burned and unburned backgrounds. Our results align with our predictions, as background matching emerged as the most employed camouflage strategy, utilized by over 30% of individuals on both types of backgrounds. This prevalence was at least five times higher than the second strategy, disruptive coloration, observed in 7.12% of the sampled individuals. We found that 51.48% of individuals have more than one camouflage strategy or defensive color trait, with the majority of combinations being background matching and disruptive coloration. This pattern translates into a close match of the arthropod community and intermediate levels of edge disruption (GabRat) in both trunk conditions. We cannot reinforce that animal size is overall related to better levels of background matching and disruptive coloration, but the relationship was observed in associations with Coleoptera and Lepidoptera groups. These finds provide support for the role of different camouflage strategies working in concert on animals in altered backgrounds, such as fire-prone environments.

To date, little attention has been paid to the ecological factors under which defensive coloration evolves, especially from a community perspective (Caro and Koneru, 2020). Here, we reinforce the assumption that background matching is the most widespread camouflage strategy in nature (Endler, 1978; Stevens and Merilaita, 2011; Michalis et al., 2017). Neotropical savannas experience seasonal variations that alter the color and texture of backgrounds, transitioning from green in the wet season to brown in the dry season (Melo et al., 2022; de Alcantara Viana, pers. obs.). Additionally, savannas can undergo unpredictable changes from heterogeneous brown to homogeneous black because of fires (Oliveira and Marquis, 2002). So why is background matching favored in such a scenario? One of the main hypotheses is that landscapes can appear more or less homogeneous depending on the scale. In many parts of the world, landscapes are described as either green or brown, which is known as the green-brown world hypothesis (Owen, 1980). This hypothesis suggests that brown-green backgrounds may be a significant condition in the evolutionary pressures driving defensive coloration, however, black backgrounds also are expected in fire-prone savannas, and it could explain why background matching is favored (Owen, 1980). Furthermore, visual search tasks for predatory organisms can be challenging in heterogeneous vegetation formations, such as transitional savannas that shift between open and closed vegetation types in a few meters. The complexity of the environment can also favor camouflage strategies that provide a compromise between different backgrounds. Thus, a generalized type of background matching that matches to some degree several brown and black backgrounds but no one in perfection (compromise) could be favored in fire-prone scenarios (Duncan and Humphreys, 1989; Nokelainen et al., 2020; Briolat et al., 2021).

Disruptive coloration is predictable to be more effective in complex environments and higher when color matching is not a feasible strategy (Price et al., 2019). However, these relationships for non-taxonomic related animals from a community perspective were never studied. We argue that the abundance of disruptive coloration as the second most common camouflage strategy is expected, as this strategy often works in conjunction with background matching and can benefit more mobile individuals, such as active predators, for example, the Salticidae spiders (Robledo-Ospina et al., 2017). These patterns related to intermediate levels of GabRat (>0.20) for the arthropod community, showing that both trunk conditions are suitable backgrounds to animals avoid bird predation (concerning just the disruptive coloration), which must be experimentally accessed. In contrast to masquerade and decoration, which rely on mimicry or the use of specific materials for construction (e.g. sticks and leaves), disruptive coloration can be favored in fire-prone scenarios as it allows for greater mobility. However, masqueraded and decorated animals need mimics objects to be functionally camouflaged, which are destroyed or diminished by fire events. In addition, here we provide strong support that different camouflage strategies and defensive colorations occur in concert in the same organisms and on high frequencies when compared to just one strategy. However, how multiple camouflage strategies working together increase prey survival is a further important question to access in experimental field predation experiments. Therefore, our results support existing literature and, to the best of our knowledge, provide the first evidence of differences in the occurrence of multiple camouflages in organisms within natural communities.

Besides 45 % of compositional dissimilarity, almost all morphospecies were found in both burned and unburned trunk conditions, but with different abundances. This suggests that even in a scenario with higher predation pressures resulting from non-adaptation to newly burned conditions (de Alcantara Viana et al. unpublished data), local extinction probably did not occur due to the existence of safe areas, as adjacent unburned areas in which morphospecies can access. Interestingly, the records of morphospecies exhibiting polymorphic coloration on different trunk conditions, include species from non-related taxonomic groups, such as *Eumiopteryx laticollis* (Mantodea, Thespidae), *Eustala* sp. (Araneae, Araneidae), *Issus* sp. (Hemiptera, Issidae), and *Ronderosia bergii* (Orthoptera, Acridiidae). While we suggest that the effects of polymorphism are related to background matching on different trunk conditions, it is important to note that color polymorphisms can coexist with other types of protective coloration (Krause-Nehring et al., 2010; Caro and Koneru, 2020). The melanism records suggest that fires are favorable environments for phenotype selection by avian predation pressures, such as those observed in industrial melanism (Cook, 2000). This can be corroborated by our color vision models, as our results showed low values of chromatic and achromatic contrasts in occupied trunks, whether burned or unburned. This result differs from de Alcantara Viana et al., (2022) study, which found differences only for the achromatic channel. However, in this study, our sampling effort was unprecedented, and many different factors from the first study may have affected the results, such as a new fire event and, potentially, strong predation pressures. Thus, arthropods are less conspicuous for predatory birds in their occupied background when compared to the other trunk condition that potentially could occupy, both at long and short distances and whether compared to burned or unburned areas (Bhagavatula et al., 2009; Siddiqi, 2004). Besides this difference, in general, our result corroborates our previous study as well as the *Biston betullaria* study conducted by Walton and Stevens (2018) and showed the importance of both visual channels in the effectiveness of background matching in arthropods communities in fire-prone savannas.

Animal size is an important factor that can affect the effectiveness of protective coloration (Caro and Koneru, 2020). As the prey size increases, the predator's discrimination ability also increases. We initially expected that larger animals would exhibit lower levels of color and luminance contrasts and higher levels of GabRat, but this observation was not consistent overall. However, our findings showed an increase in color contrasts for Coleoptera, which can favor their discriminability by predators. In contrast, the size increases are related to a decrease in luminance for Lepidoptera, with differences concerning the trunk condition (Supplementary Figure 2). The decrease in contrast is more evident for burned areas, which can be a result of differential predation (Walton and Stevens, 2018). Our disruptive coloration analysis is not positively associated with higher larger sizes, and here we cannot provide associations for real organisms that the cryptic benefit on disruptive phenotypes provides via the breaking up of the body outline is less effective at larger body sizes. Based on these results, we can argue that both luminance and disruptive coloration contrasts are important regardless of animal size. Luminance and disruptive coloration are expected to play a significant role at medium or long distances from predators (Siddiqi, 2004; Barnett and Cuthill, 2014). Therefore, animal size itself may not be the determining factor, but rather the contrast or the degree of disruptive coloration, as predatory birds have sufficient visual acuity to detect both small and large prey from a distance (Smith and Ruxton, 2021).

We conclude that fire effects can create intermediate levels of dissimilarity diversity between trunk conditions, but oppositely, the camouflage strategies are similar between conditions. We reinforce that color contrasts are shaped by trunk conditions, disruptive coloration is intermediate concerning both background conditions, and that animal size is not overall related to the camouflage metrics. We emphasize the importance of assessing the nonlethal impacts of fire on animal communities, as these effects can potentially persist for longer periods than anticipated. We argue that the colors of the landscapes impact the survival of the animals, and the fires extend beyond the immediate effects of burning. Investigating this aspect can be a crucial component of conservation programs.

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CAPÍTULO 4

Crypsis by background matching and disruptive coloration as drivers of substrate occupation in sympatric Amazonian Bark praying mantises

Manuscrito publicado no perióidco "Scientific Reports"

(https://doi.org/10.1038/s41598-023-46204-x)

Title: Crypsis by background matching and disruptive coloration as drivers of substrate occupation in sympatric Amazonian Bark praying mantises

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Abstract

Although background matching and disruptive coloration are common camouflage strategies in nature, only a limited number of studies use appropriate tools to accurately measure them in real organisms. Bark praying mantises (Liturgusidae) from the Amazon present different colour patterns that visually match the colour of whitish and greenish-brown tree trunks. All colour patterns can occur sympatrically, thus varying in the matching with the background. Here we test the functional protection of background matching and disruptive coloration for different bark praying mantises, detected using DNA barcoding. We used image analysis, visual models and a field predation experiment to investigate if the occupation of backgrounds by mantises relates to camouflage benefits against potential avian predators. Three distinct morphospecies displaying specific coloration (white, grey and green) were detected by DNA barcoding analyses. We subsequently photographed mantises and used avian visual model to calculate colour and luminance contrasts, as well as edge disruption (GabRat) against backgrounds. Data were obtained for each individual in its occupied tree trunk within local (whitish or greenish-brown trunks) and microhabitat scales (lichen or bryophyte patches), as well as comparisons with the non-occupied trunk. We also created artificial realistic white and green mantis models and placed them on matching and non-matching tree trunks in the field, measuring the search time and encounter distance required for human participants to locate models.White and grey mantises presented lower colour contrasts against occupied trunks at a local scale (whitish trunks). Oppositely, green mantises exhibited lower contrasts within a microhabitat scale (bryophyte patches) and high disruption against greenish-brown trunks. Predation experiment showed that the camouflage of white and green models against colour-matching trunks increased the search time and decreased the encounter distance of human predators.This study highlights the importance of combined camouflage strategies operating at different scales to increase individual survival against potential predators in nature. Specifically, our study addresses the functional significance of camouflage in Amazonian bark mantises, presenting a stunning study system to investigate the relationship of phylogenetically related species that use camouflage to avoid predation and hunt their prey in sympatry.

Keywords: Amazon rainforest; Avian vision; Camouflage strategies; GabRat; Predation; Habitat occupation; Mantodea.

1. Introduction

Two core determinants of the life history of predators that are not at the top of food chains are not being eaten and holding a suitable habitat for ambush and hunting their prey (Yamawaki, 2017). This "survival equation" of life is determined by making-decision for rentable hunting sites that contain available prey, lower predation risks and reduced competitive rates, as well as for backgrounds with proper physical conditions (Yamawaki, 2017; Scharf & Ovadia, 2006). The use of habitats to catch prey and reduce predation risks can be mediated by the selection of microhabitats, that is, small portions that are more suitable for a given local, with specific and distinctive ecological traits (Marshall et al., 2016).

One of the most widespread protective strategies used among prey and predators is visual camouflage (Ruxton et al., 2019). These adaptations to hide intrigued researchers since the observations made by pioneer naturalists such as Wallace (1867) as well as crossed borders for the arts, in which Thayer (1909) sets out the initial evolutionary hypothesis for camouflage. Successful camouflage combines multiple ecological conditions, such as organism coloration, ambient lightness, the sensory-cognitive capability of the observer, background contrast, and animal behaviour (Merilaita et al., 2017; Stevens & Ruxton, 2019). Camouflage includes several strategies that act in different routes in the sensory and cognitive systems and prevent detection or recognition by the viewer (Cuthill, 2019). Among these strategies, background matching and disruptive coloration are widespread in nature and can act simultaneously in the same organism (Stevens & Merilaita, 2009; Stevens & Merilaita, 2011; Caro & Koneru, 2021). Background matching occurs when body colour patterns generally match the colour, lightness, and pattern of the background, reducing the feature information between the appearance of an organism and its general or specific surroundings (Stevens & Merilaita, 2011). On the other hand, disruptive coloration is defined by the presence of highly contrasting coloration patterns that blur the outline and break up the real surface form of the organism, impairing the detection or recognition of real body configuration in the sensory system of the viewer (Cott, 1940; Stevens & Merilaita, 2009).

Amazon rain forests hold countless habitats that invertebrate predators can use as sites for both concealment and prey capture. Bark mantises (Liturgusidae, sensu Ehrmann, 2002) include a group of praying mantises strictly associated with tree bark habitats (Svenson, 2014). They have specialized morphological adaptations such as dorsoventrally body flattening for a lower profile against tree trunks and several patterns of cryptic coloration (e.g., background matching and disruptive coloration) (Wieland, 2013). In Neotropical regions, two major Liturgusidae tribes are present, namely Liturgusini, with four genera (*Corticomantis*, *Fuga*, *Liturgusa*, and *Velox*), and Hagiomantini, with the genus (*Hagiomantis*) (Svenson & Whiting, 2009; Svenson, 2014). Both tribes are highly dependent on camouflage as an anti-predatory strategy, with individuals showing preferences to occupy smooth trunks that favour running. Although praying mantises have wings, these structures are rarely used to escape from predators, except in situations where individuals are very disturbed and fly to another tree (Svenson, 2014).

Apart from the similarities in the pattern of trunk occupation, there is a significant lack of knowledge regarding the behaviour and life history of the pray mantises of these Liturgusidae tribes. We observed Liturgusidae mantises on lowland Amazonian Forest occupying both whitish tree trunks covered by random patches of lichen, and brown tree trunks covered by random patches of green bryophytes. We recorded white praying mantises exhibiting colour patterns that resembled lichen-covered tree trunks where they were exclusively found. Oppositely, we also recorded green mantises occurring in greenish-brown trees covered by random patches of bryophytes. In addition, we also found grey individuals occupying a reforested area composed of whitish trees also covered by random patches of lichens. Based on these assumptions, we aimed to investigate if the background occupation of Liturgusidae Amazonian bark praying mantises is related to camouflage benefits against potential avian predators. Neotropical praying mantises are poorly studied and difficult to identify as juveniles, therefore, we first used DNA barcode analysis to test whether the different Liturgusidae colour morphs observed in the field corresponded to distinct species, from which we detected three morphospecies associated to white, grey and green body colour patterns. Further, we used image analysis and visual modelling to test the effectiveness of background matching and disruptive coloration as possible camouflage strategies employed by bark mantises to avoid predation. To test background matching, we evaluated colour and luminance contrasts of individuals of each morphospecies to their trunks within two spatial scales: local (i.e., comparing praying-mantises colour to the colour of a broad selection of greenish-brown and whitish trunks observed in the sampling area) and microhabitat (i.e., comparing praying-mantises colour against the colour of lichen patches for the white and grey morphospecies or against the colour of bryophyte patches for the green morphospecies). In order to assess disruptive coloration, we used Gabor filters (GabRat analysis, Troscianko et al., 2017) to test the salient and coherent edges of praying mantises against their own background. We also tested colour contrasts and disruption (GabRat) of praying mantises against non-occupied backgrounds (e.g., white and grey morphospecies *vs.* greenish-brown trunks and green morphospecies *vs.* whitish trunks). We predict that individuals of the white and grey morphospecies will exhibit lower colour and luminance contrast as well higher GabRat values on whitish trunks. Differently from relatively homogeneous whitish trunks, greenish-brown trees exhibit high colour pattern heterogeneity due to the cover of bryophyte patches over the brown coloration of trunks. Based on that, we predict that individuals of the green morphospecies will show lower colour and luminance contrasts as well as higher GabRat values against bryophyte-covered tree trunks.

Finally, we aimed to test the effectiveness of camouflage by background matching employed by the individuals of the white and green morphospecies against their occupied trunks to reduce the detection of potential predators. Currently, common approaches testing similar questions use human participants as a proxy of natural predators in citizen science online games and "predation" experiments in the field (Xiao & Cuthill, 2016). Recent research has revealed no significant differences between visual processing of searching behaviour between humans and birds, despite their differences in cognitive and sensory capabilities (Xiao & Cuthill, 2016; de Alcantara Viana et al., 2022; Franklin, 2022). Therefore, we performed a field experiment with humans searching for white and green paper models of praying mantises against greenish-brown and whitish tree trunks. Our hypothesis is that prey models with similar coloration to the background (e.g., white models on whitish lichen-covered tree trunks) will lead to predators taking longer time and needed to stay at shorter distances from the subject to recognize and identify them compared to models with more contrasting coloration to backgrounds (e.g., white models on bryophyte covered greenish-brown tree trunks).

2. Material and Methods

2.1. Study site and sampling of praying mantises

The study was conducted in December 2021 and October 2022 in an area of the Amazonian rain forest at São Nicolau Farm, located in the southern of the Amazonian biome and Northwest of Mato Grosso state, Brazil (09º 51' 16" S; 58º 14' 57" W). The São Nicolau Farm holds 7.000 ha of open and dense rainforest and 2.000 ha of reforested forest and cattle pasture (Veloso et al., 1991). We extensively search for praying mantises through systematic visual scans of trunks between 0 to 3 meters high on greenish-brown (bryophyte-covered) and whitish (lichen-covered) tree trunks in natural and reforested areas of the farm. Photographs for objective measurement of praying mantis are not feasible without collections (author personal observations). Thus, we captured each individual with plastic pots and bags by cautiously holding them against the trunks before the capture. We carefully transported the individuals to the field laboratory and placed them in a freezer for 3~5 minutes to reduce their metabolism in order to obtain photos without any movement (see details below). After photography, praying mantises were euthanized and kept in absolute alcohol for molecular analyses.

We collected juvenile individuals of white (*n* = 12), green (*n* = 10) and grey (*n* = 5) colour types. White individuals occurred only in border edges of the forest and reforested areas, resting on whitish trunks of *Ficus maxima*, *Hymenaea* spp., *Croton* sp., *Anadenanthera colubrine,* and *Tabebuia* sp. covered with random patches of lichens. Green individuals were found in open and dense forest formations resting on greenish-brown tree trunks of *Acacia* sp., *Handroanthus albus* and *Senegalia polyphylla* covered with random patches of bryophytes. Grey mantises were found in whitish tree trunks of *Croton* sp. and *Tabebuia* sp. also covered with random patches of lichens in a specific reforested location (Figure 1).

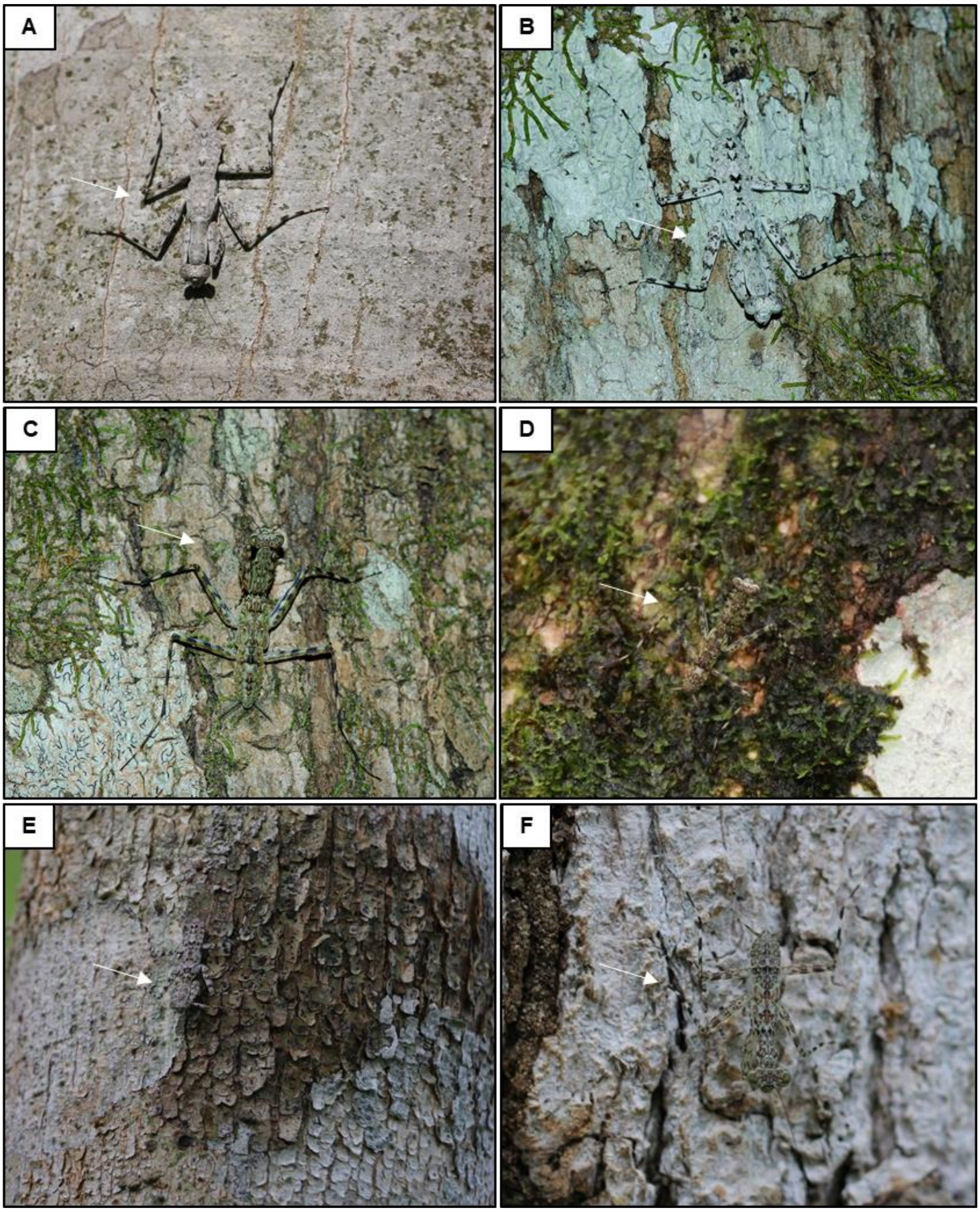

Figure 1. Background occupation of different praying-mantises morphospecies in tree trunks of the southern Amazon rainforest. Two different individuals of the white morphospecies (*Hagiomantis* sp.) resting on (A) a whitish trunk (local scale) and (B) a patch of lichen covering a whitish tree trunk (microhabitat scale). Two different individuals of the green morphospecies (*Liturgusa* sp.) resting on (C) a greenish-brown trunk (local scale) and (D) a patch of bryophyte covering a greenish-brown tree trunk (microhabitat scale). Two individuals of the grey morphospecies (Liturgusidae) resting on lichen-covered whitish trunk trees on a local trunk scale (E) and on a microhabitat scale (F).

