



**Universidade Federal De Uberlândia
Instituto De Biologia**

**Programa de Pós-Graduação em Ecologia, Conservação e
Biodiversidade**

**Cuidado parental e incidência de predadores e parasitoides de ovos
em ootecas de aranhas orbitelas (Araneae: Araneidae, Uloboridae)
em Minas Gerais, Brasil**

Adilson Quero Junior

2022

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Tese apresentada à Universidade Federal de
Uberlândia, como parte das exigências para obtenção
do título de Doutor em Ecologia, Conservação e
Biodiversidade.

Orientador

Prof. Dr. Marcelo de Oliveira Gonzaga

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RESUMO

O cuidado parental é uma característica presente em muitos animais, aumentando a sobrevivência da prole com a redução da mortalidade promovida por fatores bióticos e abióticos. Em aranhas o cuidado parental é bastante diversificado, sendo frequentemente eficiente contra alguns predadores generalistas, como formigas. Contra aves e outros predadores visualmente orientados, aranhas apresentam várias estratégias anti predação, como a camuflagem e disfarces (“masquerade”). No entanto, alguns grupos de predadores e parasitoides (e.g. vespas, dípteros e mantispídeos) apresentam espécies capazes de ultrapassar as várias linhas de defesa e atacar os ovos das aranhas. Ainda assim, pouco se sabe sobre a ocorrência e o impacto desses inimigos naturais na sobrevivência da prole de aranhas, e qual potencial efeito do cuidado parental nesse contexto. Dessa forma, nessa tese investigamos a ecologia comportamental das aranhas das famílias Araneidae e Uloboridae e de seus ovos, descrevendo os inimigos naturais de cada espécie estudada. Demonstramos que o cuidado parental em *Manogea porracea* (Araneidae) é eficiente para o aumento da sobrevivência dos ovos em uma população no Cerrado, mesmo na presença de um predador de ovos que não havia sido previamente descrito atacando a espécie. Também investigamos o cuidado maternal na aranha *Miagrammops unipus* (Uloboridae) descrevendo o seu comportamento com as ootecas, a variação nas ootecas e os ataques aos ovos por uma vespa parasitoide. Também investigamos a camuflagem nessa espécie, por meio do cálculo da correspondência na coloração entre as fêmeas, a suas ootecas e os substratos utilizados para repouso. Registramos os insetos associados a ootecas de 10 espécies de aranhas, incluindo as duas previamente mencionadas. Somente uma espécie não foi atacada por nenhum inimigo natural, a aranha invasora *Cyrtophora citricola* (Araneidae), recentemente encontrada no Brasil. Todas as outras nove espécies foram atacadas por algum predador ou parasitoide de ovos, como vespas, dípteros e mantispídeos. Por fim, descrevemos uma vespa parasitoide de aranhas atacando *C. citricola*, demonstrando o uso de um novo hospedeiro para uma vespa nativa, mas ainda sem registros de predadores ou parasitoides atacando as suas ootecas na população estudada. Muitas interações entre aranhas, suas ootecas e seus inimigos naturais foram explorados nessa tese, contribuindo para o entendimento da ecologia e evolução desses animais.

Palavras-chave: fatores de mortalidade; sobrevivência da prole; vespas parasitoides; Diptera; Mantispidae

ABSTRACT

Parental care is a trait present in many animals, increasing the offspring survival with the reduction of the mortality caused by biotic and abiotic factors. In spiders, parental care is quite diverse, being frequently efficient against some generalist predators, such as ants. For birds and other visually orientated predators, spiders have various strategies against predation, like camouflage and disguises (“masquerade”). However, some groups of predators and parasitoids (e.g. wasps, dipterans and mantispids) have species capable of surpassing the multiple lines of defense and attack the eggs of spiders. Still, little is known about the occurrence and impact of these natural enemies on offspring survival of spiders, and what potential effect parental care in this context. Thus, in this thesis we investigate the behavioral ecology of spiders from the families Araneidae and Uloboridae and their eggs, describing the natural enemies of each studied species. We demonstrate that parental care in *Manogea porracea* (Araneidae) is efficient for the survival of the eggs in a population on Cerrado, even in the presence of an egg predator that had not been described previously attacking the species. We investigated maternal care in the spider *Miagrammopes unipus* (Uloboridae) describing its behavior with the egg sacs, the variation in the egg sacs and the attack of the eggs by a parasitoid wasp. We also investigated camouflage in this species through the calculation of background matching between females, their egg sacs and substrates used to rest. We registered the insects associated with the egg sacs of 10 species of spiders, including the two previously mentioned. Only one species was not attacked by any natural enemy, the invasive spider *Cyrtophora citricola* (Araneidae), recently found in Brazil. All other nine species were attacked by some egg predator or parasitoids, such as wasps, diptera and mantispids. Finally, we describe a parasitoid wasp of the spiders attacking *C. citricola*, showing the use of a new host for a native wasp, but still without any records of predators or parasitoids attacking their egg sacs in the population studied. Many interactions between the spiders, their egg sacs and natural enemies were explored in this thesis, contributing to the understanding of the ecology and evolution of these animals.

Keywords: mortality factors; offspring survival; parasitoids wasps; Diptera; Mantispidae

INTRODUÇÃO GERAL

O cuidado parental é uma característica presente nos mais diversos grupos de animais, ocorrendo quando os pais são capazes de influenciar positivamente a sobrevivência de suas proles (Royle et al. 2012; Clutton-Brock 2019). Diversas formas de cuidado são observadas na natureza, desde a seleção de sítios para a oviposição, até a construção de ninhos e proteção dos ovos durante o seu desenvolvimento. Os pais nesses casos podem ser capazes de reduzir o comprometimento de seus ovos contra fatores bióticos ou abióticos de mortalidade. Essa forma de cuidado é bastante comum em anfíbios, por exemplo, podendo ser considerada uma forma simples de cuidado, que pode evoluir em taxa mais rápida que outros comportamentos mais complexos (Furness & Capellini 2019). Em situações de baixa sobrevivência da prole de animais na ausência dos pais, o cuidado parental deve surgir com maior frequência (Klug & Bonsall 2010) e comportamentos simples como a permanência dos pais em contato com a prole durante suas fases iniciais de desenvolvimento podem rapidamente ser selecionados e ter um impacto positivo no sucesso reprodutivo dos pais. Portanto, explorar as pressões seletivas que atuam sobre cada espécie, promovidas pelos seus principais agentes de mortalidade, pode ser essencial para entendermos a evolução e a manutenção do cuidado parental.

Muitas espécies podem ser capazes de diminuir o impacto de condições abióticas desfavoráveis, como umidade e temperatura, sobre a sobrevivência da sua prole por meio do cuidado parental. Em animais que carregam os seus ovos, os pais conseguem através da mudança de microhabitats aumentar a sobrevivência dos mesmos contra dessecação e alagamentos, por exemplo (Royle et al. 2012). No entanto, mesmo em animais que cuidam de seus ovos, mas que tem posições relativamente fixas no seu ambiente, os pais podem ser bastante eficientes em minimizar os efeitos de condições ambientais desfavoráveis. A presença de ao menos um adulto

guardando os ovos pode ser capaz de reduzir a mortalidade em condições de alta umidade, quando infecções por fungos podem ocorrer com frequência (e.g. Boos et al. 2014; Calbacho-Rosa et al. 2017). Além disso, a falta de umidade deve também afetar o desenvolvimento de ovos, e fêmeas de alguns animais são capazes de produzir ovos com maior resistência a seca nessas condições (e.g. Le Hesran et al. 2020). A dessecação é um fator de mortalidade importante, por exemplo, para ovos de sapos, que pode ser mitigado na presença de cuidado, como observado no caso da rã de vidro *Hyalinobatrachium orientale* (Lehtinen et al. 2014). No entanto, a vulnerabilidade dos ovos a essas condições deve estar relacionada à exposição dos mesmos ao seu ambiente externo. Portanto, linhas de defesa como o uso de refúgios, a produção de ovos de casca dura, e a produção de sacos de ovos, além do cuidado parental, podem ser importantes fatores que limitam o efeito negativo de condições ambientais desfavoráveis sobre os ovos de animais.

As pressões seletivas causadas por predadores e parasitoides, além de patógenos, também devem ser essenciais para a evolução do cuidado parental em animais. Para artrópodes em ambientes tropicais, onde essas interações bióticas são geralmente mais intensas, o benefício do cuidado na sobrevivência da prole tende a ser alto, mas somente quando acompanhado por linhas de defesa adicionais (Santos et al. 2017). Nesse contexto, a simples presença dos pais pode não ser suficiente para evitar a mortalidade contra certos predadores. Os ovos de aranhas, por exemplo, podem ser atacados por diversos predadores oportunistas como aves (e.g. Lockley & Young, 1993), formigas (e.g. Vieira & Romero 2008) e outras aranhas (e.g. Pasquet et al. 1997; Meira et al. 2021), além de predadores e parasitoides especializados como moscas (Gillung & Borkent 2017), mantispídeos (e.g. Redborg & Redborg 2000, Vieira & Romero 2008) e vespas (e.g. Gonzaga et al. 2015; Nascimento & Gonzaga 2016). Alguns desses inimigos naturais são capazes de atacar os ovos de forma eficiente mesmo na presença de cuidado parental e de linhas adicionais

de defesa, enquanto outros podem ter seu impacto bastante reduzido quando ambos estão presentes. A suspensão de ootecas em teias, por exemplo, é uma estratégia que pode ser eficiente contra formigas, mas não contra aves (Hieber 1992).