2.2. Molecular identification of bark mantises morphotypes

We previously used dichotomous key to separate morphotypes at the lowest possible taxonomic level (Svenson, 2014). In order to correlate morphological patterns and the genetic identity of individuals, we evaluated the barcode region of the mitochondrial encoded cytochrome c oxidase I (COI) gene (Hebert et al., 2003). The use of molecular identification allows us to evaluate if the colour pattern of individuals results from intraspecific variation or genetic differentiation between separate species.

Total genomic DNA (white morphospecie: n = 7, grey morphospecie: n =2, green morphospecie: n = 4, Table S1) was extracted according to the standard procedure of the DNeasy Blood and Tissue extraction kit (Qiagen, Hilden, Germany), then stored at -20°C. DNA concentration and quality were estimated with a UV NanoDrop spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). The mitochondrial gene cytochrome c oxidase subunit I (COI, 5′ end, ca. 640 bp) was amplified using the primers LCO-F (Folmer et al., 1994) + Nancy-R (Simon et al., 1994). The conditions for amplification were: 2 μL of genomic DNA; 2.5 μL of 5× buffer; 2.5 μL of 5% DMSO; 2 μL of MgCl2 at 25 nM; 0.4 μL of dNTP at 10 nM; 0.5 μL of each direct and reverse primer at 10 nM; 0,2 μL de Taq DNA Polymerase (Promega, Madison, WI, USA); and autoclaved deionized H_2O in sufficient quantity for 26 μL of reaction. The PCR program was set up as follow: initial denaturation at 94 °C (2 min); 34 cycles of denaturation at 94 °C (45 s); annealing at Ta°C (45 s; Table S1) and extension at 72 °C (1 min); a final extension cycle at 72 °C (7 min).

All amplified samples were run on a 1% agarose gel with 50 mM Tris–acetate (TAE) buffer (pH 7.5–7.8) to test the quality of amplification before sequencing procedures. The resulting DNA fragments were purified with the ExoSAP-IT (GE Healthcare, Bucks, UK) and sequenced with the primers used for amplifications in an ABI 3500×L automated sequencer (Life Technologies) with Big Dye Terminator v.3.1 kit (Applied Biosystems). Sequences were edited using the software Chromas v.2.6.6 (Technelysium Pty Ltd) and compared to GenBank database (Sayers et al., 2023) through BLAST (Altschul et al., 1990) to confirm their identity as COI gene of Liturgusidae.

Sequences of 640 bp were aligned with MUSCLE (Edgar, 2004) executed in Mega v11.0.13 (Tamura et al., 2021) and deposited in GenBank (Table S1). Pairwise genetic distances between individuals were estimated assuming Kimura two-parameter as nucleotide substitution model (Kimura, 1980) and used to reconstruct a neighbour-joining tree in Mega v7.0.26, using as outgroup the praying mantis *Theopompella chopardi* (Liturgusidae) (GenBank accession number: EF383918.1). Branch supports were estimated by 10,000 bootstrap replicates.

2.3. Digital photography and image analysis

2.3.1 Photography

Photography of animals and backgrounds followed standard protocols (Troscianko & Stevens, 2015). Digital photographs were taken with a Nikon D7000 camera converted to full-spectrum sensitivity by removing the UV and IR blocking filter to enable UV sensitivity and fitted with a 105-mm Micro-Nikkor lens. Human visible photographs were taken by using an ultraviolet-infrared Kolari Vision UV-IR Cut filter, allowing the capture of only visible light spectrum (from 400 to 700 nm), and UV photographs were taken with a UV pass filter (Optic Makario) allowing the capture of only ultraviolet light (from 300 to 400 nm). Changes in ambient lighting conditions were controlled by photographing one well-homogenized pellet of barium sulphate (reflecting 99% of light) placed in each image. Photographs of praying mantises and tree trunks (whitish trunks = 17; greenish-brown trunks = 12) were taken with a tripod outside of the laboratory and in the field, respectively, under natural illumination and using light diffusers and contained a scale bar in the same plane as the subjects. Trunk photographs were taken at 1 meter from the trees and 1 meter height in the North positions. All photos were taken on sunny days under diffused light with a fixed aperture of F8 (ISO 400) and saved as raw images (Troscianko & Stevens, 2015).

We used the ‘Multispectral Image Calibration and Analysis (MICA)’ toolbox, an Image-J plug-in for creating and calibrating multispectral images as well as to run all the subsequent image analyses (Troscianko & Stevens, 2015). Visible and UV photos were first aligned, with the white standards being used to equalize the pixel responses to lighting conditions, which resulted in multispectral images. After calibration, we marked regions of interest (ROIs) on images of both praying mantises and their occupied tree trunks for colour measurements (see details below) (Troscianko & Stevens, 2015). For this purpose, we marked ROIs on the dorsal surface of each praying mantis individual, excluding the appendices, and compared them with the ROIs of the tree trunks.

2.3.2 Background matching analysis

We used the Receptor Noise-Limited Model (RNL) to quantify the colour and luminance matching between praying mantises and both occupied and non-occupied backgrounds (Vorobyev & Osorio, 1998). Model calculations resulted in just noticeable differences (JNDs), which is a metric of colour/luminance discriminability between two objects by a potential viewer. Values below 1.00 indicate that the viewer is unable to discriminate the two objects, with the object detectability increasing as JND values increase (Vorobyev & Osorio, 1998; Hart, 2001). Since passerine birds are common arthropod predators and exhibit a conservative visual system across different species (Hart, 2001), we used the visual model of the blue tit (*Cyanistes caeruleus*) in our analysis. Blue tits are UV-sensitive birds and have been extensively used as models in several studies about arthropod coloration and camouflage (Nokelainen et al., 2013; Walton & Stevens, 2018; de Alcantara Viana et al., 2022). We preferred to use the D65 irradiance spectrum as a measure of incident illumination in our model (Wyszecki & Stiles, 1982) since although the sampling of praying mantises occurred in both forest and reforested areas, these landscapes are open habitats and are not considered as closed and shaded as typical Amazon Forest areas.

After modelling, we calculated achromatic (=luminance, based on double cones responses) and chromatic (=colour) contrasts of each praying-mantis morphospecies against tree trunks. We considered both the local (i.e., whole trunk, ROI defined as a square with a side of 10 cm) and microhabitat spatial scales (i.e., lichen and bryophyte patches; ROI defined as a square with a side of 2 cm) in the occupied background as well as against the mean colour and luminance JND values of non-occupied backgrounds (e.g., white and grey morphs *vs.* brown-greenish trunks and green morph *vs.* whitish trunks).

2.3.3 Disruptive coloration analysis

In order to understand how disruptive the praying-mantises morphospecies were against the different trunk types, we estimated on each image the false and coherent edges of the dorsal praying mantis' surfaces through the GabRat tool in the MICA toolbox (Troscianko & Stevens, 2015; Troscianko et al., 2017). This tool is based on the Gabor band pass filter, an angle-sensitive filter, which has an algorithm that measures the ratio of the true outline edges of mantises compared to false edges (Troscianko et al., 2017). We also used blue tit visual model and set an acuity value of 6 cycles per degree at 1 meter distance because these values closely resemble the acuity for other small avian predators that forage on trunks, as well as their initiation flight distance (Troscianko et al., 2017; Caves et al., 2018; van den Berg et al., 2020;

Wuthrich et al., 2022). We followed previous recommendation and set the sigma value of Gabor filters as 3 units (Troscianko et al., 2017). The GabRat values were calculated by randomly placing each mantis ROI against each trunk image of both occupied and non-occupied substrates over 10 different positions, without overlap, with the values being averaged subsequently to generate a single value per individual (see section 2.4). This procedure resulted in a total of 24, 10 and 20 averaged GabRat values for the white, grey and green morphospecies, respectively. Higher rates of false edges to coherent edges (i.e., higher GabRat) indicate increasing disruption of body edges and consequently greater difficulty in target detection, while lower values suggest salient coherent edges and ease of viewer detection. GabRat ranges between 0 and 1, with values below 0.20 indicating low disruption, between 0.20 and 0.40 intermediate, and above 0.40 considered highly disruptive (Troscianko et al., 2017; Price et al., 2019).

2.4. Field predation experiments

We carried out a field predation experiment using paper praying-mantis models to understand the camouflage benefits of the different Liturgusidae morphospecies on lichen and bryophyte-covered trees. For this, we created models of the white (*Hagiomantis* sp.) and green (*Liturgusa* sp.) morphospecies, as those are the most abundant and contrasting colour types in the study area. The artificial models were designed to represent the real body colour patterns of white and green bark mantises as similar as possible, except by the exclusion of their legs (Supplementary Material 1). For that, we calibrated the multispectral images of white (n = 10) and green (n = 10) praying mantises to human vision (following the same protocol to convert to bird vision) and used them to create models whose shape resembled the real silhouette and body spot patterns of each morphospecies using Adobe Photoshop (version 2.2.0). To obtain the most accurate colour for each model, we first created and printed a set of filled squares with candidate colorations for white and green models. The printed colour patches were then photographed and their RGB values were measured and compared to the reflectance values of real mantises, modelled for human vision, with the most similar values being selected for both green and white models (see Supplementary data for additional information).

Models were printed with a laser Konica Minolta - Bizhub C364 printer on waterproof photo paper and exhibited comparable size with real bark mantises (1 cm in width and 3 cm in height). In the field, models were fixed to tree trunks, using thumbtacks, which were glued to the back of models using high-resistance and quick-drying instant glue

(Superbonder). Models were inserted in pairs (one green and one white model) into each trunk in random positions, varying from 80 to 140 cm height, making them visible to all human predators. The trunks where models were placed belonged to two whitish (*Anadenanthera colubrina* and *Genipa americana*) and three greenish-brown tree species (*Acacia* sp., *Handroanthus albus* and *Senegalia polyphylla*), which were spaced at least 10 meters apart. Trees were carefully selected so that the models in subsequent trees could not be viewed at the same time by the participants.

The participants (*n* = 21) were guided through a path of pre-selected trees (*n* = 6) that randomly varied in the colour of their trunks between whitish and greenish-brown. The selected trees and the initial distance of the path (8 m) were marked to standardize the sampling effort of each participant. Before starting, all participants were instructed about the procedures of the experiment and how the models were fixed on the trees. We quantified the searching time (ST) taken by each participant to recognize each praying-mantis model as well as the encounter distance (ED) travelled to find models. To identify possible misunderstanding and guessing on the identification of models by the participant, one experienced researcher (AB, LS and GM) was positioned next to the tree to check if the target was identified. As soon as the first model was found by the participant, the researcher paused the time and recorded both the ST and ED for the first target. After that, the monitors warned the participant and restarted the time, so that the subject could approach and find the next model at the same trunk. After finding the two targets, the participant continued to the other trees until completing all the experiment path (approximate distance covered of 80 meters between the trees). The gender and age of each participant were recorded. Additional information about the experimental protocol can be found in Supplementary data and Supplementary material (Figure S 1).

2.5. Ethics statement

Fieldwork was conducted under the permission of ONF Brazil-São Nicolau Farm. Sampling collections follow the environmental Brazilian rules and were granted by the SISBIO (73795-1). The Comitê de Ética em Pesquisa (CEP) - Human Ethics Committee granted ethical approval for the human predation experiment (CEP - 65017422.5.0000.5404).

2.6. Statistical analyses

All statistical analyses were undertaken using the software R v.4.2.2 (R Core Team, 2023). We used separated linear mixed-effects models to test “colour” and “luminance” contrasts of each colour type between greenish-brown and whitish trunks as well as against lichen patches (for white and grey mantis) or bryophyte patches (for green mantis). For all models, the JND values were treated as the response variable and the trunk backgrounds as the fixed factor, with the praying mantis identity set as a random factor to control for repetitive measurements made on the same individual, because each praying mantis was compared with all backgrounds (Zuur et al., 2009). The same model structure was used to compare the GabRat values of the praying-mantis colour types between trunks, but now considering only greenish-brown and whitish trunks. We also used linear mixed-effects models to test the effectiveness of camouflage of white and green praying-mantises artificial models on greenish-brown and whitish trunks in the field predation experiment. Mixed-models were fitted separately for the two response variables (searching time – ST, encounter distance – ED), with the background (greenish-brown and whitish trunks) and the mantis model (green and white) set as fixed factors, the participant (= predator) identity as a random factor and the trunk identity as a random factor nested within background to control for data dependence, since two models of different treatments were always placed paired on the same tree. All models were fitted through the *lmer* function of the *lme4* package (Bates et al., 2015) and the associated significance tests through the *anova* function of the *lmerTest* package (Kuznetsova et al., 2017). Model residuals were checked visually for normal error distribution using q-q plots, and the homogeneity of variances was tested using the Levene test in R, for which the colour and luminance JNDs as well as the Gabrat values for all praying-mantis colour types, except the luminance contrast of the white morph and the GabRat of the grey morph, required log transformation to meet model assumptions. Similarly, the ST data of the field predation experiment also required log transformation. Finally, in the case of significant effects, we performed Tukey post hoc tests to assess differences between factor levels using the *emmeans* function of the *emmeans* package (Lenth, 2016).

3. Results

3.1. Molecular analysis: DNA barcoding

The DNA barcode analysis evidenced that the white, grey and green bark-mantis colour types are not only different species but belong to different genera (Table S2, Figure 2). After searching for similar COI sequences in the GenBank database, we identified that the white colour type belongs to the *Hagiomantis* (Serville, 1839) genus while the green morphotype to the *Liturgusa* (Saussure, 1869) genus. However, there were no matching sequences for the grey morphospecies (Liturgusidae), indicating that either the sequences for this species have not been included in the GenBank database yet or it represents a previously undescribed group of praying mantises. Anyway, for the subsequent analyses we considered the grey praying mantis as an unidentified species of the Liturgusidae family and performed all comparisons independently for the three morphospecies considering them as separate colour types.

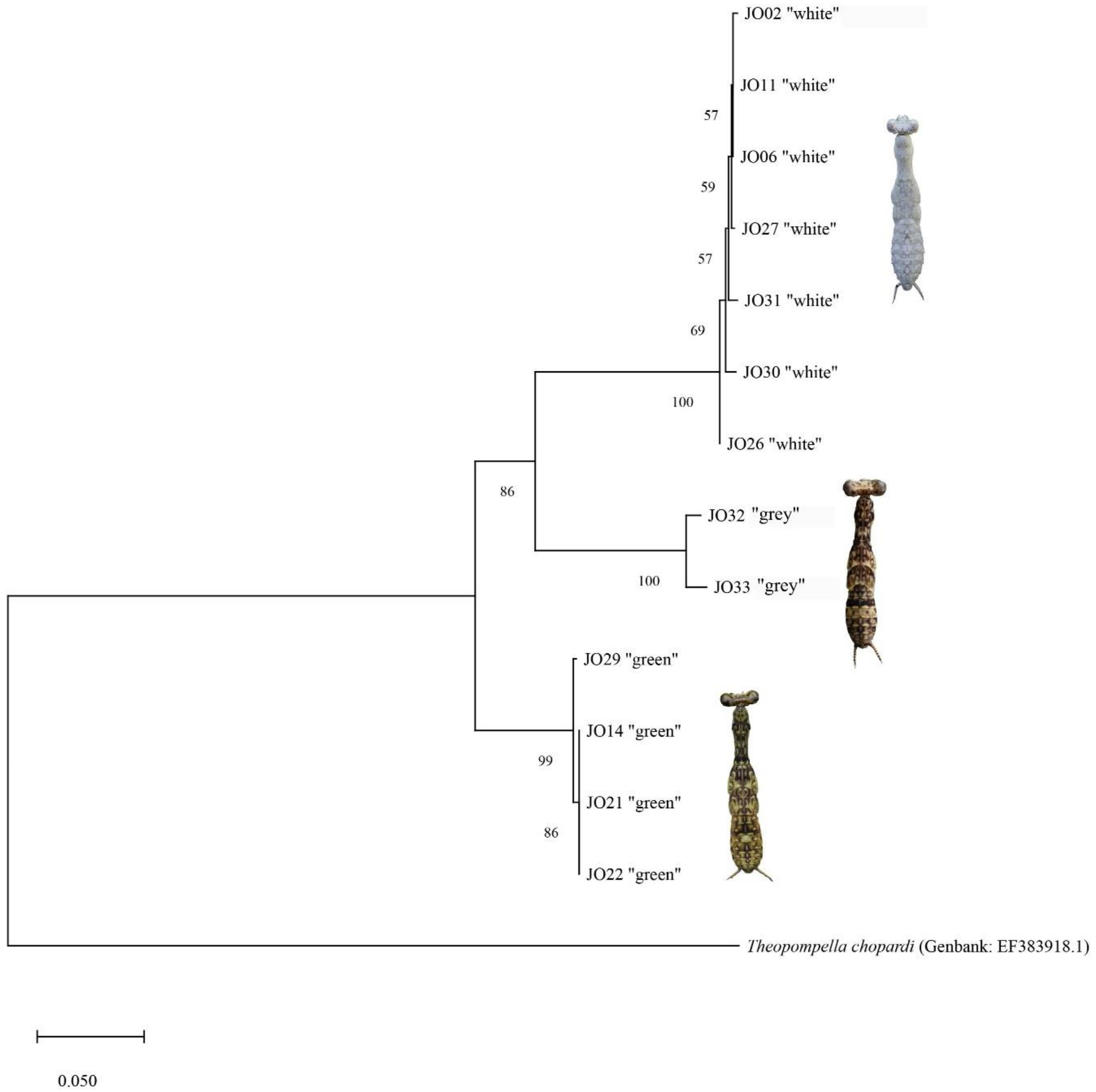

Figure 2. Neighbour-joining tree built considering Kimura two-parameter distance between praying mantis specimens and using *Theopompella chopardi* as outgroup (GenBank accession number: EF383918.1). Branch support based on 10,000 bootstrap replicates is shown.

3.2. Background matching

With the exception of the achromatic contrasts for the grey praying mantises, the colour and luminance contrasts of the other mantis morphospecies differed between trunk backgrounds (Table 1) but the scale at which individual camouflage was optimized depended on the mantis species. The individuals of the white morphospecies exhibited lower colour contrasts against whitish trunks (mean ± se: 2.20 ± 0.28), but their luminance contrasts did not vary considerably between backgrounds (Figure 2). A similar pattern was observed for the individuals of the grey morphospecies, which exhibited lower colour JNDs against whitish trunks (2.39 ± 0.37) in comparison to the other backgrounds but no differences regarding luminance contrasts. In opposition, individuals of the green morphospecies showed better chromatic and achromatic matching within the microhabitat scale, exhibiting lower colour (2.84 ± 0.27) and luminance (7.21 ± 0.92) JNDs against bryophyte patches in comparison to whole greenish-brown or whitish trunks (Figure 2).

Table 1. Background matching and disruptive coloration of praying mantis of different morphospecies against trunk backgrounds in the southern Amazon rainforest. Summary results of the analysis of variance applied to linear mixed-effects models testing differences in colour and luminance contrasts (as JNDs – just-noticeable differences) and edge disruption (as GabRat) based on the vision of the blue tit (*Cyanistes caeruleus*) between different trunk backgrounds. For all models, mantis identity was included as a random factor to control for repeated measurements made in the same individual. Significant differences are shown in bold.

		Colour JNDs			**Luminance JNDs**				**GabRat**		
	df	*MS*	*F*	*p*	*MS*	*F*	*p*	*df*	*MS*	*F*	*p*
White mantis											
Background	2	2.130	25.54	< **0.001**	47.993	3.55	**0.046**	1	0.029	0.97	0.347
Residuals	22	0.083			13.522			11	0.030		
Grey mantis											
Background	2	0.477	4.82	**0.042**	0.010	0.03	0.967	1	$2.27e^{-6}$	0.001	0.98
Residuals	8	0.099			0.291			4	0.002		

Green mantis											
Background	2	3.233	18.36	< **0.001**	4.619	17.34	< **0.001**	1	0.847	9.28	**0.014**
Residuals	18	0.176			0.266			9	0.091		

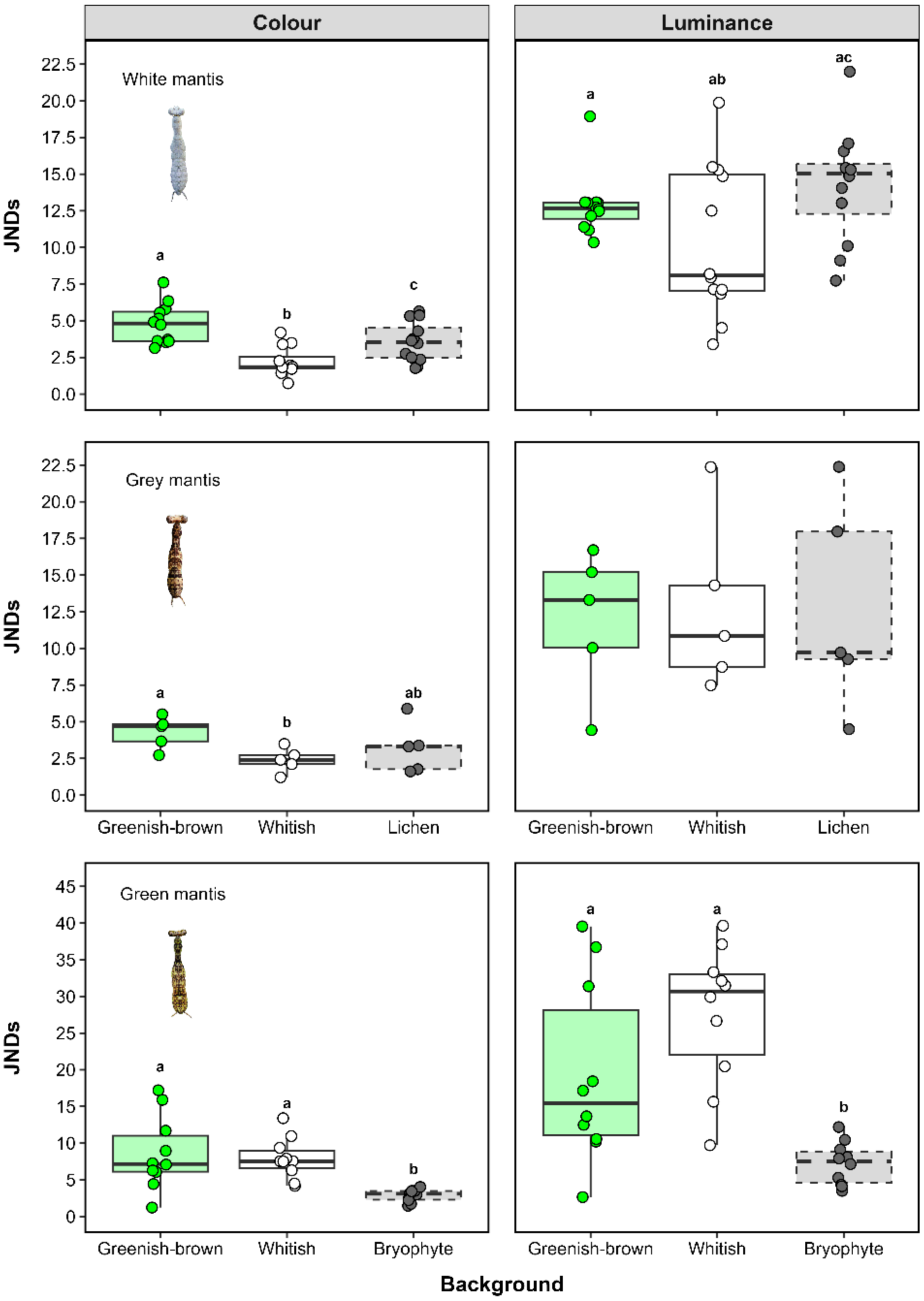

Figure 3. Colour and luminance contrasts of white (*Hagiomantis* sp.), grey (Liturgusidae) and green (*Liturgusa* sp.) praying mantises against different trunk backgrounds in the southern Amazon rainforest. Contrasts are expressed as JND (just-noticeable-differences) units based on

the vision of the blue tit (*Cyanistes caeruleus*), in which lower values indicate better matching. Here and in the next figure, boxes display medians and interquartile ranges (IQRs), whiskers represent the lowest and highest values within 1.5 × IQRs and circles represent raw data, on which a random noise was added to avoid overlap. Boxes with solid contour lines refer to trunk backgrounds (e.g., greenish-brown and whitish trunks) while boxes with dashed contour lines refer to microhabitat backgrounds (e.g., patches of lichen covering whitish trunks for white and grey mantis, and patches of bryophytes covering greenish-brown trunks for green mantis). Different letters indicate significant differences between background types ($p < 0.05$).

3.3. Disruptive coloration

Regardless of the background, individuals of the three praying mantis morphospecies exhibited intermediate levels of edge disruption (GabRat values between 0.20 and 0.40) when viewed by a potential avian predator. There was no difference in the mean GabRat of both white (*Hagiomantis* sp.) and grey mantis (Liturgusidae) between greenish-brown and whitish trunks. However, a significant effect was observed for green mantis (*Liturgusa* sp.), indicating that greenish-brown trunks promote higher levels of edge disruption when compared to whitish trunks for the individuals of this morphospecies (Table 1, Figure 4).

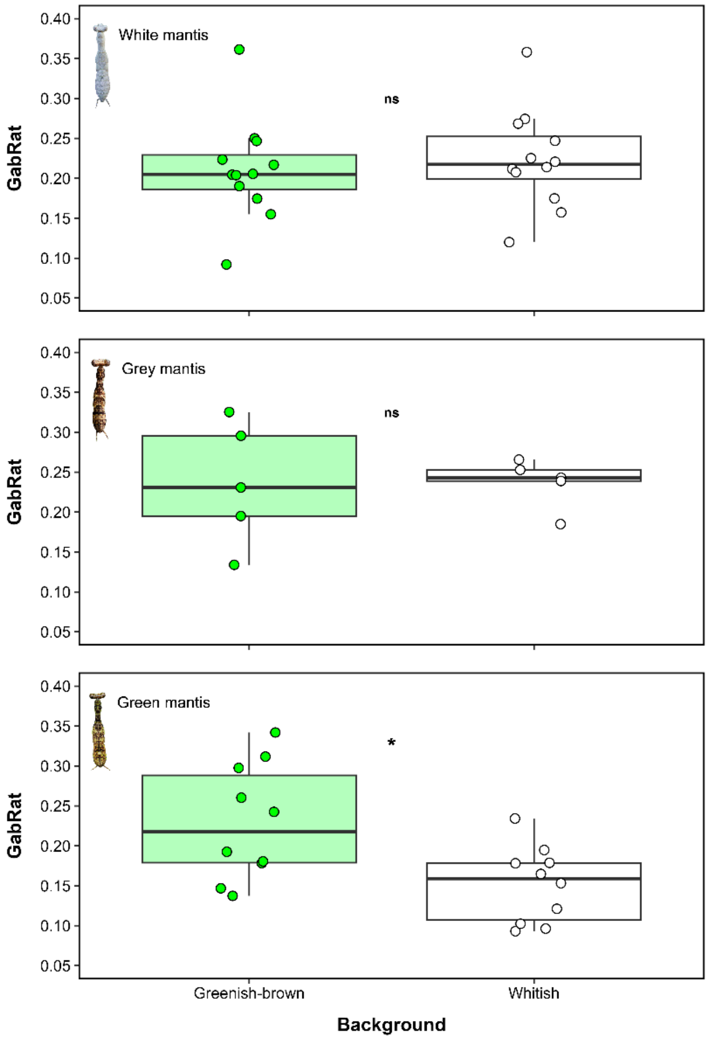

Figure 4. Edge disruption of white (*Hagiomantis* sp.), grey (Liturgusidae) and green (*Liturgusa* sp.) praying mantises against different trunk backgrounds in the southern Amazon rainforest. Edge disruption is expressed as GabRat, which is a metric comparing the ratio between false to coherent edges based on the vision of the blue tit (*Cyanistes caeruleus*), in which larger values indicate higher disruption. The asterisk (*) denotes significant differences between factor levels ($p < 0.05$), whereas *ns* indicates non-significant differences.

3.4. Field predation experiment

The time that human predators spent to find the artificial mantis models and the distance at which they spotted them differed between model types, but the direction of the effect depended on the trunk background (Table 2). The time to find green models against greenish-brown trunks was more than five times longer than to find white models against the same trunks. Similarly, the time that humans spent to find white models against whitish trunks was more than three times shorter than to find green models against similar trunks (Figure 5). An opposite pattern was observed for the encounter distance, at which more camouflaged models (i.e., green models against greenish-brown trunks and white models against whitish trunks) required shorter distances to be found by humans compared to those more conspicuous (Figure 5).

Table 2. Predation experiment using humans as predators searching for mantis models against trunk backgrounds in the southern Amazon rainforest. Summary results of the analysis of variance applied to linear mixed-effects models testing differences in the searching time (in seconds) and the encounter distance (in meters) of human predators "hunting" realistic praying mantis paper models resembling white (*Hagiomantis* sp.) and green (*Liturgusa* sp.) morphospecies against different trunk backgrounds (greenish-brown and whitish trunks). For both models, predator and tree identity were included as random factors to control for data dependence. Significant differences are shown in bold.

		Searching time (s)			**Encounter distance (m)**		
	df_{num} / df_{den}	*MS*	*F*	*p*	*MS*	*F*	*p*
Background	1/4	0.016	0.16	0.713	7.413	3.11	0.152
Mantis model	1/224	0.363	3.49	0.063	14.829	6.23	**0.013**
Background * Mantis model	1/224	24.896	239.47	< **0.001**	747.272	313.84	< **0.001**

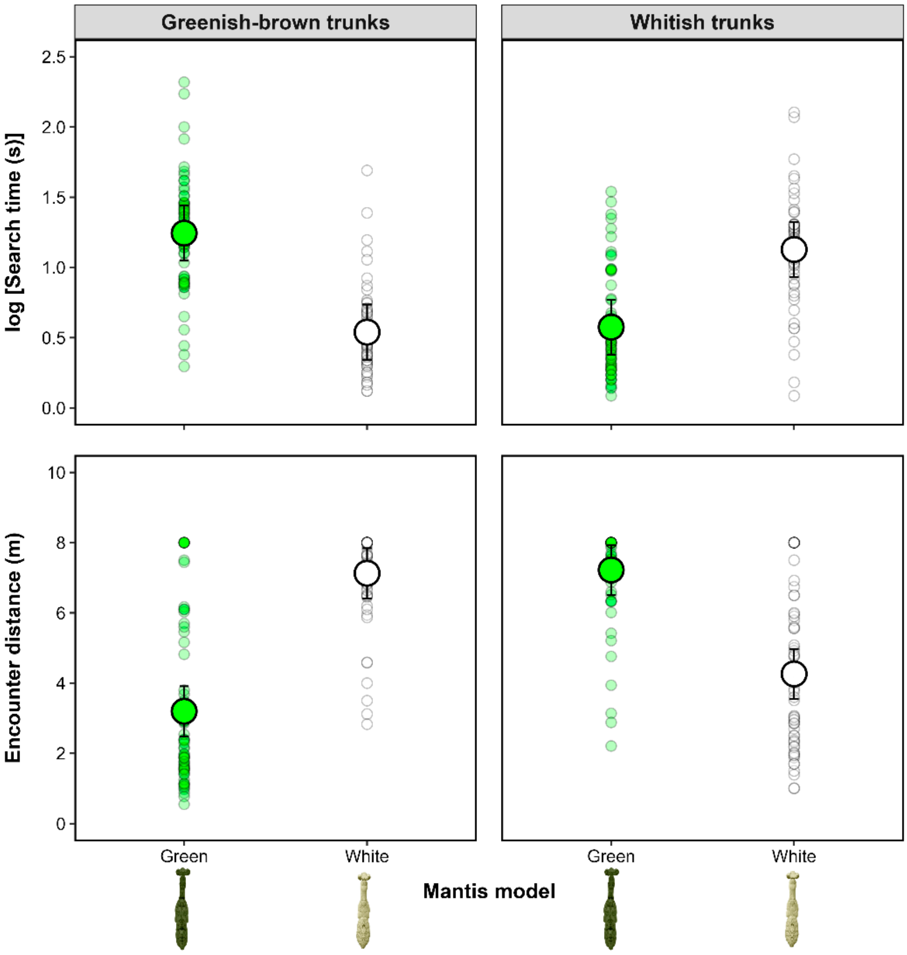

Figure 5. Searching time (log-transformed, in seconds) and encounter distance (in meters) of human predators "hunting" paper models resembling green (*Liturgusa* sp.) and white (*Hagiomantis* sp.) praying mantis morphospecies against greenish-brown and whitish trunk backgrounds in the southern Amazon rainforest. The big circles indicate mean values while the whiskers represent upper and lower confidence intervals (CI 95%) estimated from the mixed-effects model (see details in the main text). Small circles represent raw data.

4. Discussion

In this study, we integrate the use of colour analysis and experiment in the field to assess how bark praying-mantises of different morphospecies may employ distinct camouflage strategies to occupy variable backgrounds in the Amazon rainforest. Our results strongly suggest local and microhabitat adaptations between praying mantis body colour patterns and their trunk background. Ultimately, the combination between increased colour matching and disruptive coloration promotes efficient camouflage for praying mantises, as it increases search time and decreases encounter distances by potential predators.

As predicted, the different praying-mantises morphospecies vary in their level of background matching and disruptive coloration among background types, with better adjustments favouring, in the major scenarios, local and microhabitat adaptations. It is important to point out that most tree trunks in the Amazonian Forest are highly heterogeneous regarding their colour pattern (Figure 1), with bryophyte and lichen patches randomly distributed over trunks, resulting in colour and pattern changes within a few centimeters. Even though praying mantises are very fast, frequently shifting from the bottom to the top of the tree in a few seconds (authors, *personal observations*), the individuals of the three morphospecies we study here still exhibit very low mean JND values and high GabRat values against fixed points in trunk backgrounds. However, in the case of green praying mantis (*Liturgusa* sp.), for example, the lack of a representative microhabitat patch (i.e., bryophytes in greenish-brown trunks) may lead to a poor local background matching. Moreover, the mean chromatic contrast of all mantis morphospecies against the occupied trunks regardless of the scale was broadly < 3.00 JND, indicating that avian predators will have difficulty to detect praying mantis against their trunks under natural light conditions. The trunk-dwelling lifestyle of these animals may also reinforce that natural selection would favour highly efficient luminance matching against potential predators in Liturgusidae praying mantis. However, under medium and long distances, the praying mantises of all morphospecies would be easily detectable from potential predators, as the mean luminance value for all combinations are consistently larger than 3 units. Luminance contrasts are though very variable, varying from low (~ 3 JND) to very high (> 30 JND units) between trunk conditions and morphospecies, which indicates a high heterogeneity in the brightness of trunk backgrounds.

Only few studies have quantified the role of microhabitat behavioural selection as a strategy to improve individual camouflage (Kang et al., 2012; Marshall et al., 2016, Gómez et al., 2018; Green et al., 2019). In order to be successful and increase prey survival, camouflage may benefit from prey habitat choices towards matching backgrounds within broad or fine scales (Ruxton et al., 2019; Stevens & Ruxton, 2019). For example, artificial models of lichen moths *Declana atronivea* presented higher survival rates against avian predators when models were fixed on specific positions of tree trunks composed by lichens but had lower survival on bark lichen-free substrates (Mark et al., 2022). These results can be related to our study system, as green praying mantis (*Liturgusa* sp.) exhibited improved background matching in terms of both colour and luminance contrasts against bryophyte patches (microhabitat scale). However, differently from moths, praying mantises are predators and very mobile animals, so the challenge to match specific positions of trunks is even higher, as they use trunks not only to rest but also for foraging (Pembury Smith & Ruxton, 2020). Considering that bryophyte patches are randomly distributed over trunks and green praying mantises exhibited a high match to this microhabitat, it is also possible that individuals of this species could benefit from a masquerade camouflage strategy (Skelhorn et al., 2010). When occupying portions of the trunks without bryophyte cover, green mantis could be detected by predators but being recognized as a small patch of bryophyte, especially when viewed in a flat position and at long distances by a generalist avian predator (but see Mark et al., 2022). New studies may indicate whether praying mantises can optimize their colour matching by selecting microhabitat patches and orient the body to specific positions to improve concealment or if individuals would remain immobile close to those patches, which would make them similar to bryophyte or lichen patches and favour a masquerade strategy.

Disruptive coloration is one of the most widespread camouflage strategies in nature and has been studied for a long time (Cuthill et al., 2005; Cuthill et al., 2017). However, only recently, new tools allowed us to quantify animal disruption and how this strategy is affected by different backgrounds, but with seldom examples across taxa (Troscianko et al., 2017; Ramírez-Delgado & Castillo, 2020; Bu et al., 2020; Castillo & Tavera, 2022; Wuthrich et al., 2022). In addition, several studies show that disruptive coloration can operate simultaneously or independently to background matching, as the high contrast of markings especially in the body edges promotes advantages less dependent on the background similarity (Cuthill et al., 2005; Schaefer & Stobbe, 2006; Fraser et al., 2007). Our results suggest that disruptive coloration can favour green mantises (*Liturgusa* sp.) on a local scale when the level of background matching is poor. Similar outcomes were observed for juvenile shore crabs (*Carcinus maenas*), in which camouflage has seen to be improved by increasing edge disruption when background matching was not highly effective (Price et al., 2019). Similarly, white (*Hagiomantis* sp.) and grey praying mantis (Liturgusidae) presented intermediated degrees of edge disruption against both whitish and greenish-brown trunks, which reinforces the less dependence of this strategy on substrate types and its high efficiency against detection in heterogeneous backgrounds (Schaefer & Stobbe, 2006).

Field predation experiments have been considered as important tools to determine the protective role of animal coloration in natural settings (Cuthill et al., 2005; Xiao & Cuthill, 2016). These experiments are important because they allow researchers to change, add or subtract a given colour trait and test it in real or simulated situations (Kjernsmo & Merilaita, 2013; Murali et al., 2021). Here, we used experimental models to test the camouflage efficiency of realistic praying-mantis models against natural backgrounds in the Amazonian Forest for the first time. Differently from recent studies (Mark et al., 2022; Walton & Stevens, 2018), we purposely did not insert the models directly to lichen or bryophyte patches but in random locations on the trunk, given that our observations indicated that mantises usually remain in the same trunk portions, changing their position only when disturbed. Therefore, in our experiment we show that from a local tree-type scale, the camouflage of green praying mantises (*Liturgusa* sp.) against greenish-brown trunks is efficient to reduce predation risks, even though the visual models indicated better matching to bryophyte microhabitat. In addition, white praying mantises (*Hagiomantis* sp.) also presented higher survival against whitish trunks, which matches the visual model contrasts. Since the models we created sought to mimic the silhouette and the colour patterns of real praying mantises as much as possible, some level of disruptive coloration was maintained during the experiment and cannot be dissociated from our results. However, it is important to note that we used humans as predators because several other studies have provided evidence about the similarity in predation response to artificial targets between human and avian predators, which allow us to discuss the evolutionary forces shaping animal coloration in nature (de Alcantara Viana et al., 2022; Xiao & Cuthill, 2016). Other predators, such as invertebrates, possess different visual systems and may differ in predatory patterns (Karpestam et al., 2016). Therefore, our field experiment is one of the few studies to date testing how different morphospecies can use different concealment strategies (i.e., background matching over local and microhabitat scales and disruptive coloration) to evade detection in heterogeneous environments such as the Amazon rainforest.

Although Liturgusidae praying mantises are known to be highly dependent on camouflage due to their trunk–dwelling lifestyle, to our knowledge, no study has objectively quantified the degree of camouflage of these important predators considering their natural backgrounds (Svenson, 2014). We reinforce the importance of molecular tools (e.g., DNA Barcoding) to minimize research bias and identification mistakes, especially in juvenile individuals and in studies with unexplored taxonomic groups. Our study shows that different morphospecies, despite the similarity in morphology and behaviour, can use different camouflage strategies that are highly effective on a local and microhabitat scale to avoid their predators. The Liturgusidae family contains 19 genera of praying mantises around the world, with all the described species being highly dependent on tree trunks to forage and avoid predation (Patel et al., 2016). Therefore, the increased camouflage effectiveness of these praying mantises associated to their tree-dwelling lifestyle suggests a high irradiation process for the occupation of different forest habitats mediated by natural selection, which provides a new future research area (Svenson & Whiting, 2009).

In conclusion, we bring new evidence of the use of different camouflage strategies by praying-mantis of different species that are virtually unknown in the Amazonian Forest. The high diversity and heterogeneity of colour patterns of tropical rainforests habitats can select for a diversity of animal adaptive responses, ranging from optimal camouflage to local and microhabitat scales. Furthermore, our study opens a research avenue for new studies testing hypotheses on local adaptation in highly heterogeneous environments, such as Amazonian trees, as well as potential adaptations for active habitat choice and ontogenetic or substrate-dependent colour change in praying-mantises (Green et al., 2019; Nokelainen et al., 2019). Our study is one of the few to integrate field predation experiment with realistic prey based on vision models, to compare background matching and disruptive coloration camouflage strategies and to access the functional level of such strategies against different backgrounds, which ultimately presents an ideal system for further investigation about the adaptive value of camouflage strategies under sympatric conditions. Furthermore, our study reveals a wide and underexplored field of research about the evolutionary and ecologic processes shaping camouflage diversification in natural systems, widening the knowledge on the diversity of cryptic species so far unknown at unexplored ecosystems.

Acknowledgments

We would like to thank IB-Unicamp for the logistic support and ONF Brasil-São Nicolau Farm for fieldwork support, accommodation, and work permissions. Financial support came from the Coordination for the Improvement of Higher Education Personnel (CAPES-CAPES#88887.338483/2019-00) to João Vitor de Alcantara Viana. Gustavo Q. Romero was supported by FAPESP (grant nos. 2018/12225-0 and 2019/08474-8), CNPq-Brazil productivity grant, and funding from the Royal Society, Newton Advanced Fellowship (grant no. NAF/R2/180791). RCD. is funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP: #2019/01934-3 and #2022/00946-0) through a postdoctoral fellowship. PAPA was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - #2017/26243-8).

Author Contributions:

J.V.A.V., G.Q.R., R.C.D designed the study. J.V.A.V., G.Q.R., P.A.P.A, A.B., G.B., and L.S, conducted fieldwork and data collection. C.R.O.L. conducted the molecular analysis and wrote the molecular analysis section. J.V.A.V. wrote the first draft of the manuscript. R.C.D. checked the results. CV. contributed to manuscript revisions. All authors read and approved the final manuscript.