Em aranhas o cuidado parental é bastante diversificado e geralmente oferecido somente pelas mães (Yip & Rayor 2014), com raras exceções, nas quais ocorrem o cuidado paternal ou biparental (e.g. Moura et al. 2017). As fêmeas produzem ootecas que variam em complexidade estrutural, desde poucos fios de seda para simplesmente manter os ovos juntos (e.g. Pholcidae, Huber & Eberle 2021) até ootecas com múltiplas camadas de seda de diferentes tipos, que podem agir como barreiras contra inimigos naturais generalistas (Austin 1985). A coloração desses sacos de ovos também é bastante variável, mas em muitas espécies eles apresentam aparência amarronzada, o que pode diminuir a conspicuidade dos mesmos para potenciais predadores guiados pela visão (Barrantes et al. 2013). Algumas aranhas selecionam locais específicos para produzir e abandonar as suas ootecas, como em folhas (Fig. 1.A) e ramos da vegetação (Fig. 1.B), ou até mesmo em caules de árvores (obs. pessoal) e no solo junto a serra pilheira (e.g. da Ponte et al. 2021). Muitas outras espécies de aranhas guardam (Fig. 1.C) e/ou carregam (Fig. 1.D) as ootecas até a sua eclosão. Nesses casos, existe uma associação entre a fêmea e prole categorizada como subsocial transiente (Yip & Rayor 2014), na qual após a eclosão dos ovos ocorre por curto período pré-dispersão, geralmente apenas durante o primeiro ínstar fora da ooteca da prole (Fig. 1.E). Já nas espécies com comportamento subsocial, o cuidado parental é estendido e as mães podem viver associadas com a sua prole por vários ínstares (e.g. Gonzaga & Leiner 2012), ou até mesmo manter essa associação até a sua morte, quando seus filhotes podem se alimentar de seu corpo (Fig. 1.F), fenômeno conhecido como matrifagia. (e.g. Dias et al. 2021).

A eficiência do cuidado parental, ou seja, o impacto do cuidado na sobrevivência dos ovos,

já foi investigado para algumas espécies de aranhas que guardam as suas ootecas (e.g. Vieira & Romero 2008; Gonzaga & Leiner 2012; Zanatta et al. 2016; Moura et al. 2017). No entanto, pouco se sabe sobre a variação no tempo e no espaço nos fatores de mortalidade que afetam a sobrevivência da prole e como isso pode influenciar o cuidado parental oferecido. Entender o impacto de agentes de mortalidade das aranhas adultas no seu ambiente também se torna importante nesse contexto, já que a remoção dos pais pode ter um efeito significativo na sobrevivência dos ovos de algumas espécies (e.g. Zanatta et al. 2016, Moura et al. 2017). Ainda assim, as ootecas podem também ser consumidas juntamente com as aranhas adultas.

Uma das formas que aranhas e outros animais evoluíram para reduzir a predação foram estratégias visuais anti-predação. As principais formas de evitar a atenção de predadores visualmente orientados em aranhas são a camuflagem por correspondência de cor com o plano de fundo, a coloração disruptiva e o “masquerade” (disfarce), no qual animais aparentam ser objetos inanimados de seu ambiente (Robledo-Ospina & Rao 2022). Tanto os indivíduos jovens e adultos quanto as ootecas devem obter benefícios na sua sobrevivência por apresentarem essas estratégias, podendo atualmente ser testado se os seus predadores visualmente orientados são capazes de distinguir as suas cores das cores dos substratos que utilizam (e.g. Xavier et al. 2018). No entanto, os comportamentos podem ter um papel essencial na eficiência dessas estratégias, com algumas espécies sendo capazes de selecionar os seus planos de fundo, ou ajustar as suas posições corpóreas para se tornarem menos evidentes, por exemplo (Stevens & Ruxton, 2019). Ainda assim, a diversidade, frequência de ataque e o impacto dos diversos predadores de aranhas e de seus ovos ainda é pouco conhecida. No caso das aves, se elas não forem capazes de distinguir as cores entre as aranhas e suas ootecas com os seus substratos, é possível que o seu impacto seja bastante limitado, principalmente quando esses animais e seus ovos são recursos pouco valiosos, como

aranhas muito pequenas ou ootecas com poucos ovos. Formigas, por outro lado, podem localizar aranhas por meio de sinais químicos e não visuais, não sendo impactadas por estratégias de defesa visual. Ainda assim, o potencial de ataque por formigas em casos com barreiras mecânicas, como teias complexas, pode ser pequeno já que essas teias devem limitar o seu acesso às aranhas e/ou às suas ootecas, caso estejam suspensas.

Já no caso de predadores e parasitoides especializados em consumir aranhas ou seus ovos, essas e outras estratégias de defesa devem ter um impacto limitado ou até ausente na sobrevivência das aranhas. Diversos insetos são agentes de mortalidade para aranhas e seus ovos, como os mantispídeos (Neuroptera: Mantispidae). Em suas fases larvais esses insetos são predadores de ovos de aranhas, podendo penetrar ootecas diretamente ou se associarem as fêmeas até o momento da produção das ootecas, quando eles se movem para dentro das mesmas (Redborg 1998). Moscas também são inimigos naturais de ovos de aranhas, e em alguns casos de juvenis e adultos, com algumas famílias de Diptera podendo apresentar esses hábitos. No entanto, para a maior parte das espécies que atacam ootecas ainda não se sabe se as suas larvas atuam como predadores ou parasitoides de ovos (Gillung & Borkent 2017). Por fim, uma grande diversidade de vespas de várias famílias atacam aranhas e seus ovos. No caso de vespas associadas as ootecas de aranhas, elas podem atuar como predadores (e.g. Nascimento & Gonzaga 2016) e parasitoides de ovos (e.g. Gonzaga et al. 2015), ou até mesmo hiperparasitoides, atacando outros insetos que se alimentam dos ovos das aranhas (e.g. Miranda et al. 2020). Já no caso de vespas que atacam as próprias aranhas e não os seus ovos, algumas são caçadoras (e.g. Sphecidae), paralisando e capturando indivíduos jovens e adultos e os levando para seus ninhos onde suas larvas se alimentam dos mesmos (e.g. Gonzaga & Vasconcellos-Neto 2005). Outras vespas paralisam aranhas em suas próprias teias e depositam os seus ovos nas mesmas, os quais se desenvolvem se alimentando do

corpo de seus hospedeiros (e.g. Gonzaga & Sobczak 2007).

O primeiro passo para se entender o impacto dos agentes de mortalidade oportunistas e especializados na sobrevivência de aranhas e de seus ovos é investigar qual a frequência de ataque nos indivíduos e nas ootecas em seu ambiente natural. Além disso, a especificidade das interações entre predadores, parasitoides e as aranhas também deve ser explorada. Até o momento muitas interações com parasitoides foram descritas com somente uma espécie hospedeira, mas é possível que os mesmos utilizem várias espécies de aranhas como hospedeiro. Portanto, trabalhos que descrevem esses tipos de interações são importantes, já que para a maior parte das espécies de aranhas essas interações ainda não foram investigadas. Ao mesmo tempo, variações populacionais e temporais nas frequências de ataque dentro de uma única espécie podem ser relevantes e influenciar na história de vida das aranhas em diferentes contextos. Nas espécies com cuidado parental oferecido a suas ootecas, essas variações também podem influenciar na duração do cuidado, na sobrevivência da prole e nos benefícios obtidos pela guarda de ovos.

Nesse contexto, a presente tese teve como objetivo investigar questões relacionadas a ecologia do cuidado parental em espécies de aranhas construtoras de teias da família Araneidae e Uloboridae. No primeiro capítulo, testamos a eficiência do cuidado parental em uma espécie de aranha com cuidado biparental no Cerrado, *Manogea porracae* (Araneidae), descrevendo os agentes de mortalidade para os seus ovos, os papéis do cuidado e comparamos a eficiência do cuidado parental com uma população previamente investigada, mas em um ambiente homogêneo de produção de Eucalipto. No segundo capítulo descrevemos o cuidado maternal, os parasitoides associados às ootecas, e as estratégias visuais anti-predação na espécie de aranha, *Miagrammopes unipus* (Uloboridae). Testamos a correspondência de cor com o seu plano de fundo (substrato) sob a perspectiva de predadores visualmente orientados através de uma análise de modelagem visual.

No terceiro capítulo investigamos qual a diversidade de insetos que são encontrados associados as ootecas de 10 espécies de aranhas em três áreas no estado de Minas Gerais. Nas espécies amostradas duas vezes também comparamos as frequências entre amostras e discutimos a respeito da variação encontrada. No quarto capítulo descrevemos a inclusão de uma nova aranha hospedeira para a vespa parasitoide *Hymenoepimecis bicolor* que ataca aranhas construtoras de teias. A aranha *C. citricola* é uma espécie introduzida a pouco tempo no Brasil, e esse novo registro demonstra que parasitoides podem ser capazes de incluir novas espécies como hospedeiros mesmo após um tempo relativamente curto de exposição aos mesmos. No entanto, predadores ou parasitoides de ovos não foram encontrados até o momento. Por fim, nos quatro capítulos dessa tese a diversidade de predadores e/ou parasitoides de aranhas e de seus ovos é investigada com diversas novas interações sendo registradas pela primeira vez.