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CAPÍTULO 5

Physiological color change in the tree frog (*Pithecopus hypochondrialis*) as a mechanism of nocturnal camouflage

Manuscrito sob revisão no periódico "The American Naturalist"

Title: Physiological color change in the tree frog (*Pithecopus hypochondrialis*) as a mechanism of nocturnal camouflage

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Abstract: Animals possess several mechanisms to achieve camouflage, with color change being an adaptation that enhances the effective use of multiple habitats. Nevertheless, the mechanisms driving color change in nocturnal animals remain largely non-understood. Here, we conducted a background-induced color change experiment and examined whether altering backgrounds for a tree frog species (*Pithecopus hypochondrialis*) could trigger color change that results in an effective background matching against the visual system of owls. Our experiment reveals that the tree frogs can undergo multiple color changes across grass and leaf-litter backgrounds in low-light conditions. The color changes lead to diminished color contrasts, potentially making tree frogs less conspicuous to owl predation. Our findings shed light on the significance of short-term color adaptation in response to different backgrounds under nocturnal conditions. This adaptation may culminate in effective camouflage through background matching with various backgrounds, offering rapid protection against predation.

Keywords: Amphibian, Avian, Background-matching, Coloration, Crypsis, Night, Predator, Prey.

1. Introduction

Coloration plays a fundamental role in the life history of animals, ranging from sexual selection, thermoregulation, and social differentiation to predator-prey interactions (Cuthill et al., 2017). Traditionally, color signals have been studied from a daylight perspective under various illuminations, such as those found in shadowed forest environments and open field habitats (San-Jose et al., 2019). It is commonly assumed that many color displays evolve in concert with a diurnal lifestyle, as there is at least a million-fold increase in available light information in open daylight when compared to nighttime conditions (Johnsen et al., 2006). This allows for more a efficient transmission of color-based information, increasing the likelihood of detecting visual stimuli across a diverse range of taxa. Indeed, nocturnal color vision itself was considered rare in nature due to sensitivity loss (Jacobs, 1993). Recent evidence, however, suggests that color is an important dimension of visual perception for many taxa, including nocturnal animals, and it is more widespread than initially thought (Kelber and Roth, 2006; Stöcklc and Foster, 2022).

Many nocturnal animals are capable of color and luminance perception under low illumination due to morphological adaptations such as large eyes and pupils in combination with short focal lengths (Warrant, 2004). Moonlight — being reflected sunlight — is the dominant source of nocturnal light in most terrestrial habitats. Its intensity, however, varies over time and across weather conditions and habitats (Johnsen et al., 2006), which shapes a suite of animal activities, including migratory orientation, hunting behavior, as well anti-predatory defenses (Foster et al., 2019). In terms of primary prey defenses, camouflage is considered the most commonly employed anti-predatory strategy in nature (Stevens and Merilaita, 2011), and it includes several strategies that target the visual systems to prevent recognition and detection. Compared to diurnal organisms, however, the mechanisms of camouflage among nocturnal species are far less studied (Cuthill, 2019).

One of the many adaptations used by organisms to mediate visual camouflage is color change, which enables the organism to effectively occupy different habitats (Duarte et al. 2017). Animals can undergo color change through morphological and physiological mechanisms. Morphological color change is characterized by the production, degradation, or chemical modification of pigments, which typically takes place over an extended period of time, such as during ontogeny (Insausti & Casas, 2019). In contrast, physiological mechanisms of color change are generally driven by specialized color-changing cells that modify light reflectance through intracellular pigment movement. These processes are often triggered by a change in external conditions such as detection of predators or a change in abiotic conditions (Umbers et al., 2014). The timescale of physiological mechanisms that drive color change is relatively brief, ranging from several days (Choi and Jang, 2014) to hours, minutes, or even seconds (Kinderman et al., 2014). However, the extent to which both long and short-term color changes contribute to nocturnal camouflage in animals remains largely unknown.

While many amphibians are recognized for their color-changing abilities, and discussions about their ecological function have spanned nearly a century, there remain significant gaps in explaining the adaptive value of this phenomenon (Rudh et al., 2013). Kang et al. (2016) showed that Hyla japonica tree frogs are capable of changing their color in response to both substrate luminance and color pattern to achieve camouflage. In addition, H. japonica can also change color during nighttime to facilitate diurnal color adjustments, thus lowering the associated physiological costs (Kang et al., 2016). However, the mechanisms by which color change may enhance camouflage against nocturnal predators in low-light conditions requires thorough examination. Utilizing amphibians as a study system presents a valuable opportunity to address this significant gap in our understanding.

Recently, we observed an example of a potential nocturnal camouflage through a short-term physiological color change in the Neotropical Hylidae tree frog species, Pithecopus hypochondrialis. Near one of our focal study pools, we came across individual tree frogs that seemed to blend perfectly with a green leaf amidst the grass, showcasing effective camouflage.

Additionally, we observed other conspecific tree frogs calling in close proximity. These tree frogs, however, exhibited a brown coloration, which allowed them to blend seamlessly with the leaf-litter surrounding the palm swamp (Figure 1).

These field observations prompted us to experimentally test the role of short-term physiological mechanisms as drivers of nocturnal camouflage in P. hypocondrialis. While birds and snakes are among the primary predators of tropical amphibians, birds primarily rely on visual cues, while snakes rely more on chemical cues to locate their prey (Toledo et al., 2007). We therefore aimed to conduct a field experiment to test whether individuals of P. hypochondrialis are capable of undergoing rapid background color matching in their grass and leaf-litter habitats, which would support the prediction of short-term physiological color change as a mechanism of nocturnal camouflage.

We conducted a background-induced change experiment to test whether altering the substrate affects the tree frog's coloration, resulting in camouflage through background color matching. We quantified the degree of color matching using the visual system of a nocturnal avian predator, the Tawny Owl (*Strix aluco*). We hypothesized that following exposure to the leaf-litter background, the green tree frogs would adopt a brown coloration that minimizes color contrast for the owl's visual system. We anticipated a comparable color shift in brown tree frogs later exposed to grass background. When tree frogs are repeatedly exposed to the same type of background (eg. grass to grass or litter to litter), however, we predict no significant change in coloration or visual contrast.

Figure 1. Schematic illustration based on our field observations showing individuals of the species *Pithecopus hypochondrialis* displaying nocturnal color change according to their background (grass and leaf-litter) in Palm swamps at Panga Ecological Reserve, Uberlandia city, Minas Gerais state, Brazil. Illustration by Felipe Capoccia.

2. Methods

2.1. Field collections and background-induced color change experiment

We carried out active nocturnal collections of Pithecopus hypochondrialis individuals (n = 9) in Palm swamps at Panga Ecological Reserve, Uberlândia-MG, Brazil (19º11’40’’S, 48º19’06’’W) (Supplementary figure 1 and 2). Sampled tree frogs were individually housed in plastic bags and transported to a nearby field station (approximately 1 km away from the collection site). For the experiment, we used circular transparent arenas (27 cm diameter x 20 cm height) with background substrates of grass or leaf-litter sampled from the same path where individuals were captured. One day prior to the experiment, we used a subsample of the tree frogs to perform initial tests on the effect of daytime luminosity on color change, by placing each individual tree frog into the arenas under natural daylight conditions (D65). After a period of 30 minutes in the arenas, however, no color changes were observed in individual tree frogs. Thus, after this first observation and considering that P. hypochondrialis is a nocturnal species, we acclimated all experimental individuals to dark-room conditions before the onset of the experiment. We kept our experimental dark room at 24 °C, matching the approximate natural local temperature.

We collected individual data on initial reflectance prior to the experiment and after tree frogs were placed on each of the two experimental arenas with distinct background substrates (Fig. 2). We aimed the probe of a reflectance spectrophotometer (Jaz; Ocean Optics Inc., Dunedin, FL, USA) at a 45° angle, at a distance of approximately 2 mm, and recorded the spectral reflectance at a circular point (2 mm in diameter) of light spanning 300 to 700 nm from the dorsal portions of an individual's head, thorax, and abdomen, as well as grass and leaf-litter substrate backgrounds (grass: n = 10; leaf-litter: n = 10). Three paired measurements taken for each tree frog and background were aggregated into individual mean values for the analyses, thus mitigating the potential impact of measuring errors. We calibrated our spectrometer before taking reflectance measurements using a standard whiteboard of 99% reflectance (Spectralon Wavelength Calibration Standards – Labsphere®).

Our experiment of background-induced color change consisted of randomizations of the initial substrate (leaf-litter or grass) in which individual tree frogs were placed. Each tree frog was exposed to a distinct substrate background for a period of 25 minutes. After this period, we carefully removed individual tree frogs and recorded their spectral reflectance, as described above. Then, individuals were placed in arenas with another background, with each individual ultimately being exposed to all possible combination of background change in a random order. For example: (1) grass ➔ leaf-litter, (2) leaf-litter ➔ grass, and their respective controls (3) grass ➔ grass and (4) leaf-litter ➔ leaf-litter (Figure 2; Supplementary figure 3). To calibrate each individual measurement, we also quantified the reflectance of each individual tree frog using on standard whiteboard background with 99% reflectance.

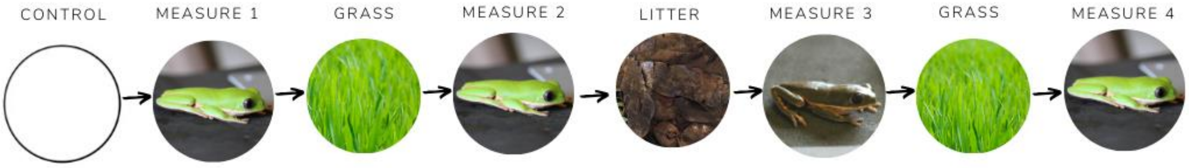

Figure 2. Flowchart demonstrating an example of experimental manipulations involving a background-induced color change in *Pithecopus hipochondrialis*. Controls consisted of frogs sequentially exposed to the same background, either grass ➔ grass or leaf-litter ➔ leaf-litter.

2.2. Spectral descriptors and background-induced color change

We used spectral measures of ‘hue’ and ‘saturation’ as explanatory variables in Generalized Linear Models (GLMs) predicting experimentally induced changes in reflectance in our focal individual tree frogs. In accordance with Kemp et al. (2015), we defined "hue" for each tree frog as the wavelength at which the peak reflectance was recorded. In terms of "saturation," we utilized a descriptive approach that involved dividing the spectrum into four equal regions. The coordinates of each sample were transformed into a segment-based colorspace, and the saturation was measured as the distance of each point from the achromatic center of this colorspace (see Montgomerie 2006). First, we calculated marginal means estimates for the effect of each experimental translocation across or within visual backgrounds (grass to grass, grass to leaf-litter, leaf-litter to leaf-litter, and leaf-litter to grass). We thus used hue and saturation as response variables, in turn, in GLMs with gaussian distribution and identify link function. We visually inspected residual distribution extracted from each GLM to validate normality assumptions. We used ‘emmeans’ (Lenth 2023) and ‘stats’ packages in R (v 4.2.0; R Core Team, 2021) for all statistical analyses.

2.3. Visual modelling and frog conspicuousness

To estimate the viewer-subjective conspicuousness of tree frogs against grass and leaf-litter backgrounds, we used the log-linear receptor-noise-limited model (Vorobyev & Osorio, 1998; Vorobyev et al., 1998) with a tetrachromatic avian visual phenotype obtained from the Tawny Owl (Hoglund et al. 2009), as nocturnal birds are likely to be key predators of our focal tree frog species. This model allows for the estimation of noise-weighted chromatic (hue/saturation; ΔS) and achromatic (luminance, or subjective ‘brightness’; ΔL) distances between color patches, with distances falling below a theoretical ‘threshold’ (often referred to as a ‘Just-Noticeable Distance’ - JND) likely to represent patches that are near indistinguishable to the viewer. These thresholds have been behaviorally validated in a few species, including our hypothesized viewer. In such cases, a theoretical value of one is typically adopted as a tentative limit to discrimination, which we also adhere to here (Kemp et al. 2015).

Following initial visual modeling, we estimated the conspicuousness of tree frogs against distinct backgrounds using a bootstrap procedure (Maia & White, 2017). For a given run, we sampled points from each group (tree frogs and backgrounds) equal to the size of the original group, with replacement, and calculated the distance between the center of each distribution. We repeated this process 1000 times, generating a distribution of subjective chromatic and achromatic distances between groups, from which we calculated a mean chromatic (ΔS) and achromatic (ΔL) distance and 95% confidence interval. We then inspected this interval to predict the discriminability of groups in color space. As noted above, if the value contained or was entirely less than the threshold value of one, then the patches were predicted to be indiscriminable to a nocturnal avian viewer under ideal conditions (Siddiqi, Cronin & Loew, 2004). Conversely, intervals lying above this threshold suggested that such color patches should be increasingly conspicuous to such viewers (Fleishman et al., 2016). We used the package ‘pavo’ (v. 2.9.0; Maia et al., 2019) in R (v 4.2.0; R Core Team, 2021) for all spectral processing and visual modelling.

3. Results

3.1. Tree frog coloration and background-induced color change experiment

The reflectance profile of tree frogs when at rest on grass was characterized by a sharp peak in the 540-550 nm (‘green’) range, with a maximum reflectance of ca. 40-50% (Fig. 3-b). Tree frogs on leaf-litter backgrounds showed instead much less saturation and showed only a weak reflectance peak in the same region, which was otherwise dominated by relatively greater contributions of longer wavelengths (> 650 nm; Fig. 3-a). Tree frogs translocated between distinct background substrates showed rapid color shifts. Specifically, tree frogs moved from grass to leaf-litter background showed a significant decrease in saturation (est. = -2578 ± 590, $t = 4.369$, $p < 0.001$) and an increase in their dominant wavelength (or ‘hue’; est. = 87.2 ± 27.9, $t = -3.126$, $p = 0.022$) over the 25-minute period when compared to the grass-to-grass control treatment. We detected the opposite effect when moving individual tree frogs from leaf-litter to grass arenas, with an increase in saturation (est. = 1509 ± 650, $t = 2.322$, $p = 0.012$) and a decrease in the dominant wavelength toward the green region of the spectrum (est. = -129.9 ± 4.26, $t = -4.226$, $p = 0.002$), when compared to those moved to and from leaf-litter to leaf-litter control treatment.

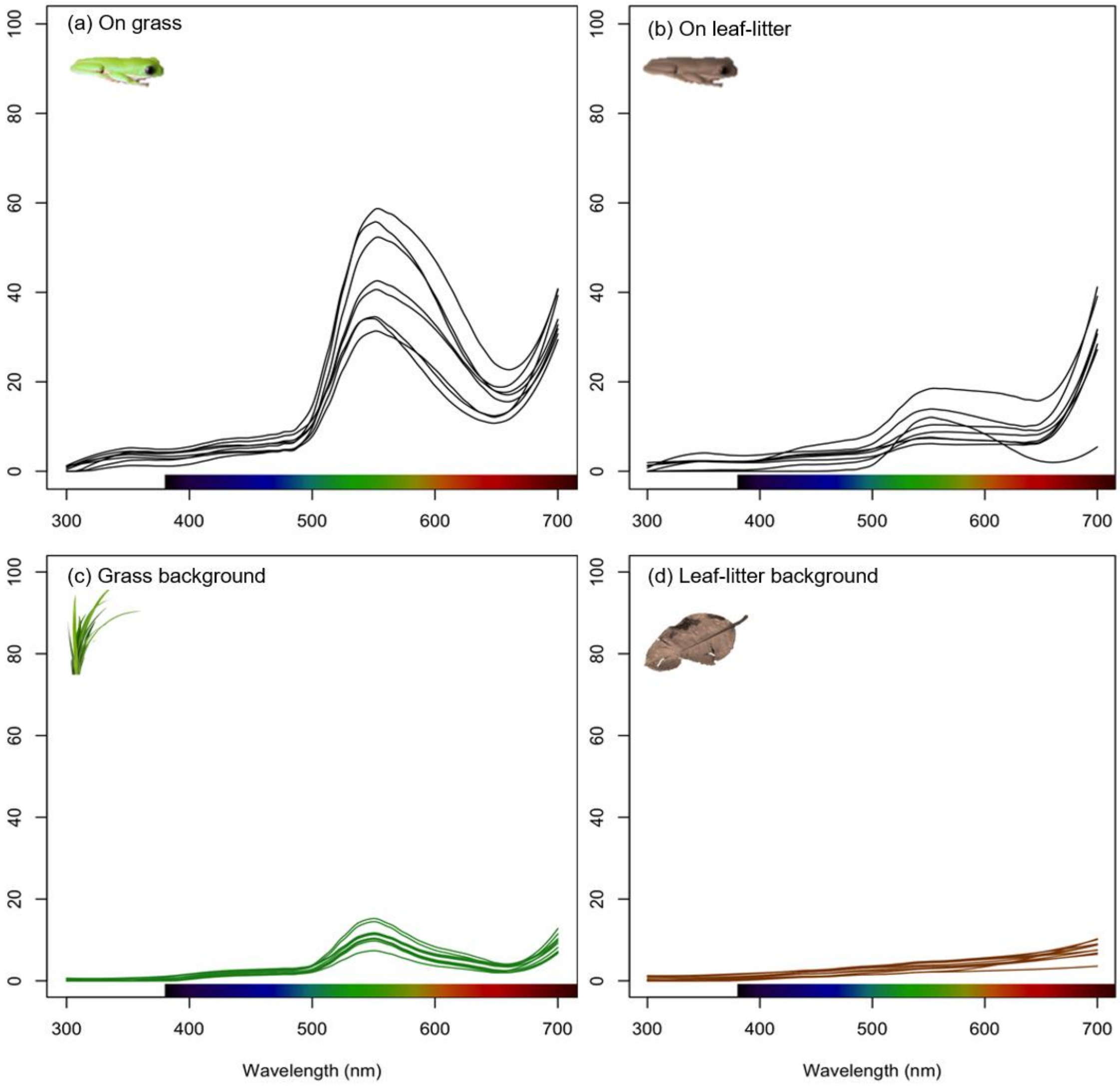

Figure 3. Reflectance spectra of frogs and the substrates during experimental trials. Panel (a) denotes frog spectral reflectance after experimental individuals remained on grass backgrounds for 25 minutes, while (b) depicts frogs exposed to leaf-litter for 25 minutes. Panels (c) and (d) depict the spectral reflectance of background substrates grass and leaf-litter.

3.2. Tree frog conspicuousness to putative predators

We identified a distinct shift in the subjective conspicuousness of tree frogs in response to background exposure. Our visual modelling predicts that tree frogs placed on green, grassy background should typically be indistinguishable against said background (95% CI dS = 0.89 – 1.98), although they should be relatively more conspicuous against the alternate leaf-litter substrate (dS = 1.47 – 2.04; Fig. A). Conversely, tree frogs initially exposed to brown, leaf-litter backgrounds should indistinguishable against leaf-litter background (dS = 0.200 –

1.501), but relatively conspicuous amidst the alternative green grass background (dS = 1.599 – 3.144). This was true of chromatic contrasts only, as modelling suggests that tree frogs should generate significant achromatic contrast independent of their adaptation or resting backgrounds, apart from 'brown' (leaf-litter adapted) tree frogs resting on grass (Fig. b), whose luminance contrast likely falls below-threshold.

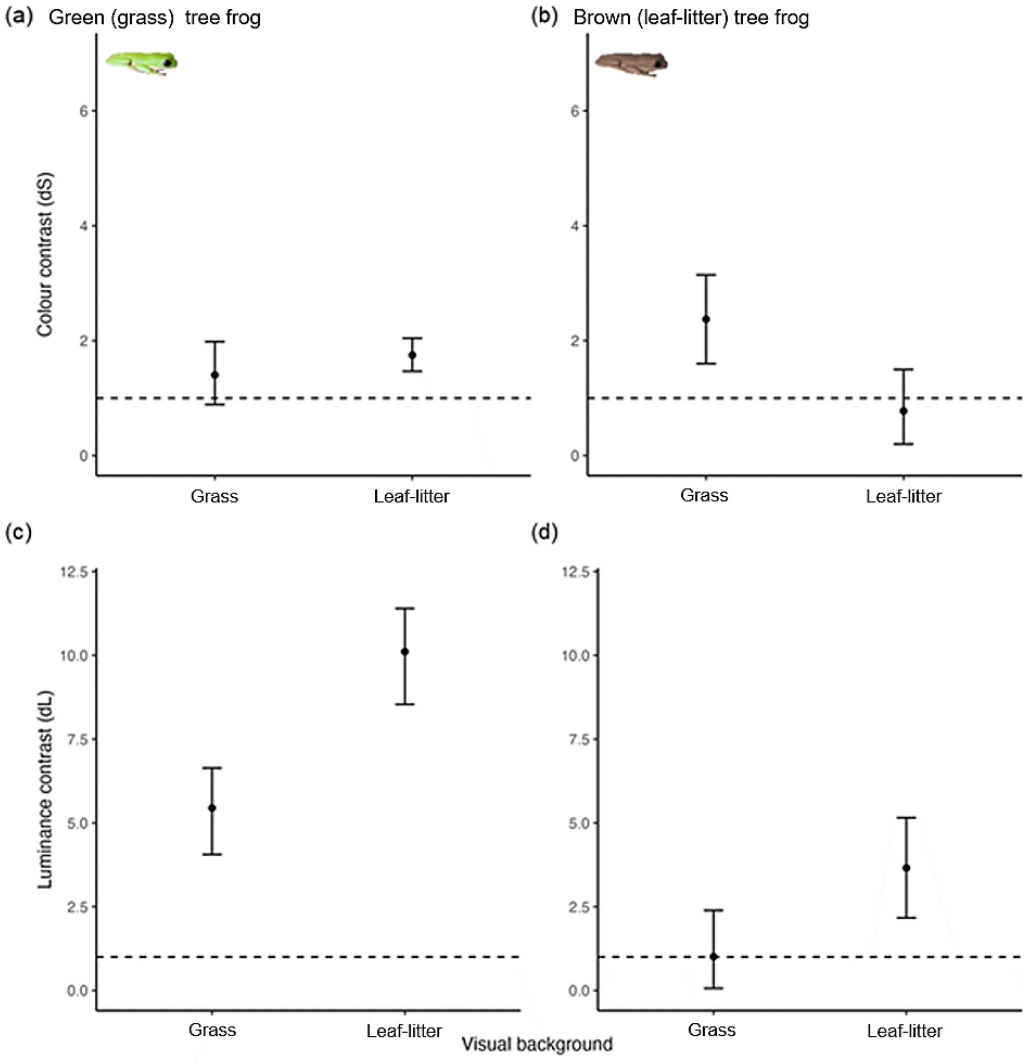

Figure 4. Panels (a) and (b) show chromatic (ΔS) and panels (c) and (d) show achromatic (ΔL) contrasts of experimental frogs against their visual backgrounds. The left plots show the contrast of frogs over grass backgrounds and right shows the contrasts of frogs over leaf-litter backgrounds (right plots) as modeled according to the visual acuity of a Tawny Owl. Points and lines represent mean values and the bootstrapped 95 % confidence intervals.