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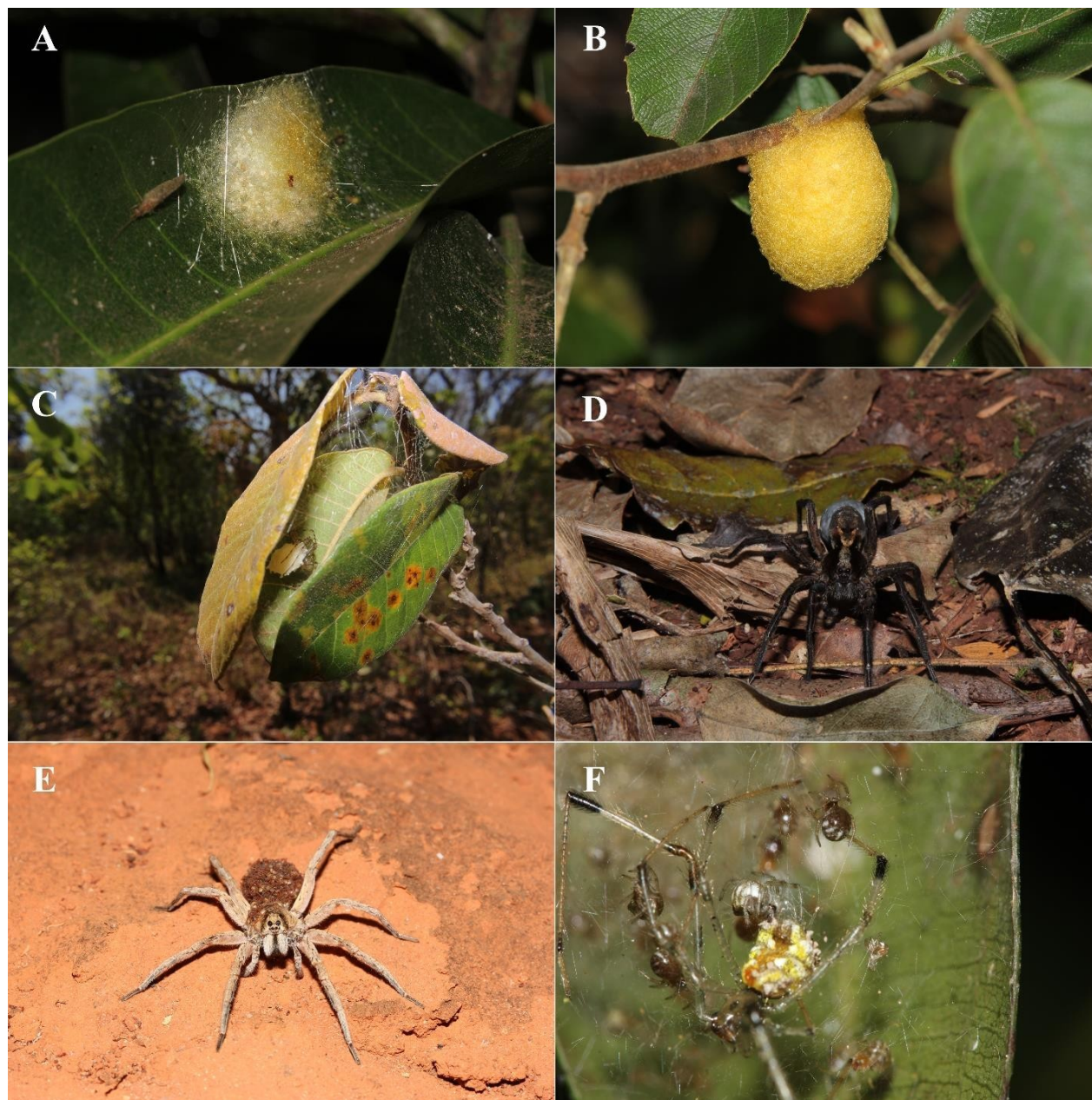
Figuras

Fig. 1. Ootecas e cuidado maternal em aranhas. A e B. Ootecas abandonadas na vegetação (Araneidae). C. Fêmea guardando sua ooteca em seu refúgio (Oxyopidae). D. Fêmea carregando sua ooteca (Lycosidae). E. Fêmea carregando seus filhotes recém eclodidos (Lycosidae). F. Fêmea morta sendo atacada por seus filhotes (Theridiidae).

Capítulo 1

Offspring mortality factors and parental care efficiency of the spider *Manogea porracea* (Araneidae) in the Brazilian savanna



Abstract

The expressions of parental care can vary in space and time because factors driving offspring mortality depend on variable abiotic and biotic conditions. *Manogea porracea* is a spider that exhibits biparental care and widespread distribution in the Neotropical region. Parents can protect their offspring against abiotic conditions that may damage their webs, or compromise their egg sacs, and against araneophagic spiders that prey on their eggs. However, the current knowledge of the parental care in the species is restricted to a population located in areas covered by *Eucalyptus* plantations. This study investigates parental care efficiency in a new ecological context, a protected area of Brazilian savanna. We described the substrates in which *M. porracea* built webs and the agents of egg mortality. We also performed a parent removal experiment to test the effect of parental care on the integrity of nursery webs, offspring survival, and the presence of egg predators. Spiders built webs at variable heights and many distinct substrates but were mainly associated with the bromeliad *Ananas ananassoides*. We found two previously reported egg predators, *Faiditus caudatus* and *Argyrodes elevatus* (Araneae Theridiidae), and a new one emerging from the egg sacs, the insect *Zeugomantispa virescens* (Neuroptera Mantispidae). The presence of parents in the webs of *M. porracea* prevented the webs from collapsing and effectively increased offspring survival. However, the occurrence of egg predators was not reduced by the presence of parents. Overall, parental care increased offspring survival in both populations, but variation in biotic and abiotic factors between areas influenced offspring mortality and the efficiency of parental behaviors. Our results emphasize the importance of investigating multiple populations in behavioral studies of parental care.

Keywords: microhabitat selection, web integrity, egg guarding, egg attendance, population variation

INTRODUCTION

Parental care is a set of behaviors adopted by parents that increase offspring fitness (Royle et al. 2012). It can be expected to evolve when life history conditions favor it, such as scenarios of high egg death rate and low juvenile survival in the absence of care (Klug & Bonsall 2010). Females usually invest more in parental activities to increase their fitness, while males tend to benefit more from investing in mating with multiple partners (conventional sex roles; Janicke et al. 2016) but this relationship might be different in species with reversed sex roles (e.g. Jones et al. 2000). The view of sexual selection as a process shaping categorical sex roles has been challenged recently (Roughgarden 2015; Shuker & Kvarnemo 2021), mainly because females can benefit from multiple copulations; thus, increasing the intensity of sexual selection in polyandrous females (Winkler et al. 2021). Males can also benefit by being the lone caregivers to their offspring in two scenarios: (1) when exclusive paternal care increases the attraction of mates and, consequently, the success of male fertilization (e.g. Machado et al. 2004; Buzatto & Machado 2009); and (2) when parental care reduces mating success, but new mating opportunities are scarce, and offspring survival is reduced in the absence of the parent (e.g. Ringler et al. 2015; Moura et al. 2017). Thus, biparental care or exclusive male care can evolve.

Parental care may vary depending on biotic and abiotic factors both between and within species. The biotic factors can be separated into social components, such as the period of association between the siblings and/or their parents, and nonsocial ones, such as the impact of predators, parasitoids, and pathogens (Royle et al. 2014; Santos et al. 2017). An example of social components is observed in the burying beetle *Nicrophorus vespilloides* (Silphidae). The time that males spend providing parental care depends on the presence of females, because, when females are absent, males increase parental investment (Royle et al. 2014). In an example of the effects of

nonsocial components, long-tailed sun skink females, *Eutropis longicaudata* (Squamata Scincidae), exhibited parental care under the experimental condition of high predation risk. However, they usually experience low predation risk under non-experimental conditions and do not provide parental protection (Huang et al. 2013; Pike et al. 2016). The females of *Phymaturus spectabilis* (Squamata Liolaemidae) can also change their behaviors in response to the type of predator, exhibiting different aggressive displays for distinct predator species during offspring protection (Cabezas-Cartes et al. 2018). Distinct biotic and abiotic factors may determine different parental behavioral strategies; thus, parental care often varies depending on the ecological context (e.g. Badyaev & Ghalambor 2001; Barbasch & Buston 2018; Barbasch et al. 2020).

The effectiveness of parental care in increasing offspring survival can also change according to seasonal and annual variation in abiotic factors. For example, in the grass frog *Hyalinobatrachium orientale* (Centrolenidae), the experimental removal of males showed that offspring survival decreased in the dry season, mainly due to desiccation, and in the wet season, due to both desiccation and predation (Lehtinen et al. 2014). In the case of extreme drought, however, parental care in frogs can become irrelevant since parents may not protect their offspring from it (Rocha et al. 2021). Hence, the effects of parental care on increasing offspring survival may vary in different ecological contexts. Thus, studies exploring the variation of parental care at different spatial and temporal scales can improve our understanding of factors that influence offspring mortality and the efficiency of parental care in animals.

For arthropods, mortality of unprotected offspring and the benefits of parental care are higher in tropical than in temperate regions (Santos et al. 2017). Therefore, spatial scales have important effects on the efficiency of parental care and on the variation of offspring mortality, which may be greater in species with a wide distribution within tropical regions, because they may

face different biotic and abiotic factors. Beyond offspring attendance, additional lines of defense provided by parents further reduced offspring mortality against natural enemies in tropical regions (Santos et al. 2017). In spiders, females usually offer different types of parental protection for their offspring in addition to patrolling (Yip & Rayor 2014). The mothers can produce some kinds of silk structures (i.e. egg sacs), varying from just a few threads binding eggs together to multiple layers of silk, that can act as a barrier against egg predators and parasitoids (Austin 1985; Hallas & Jackson 1986). Females can guard the egg sacs and, in some cases, remain in contact with their offspring (for a short or long interval), interacting aggressively against predators (Gonzaga & Leiner 2013; Yip & Rayor 2014). They can also carry the egg sacs with their chelicerae, attach them to their spinnerets (Barth 2002), or suspend the egg sacs on nursery webs (Hieber 1992). However, even within the silk layers of egg sacs, spider's eggs can still be preyed upon and/or parasitized. Araneophagic spider species (Meira et al. 2021), wasps (Finch 2005; Moura et al. 2021), a few families of Diptera (Gillung & Borkent 2017), and the Mantispidae family of Neuroptera (Berti-Filho et al. 2002) are among the primary sources of egg mortality in spiders. In addition, during parental care, adult spiders can also be attacked by their own predators, such as araneophagic spiders (Meira et al. 2021), parasitoids (Gonzaga et al. 2015), and hunting wasps (Gonzaga & Vasconcellos-Neto 2005; Moura et al. 2019a). These adult mortality agents may, ultimately, affect the duration of offspring attendance and, consequently, increase the mortality of offspring.

Manogea porracea (C.L. Koch 1838) (Araneidae) is the only solitary spider with biparental care known to date (Moura et al. 2017). In the absence of females, males may care for the offspring, repair the web structure, reduce moisture after rains and protect the egg sacs against araneophagic spider species (Moura et al. 2017, 2019b). These araneophagic predators can also attack and prey

on *M. porracea* adults, steal prey from their webs, or steal egg sacs to prey on the eggs, but parents are also capable of attacking and consuming these potential predators (Moura et al. 2017). There is no synergetic effect of biparental care as just the action of the parent male or the parent female is enough to increase offspring survival at similar levels to that observed when both parents are present (Moura et al. 2017). *M. porracea* can be found from Argentina to Panama (Levi 1997). However, all studies on parental behaviors of *M. porracea* were conducted in a single population located on a *Eucalyptus* plantation in Southeastern Brazil (Moura et al. 2017, 2019b, 2020a, 2021). In a heterogeneous area covered by native vegetation, adults may experience many different abiotic and biotic conditions, such as distinct habitats to build their webs and different species of predators and parasitoids. These variable conditions between areas may influence the efficiency of parental care. For example, canopy coverage in the *Eucalyptus* plantation is the same for most webs, similarly exposed to rain, wind, and light. Furthermore, the webs are usually located in different trees, 3 m apart from each other, typically close to the ground (Moura et al. 2017). For predators and parasitoids, the uniform distribution of trees may facilitate host web location and the migration to other webs after consuming *M. porracea* adults or their eggs. In an area with native vegetation, the composition and impact of predators and parasitoids may be distinct and could affect offspring survival and the efficiency of parental care in different ways compared to the population found in the *Eucalyptus* plantation.

In this study, we described the substrates in which *M. porracea* build webs on a protected area of Brazilian savanna to compare with those used in the *Eucalyptus* plantation. As the Brazilian savanna presents a great habitat heterogeneity, ranging from grasslands to forest formations, the variation in biotic and abiotic factors may influence the integrity of the webs and the efficiency of parental care. We then investigated the sources of egg mortality and the efficiency of parental care.

We hypothesized that parents could maintain web integrity and reduce offspring mortality during egg development in this population. Thus, we experimentally manipulated the presence of parents on the web to test the predictions that (1) the number of intact nursery webs and (2) the number of spiderlings that hatched per egg sac during the experiment will be higher in the presence of parents, and (3) the number of predators on the webs, or within egg sacs, will be higher in the absence of parents.

MATERIAL AND METHODS

Study area

We performed the data collection in the “Estação Ecológica de Pirapitinga” (EEP; 18°21'18"S, 45°19'19"W), a federal conservation unit situated close to the city of Três Marias, in the State of Minas Gerais, Brazil. The reserve is a fluvial peninsula within the Três Marias dam reservoir, at the upper part of the São Francisco river. Still, it can become an island depending on the water level. The EEP has an area of 1,384 ha and is accessed only by researchers and students during educational programs. The reserve is composed of Brazilian savanna (Cerrado), with heterogeneous habitats ranging from grasslands to forest formations. The climate is Aw in Köppen's classification, with rainy summers and dry winters (Alvares et al. 2013). Major fires have not been registered in the area for a few decades, allowing researchers to explore an environment without the impact of fire, which is rare for most Cerrado areas.

Study species

Manogea porracea is a spider that builds dome-shaped orb webs within a tridimensional structure and provides parental care for its egg sacs and spiderlings (Fig. 1A; Moura et al. 2017).

This species produces multiple egg sacs during their reproductive period. Their webs can be found in the field with up to seven egg sacs (A. Quero pers. obs.). Populations of *M. porracea* are present from Argentina to Panama in many different environments (Levi 1997), and their reproductive behaviors (Moura & Gonzaga 2017; Moura et al. 2020b) and parental care (Moura et al. 2017, 2019b, 2020a, 2021) were previously investigated in a population found in a *Eucalyptus* plantation situated in Estrela do Sul, MG, Brazil.

Data collection and experimental design

We conducted field observations between November and December 2018, 2019, and 2020. During the expeditions, we searched and marked webs close to the trails in the EEP. We recorded the height of webs (from the center of the webs to the ground), the type of substrate in which spiders built them, and the number of males, females, egg sacs, spiderlings, and araneophagic spiders. In 2019, we performed the parental removal experiment to evaluate the effect of parental care on the integrity of the webs, the loss of egg sacs, the presence of egg predator spiders, and offspring survival. We marked 30 webs with one or both parents with at least one egg sac for the experiment, separating 15 webs for the group with parental care (control with both parents, so parental care would still be provided if one parent died during the experiment) and 15 webs for the group without care (a treatment where parents would be removed). We selected webs with a similar number of egg sacs between both groups (Mann-Whitney test: $U = 98.5$, $n = 30$, $P = 0.560$). The total number of egg sacs found on the 30 webs was 67 (control = 32, treatment = 35), and webs contained from one to four egg sacs.

We carefully removed the parents from the webs of the group without parental care, avoiding damage to the support threads of their webs. We gently touched the web with sticks found

in its surroundings, inducing each individual to move from its resting position to the periphery of the horizontal sheet so that we could capture it using a 50 mL tube. We repeated this procedure for the group with parental care but allowed the spiders to return to the resting position without being captured. We conducted this experiment for 9 days and checked the webs every 3 days to record web integrity, the number of egg sacs, parents, and araneophagic spiders that prey on *M. porracea* eggs. Web integrity was defined as a condition in which the web has most of its tridimensional structure and can maintain the suspension of the egg sacs. We considered that the webs collapsed when they lost most of their three-dimensional structure and, therefore, are unable to maintain the suspension of the egg sacs.

After 9 days from the beginning of the experiment, we collected each egg sac from the remaining webs and isolated each one in a test tube closed by a net. We maintained the egg sacs in the tubes for up to 30 days and observed every 3 days if eggs or predators hatched. After this period, we opened all the egg sacs to count the number of spiderlings and natural enemies that remained inside them. We defined clutch size as the total number of emerged spiderlings and those fully developed within the egg sacs. We excluded from the analysis the egg sacs that were empty, meaning that spiderlings, or predators, emerged before the collection. For the analysis of offspring survival, we also excluded any new egg sacs laid by females in the control group during the experiment and egg sacs whose oviposition order could not be traced back based on hatch date in webs that contained multiple egg sacs.

Statistical analysis

We conducted field observations between November and December 2018, 2019, and 2020. During the expeditions, we searched and marked webs close to the trails in the EEP. We recorded

the height of webs (from the center of the webs to the ground), the type of substrate in which spiders built them, and the number of males, females, egg sacs, spiderlings, and araneophagic spiders. In 2019, we performed the parental removal experiment to evaluate the effect of parental care on the integrity of the webs, the loss of egg sacs, the presence of egg predator spiders, and offspring survival. We marked 30 webs with one or both parents with at least one egg sac for the experiment, separating 15 webs for the group with parental care (control with both parents, so parental care would still be provided if one parent died during the experiment) and 15 webs for the group without care (a treatment where parents would be removed). We selected webs with a similar number of egg sacs between both groups (Mann-Whitney test: $U = 98.5$, $n = 30$, $P = 0.560$). The total number of egg sacs found on the 30 webs was 67 (control = 32, treatment = 35), and webs contained from one to four egg sacs.