4. Discussion

Rapid color change is a widespread phenomenon in nature, with examples in cephalopods, fish, chameleons, and amphibians (Fingon and Casas, 2018). Several studies have demonstrated the relationship between rapid cephalopod and amphibian color change in support of camouflage strategies, such background matching and disruptive coloration (Mäthger et al.2008; Hanlon et al., 2009; Kang et al. 2016). Even though color change in amphibians may also be effective for thermoregulation (Park et al., 2023), our background-induced color change experiment revealed a short-term physiological color change in the tree frog species Pithecopus hypochondrialis as a mechanism of nocturnal camouflage. We demonstrated a decrease in color contrast when our focal tree frogs switched backgrounds, with contrast values for chromatic contrast (ΔS) falling below the discriminable threshold of owl predators. Unlike Kang et al. (2016), our experiment showed that P. hypochondrialis are capable of performing multiple color changes during dark conditions, thereby adjusting their coloration to reduce contrast depending on their background. Taken together, these results are consistent with the hypothesis of adaptive background-matching camouflage in nocturnal conditions, in that tree frogs appear able to change their color multiples times to match the color properties of the backgrounds on which they rest, hence minimizing their chromatic conspicuousness to avian predators.

In amphibians, physiological color change mechanisms are characterized by the reorganization of pigment cells in organelles (chromophores), which, in general, leads to higher luminance contrast changes (Sköld et al. 2012; Teyssier et al. 2015). These luminance changes are predicted to be important to animals that bear dynamic color signals, which vary from conspicuous during social interactions to cryptic, depending on environmental cues (Whiting et al. 2022). Stegen et al (2004), for example, performed a background change experiment testing the rate of color change in the tree frog Hyla regilla, and detected a slower change rate when the individuals were exposed to a brown background than when exposed to a green background. While our study did not delve into the timing of color change, we did manage to control for the duration of individual exposure to different backgrounds. Our informal observations, however, suggest that color change occurred rapidly (– ca. 5 minutes), aligning with the working hypothesis of physiological color change (Kang et al. 2016). The physiological cost of the observed rapid and successive color changes, however, is an open question worthy of further investigation.

In terms of conspicuousness, substrate switching did not lead to background-specific differences in luminance in green and brown tree frogs on grass and leaf-litter, but rather led to a general reduction in contrast. This suggests that tree frogs, when exhibiting brown coloration, are more difficult to be discriminated against over long distances for avian predators in both grass and leaf-litter backgrounds, as this channel is predominantly used at medium and long distances by avian predators (Hart, 2001). However, the diminished color contrasts in low-light conditions imply that intense predation pressures during nighttime drive rapid color changes, potentially reducing the likelihood of predation. Furthermore, it is important to note that moonlight is not constant over time, and light will vary among sites (for example urban vs natural areas, close vs open canopy), which may also affect the spotting and discriminability of P. hipocondryalis by their predators.

Animals are capable of identifying predation risks and behaviorally adjusting to avoid predators, such as altering habitat use or, in some situations, drastically altering their coloration. A recent study showed that Jackson's chameleons (Trioceros jacksonii xantholophus), in their natural range (Kenya), are capable of changing their color to become even more cryptic when predators are present (Whiting et al. 2022). Although not related to chameleons, nocturnal tree frogs might use other cues to match their coloration to the substrate on which they rest. We suggest that further studies should be carried out to elucidate which environmental cues in nocturnal environments, such as predator vocalization, can induce color changes in P. hipochondrialis.

Nocturnal habits promote biodiversity due to habitat segregation and species coexistence in the wild (Begon, 2021). With this in mind, studies that elucidate the natural history of nocturnal animals could significantly enhance our ability to understand how the biodiversity of understudied nocturnal species, as well as predator-prey interactions, evolve and are shaped during nighttime. Our findings highlight the importance of rapid nocturnal color change as a short-term physiological mechanism of camouflage in tropical amphibians.

Acknowledgments

We thank the Universidade Federal de Uberlândia (UFU) and Reserva Ecológica do Panga for the sampling permitions and for their support during fieldwork. This work was funded by the Coordenação de Aperfeicoamento de Pessoal de Nivel Superior, CAPES (JVAV –grant no. 88887.338483/2019-00), Programa de Pos Graduacao em Ecologia, Instituto de Biologia, and Universidade Estadual de Campinas (UNICAMP).

Ethics

All capture and handling of individuals of *P. hipochondrialis* were approved by the competent Brazilian legislation (SISBIO #86439-1) and the University Ethics Committee

(CEUA-UNICAMP: #6158-1/2022). All individuals were released at their capture location after the short restraining period.

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ANEXOS CAPÍTULO I

Electronic Supplementary material (ESM) 1 from "Predator responses to prey camouflage strategies: a meta-analysis"

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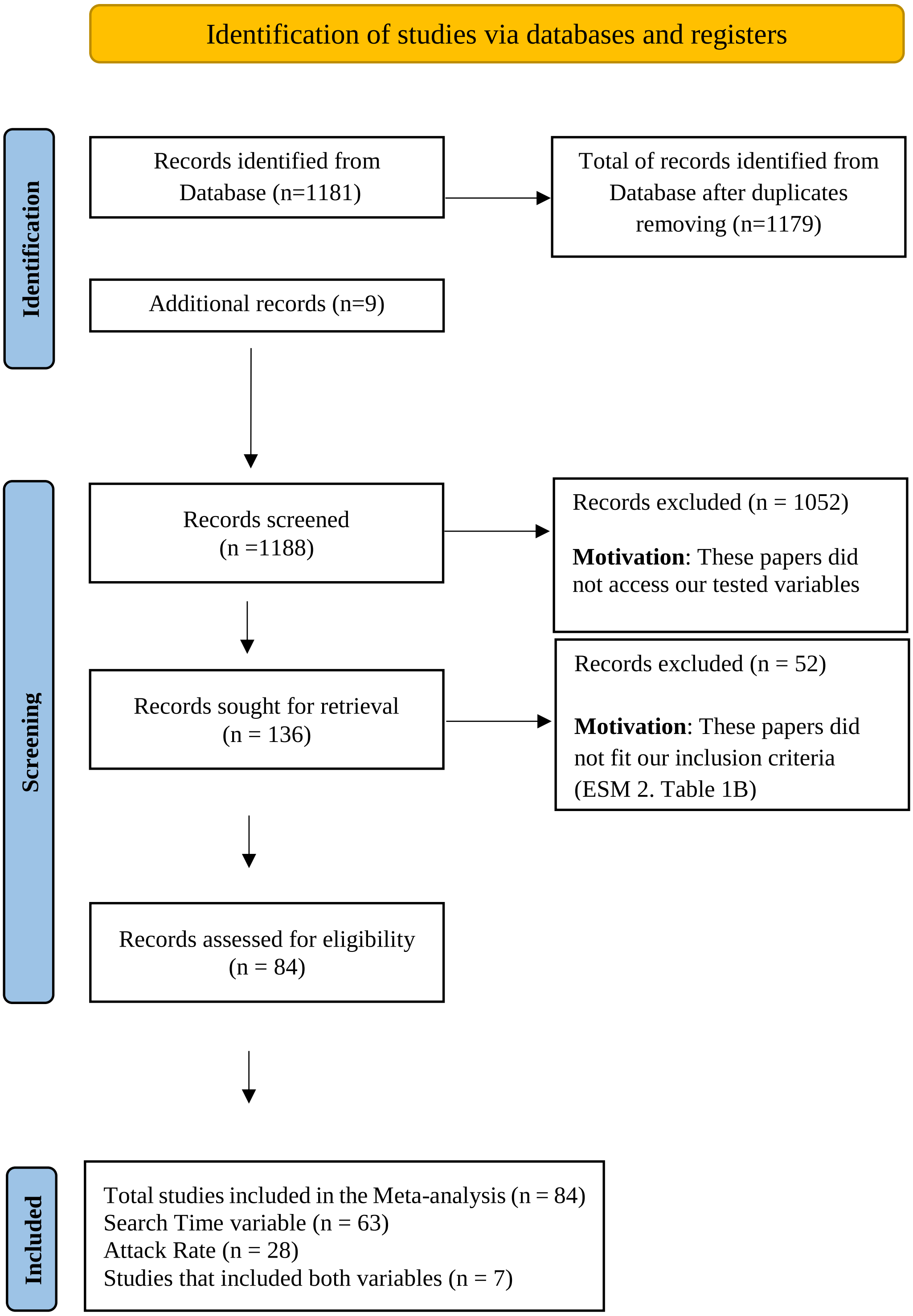
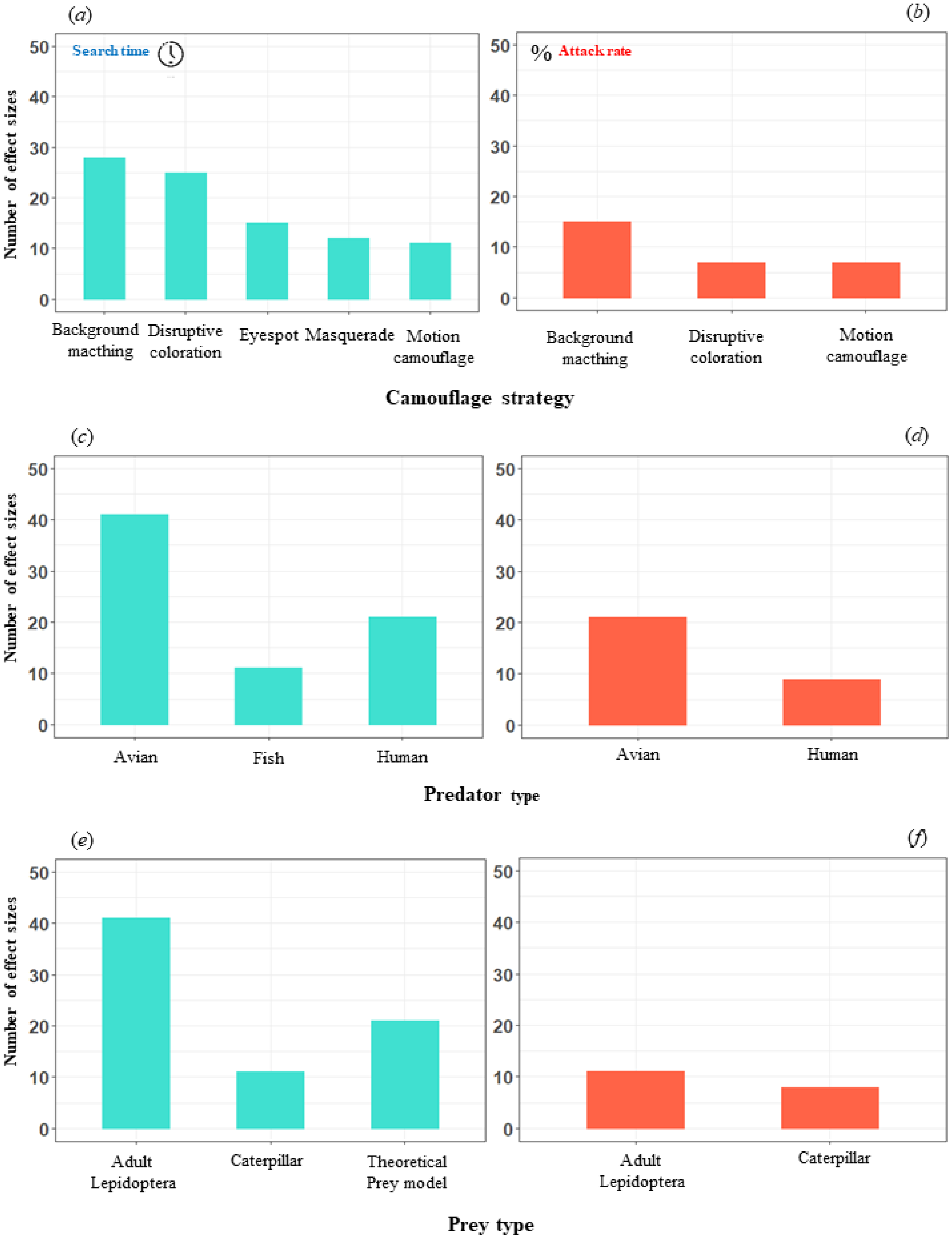
Supplementary material 1. Figure S4. Funnel plot of the overall effect size (ln*R*) for the mean percentage of attacked camouflaged models.



S1-Figure 1: PRISMA flow diagram describing the systematic literature search strategy about the effects of the different camouflage strategies on prey protection.



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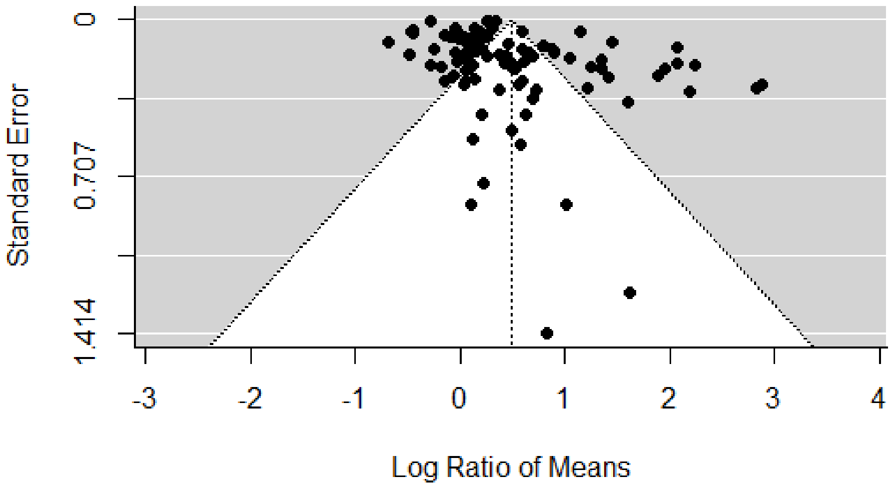
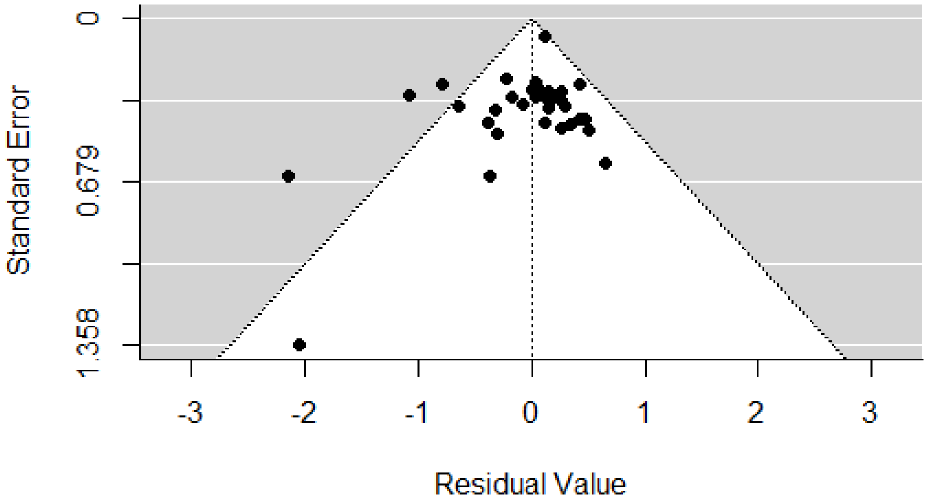
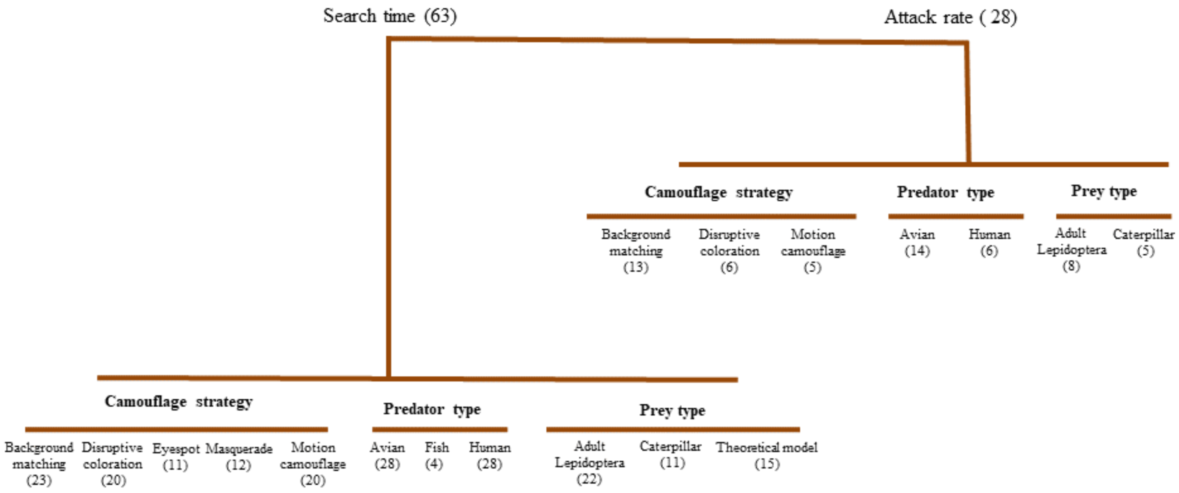


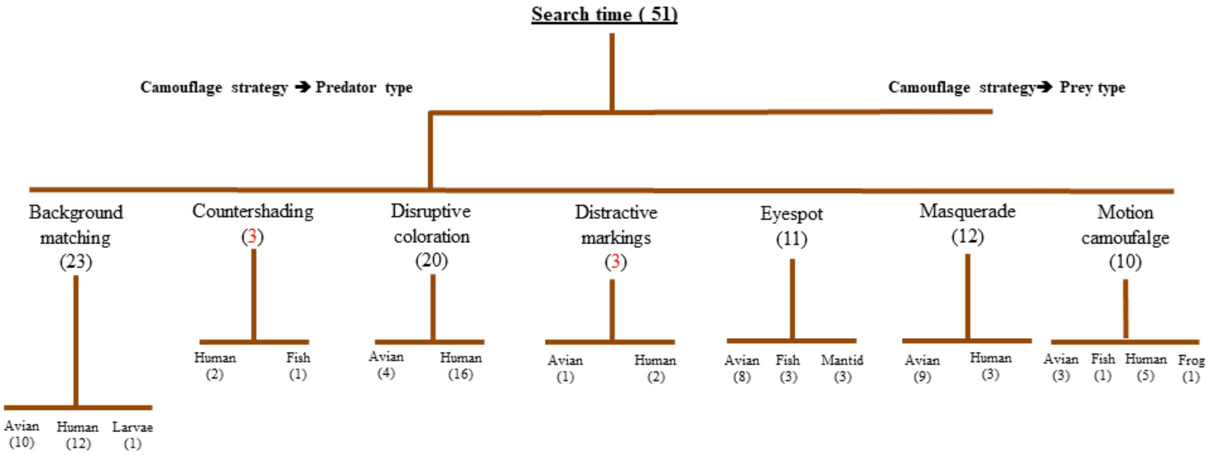
S1. Figure 3. Funnel plot of the overall effect size (ln*R*) for the search time of the camouflaged prey by predators.

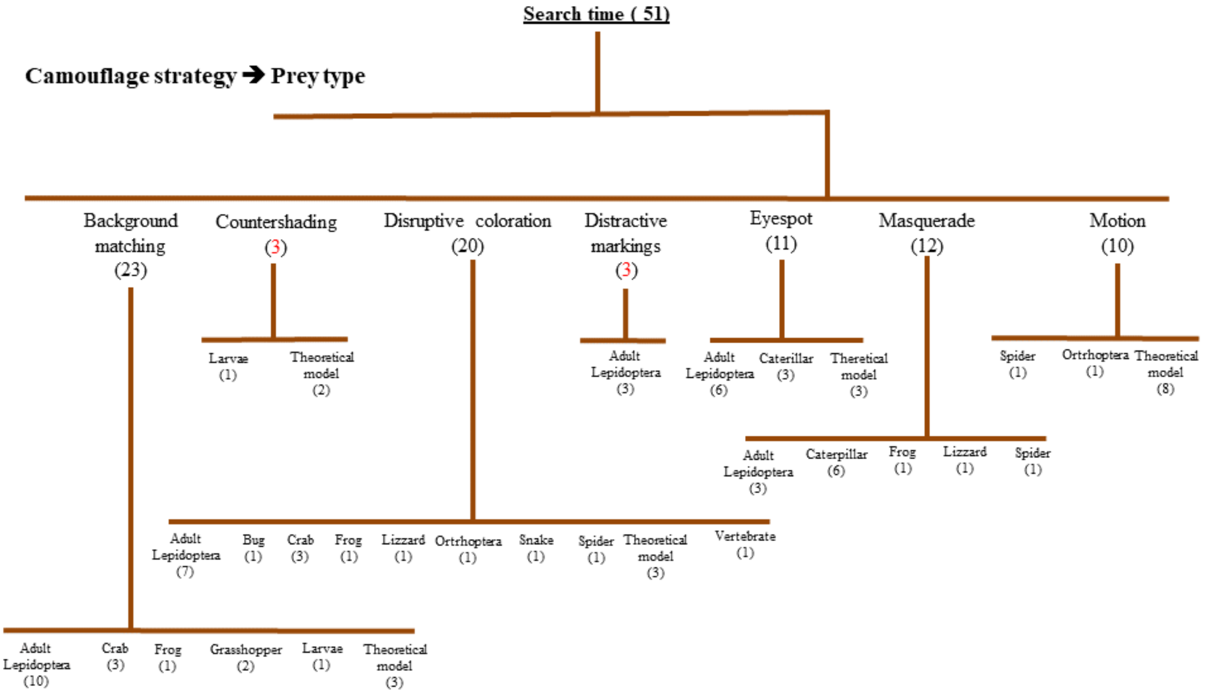


S1. Figure 4. Funnel plot of the overall effect size (ln*R*) for the mean percentage of attacked camouflaged models.