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RESULTS

Habitat features

Manogea porracea individuals (Fig. 1A) built their webs at an average height of 59.83 ± 5.09 cm [18–171 cm] ($\bar{X} \pm \text{SD}$ [min.–max.]) and were associated with distinct types of substrates (Fig. 2; $n = 79$ webs). The spiders used grasses, shrubs, and trees (both dead and alive) to construct their webs. Exposure to the sun varied between webs occupying areas with distinct canopy cover. Webs were more often found in some substrates than others ($\chi^2 = 184.05$, $\text{df} = 8$, $P < 0.001$). The most common substrates used were *Ananas ananassoides* (Bromeliaceae), a pineapple plant (Fig. 1B), and *Miconia albicans* (Melastomataceae). Both of these plant species are native to the Cerrado. Webs associated with tougher substrates, like *A. ananassoides*, moved less due to winds than webs associated with more unstable substrates, such as grasses.

Web integrity

There was no interaction between the presence of the parents and substrate types in our model (interaction: $\chi^2 = 1.130$, $df = 1$, $P = 0.288$; parental care: $\chi^2 = 5.661$, $d.f. = 1$, $P = 0.017$). Since the model with the interaction term ($AICc = 36.53$) was equivalent to the model without it ($AICc = 34.98$), we removed the interaction and estimated parameters for the most parsimonious model. Webs were less likely to collapse in the presence of the adults than webs without any parent ($\chi^2 = 6.686$, $df = 1$, $P = 0.010$). However, the frequencies of webs that collapsed or maintained their integrity in the pineapple plant *A. ananassoides* and other types of substrates were not different ($\chi^2 = 0.015$, $df = 1$, $P = 0.901$). Only one web collapsed in the presence of parents (6.6%), losing all of its egg sacs. The other 14 webs with parental care were intact. In the group without parents, seven webs collapsed (46.6%), while eight webs remained functional. Two of these seven collapsed webs without parents still had one egg sac present, but they lost most of the tridimensional structure that allows the suspension of the egg sacs. In the other five collapsed webs without parents, all the egg sacs were lost and the webs collapsed.

Predator species

Two araneophagic spider species consumed *M. porracea* eggs outside the egg sacs (hereafter, exogenous predator), while one insect species consumed eggs from inside the egg sacs (hereafter, endogenous predator). *Argyrodes elevatus* (Taczanowski 1873) and *Faiditus caudatus* (Taczanowski 1874) (Theridiidae; Fig. 1C) were the exogenous predators. *A. elevatus* was found in three webs and *F. caudatus* in four webs. Both species never occurred together in the same web, but two *F. caudatus* were found twice occupying the same web. The Neotropical insect

Zeugomantispa virescens (Rambur 1842) (Neuroptera Mantispidae) was the endogenous predator (Fig. 1D).

The frequency of the exogenous predators (araneophagic Theridiidae spiders; Fig. 1C) was not associated with the presence of parents ($\chi^2 = 1.677$, $df = 1$, $P = 0.396$), and their abundance was generally low, as we found these predators in only five webs without parents (33.3%) and two webs with parents (13.3%). However, they were more likely to occur on collapsed webs than on intact ones ($\chi^2 = 9.354$, $df = 1$, $P < 0.01$). Out of the eight collapsed webs, five of them had these exogenous predators (62.5%). Meanwhile, only two out of 22 intact webs (9.1%) had predator spiders associated with them.

The endogenous egg predator *Z. virescens* (adult insect: Fig. 3A; larvae: Fig. 3B) occurred in 10 (14.5%) of the 69 egg sacs. The insect emerged from four out of 15 egg sacs under no care (26.6%) and from six out of 54 egg sacs under parental care (11.1%). The presence of parents in the webs did not reduce the number of these egg predators ($\beta_1 = 1.47$, 95% CI = - 0.42 to 4.48, $\chi^2 = 1.34$, $df = 1$, $P = 0.25$, Fig. 4), but their number increased with the number of egg sacs ($\beta_1 = 0.57$, 95% CI = 0.08 to 1.34, $\chi^2 = 4.31$, $df = 1$, $P = 0.04$). In all the egg sacs attacked by this egg predator, no spiderlings survived as they preyed on all eggs present.

Offspring survival

The total number of egg sacs found in the 24 webs that made it through the 9 experimental days, with at least one egg sac remaining in the web, changed both in the presence and absence of parental care. All of the 14 remaining webs with parents increased from one to four new egg sacs, and 11 of them still had both parents, while the male was missing in three webs. Of the 10 remaining webs without parents, six still had the same number of egg sacs, while four had lost

from one to two egg sacs compared to the beginning of the experiment. In the statistical analysis, the final number of egg sacs used was 29, of which 15 were under no care and 14 were under parental care.

The presence of parents in the webs increased offspring survival by 6.7 times compared to the group where parents were removed ($\chi^2 = 11.182$, $df = 1$, $P < 0.001$; Fig. 5). The average number of spiderlings per egg sac with parents was 15.4 ± 10.3 [0–32], while only 2.3 ± 3.4 [0–8] spiderlings emerged in the group without parents. Furthermore, fully developed spiderlings were found both outside and inside the egg sacs under parental care, but no spiderlings were located outside the egg sacs in absence of parental care. All eggs from egg sacs under parental care hatched, except when attacked by *Z. virescens*. Meanwhile, all egg sacs in the treatment group had eggs that did not hatch.

DISCUSSION

Variation in parental care efficiency between populations is still poorly studied. The benefits of parental care, as well as its effectiveness, might be higher in “harsh environments” because offspring require more effort from parents (Wilson 1975). On a macroecological scale, there is evidence that the benefits of parental care for arthropod species is higher in the tropical region, where biotic interactions with natural enemies are likely to be more intense and additional lines of defense are essential to increase offspring survival (Santos et al. 2017). Predation pressure in particular can be an important driver in this process, and parents can adjust their protective behaviors depending on the presence of different predator species (Huang et al. 2013; Pike et al. 2016; Cabezas-Cartes et al. 2018). Abiotic factors can also have strong impacts on offspring survival, such as egg desiccation (Lehtinen et al. 2014) and excess humidity, which can favor

fungus infections (Calbacho-Rosa et al. 2017). In this study, we quantified offspring mortality and the efficiency of parental care in a new population of *M. porracea*. For the first time, we recorded a new natural enemy inside the egg sacs, the endogenous egg predator *Z. virescens*. Overall, parents increased offspring survival, but did not reduce the presence of predators in the webs or egg sacs. Even in this scenario, the efficiency of parental care was higher than in the previously studied population (Moura et al. 2017). The damages to the nursery webs of *M. porracea* were effectively repaired by parents in both populations (Moura et al. 2017). Variation in both biotic and abiotic factors between *M. porracea* populations and the period in which experiments were performed in both populations may explain the differences in the magnitude of the efficiency of parental care. We discuss these differences in more detail in the following paragraphs.

Although *M. porracea* has a widespread distribution in the Neotropics (Levi 1997), few details about the environmental conditions and natural enemies that influence offspring survival in distinct populations are known. All the studies on its biology have been conducted in a *Eucalyptus* plantation in Southeastern Brazil (Moura & Gonzaga 2017; Moura et al. 2017, 2019b, 2020a, 2020b, 2021). In the study area, canopy coverage varied significantly from open to forest areas with some canopy cover. This great heterogeneity is usually found in the Cerrado biome. Therefore, the webs were exposed to more variable conditions of light, wind, and rain exposure. They were built, on average, almost 60 cm from the ground and were built mainly on one plant species: *A. ananassoides* (Bromeliaceae). This pineapple plant seems to provide good stability for attaching the web supporting threads. In the previously studied population, *M. porracea* built their webs closer to the ground, using branches of *Eucalyptus* and litter to attach supporting threads (Moura et al. 2017). Overall, even in a more variable environment with an additional egg predator species, parents efficiently increased offspring survival.

The presence of adults assured the integrity of most webs in this population, similarly to what was observed in webs attached to *Eucalyptus* branches in the other population (Moura et al. 2017). Almost half of the nursery webs without parents collapsed (47.7%), a proportion similar to that found in the *Eucalyptus* plantation (46.6%; Moura et al. 2017). Meanwhile, only a single web collapsed when adults were present, likely due to the fall of a branch during a storm. More stable substrates may also reduce the damage caused to the webs due to wind and rain. However, the type of substrate did not influence the integrity of the webs in our field experiment. Since we observed webs only during the experiment (9 days) and the breeding season can last 2 to 4 months, the type of substrate may still influence the integrity of the webs, but against moderate damage and not against more intense events, such as storms and the fall of branches. During the experiment some storms that occurred may have caused webs to collapse independent of the stability of its substrates. Therefore, *M. porracea* adults can efficiently repair most gradual damage to their nursery webs in both environments studied so far, the homogeneous *Eucalyptus* plantations and the heterogeneous Brazilian savanna. This is important because intact tridimensional webs keep the egg sacs suspended in silk, which can limit the access of potential egg predators, such as ants (e.g. Hieber 1992), and can also protect the parents from their predators, such as hunting wasps (e.g. Blackledge et al. 2003). In addition to web maintenance, egg attendance in *M. porracea* could protect egg sacs against excessive moisture after heavy rain. The specific ways in which moisture can influence egg development, potential fungal infections, and the effects of parental care on this process still need to be evaluated.