Supplementary material 3. Hierarchical representation of the moderators tested, and number of studies in each category in parentheses. Only subgroups up to 4 articles was used in subgroup analysis (red letters indicate the subgroups that was used only for global analisys and did not enter in subgroup analysis).

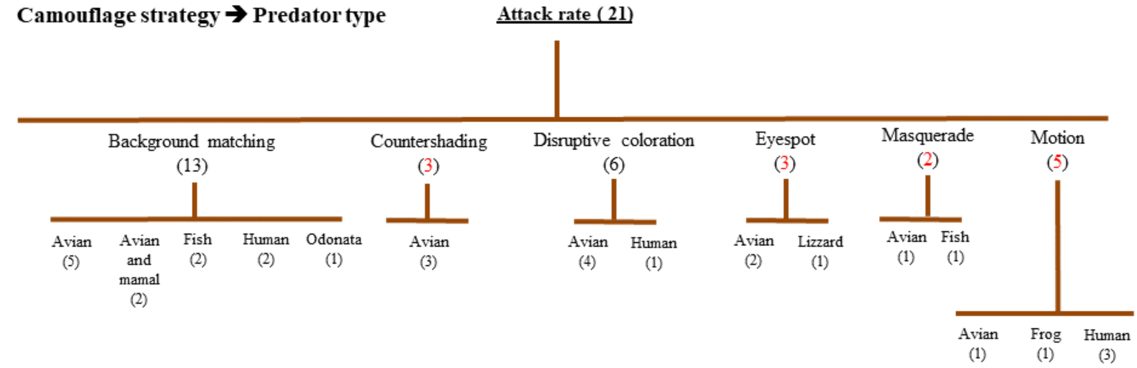



Search time (51)
Camouflage strategy ➔ Predator type
Camouflage strategy➔ Prey type
Background matching (23)
Countershading (3)
Disruptive coloration (20)
Distractive markings (3)
Eyespot (11)
Masquerade (12)
Motion camoufalge (10)
Avian (10)
Human (12)
Larvae (1)
Human (2)
Fish (1)
Avian (4)
Human (16)
Avian (1)
Human (2)
Avian (8)
Fish (3)
Mantid (3)
Avian (9)
Human (3)
Avian (3)
Fish (1)
Human (5)
Frog (1)

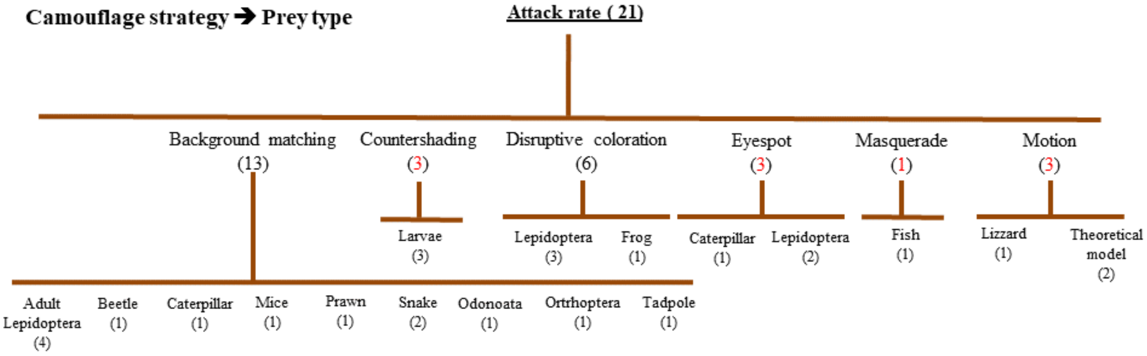

Search time (51)
Camouflage strategy ➔ Prey type
Background matching (23)
Countershading (3)
Disruptive coloration (20)
Distractive markings (3)
Eyespot (11)
Masquerade (12)
Motion (10)
Larvae (1)
Theoretical model (2)
Adult Lepidoptera (3)
Adult Lepidoptera (6)
Caterillar (3)
Theretical model (3)
Spider (1)
Ortrhoptera (1)
Theoretical model (8)
Adult Lepidoptera (3)
Caterpillar (6)
Frog (1)
Lizzard (1)
Spider (1)
Adult Lepidoptera (7)
Bug (1)
Crab (3)
Frog (1)
Lizzard (1)
Ortrhoptera (1)
Snake (1)
Spider (1)
Theoretical model (3)
Vertebrate (1)
Adult Lepidoptera (10)
Crab (3)
Frog (1)
Grasshopper (2)
Larvae (1)
Theoretical model (3)

Supplementary material 3. Hierarchical representation of the moderators tested, and number of studies in each category in parentheses.

Camouflage strategy ➔ Predator type



Camouflage strategy ➔ Prey type



Electronic Supplementary material (ESM) 6 from "Predator responses to prey camouflage strategies: a meta-analysis"

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Supplementary Table 3. Multiple Comparisons of Means: Tukey Contrasts (Adjusted p values reported -- bonferroni method) – Search time variable

Supplementary Table 4. Multiple Comparisons of Means: Tukey Contrasts (Adjusted p values reported -- bonferroni method) -Attack rate variable

Supplementary Table 1.

Mixed-effects model -Search time of the camouflaged prey by predators						
Moderator- Strategy	**estimate**	**se**	**zval**	**pval**	**ci.lb**	**ci.ub**
Background matching	0.4279	0.105	4.0766	<.0001	0.2221	0.6336
Disruptive coloration	0.4217	0.1146	3.6781	0.0002	0.197	0.6464
Eyespot	0.1154	0.1467	0.7867	0.4314	-0.1721	0.4029
Masquerade	1.3916	0.1813	7.6773	<.0001	1.0363	1.7469
Motion camouflage	0.0444	0.1648	0.2696	0.7875	-0.2786	0.3675
Moderator- Predator type						
Avian	0.3765	0.1029	3.6604	0.0003	0.1749	0.5781
Fish	0.8314	0.3667	2.2672	0.0234	0.1127	1.5501
Human	0.6043	0.1092	5.5357	<.0001	0.3905	0.8187
Moderator- Prey type						
Adult Lepidoptera	0.1333	0.0929	1.4346	0.1514	-0.0488	0.3155
Caterpillar	1.341	0.1952	6.8684	<.0001	0.9583	1.7237
Theoretical model	0.5741	0.1298	4.4237	<.0001	0.3197	0.8284

Supplementary Table 2.

Mixed-effects model -Attack rate of attacked camouflaged prey models

Moderator- Strategy	**estimate**	**se**	**zval**	**pval**	**ci.lb**	**ci.ub**
Background matching	-0.3071	0.0889	-3.4538	0.0006	-0.4814	-0.1328
Disruptive coloration	-0.2827	0.1315	-2.1505	0.0315	-0.5404	-0.025
Motion camouflage	-0.3757	0.1335	-2.8146	0.0049	-0.6373	-0.1141
Moderator- Predator type						
Avian	-0.3151	0.1041	0.0025	-0.519	-0.5191	-0.1111
Human	-0.3568	0.1501	0.0174	-0.651	-0.6509	-0.0626
Moderator- Prey type						
Adult Lepidoptera	-0.1564	0.1121	-1.3947	0.1631	-0.3762	0.0634
Caterpillar	-0.4165	0.1606	-2.5931	0.1623	-0.7313	-0.1017

Supplementary Table 3.

Multiple Comparisons of Means: Tukey Contrasts (Adjusted p values reported -- bonferroni method) - Search time variable

Linear Hypotheses:	Estimate Std.	Error	z value	Pr(>|z|)
Moderator	**Camouflage strategy**			
Disruptive coloration - Background matching	-0.006191	0.155429	-0.04	1.0000
Eyespot - Background matching	-0.312447	0.180367	-1.732	0.832
Masquerade - Background matching	0.963759	0.209455	4.601	<0.01
Motion - Background matching	-0.383419	0.1954	-1.962	0.497
Eyespot - Disruptive coloration	-0.306256	0.186172	-1.645	1.0000
Masquerade - Disruptive coloration	0.96995	0.214473	4.522	<0.01
Motion - Disruptive coloration	-0.377228	0.200771	-1.879	0.603
Masquerade - Eyespot	1.276205	0.23318	5.473	<0.01
Motion camouflage - Eyespot	-0.070972	0.220642	-0.322	1.0000
Motion camouflage - Masquerade	-1.347177	0.244994	-5.499	<0.01
Moderator	**Predator type**			
Fish - Avian	0.4549	0.3808	1.1940	0.6970
Human - Avian	0.2281	0.15	1.5210	0.3850
Human - Fish	-0.2268	0.3826	-0.5930	1.0000
Moderator	**Prey type**			
Adult Lepidoptera - Caterpillar	-1.1966	0.2070	-5.7800	<0.01
Theoretical model - Caterpillar	-0.7561	0.2228	-3.3490	<0.01
Theoretical model - Adult Lepidoptera	0.4405	1587.0000	2.7760	0.0116

Supplementary Table 4.

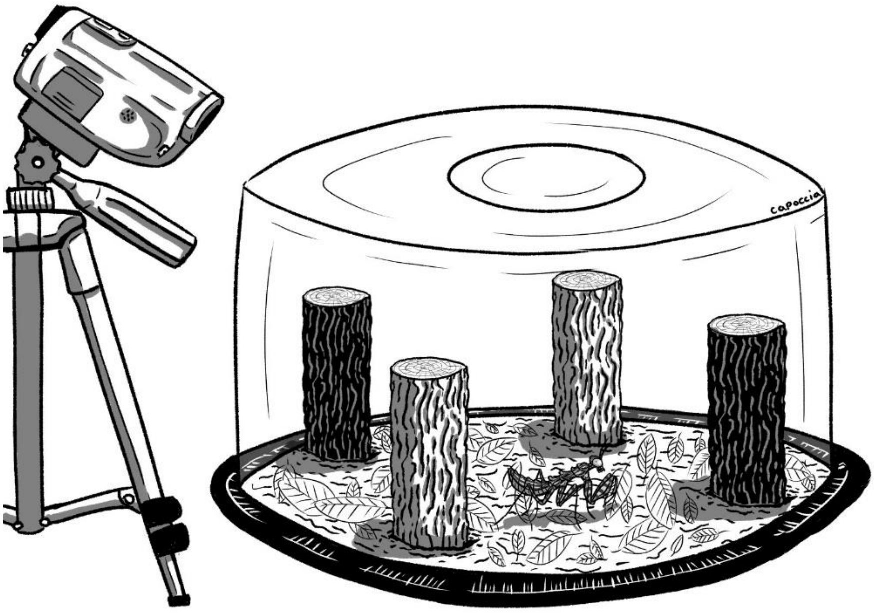
Multiple Comparisons of Means: Tukey Contrasts (Adjusted p values reported -- bonferroni method) -Attack rate variable

Linear Hypotheses:	**Estimate Std.**	**Error**	**z value**	**Pr(>|z|)**
Moderator		**Camouflage strategy**		
Background matching - Disruptive coloration	0.02441	0.15872	0.154	1.000
Motion camouflage - Background matching	-0.06854	0.16038	-0.427	1.000
Motion camouflage - Disruptive coloration	-0.09296	0.18735	-0.496	1.000
Moderator		**Predator type**		
Avian - Human	-0.04163	0.18263	-0.228	0.82
Moderator		**Prey type**		
Adult Lepidoptera - Caterpillar	0.2601	0.1959	1.328	0.184

ANEXOS CAPÍTULO II

Differential survival and background selection in arthropod camouflage strategies in fire-prone environments

Supporting information



Supporting information - Figure 1. Arthropod background selection experimental setup. Each arthropod specimen was inserted into the arena with two branches of burned (black) and unburned (white) trunks of *Qualea grandiflora* for 6 hours duration.

Supporting information - Figure 2. Human predation experiments. Each trunk receives a random pair of theoretical moth prey targets. The prey types were from left to right: background colour matching and pattern; disruptive coloration; brown matching and black targets.

ANEXOS CAPÍTULO III

Post-fire effects on the camouflage strategies of Cerrado arthropods

Supplementary tables.

Supplementary table 1. Detailed classification of arthropods collected in the study by taxonomic level.

Order	Family	Genus	Species
Aranae	Araneidae	*Eustala*	-
Aranae	Araneidae	*Parawixia*	-
Aranae	Araneidae	*Wagneriana*	*Wagneriana jacaza*
Aranae	Ctenidae	*Parabatinga*	-
Aranae	Hersillidae	*Iviraiva*	*Iviraiva argentina*
Aranae	Thomisidae	*Misumenops*	-
Aranae	Trechaleidae	*Syntrechalea*	*Syntrechalea brasilia*
Aranae	Salticidae	*Breda*	*Breda modesta*
Aranae	Salticidae	*Chira*	-
Aranae	Salticidae	*Corythalia*	-
Aranae	Salticidae	*Freya*	-
Aranae	Salticidae	*Hypaeus*	-
Aranae	Salticidae	*Titanattus*	-
Aranae	Selenopidae	*Selenops*	-
Coleopetra	Cerambycidae	Onychocerus	-
Coleopetra	Curculionidae	*Heilipodus*	-
Hemiptera	Cicadellidae	*Ciminius*	-
Hemiptera	Cicadellidae	*Crepluvia*	-
Hemiptera	Derbidae	*Mysidia*	-
Hemiptera	Fulgoridae	*Cyrpoptus*	-
Hemiptera	Issidae	*Issus*	-
Hemiptera	Rhopalidaea	*Jadera*	*Jadera sanguinolenta*
Lepidopera	Erebidae	*Saurita*	-
Lepidopera	Noctuidae	*Spodopera*	-
Lepidopera	Tineidae	*Phereoeca*	-
Lepidopera	Tineidae	*Tinea*	-
Lepidopera	Pyralidae	*Plodia*	-
Lepidopera	Noctuidae	*Hypena*	-
Lepidopera	Noctuidae	*Helicoverpa*	-
Mantodea	Thespidae	*Eumiopteryx*	*Eumiopteryx laticollis*
Mantodea	Liturgusidae	*Liturgusa*	-
Orthoptera	Acrididae	*Ronderosia*	*Ronderosia bergii*

Orthoptera	Acrididae	*Schistocerca*	-
Orthoptera	Gryllidae	*Eneopteryx*	*Eneopteryx surinamensis*

Supplementary material - table 2. Pairwise contrasts applied to GLM analysis of the occurrence of camouflage strategies on individuals of arthropod community. Abbreviated names are related to the following camouflage strategies. Apo = Aposematism; Bm = Background matching; Dis = Disruptive coloration; Dec = Decoration; Dist = Distractive marks; Masq =Masquerade; Mim = Mimicry.

Pairwise contrasts			**estimate**	**SE**	**df**	**t.ratio**	**p.value**
Apo_Bm	-	Apo_Bm_Dis	-0.3891	0.444	13	-0.876	0.9994
Apo_Bm	-	Bm	-2.0115	0.444	13	-4.531	**0.0223***
Apo_Bm	-	Bm_Dec_Masq	-0.4771	0.444	13	-1.075	0.9957
Apo_Bm	-	Bm_Dis	-2.0255	0.444	13	-4.562	**0.0212***
Apo_Bm	-	Bm_Dis_Dist	-0.6611	0.444	13	-1.489	0.9474
Apo_Bm	-	Bm_Dis_Masq	-0.588	0.444	13	-1.324	0.9767
Apo_Bm	-	Bm_Dist	-0.588	0.444	13	-1.324	0.9767
Apo_Bm	-	Bm_Dist_Masq	-0.1505	0.444	13	-0.339	1
Apo_Bm	-	Bm_Masq	-1.2274	0.444	13	-2.765	0.3409
Apo_Bm	-	Dec_Masq	-0.3495	0.444	13	-0.787	0.9998
Apo_Bm	-	Dis	-1.3337	0.444	13	-3.004	0.2469
Apo_Bm	-	Masq	-0.7955	0.444	13	-1.792	0.8467
Apo_Bm	-	Mim	-0.3891	0.444	13	-0.876	0.9994
Apo_Bm_Dis	-	Bm	-1.6224	0.444	13	-3.654	**0.0927**
Apo_Bm_Dis	-	Bm_Dec_Masq	-0.088	0.444	13	-0.198	1
Apo_Bm_Dis	-	Bm_Dis	-1.6365	0.444	13	-3.686	**0.0882**
Apo_Bm_Dis	-	Bm_Dis_Dist	-0.272	0.444	13	-0.613	1
Apo_Bm_Dis	-	Bm_Dis_Masq	-0.199	0.444	13	-0.448	1
Apo_Bm_Dis	-	Bm_Dist	-0.199	0.444	13	-0.448	1
Apo_Bm_Dis	-	Bm_Dist_Masq	0.2386	0.444	13	0.537	1
Apo_Bm_Dis	-	Bm_Masq	-0.8383	0.444	13	-1.888	0.8026
Apo_Bm_Dis	-	Dec_Masq	0.0396	0.444	13	0.089	1
Apo_Bm_Dis	-	Dis	-0.9447	0.444	13	-2.128	0.6763

Apo_Bm_Dis	-	Masq	-0.4065	0.444	13	-0.915	0.999
Apo_Bm_Dis	-	Mim	0	0.444	13	0	1
Bm	-	Bm_Dec_Masq	1.5344	0.444	13	3.456	0.1265
Bm	-	Bm_Dis	-0.014	0.444	13	-0.032	1
Bm	-	Bm_Dis_Dist	1.3504	0.444	13	3.042	0.2342
Bm	-	Bm_Dis_Masq	1.4235	0.444	13	3.206	0.1845
Bm	-	Bm_Dist	1.4235	0.444	13	3.206	0.1845
Bm	-	Bm_Dist_Masq	1.861	0.444	13	4.192	**0.0389***
Bm	-	Bm_Masq	0.7841	0.444	13	1.766	0.8576
Bm	-	Dec_Masq	1.662	0.444	13	3.743	**0.0804**
Bm	-	Dis	0.6778	0.444	13	1.527	0.9383
Bm	-	Masq	1.216	0.444	13	2.739	0.3523
Bm	-	Mim	1.6224	0.444	13	3.654	**0.0927**
Bm_Dec_Masq	-	Bm_Dis	-1.5484	0.444	13	-3.488	0.1204
Bm_Dec_Masq	-	Bm_Dis_Dist	-0.184	0.444	13	-0.414	1
Bm_Dec_Masq	-	Bm_Dis_Masq	-0.1109	0.444	13	-0.25	1
Bm_Dec_Masq	-	Bm_Dist	-0.1109	0.444	13	-0.25	1
Bm_Dec_Masq	-	Bm_Dist_Masq	0.3266	0.444	13	0.736	0.9999
Bm_Dec_Masq	-	Bm_Masq	-0.7503	0.444	13	-1.69	0.8874
Bm_Dec_Masq	-	Dec_Masq	0.1276	0.444	13	0.287	1
Bm_Dec_Masq	-	Dis	-0.8566	0.444	13	-1.929	0.7823
Bm_Dec_Masq	-	Masq	-0.3184	0.444	13	-0.717	0.9999
Bm_Dec_Masq	-	Mim	0.088	0.444	13	0.198	1
Bm_Dis	-	Bm_Dis_Dist	1.3644	0.444	13	3.073	0.2239
Bm_Dis	-	Bm_Dis_Masq	1.4375	0.444	13	3.238	0.1761
Bm_Dis	-	Bm_Dist	1.4375	0.444	13	3.238	0.1761
Bm_Dis	-	Bm_Dist_Masq	1.875	0.444	13	4.223	**0.0369***
Bm_Dis	-	Bm_Masq	0.7981	0.444	13	1.798	0.8442
Bm_Dis	-	Dec_Masq	1.6761	0.444	13	3.775	**0.0765**
Bm_Dis	-	Dis	0.6918	0.444	13	1.558	0.9299
Bm_Dis	-	Masq	1.23	0.444	13	2.77	0.3384
Bm_Dis	-	Mim	1.6365	0.444	13	3.686	**0.0882**
Bm_Dis_Dist	-	Bm_Dis_Masq	0.0731	0.444	13	0.165	1

Bm_Dis_Dist	-	Bm_Dist	0.0731	0.444	13	0.165	1
Bm_Dis_Dist	-	Bm_Dist_Masq	0.5106	0.444	13	1.15	0.9924
Bm_Dis_Dist	-	Bm_Masq	-0.5663	0.444	13	-1.276	0.9825
Bm_Dis_Dist	-	Dec_Masq	0.3116	0.444	13	0.702	0.9999
Bm_Dis_Dist	-	Dis	-0.6726	0.444	13	-1.515	0.9413
Bm_Dis_Dist	-	Masq	-0.1344	0.444	13	-0.303	1
Bm_Dis_Dist	-	Mim	0.272	0.444	13	0.613	1
Bm_Dis_Masq	-	Bm_Dist	0	0.444	13	0	1
Bm_Dis_Masq	-	Bm_Dist_Masq	0.4375	0.444	13	0.985	0.9981
Bm_Dis_Masq	-	Bm_Masq	-0.6394	0.444	13	-1.44	0.9579
Bm_Dis_Masq	-	Dec_Masq	0.2386	0.444	13	0.537	1
Bm_Dis_Masq	-	Dis	-0.7457	0.444	13	-1.68	0.8912
Bm_Dis_Masq	-	Masq	-0.2075	0.444	13	-0.467	1
Bm_Dis_Masq	-	Mim	0.199	0.444	13	0.448	1
Bm_Dist	-	Bm_Dist_Masq	0.4375	0.444	13	0.985	0.9981
Bm_Dist	-	Bm_Masq	-0.6394	0.444	13	-1.44	0.9579
Bm_Dist	-	Dec_Masq	0.2386	0.444	13	0.537	1
Bm_Dist	-	Dis	-0.7457	0.444	13	-1.68	0.8912
Bm_Dist	-	Masq	-0.2075	0.444	13	-0.467	1
Bm_Dist	-	Mim	0.199	0.444	13	0.448	1
Bm_Dist_Masq	-	Bm_Masq	-1.0769	0.444	13	-2.426	0.5093
Bm_Dist_Masq	-	Dec_Masq	-0.199	0.444	13	-0.448	1
Bm_Dist_Masq	-	Dis	-1.1832	0.444	13	-2.665	0.3864
Bm_Dist_Masq	-	Masq	-0.645	0.444	13	-1.453	0.9553
Bm_Dist_Masq	-	Mim	-0.2386	0.444	13	-0.537	1
Bm_Masq	-	Dec_Masq	0.8779	0.444	13	1.977	0.7578
Bm_Masq	-	Dis	-0.1063	0.444	13	-0.239	1
Bm_Masq	-	Masq	0.4319	0.444	13	0.973	0.9983
Bm_Masq	-	Mim	0.8383	0.444	13	1.888	0.8026
Dec_Masq	-	Dis	-0.9842	0.444	13	-2.217	0.6261
Dec_Masq	-	Masq	-0.446	0.444	13	-1.005	0.9977
Dec_Masq	-	Mim	-0.0396	0.444	13	-0.089	1
Dis	-	Masq	0.5382	0.444	13	1.212	0.9883

Dis	-	Mim	0.9447	0.444	13	2.128	0.6763
Masq	-	Mim	0.4065	0.444	13	0.915	0.999

ANEXOS CAPÍTULO IV

Supplementary material 1.