In the webs of *M. porracea* and other spiders, the two exogenous egg predators, *F. caudatus* and *A. elevatus*, usually behave as kleptoparasites, stealing prey from their hosts (Whitehouse et al. 2002; Su & Smith 2014; Moura et al. 2017). However, Meira et al. (2021) showed that the

consumption of egg sacs and hosts by these invaders is probably also frequent, as their isotopic signature is similar to that of other araneophagic spiders. Egg predation by *Argyrodes* was also observed on webs of *Cyrtophora citricola* (Araneidae). This invader species attacks egg sacs when host females are absent, with eggs becoming their primary food source even though they continue capturing prey items intercepted by the webs (Pasquet et al. 1997). In this study, the incidence of the exogenous egg predators *F. caudatus* and *A. elevatus* was similar in webs with and without parental care while in the *Eucalyptus* population they were more likely to be found on webs without parents, or with just the male, when compared to those webs with both parents present (Moura et al. 2017). Even so, these araneophagic spiders might adjust their strategy according to the presence of *M. porracea* adults. If parents are on the web, they might focus on stealing prey. When parents are absent, however, they might consume the egg sacs as there are no risks associated with this behavior. We also found egg predators on collapsed webs (without parents) more often than in intact ones. Eventually, all webs without egg attendance collapse, increasing the chances of the egg sacs being exposed to potential predators.

Parental care in *M. porracea* has a significant impact on offspring survival, as egg sacs from webs in which parents were present had on average 6.7 times more spiderlings than webs from which parents were removed. In the *Eucalyptus* plantation, about 2.8 times more spiderlings per egg sac were found in the webs with parental care than webs without parents (Moura et al. 2017). Even though parental care is efficient in both populations, the higher efficiency in the newly studied area is probably due to a greater variation in the number of offspring between groups. Fewer spiderlings survived on egg sacs from webs without parents as many eggs stopped developing. Meanwhile, all eggs from webs with parental care developed into spiders or were preyed upon. The lower survival on webs without parental care was potentially due to excessive

humidity on the egg sacs after rainy days before their collection. Differences in the time of the year when experiments were conducted in each population may also have played a role, as more rain falls in November than in April. The parental action of adult spiders removing the excess moisture from egg sacs could be capable of preventing egg mortality. We found no sign of fungal infections on the eggs of *M. porracea*, likely due to the silk on the egg sacs acting as an effective barrier between the eggs and the outside. On other spiders with eggs more exposed to the outside, parental action might also prevent fungal infections in scenarios of high humidity (e.g. Calbacho-Rosa et al. 2017). In the group with parental care, the parents may have removed water from the egg sacs before their collection, while the egg sacs on webs without parents would depend on favorable weather conditions to dry the water around them. At the same time, the presence of the exclusive predator *Z. virescens* decreases offspring survival as they consume all eggs in the egg sacs they are present.

The insect *Z. virescens* occurs in all regions of Brazil (Machado & Rafael 2010) and its host records indicate a preference for web-building spiders, especially in the family Araneidae (Snyman et al. 2020). Here, we recorded a new interaction, with *M. porracea*. These egg predators were found in multiple egg sacs and the presence of parents in the webs did not affect the presence of *Z. virescens* on their egg sacs. Parental care in *M. porracea* might not be efficient in reducing the occurrence of these specialized egg predators on their egg sacs, as observed in other species of spiders from different families (e.g. Fink 1986; Vieira & Romero 2008). Their first instar larvae can adopt two strategies: direct penetration of the egg sac or spider boarding. Some species are specialists, adopting an obligate strategy, while others can employ both strategies depending on what their larvae find first (Redborg 1998). Adult *M. porracea* females were never seen in the field with Mantispidae larvae attached to them, which could indicate that *Z. virescens* larvae penetrate

the egg sacs directly, without boarding on the adult spiders. However, in the spider *Psecas chapoda* (Salticidae), also attacked by *Z. virescens*, no evidence was found of holes in the egg sacs that correspond to the Mantispidae entrance, raising the possibility of a boarding life cycle for this insect (Vieira & Romero 2008). The presence of holes on *M. porracea* egg sacs and their possible association with Mantispidae larvae need to be investigated. Still, *Z. virescens* larvae were not yet found on spiders in the field and seem to have no interest in spiders in confined spaces, indicating the possibility of them being obligate egg sacs penetrators (Snyman et al. 2020). In spiders that produce hundreds of eggs, the impact of these insects per egg sac might be small, as many spiderlings might still hatch (Berti-Filho et al. 2002). However, this is not the case with *M. porracea*. The Mantispidae predator consumed all eggs within the egg sacs probably because they are nearly sufficient to provide the energy necessary for completing their development. Nevertheless, *M. porracea* females produce multiple egg sacs, up to seven in the studied area, which likely decreases the overall impact of these egg predators on spider fitness and at a population level.

With the natural variation in biotic factors acting on different populations of a widespread species such as *M. porracea*, the ecological context of parental care might be dynamic, and its benefits may vary between regions (Santos et al. 2017). The exogenous egg predators, *F. caudatus* and *A. elevatus*, were found in both populations studied so far and might be associated with *M. porracea* throughout their distribution. On the other hand, the Mantispidae egg predator *Z. virescens* was unique to the Brazilian savanna population and might not be present or might be very rare in the *Eucalyptus* population. As previously discussed, our results suggest that *M. porracea* adults are ineffective in guarding their egg sacs against *Z. virescens*. Unlike araneophagic spiders and generalist predators that can be a constant threat to the egg sacs, Mantispidae larvae

could only require a single interaction with the egg sacs to efficiently penetrate and remain concealed within. Instead of preventing the access of these specialized predators, perhaps the best strategy to minimize the impact on parental fitness is to divide egg production into several egg sacs with variable clutch sizes (i.e. bet-hedging strategy; Marshall et al. 2008). In the spider *Philodromus vulgaris* (Philodromidae), their first and larger egg sacs were often attacked by the Mantispidae *Mantispa uhleri*. However, their second smaller egg sacs were never attacked by these predators. The construction of a second egg sac in this spider could be a strategy that might have evolved, at least in part, to decrease the impact of these egg predators (Redborg & Redborg 2000). In another example, *Latrodectus geometricus* (Theridiidae) females usually produce several egg sacs, and those with fewer eggs are generally more attacked by wasps (Moura et al. 2021). Additional studies are still necessary to investigate the susceptibility of webs and egg sacs of *M. porracea* containing different clutch sizes to attacks by *Z. virescens* and other predators.

In conclusion, we showed that different populations of *M. porracea* can experience distinct ecological contexts with variable biotic and abiotic conditions that could affect parental care and offspring survival. Even so, parents efficiently maintained the integrity of webs and increased offspring survival in the Brazilian savanna population, as observed in the *Eucalyptus* plantation. The presence of *Z. virescens* represents a novel interaction found in the studied population. It indicates that, in other areas, the composition, abundance, and impact of egg predators may vary. This variation might represent an important aspect determining particularities in protective behaviors and could lead to different outcomes for parental care in distinct populations.

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Figures



Fig. 1. (A) A web of *Manogea porracea* with a male (upper arrow), two egg sacs (middle arrow) and a female (lower arrow). (B) The habitat of the spiders showing the most commonly used plants for web construction *Ananas ananassoides* and *Miconia albicans*. (C) A *Faiditus caudatus* female, an exogenous egg predator, with an egg sac that it was preying upon. (D) A adult *Zeugomantispa virescens*, an endogenous egg predator recently emerged from the attacked egg sac. Scales: 5 mm.