Crypsis by background matching and disruptive coloration as drivers of substrate occupation in sympatric Amazonian Bark praying mantises

Supplementary tables.

Table S1. Individuals of praying mantis sampled in the Amazonian Rainforest included in cytochrome c oxidase I (COI) Barcode analysis. Annealing temperature for PCR amplification and GenBank accession number are provided.

Morphotype Specimen	Colour morph	Annealing temperature (°C)	GenBank acession number
Hagiomantis sp.	White	50	OR073655
Hagiomantis sp.		50	OR073656
Hagiomantis sp.		50	OR073657
Hagiomantis sp.		45	OR073658
Hagiomantis sp.		45	OR073659
Liturgusa sp.	Green	50	OR073660
Liturgusa sp.		50	OR073661
Liturgusa sp.		50	OR073662
Liturgusa sp.		45	OR073663
Liturgusidae sp.	Grey	45	OR073664
Liturgusidae sp.		54	OR073665
Liturgusidae sp.		45	OR073666
Liturgusidae sp.		54	OR073667
Liturgusidae sp.		45	OR073668

Table S2. Pairwise Kimura two-parameter distance of praying mantis considering 640 bp cytochrome c oxidase I (COI) sequences. Values in bold are showing distance higher than 3%, indicating that individuals with different colour do not belong to the same species (Hebert et. al 2003).

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 - JO02_"white"	-												
2 - JO06_"white"	0.002	-											
3 - JO11_"white"	0.002	0.000	-										
4 - JO26_"white"	0.009	0.007	0.007	-									
5 - JO27_"white"	0.004	0.002	0.002	0.009	-								
6 - JO30_"white"	0.009	0.007	0.007	0.007	0.009	-							
7 - JO31_"white"	0.007	0.005	0.005	0.009	0.005	0.009	-						
8 - JO14_"green"	**0.181**	**0.179**	**0.179**	**0.174**	**0.181**	**0.179**	**0.181**	-					
9 - JO21_"green"	**0.181**	**0.179**	**0.179**	**0.174**	**0.181**	**0.179**	**0.181**	0.000	-				
10 - JO22_"green"	**0.181**	**0.179**	**0.179**	**0.174**	**0.181**	**0.179**	**0.181**	0.000	0.000	-			
11 - JO29_"green"	**0.179**	**0.177**	**0.177**	**0.172**	**0.179**	**0.177**	**0.179**	0.004	0.004	0.004	-		
12 - JO32_"grey"	**0.185**	**0.183**	**0.183**	**0.178**	**0.185**	**0.183**	**0.185**	**0.142**	**0.142**	**0.142**	**0.142**	-	
13 - JO33_"grey"	**0.187**	**0.185**	**0.185**	**0.180**	**0.183**	**0.185**	**0.181**	**0.148**	**0.148**	**0.148**	**0.148**	0.014	-

Supplementary Figure 1.

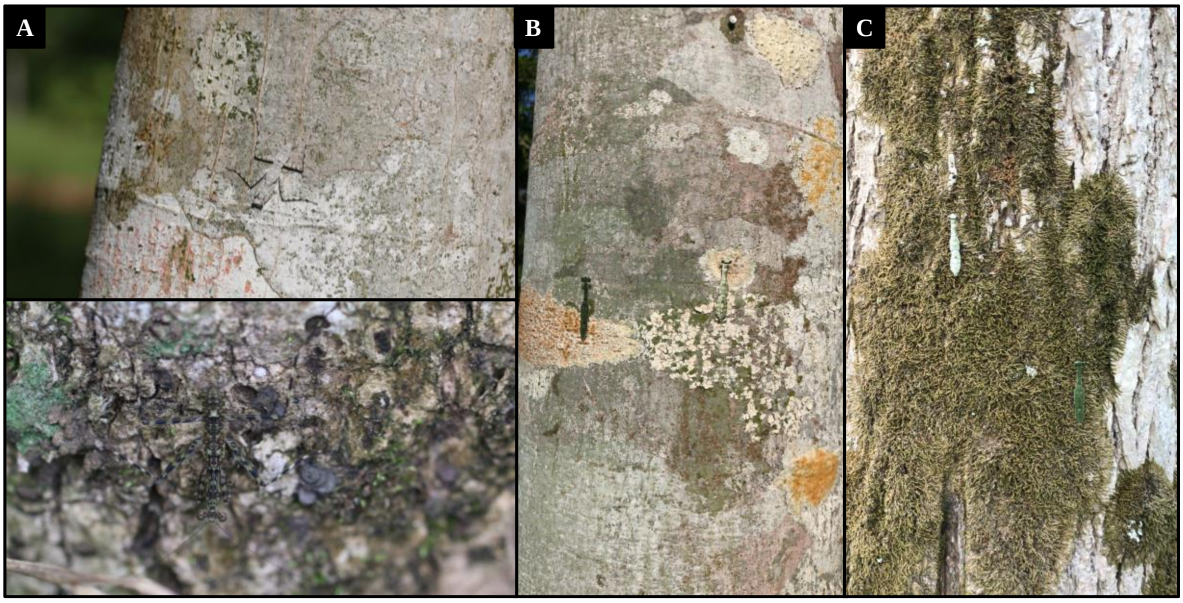
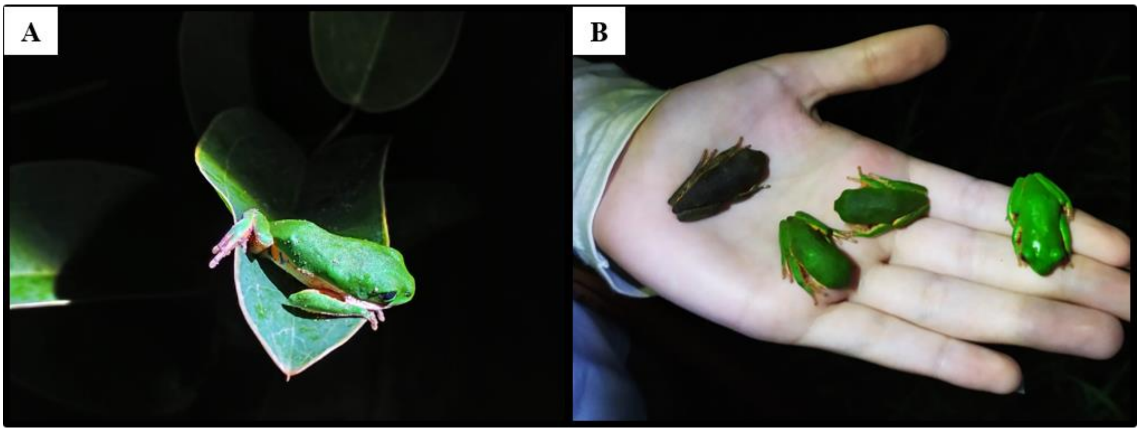


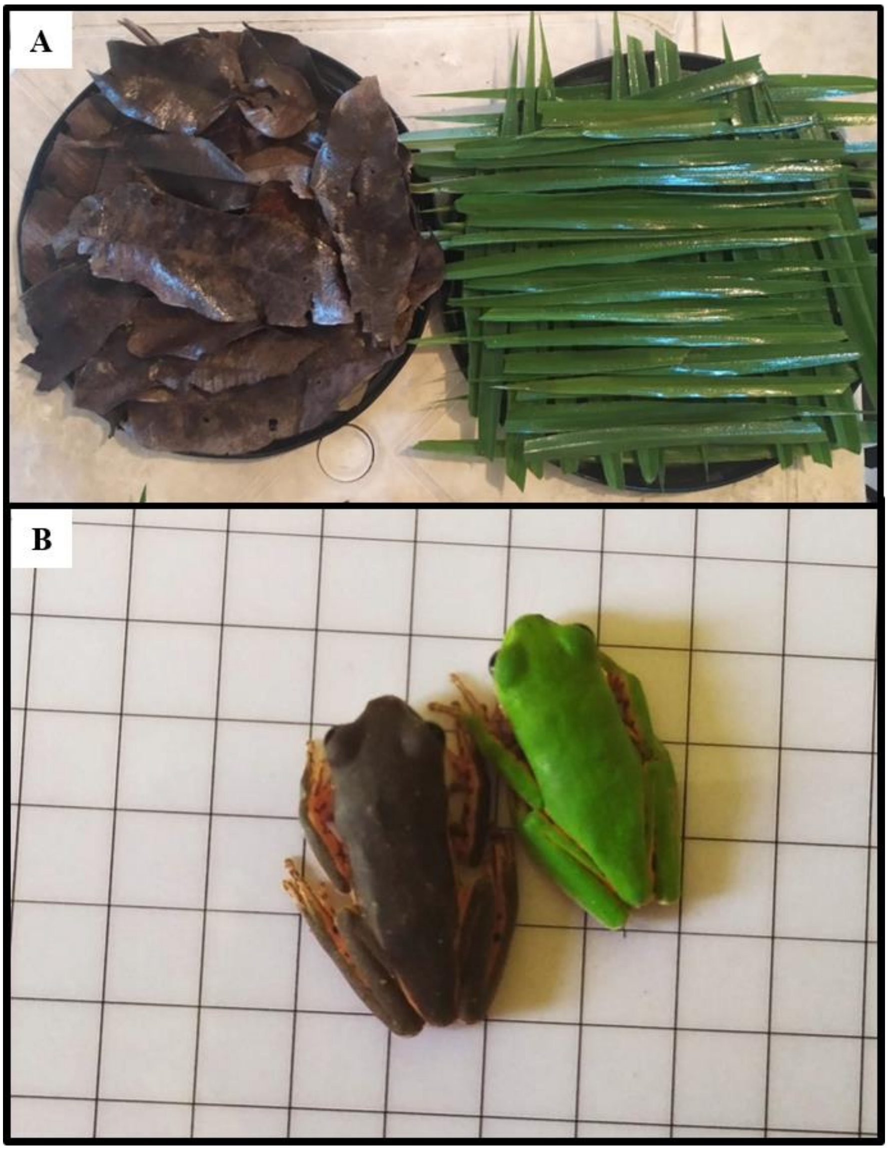
Figure S1. Field predation experiment. The photos depict the field predation experiment with human predators in the southern Amazon rainforest. Picture A) shows in the upper panel the white morphospecies (*Hagiomantis* sp.) resting on whitish trunks and in the below panel, the green morphospecies (*Liturgusa* sp.) resting on greenish-brown trunks. Panel B shows the pair models of paper mantis (white and green) placed on whitish trunks covered by lichens. Panel B shows the pair models of paper mantis (white and green) placed on greenish-brown trunks covered by random patches of bryophytes. In the experiment, the pair of paper model mantis were randomly placed on both trunk types.

ANEXOS CAPÍTULO V

Supplementary Figures



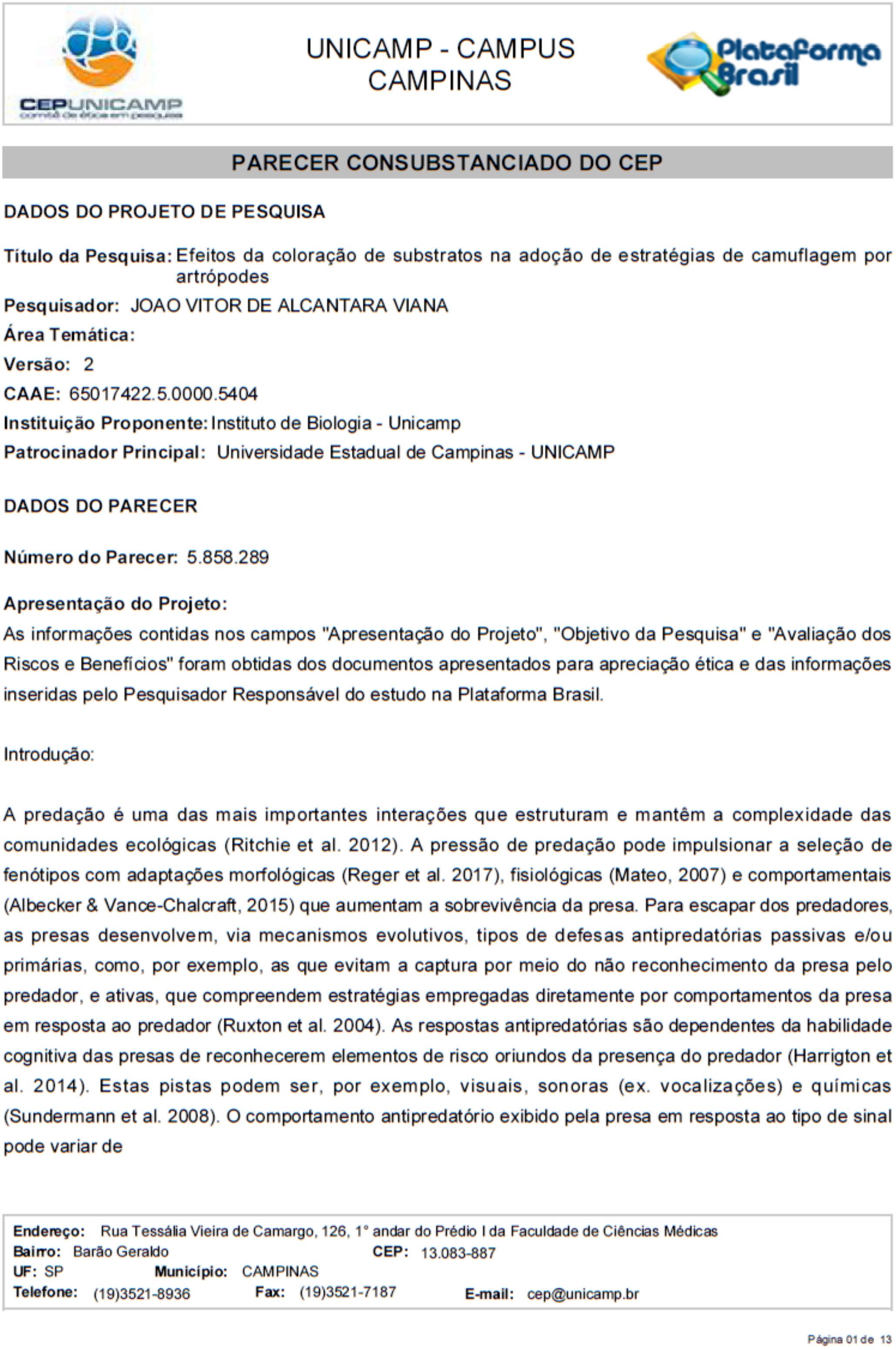
Supplementary Figure 1. Field observations showing individuals of the species *Pithecopus hypochondrialis* displaying nocturnal color change. A) An individual resting on a green leaf. B) Individuals displaying different colors - brown and green.



Supplementary Figure 2. Experimental procedure showing the preparation of experimental arenas with backgrounds (grass and leaf-litter) (A), as well individuals of the species *Pithecopus hypochondrialis* displaying physiological color change according to their experimental backgrounds (B).

ANEXO VI

Aprovação para estudos envolvendo Humanos



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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeitos da coloração de substratos na adoção de estratégias de camuflagem por artrópodes

Pesquisador: JOAO VITOR DE ALCANTARA VIANA

Área Temática:

Versão: 2

CAAE: 65017422.5.0000.5404

Instituição Proponente: Instituto de Biologia - Unicamp

Patrocinador Principal: Universidade Estadual de Campinas - UNICAMP

DADOS DO PARECER

Número do Parecer: 5.858.289

Apresentação do Projeto:

As informações contidas nos campos "Apresentação do Projeto", "Objetivo da Pesquisa" e "Avaliação dos Riscos e Benefícios" foram obtidas dos documentos apresentados para apreciação ética e das informações inseridas pelo Pesquisador Responsável do estudo na Plataforma Brasil.

Introdução:

A predação é uma das mais importantes interações que estruturam e mantêm a complexidade das comunidades ecológicas (Ritchie et al. 2012). A pressão de predação pode impulsionar a seleção de fenótipos com adaptações morfológicas (Reger et al. 2017), fisiológicas (Mateo, 2007) e comportamentais (Albecker & Vance-Chalcraft, 2015) que aumentam a sobrevivência da presa. Para escapar dos predadores, as presas desenvolvem, via mecanismos evolutivos, tipos de defesas antipredatórias passivas e/ou primárias, como, por exemplo, as que evitam a captura por meio do não reconhecimento da presa pelo predador, e ativas, que compreendem estratégias empregadas diretamente por comportamentos da presa em resposta ao predador (Ruxton et al. 2004). As respostas antipredatórias são dependentes da habilidade cognitiva das presas de reconhecerem elementos de risco oriundos da presença do predador (Harrigton et al. 2014). Estas pistas podem ser, por exemplo, visuais, sonoras (ex. vocalizações) e químicas (Sundermann et al. 2008). O comportamento antipredatório exibido pela presa em resposta ao tipo de sinal pode variar de

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acordo com a identidade e comportamento de caça do predador, gerando desde aumento de vigilância (Suraci et al. 2016), comportamentos evasivos (Dukas, 2001) ou mesmo seleção de hábitats adequados (Stevens & Ruxton , 2018; Tavares et al. 2018). Uma das formas mais amplamente utilizadas por presas para escapar de predadores visualmente orientados é a utilização de padrões de coloração que reduzem a conspicuidade (Thayer, 1909; Cott, 1940; Troscianko et al. 2016). Camuflagem pode ser definida como colorações e padrões corporais, além de outras adaptações morfológicas que diminuem a probabilidade de detecção ou reconhecimento da presa pelo predador (Merilaita et al. 2017). Esse mecanismo antipredatório é um termo ecológico amplo e compreende 16 tipos de estratégias que agem de formas variadas ao explorar a capacidade da presa em distorcer a percepção do predador (Merilaita et al. 2017). A camuflagem pode agir selecionando pontos fracos nos mecanismos de percepção sensorial e cognitivo do predador, sendo a detecção e reconhecimento advindas do balanço entre informação de sinal e de ruído de um alvo. Como exemplo, existem estratégias de camuflagem que dificultam o reconhecimento das bordas corporais de um animal, como a correspondência de pano de fundo, em que os animais se correspondem em cor e brilho com uma amostra aleatória do pano de fundo (Merilaita et al. 2017). Outras, no entanto, dependem menos do pano de fundo e funcionam aumentando o ruído, ao criar bordas corporais contrastantes falsas nos animais que se cruzam com os reais, como a coloração disruptiva, que é uma das mais efetivas estratégias de camuflagem (Cuthill et al. 2005; Stevens & Merilaita, 2009). Um aspecto importante a ser considerado no sucesso da utilização das estratégias de camuflagem é o substrato utilizado, pois ainda que em determinado substrato possa ser críptico, em outro o animal pode ser altamente conspícuo (Vieira et al. 2017). Deste modo, presas que exibem camuflagem devem selecionar substratos que melhor se correspondam em coloração e brilho, ou que facilitem a exibição de estratégias que reduzem as chances de reconhecimento e detecção a predadores visualmente orientados (Stevens & Ruxton, 2018). Tendo isso em vista, nosso trabalho é composto por dois capítulos que buscam entender o efeito da coloração dos substratos na camuflagem de diferentes modelos biológicos em dois biomas distintos: Amazônia e Cerrado.
Hipótese: Esperamos que modelos de animais contrastantes quanto a cor de tronco em que forem inseridos (ex. modelo melânico em tronco não queimado) sejam encontradas mais rapidamente e em distâncias maiores nesses troncos do que comparados a troncos em que estão camuflados (ex. modelo melânico em tronco queimado). Também acreditamos que os modelos camuflados disruptivos e de coloração de correspondência e de padrão sejam mais efetivos aos se camuflarem dos predadores humanos do que comparados aos modelos marrons em troncos não queimados. Em troncos queimados acreditamos que os

modelos melânicos serão mais efetivos quanto ao tempo de busca e a distância de encontro do que modelos marrons, disruptivos ou de correspondência de fundo, aumentando o tempo e diminuindo a distância que cada participante terá que percorrer para encontrar o alvo.