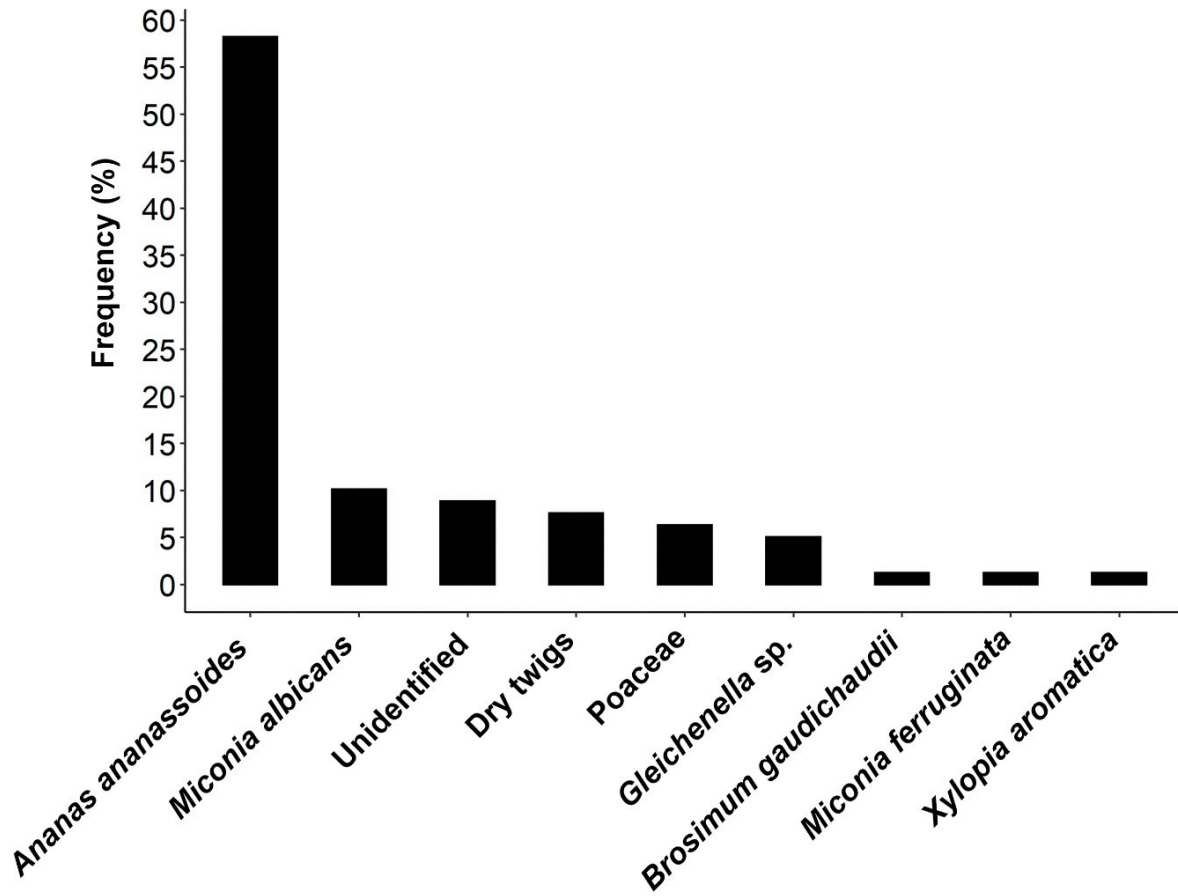


Fig. 2. Frequency of substrates used by *M. porracea* females to build and anchor their webs in the field (n = 79 webs).

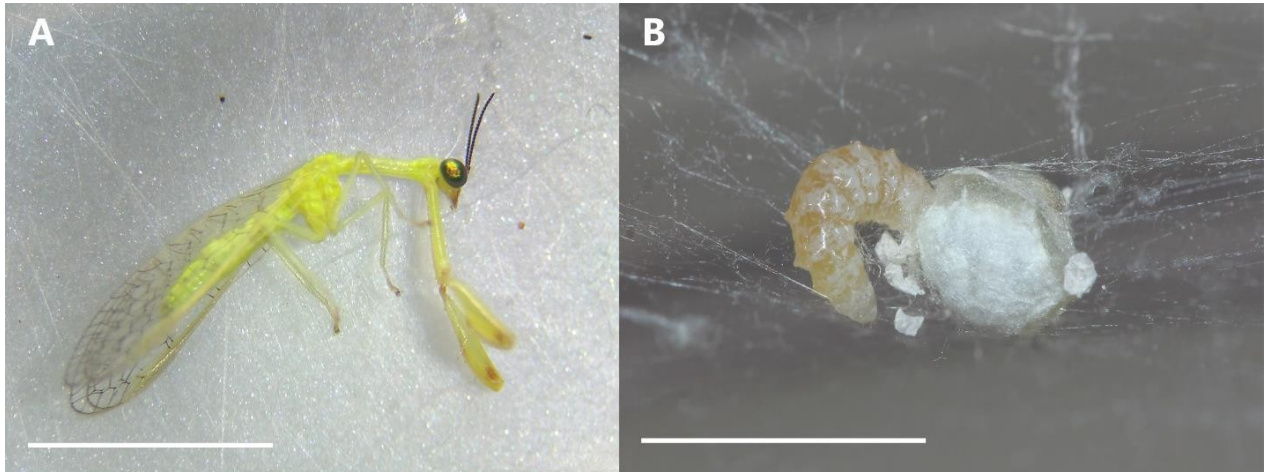


Fig. 3. (A) The adult of the endogenous (to the egg sac) egg predator *Z. virescens* (Neuroptera: Mantispidae); (B) The larvae of *Z. virescens* preying upon the eggs off the egg sac it was associated with. Scales: 5 mm.

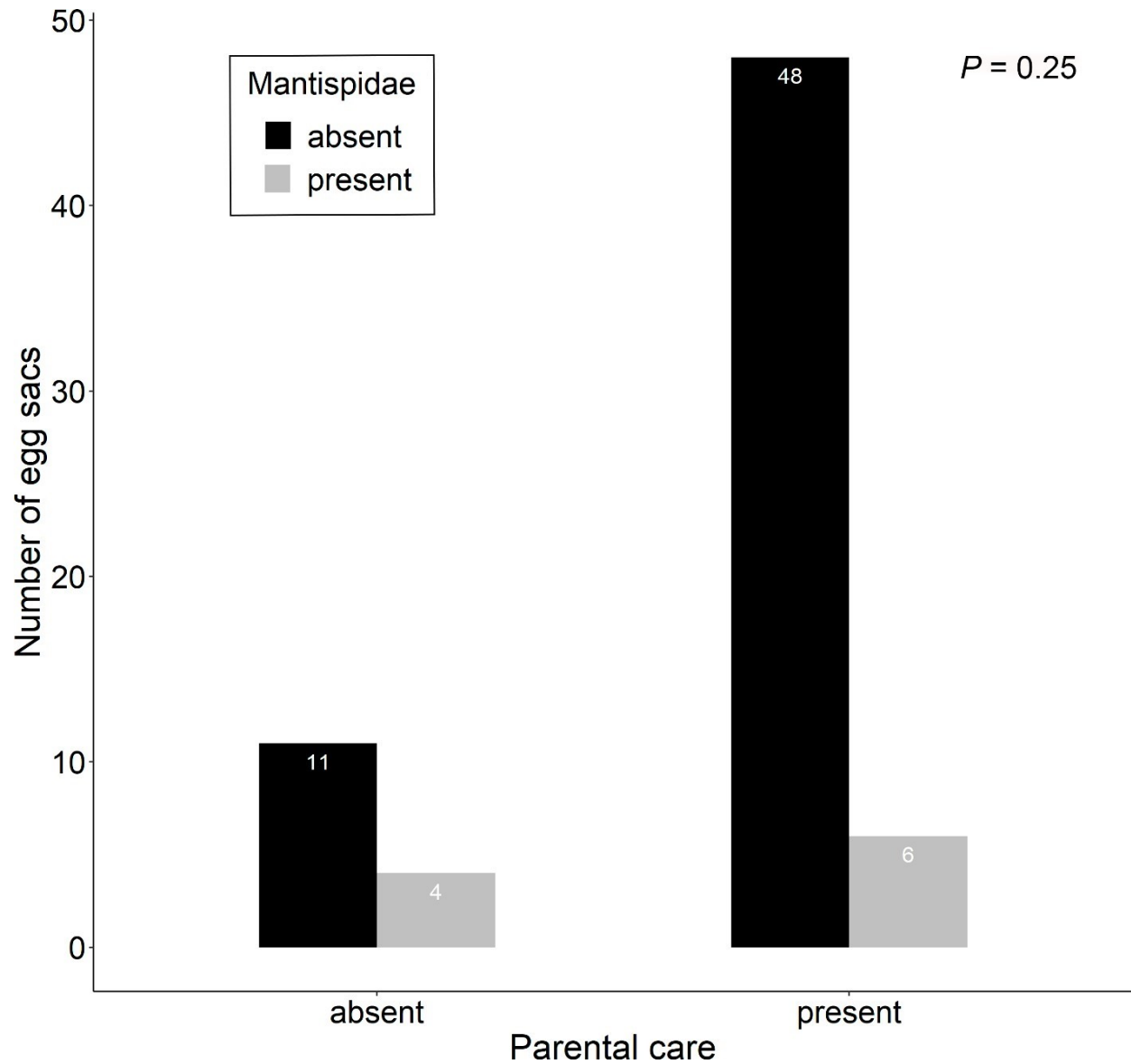


Fig. 4. Number of egg sacs of *M. porracea* attacked and not attacked by the endogenous egg predator *Z. virescens* in the presence and absence of parents on the webs.