Metodologia:

Métodos - Capítulo 1 Nosso estudo abordará as questões acima citadas de modo semelhante ao feito por Michalis et al. (2017); Xiao & Cuthill (2016) e Cuthill et al. (2005) e (2006). Pediremos para que participantes (predadores) andem por trilhas de manejo e reflorestamento da Fazenda São Nicolau para procurarem por louva-a-deus artificiais de papel fixados em troncos claros de figueira-branca e marrons-esverdeados em troncos de Ipê. Os modelos de louva-a-deus artificiais serão impressos em papel fotográfico (à prova d'água) e terão aproximadamente 1.5 cm de largura por 3 cm de comprimento. Os modelos serão fixados por tachinhas, colados com superbonder e inseridos aos pares, isto é um Hagiomantis sp e um Liturgusa sp. em troncos brancos de figueira-branca e marrons-esverdeados de Ipê. Os modelos serão inseridos aproximadamente à altura do peito e no mesmo plano de visão. Para a fixação, nós inseriremos a tachinha no tronco e posteriormente colaremos o modelo sobre ela com superbonder, de modo que apenas o louva-a-deus fique visível. Os pares de modelos deverão estar espaçados entre si para não se sobreporem. A altura de inserção de cada modelo será anotada na ficha de montagem de experimento (se necessária alguma observação usar a parte destinada a posição geográfica: N, S, L, O). Utilizaremos no mínimo 10 arvores brancas e 10 arvores marrons-esverdeadas espaçadas entre si por pelos menos 10 metros em trilhas não lineares, de modo que os modelos de árvores subsequentes não possam ser visualizados ao mesmo tempo. Os participantes percorrerão duas trilhas principais acompanhados pelo pesquisador, sendo uma com troncos brancos compostos por figueiras e a outra com marrons-esverdeados compostos por Ipês. Os participantes serão orientados a permanecerem com os rostos abaixados para que não vejam os modelos antes do tempo inicial do experimento. Ao chegarem na distância inicial do experimento (7.5 metrs metros), os participantes serão avisados de que dois tipos de modelos de presas estarão em uma árvore à sua frente (que serão mostrados brevemente anteriormente ao início do experimento). O tempo que cada pessoa demorará para achar cada espécie de louva-a-deus será quantificado, assim como a distância que ele encontrou o alvo fixado na árvore, isto é, da distância inicial do experimento até arvore com o modelo. Para evitarmos que os participantes tentem adivinhar sobre localização dos modelos, usaremos apontadores a lazer que meçam a distância e que indiquem o local dos mesmos. Após achar os dois alvos o participante seguirá para as demais árvores até completar o experimento em ambas as áreas do experimento. Estipularemos o intervalo de idade dos participantes entre 18 e 50 anos, dado que acima da idade máxima a visão humana aumenta em chance de apresentar condições oftalmológicas desfavoráveis ao intuito da pesquisa. Buscaremos também balancear entre participantes do sexo masculino e feminino. Também pediremos para que usem óculos, caso os mesmos apresentem alguma condição oftalmológica. O sexo e a idade de cada participante serão anotados. Métodos - Capítulo 2 Nosso estudo abordará as questões acima citadas de modo semelhante ao feito por Xiao & Cuthill (2016). Pediremos para que 23 participantes andem por trilhas de Cerrado para procurarem por presas artificiais de papel fixadas em troncos queimados e não queimados de Qualea grandiflora. A escolha por Q. grandiflora deve-se ao fato de ser uma planta muito comumente encontrada em áreas de Cerrado e resistente a queimadas. Usaremos quatro tipos de presas artificiais, isto é, modelos triangulares que representem genericamente mariposas (Lepidoptera), sendo elas: correspondência em coloração com troncos não queimados (marrons), modelos melânicos, modelos que se correspondam em coloração e em padrão de forma e textura do tronco (coloração de correspondência de pano de fundo e substrato) e modelos disruptivos. As presas marrons, disruptivas e coloração de correspondência de fundo.

Critérios de inclusão e exclusão:

Critério de Inclusão:

Não informado.

Critério de Exclusão:

Não informado.

Objetivo da Pesquisa:
Objetivo Primário:

O objetivo do capítulo 1 é testar se a ocorrência de Hagiomantis sp. em troncos brancos e Liturgusa sp. em troncos marrons-esverdeados se relacionam com efetividade de camuflagem das espécies em seus respectivos troncos de ocupação. Além disso, também queremos testar se o uso de micro -habitat pelas espécies em seus troncos, isto é, Hagiomantis sp. sobre liquens e Liguturgusa sp. sobre musgos, confere maior valor protetivo contra a predação do que a ocorrência aleatória nos troncos ocupados. O objetivo do capítulo 2 é é entender como as queimadas afetam a sobrevivência de organismos que usam troncos para se camuflarem dos seus predadores em ambientes de Cerrado pós-fogo.

Objetivo Secundário:

Não informado.

Avaliação dos Riscos e Benefícios:
Segundo informações do pesquisador:

Riscos:

Pediremos para que os participantes usem Equipamentos de proteção individual(EPIs) para o experimento em campo, tais como perneiras, botas e camisas longas e fechadas.

Benefícios:

Não haverá remuneração para os participantes. O transporte dos participantes será realizado pela equipe de pesquisa.

Comentários e Considerações sobre a Pesquisa:
Este protocolo se refere ao Projeto de Pesquisa intitulado "Efeitos da coloração de substratos na adoção de estratégias de camuflagem por artrópodes", cujo pesquisador responsável é o aluno João Vitor de Alcantara Viana, do Programa de Pós-graduação em Ecologia, do Instituto de Biologia da UNICAMP, sendo a equipe de pesquisa composta pela Dra Carolina Lambertini, Anna Luiza Oliveira Martins e Felipe Capoccia Coelho. Consiste em um projeto de pesquisa de doutorado, sob orientação do Prof. Dr. Gustavo Quevedo Romero. A Instituição Proponente é o Instituto de Biologia da UNICAMP. Segundo as Informações Básicas do Projeto, a pesquisa tem orçamento estimado em R$7.800,00 (sete mil e oitocentos reais), com financiamento do tipo secundário pela

Universidade Estadual de Campinas - UNICAMP, e o cronograma apresentado contempla coleta dos dados com início em fevereiro de 2023 e término em fevereiro de 2023. Serão obtidos ao todo dados de 53 participantes, divididos em 2 grupos/capítulos da pesquisa: o experimento da Amazônia será realizado na Fazenda São Nicolau – ONF Brasil, em Cotriguaçu (MT), onde 30 participantes (moradores do município) buscarão por 10 tipos de cada modelo artificial (Hagiomantis sp e Liturgusa sp.) em 20 árvores de uma área queimada e 20 árvores de outra área próxima que não foi queimada; e o experimento do Cerrado será realizado na Reserva Ecológica do Panga, RPPN pertencente a Universidade Federal de Uberlândia – MG, onde 23 participantes (moradores do município) buscarão por 10 tipos de cada modelo artificial de papel em formatos de mariposas em 20 árvores de uma área queimada e 20 árvores de outra área próxima que não foi queimada.

Considerações sobre os Termos de apresentação obrigatória:
Foram analisados os seguintes documentos de apresentação obrigatória:

1 - Folha de Rosto Para Pesquisa Envolvendo Seres Humanos: Foi apresentado o documento "FolhadeRosto_Joao.pdf" de 09/11/2022 09:35:35, devidamente preenchido, datado e assinado.

2 - Projeto de Pesquisa: Foram analisados os documentos “Projeto_Realcados.pdf” de 28/12/2022 23:28:32, “Projeto_Etica_joao.pdf” de 28/12/2022 23:10:24 e "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1905870.pdf" de 28/12/2022 23:30:56. Adequado.

3 - Orçamento financeiro e fontes de financiamento: Informações sobre orçamento financeiro incluídas nos documentos “Projeto_Realcados.pdf” de 28/12/2022 23:28:32, “Projeto_Etica_joao.pdf” de 28/12/2022 23:10:24 e "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1905870.pdf" de 28/12/2022 23:30:56. De acordo com o pesquisador o apoio financeiro será do tipo secundário pela Universidade Estadual de Campinas - UNICAMP. Adequado.

4 - Cronograma: Informações sobre o cronograma incluídas nos documentos “Projeto_Realcados.pdf” de 28/12/2022 23:28:32, “Projeto_Etica_joao.pdf” de 28/12/2022 23:10:24 e "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1905870.pdf" de 28/12/2022 23:30:56. Adequado.

5 - Termo de Consentimento Livre e Esclarecido: foram apresentados os documentos “TCLE_Cap_Cerrado.pdf” de 28/12/2022 17:27:53, “TCLE_Cap_Amazonia.pdf” de 28/12/2022 23:

21:16, “TCLE_Amazonia_Realcados.pdf” de 28/12/2022 23:26:20 e “TCLE_Cerrado_Realcados.pdf” de 28/12/2022 23:27:57. Adequado.

6 - Currículo do pesquisador principal e demais colaboradores: Foram analisados os documentos “AtestadoMatricula.pdf” de 01/11/2022 13:42:50, “Projeto_Realcados.pdf” de 28/12/2022 23:28:32, “Projeto_Etica_joao.pdf” de 28/12/2022 23:10:24 e "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1905870.pdf" de 28/12/2022 23:30:56. Adequado.

7 - Outros documentos que acompanham o Protocolo de Pesquisa:
- “Autorizacao_Exp_fora_da_Unicamp_Reserva_Panga.pdf” de 11/10/2022 15:42:24 e “Autorizacao_amazonia.pdf” de 29/11/2022 12:18:38: declarações de concordância com realização da pesquisa.
- “Carta_Resposta.pdf” de 28/12/2022 23:23:56: carta com respostas às pendências.

Conclusões ou Pendências e Lista de Inadequações:
Lista de inadequações e pendências emitidas no parecer CEP nº: 5.782.973:

1. Quanto ao Termo de Consentimento Livre e Esclarecido, segundo os documentos “TCLE_Amazonia.pdf” de 08/11/2022 20:07:34 e “TCLE_joao.pdf” de 08/11/2022 20:10:24: 1.1. No documento das Informações Básicas da Plataforma Brasil consta no campo “Projeto de Pesquisa” o título “Efeitos da coloração de substratos na adoção de estratégias de camuflagem por artrópodes”. Porém, no documento do TCLE “TCLE_Amazonia.pdf” consta como “Título da pesquisa” o texto: “Efeitos da coloração de substratos na camuflagem de duas espécies de Louva-adeus da Amazônia”; e no documento do TCLE “TCLE_joao.pdf” consta como “Título da pesquisa” o texto: “Efeito ecológico do pós-fogo na exibição de estratégias de camuflagem e seleção de hábitat por organismos de Cerrado”. Solicita-se esclarecimento e padronização das informações em ambos os documentos.

Resposta: O presente estudo intitulado “Efeitos da coloração de substratos na adoção de estratégias de camuflagem por artrópodes” é composto por dois capítulos complementares que são execeutados em dois biomas distintos, por isso cada capítulo recebe um segundo título, que também é identificado no TCLE. . Alteramos isso nos TCLE, de modo a manter o título original da pesquisa e identificar também o capítulo do qual o mesmo se trata. Por favor, checar nos TCLE referents a cada bioma (Amazônia e Cerrado), arquivos denominados “TCLE_ Cap.1_Amazonia” e “TCLE_Cap.2_Cerrado”.

Análise: PENDÊNCIA ATENDIDA.

1.2. No documento “TCLE_Amazonia.pdf” na seção “Benefícios” consta “Não haverá remuneração para os participantes”. Entende-se dessa forma que não haverá benefícios diretos aos participantes. Assim, solicita-se incluir nessa seção que “A pesquisa não terá benefícios diretos ao participante”, ou algo semelhante, no documento.

Resposta: Os documentos doram alterados de acordo com a orientação do presente parecer. Por favor, checar nos TCLE.

Análise: PENDÊNCIA ATENDIDA.

1.3. No documento “TCLE_joao.pdf” na seção “Benefícios” consta “Não haverá remuneração para os participantes. Buscaremos e levaremos os participantes as suas residências ou locais de fácil acesso para locomoção dos mesmos. Também ofereceremos água em abundância e alimentação aos participantes”. Entende-se dessa forma que não haverá benefícios diretos aos participantes. Assim, solicita-se incluir nessa seção que “A pesquisa não terá benefícios diretos ao participante”, ou algo semelhante, no documento.

Resposta: Os documentos doram alterados de acordo com a orientação do presente parecer. Por favor, checar nos TCLE.

Análise: PENDÊNCIA ATENDIDA.

1.4. Nos dois documentos de TCLE na seção “Acompanhamento e assistência” consta “A pesquisa não tem o intuito de acompanhar os participantes, dado que cada pessoa só participará apenas uma vez. Os participantes poderão pedir os resultados da pesquisa após as análises estatísticas e gráficas. Também forneceremos os resultados da pesquisa via publicação em periódicos internacionais da área de Ecologia, quando solicitado”. Porém, a Resolução CNS N° 466 de 2012 define dano associado (ou decorrente) da pesquisa o “agravo imediato ou posterior, direto ou indireto, ao indivíduo ou à coletividade, decorrente da pesquisa" (item II.6). Ainda no item V.6, a citada Resolução define que "O pesquisador, o patrocinador e as instituições e/ou organizações envolvidas nas diferentes fases da pesquisa devem proporcionar assistência imediata, nos termos do item II.3, bem como responsabilizarem-se pela assistência integral aos participantes da pesquisa no que se refere às complicações e danos decorrentes da pesquisa". Assim, o TCLE deve assegurar, de forma clara e afirmativa, que o participante de pesquisa receberá a assistência integral e imediata, de forma gratuita (pelo patrocinador), pelo tempo que for necessário em caso de danos decorrentes da pesquisa. Solicita-se esclarecimento e adequação dessa questão nos respectivos documentos.

Resposta: Os documentos doram alterados de acordo com a orientação do presente parecer. Por favor, checar nos TCLE.

Análise: PENDÊNCIA ATENDIDA.

1.5. Não foi declarado nos dois documentos de TCLE o item referente ao direito à indenização para o participante da pesquisa. Solicita-se inclusão dessa informação. A Resolução CNS N° 466 de 2012 (item IV.3) define que "os participantes da pesquisa que vierem a sofrer qualquer tipo de dano resultante de sua participação na pesquisa, previsto ou não no Termo de Consentimento Livre e Esclarecido, têm direito à indenização, por parte do pesquisador, do patrocinador e das instituições envolvidas nas diferentes fases da pesquisa" (item V.7). Cabe enfatizar que a questão da indenização não é prerrogativa da Resolução CNS N° 466 de 2012, estando originalmente prevista no Código Civil (Lei 10.406 de 2002), sobretudo nos artigos 927 a 954, dos Capítulos I (Da Obrigação de Indenizar) e II (Da Obrigação de Indenizar), Título IX (Da Responsabilidade Civil). Adequar o TCLE segundo a estrutura básica apresentada na página do Comitê de Ética da UNICAMP: https://www.prp.unicamp.br/sites/default/files/2019/cep/3_estrutura_basica_tcle-versao-2019novembro06-disponivel_site.docx

Resposta: Incluímos o direito à indenização para o participante da pesquisa. De acordo com a Resolução CNS N° 466 de 2012(item IV.3) em ambos TCLE. Por favor, checar nos TCLE.

Análise: PENDÊNCIA ATENDIDA.

1.6. A UNICAMP e os órgãos de fomento solicitam a inserção dos dados anonimizados em repositório de dados. Diante disso, para esclarecimentos dos participantes da pesquisa, solicitamos que seja inserido nos TCLEs, abaixo do item "Ressarcimento e Indenização", o seguinte texto: “Tratamento dos dados: Esta pesquisa prevê o armazenamento dos dados coletados em repositório de dados, em local virtual de acesso público, com o objetivo de possível reutilização, verificação e compartilhamento em trabalhos de colaboração científica com outros grupos de pesquisa. Sua identidade não será revelada nesses dados, pois os dados só serão armazenados de forma anônima (isto é, os dados não terão identificação), utilizando mecanismos que impeçam a possibilidade de associação, direta ou indireta com você. Cabe ressaltar que quem compartilhar os dados também não terá possibilidade de identificação dos participantes de quem os dados se originaram. Sendo assim, não haverá possibilidade de reversão da anonimização”.

Resposta: Os documentos doram alterados de acordo com a orientação do presente parecer. Por favor, checar nos TCLE.

Análise: PENDÊNCIA ATENDIDA.

1.7. Na seção “Contato” do TCLE consta o texto “Em caso de dúvidas sobre a pesquisa, você poderá entrar em contato com os pesquisadores: Pesquisador responsável Nome: Me. João Vitor de Alcantara Viana Função: Doutorando Endereço Profissional: Universidade Estadual de Campinas, Instituto de Biologia. Rua Monteiro Lobato 255, IB, Departamento de Biologia Animal Cidade Universitária 13083862 - Campinas, SP - Brasil”. Solicita-se a inclusão de um número de telefone ou de um endereço eletrônico (e-mail) do pesquisador responsável, caso o participante sinta a necessidade de contato direto ou urgente com o mesmo.

Resposta: Incluímos o email do pesquisador responsável, de acordo com a orientação do presente parecer. Por favor, checar nos TCLE. Ressaltamos que o cronograma de pesquisa foi alterado para início no dia 13 de Fevereiro de 2023 com término em 23 de Fevereiro de 2023. Por favor ver alteração no projeto detalhado.

Análise: PENDÊNCIA ATENDIDA.

Considerações Finais a critério do CEP:

- O participante da pesquisa deve receber uma via do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (quando aplicável).

- O participante da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (quando aplicável).

- O pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado. Se o pesquisador considerar a descontinuação do estudo, esta deve ser justificada e somente ser realizada após análise das razões da descontinuidade pelo CEP que o aprovou. O pesquisador deve aguardar o parecer do CEP quanto à descontinuação, exceto quando perceber risco ou dano não previsto ao participante ou quando constatar a superioridade de uma estratégia diagnóstica ou terapêutica oferecida a um dos grupos da pesquisa, isto é, somente em caso de necessidade de ação imediata com intuito de proteger os participantes.

- O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo. É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

- Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas e aguardando a aprovação do CEP para continuidade da pesquisa. Em caso de projetos do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma, junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial.

- Relatórios parciais e final devem ser apresentados ao CEP, inicialmente seis meses após a data deste parecer de aprovação e ao término do estudo.

-Lembramos que segundo a Resolução 466/2012 , item XI.2 letra e, “cabe ao pesquisador apresentar dados solicitados pelo CEP ou pela CONEP a qualquer momento”.

-O pesquisador deve manter os dados da pesquisa em arquivo, físico ou digital, sob sua guarda e responsabilidade, por um período de 5 anos após o término da pesquisa.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1905870.pdf	28/12/2022 23:30:56		Aceito
Brochura Pesquisa	Projeto_Realcados.pdf	28/12/2022 23:28:32	JOAO VITOR DE ALCANTARA VIANA	Aceito
Declaração de Pesquisadores	TCLE_Cerrado_Realcados.pdf	28/12/2022 23:27:57	JOAO VITOR DE ALCANTARA VIANA	Aceito
Solicitação registrada pelo CEP	TCLE_Amazonia_Realcados.pdf	28/12/2022 23:26:20	JOAO VITOR DE ALCANTARA VIANA	Aceito
Declaração de Pesquisadores	Carta_Resposta.pdf	28/12/2022 23:23:56	JOAO VITOR DE ALCANTARA VIANA	Aceito
Outros	TCLE_Cap_Amazonia.pdf	28/12/2022 23:21:16	JOAO VITOR DE ALCANTARA VIANA	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Etica_joao.pdf	28/12/2022 23:10:24	JOAO VITOR DE ALCANTARA VIANA	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Cap_Cerrado.pdf	28/12/2022 17:27:53	JOAO VITOR DE ALCANTARA VIANA	Aceito
Outros	Autorizacao_amazonia.pdf	29/11/2022 12:18:38	JOAO VITOR DE ALCANTARA VIANA	Aceito
Folha de Rosto	FolhadeRosto_Joao.pdf	09/11/2022 09:35:35	JOAO VITOR DE ALCANTARA VIANA	Aceito
Outros	AtestadoMatricula.pdf	01/11/2022 13:42:50	JOAO VITOR DE ALCANTARA VIANA	Aceito
Declaração de concordância	Autorizacao_Exp_fora_da_Unicamp_Reserva_Panga.pdf	11/10/2022 15:42:24	JOAO VITOR DE ALCANTARA VIANA	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CAMPINAS, 19 de Janeiro de 2023

Assinado por:
Renata Maria dos Santos Celeghini
(Coordenador(a))

ANEXO VII

Declaração de direitos autorais

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **" Camuflagem sob diferentes contextos e perspectivas: padrões gerais, mecanismos e efeitos do substrato utilizado"**, não infringem os dispositivos da Lei n.º 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 25 de novembro de 2023

Assinatura: João Vitor de Alcantara Viana
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