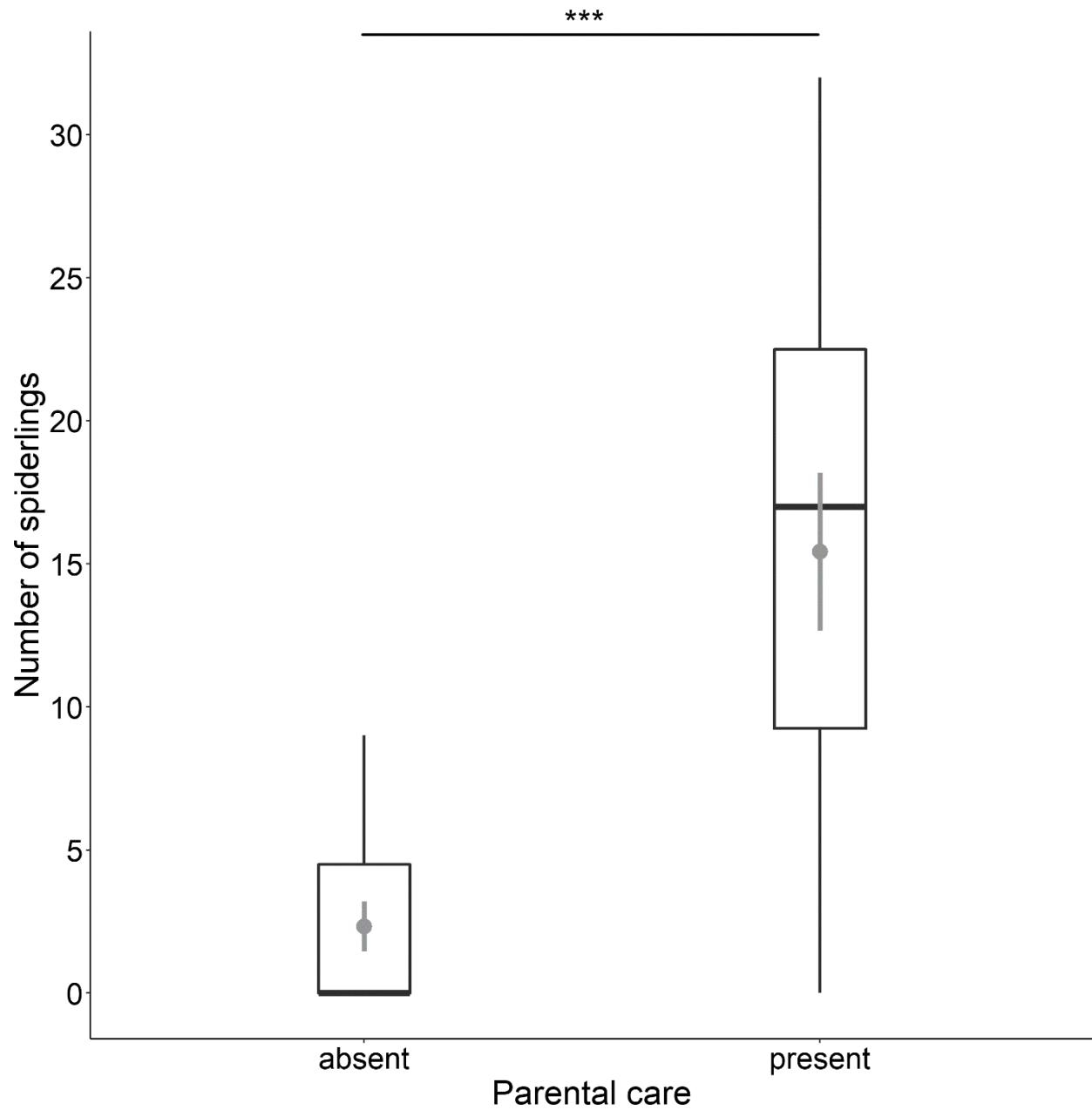


Fig. 5. Number of spiderlings of *M. porracea* that hatched per egg sac on the webs where parental care was present and absent. (Boxplot with range and median, internal line and circle represents mean and standard deviation).

Capítulo 2

**Maternal care and camouflage in the spider *Miagrammopes unipus*
(Uloboridae): egg sac guarding, agents of mortality and color
matching**



Abstract

Maternal care in spiders that guard egg sacs can be an important feature to increase their offspring survival, as spiders and their eggs are attacked by many distinct predators and parasitoids, such as birds and wasps. A common strategy to avoid predation by visually orientated animals is camouflage. Some spiders present an anti-predator strategy, influenced by the visual systems of their predators, called masquerade, in which they resemble inanimate objects from their surroundings, such as twigs and bird droppings. Females of the spider *Miagrammopes unipus* (Uloboridae) guard their egg sacs and stay in a cryptic position during the day, resembling the twigs found in their environment. In this study we describe maternal behavior and camouflage strategies in this species, testing for color matching between the spiders, their egg sacs, and the twigs used as substrate by using reflectance spectrophotometry, and visual modeling analysis. Adult females with their egg sacs are found in a cryptic and/or masquerade position during the day. At night, they are found hanging from their reduced webs holding their egg sacs. The average number of eggs per egg sac was 18.2, but significant variation was observed in the field. The parasitoid wasp *Baeus* sp. (Platygastridae: Scelioninae) was found attacking the egg sacs of *M. unipus*. We found that the potential predators of these spiders and their egg sacs, birds and hymenopterans, were able to distinguish the color between adult females, their egg sacs and the thin branches where they were located. However, considering the variation of twigs available in the habitat and the constant change of substrates, the visual recognition of spiders and their egg sacs is probably compromised in many occasions. We showed how, during maternal care, spiders adjust their behavior, focusing on guarding the egg sacs and including them as part of their camouflage strategy. Our study also highlights the importance of parental behaviors for camouflage in some animals like *M. unipus*.

Keywords: egg attendance, predators, parasitoids, masquerade, visual modeling analysis, visual antipredator strategy

INTRODUCTION

Parental care is a common feature in many spider families, varying significantly in the complexity of behaviors involved (Yip & Rayor 2014). Male spiders usually do not invest in parental care and do not take part in any activity of protection or food provisioning of spiderlings, with just a few exceptions known to date (Kürpick 2002; Moura et al. 2017). Adult females, however, produce egg sacs and, in many cases, stay associated with them until their offspring hatch and disperse, which happens usually shortly after their emergence. This type of parental care is called transient subsocial behavior, but some species may also present subsocial behavior, providing care to their offspring after they emerge from the egg sacs for an extended period (Yip & Rayor 2014). Still, many spiders just produce egg sacs, abandoning them in specific locations, such as leaves (e.g. Sandoval 1987) and on the ground, often associated with leaf litter (e.g. da Ponte et al. 2021). The variation in the colors of egg sacs found in some species seem to reduce their conspicuousness against their background. When no matching of color is observed, the low light in some environments can help camouflage the spiders' egg sacs (Barrantes et al. 2013). However, on species in which females stay guarding egg sacs during their development, parental care may be essential to increase offspring survival as the eggs of spiders and other arthropods are valuable resources to many natural enemies (Santos et al. 2017). Without parental attendance, the egg sacs could be much more accessible to their potential agents of mortality.

Adult and juvenile spiders can also be preyed upon by many different predators and parasitoids. Some potential predators are wasps (Coville 1987; Gonzaga & Vasconcellos-Neto 2005a; Eberhard & Gonzaga 2019), birds (Gunnarsson 2007), and araneophagic spiders (e.g. Jackson & Whitehouse, 1986; Wilcox & Jackson, 1998; Meira et al. 2021). At the same time, parasitoids, such as wasps and flies, are also known to attack spiders (e.g. Sobczak et al. 2009;

Gillung and Borkent 2017). The eggs of spiders have a variety of potential predators and parasitoids of their own. Insects, such as wasps (e.g. Margaría et al. 2006; Nascimento & Gonzaga 2016), flies (Gillung & Borkent 2017), and mantispids (Snyman et al. 2020) are common enemies found within egg sacs. Besides these specialized agents of mortality, some generalist predators, such as ants (Santos et al. 2017) and birds (e.g. Hieber 1992; Lockley & Young 1993), may also attack spider's egg sacs. Due to this diversity of predators and parasitoids associated with spiders and their eggs, many defense strategies have evolved to reduce the potential impact of these natural enemies on their survival.

Anti-predator strategies against visually oriented predators are present in many spiders, with varying degrees of conspicuousness and honesty (Robledo-Ospina & Rao 2022). Some behaviors, such as adjusting the body positions and background choices, can be important to improve their camouflage (Stevens & Ruxton 2019). Some animals' morphology and colors may also resemble objects in their environment, a strategy called masquerade. Individuals using these strategies can be separated into 'element' and 'object' imitators (Skelhorn et al. 2010). Object imitators resemble more specific and less common objects, such as bird droppings (Liu et al. 2014), and do not depend on color matching with their background. Meanwhile, element imitators resemble common elements of their environment, such as twigs (Xavier et al. 2018) and leaves (Kuntner et al. 2016), obtaining additional benefits from being cryptic and matching their background color (Skelhorn et al. 2010). For spiders, the mortality pressure promoted by birds and wasps, that are essentially visually orientated predators, may have led to the independent emergence of masquerade defenses in many species, from various families.

The web-building spider family Uloboridae contains genera that provide maternal care, such as *Uloborus* (e.g. Nascimento & Gonzaga 2016) and *Miagrammopes* (Opell 1989b). Spiders

of this family show significant variation in the types of webs they build (Opell 1994), their morphology (Opell 1989a), and their resting postures (Opell & Eberhard, 1983). In the genus *Miagrammopes*, spiders build reduced webs composed of just one or a few threads (Opell 1978) and, when adult females are caring for their egg sacs, they may not build capture webs. They remain exclusively guarding their egg sacs through egg development (Opell 1984). Spiders in this genus can produce egg sacs with colors similar to their own bodies (e.g., Opell 1989) and may assume a cryptic posture during the day together with their egg sacs, having a continuous twig-like appearance making it hard to distinguish them from twigs. In the spider *M. unipus* (Chickering 1968), females can be found during the night hanging with their egg sacs from their reduced webs. However, during the day they assume a cryptic body position, staying close to the twigs their webs are attached to. The similarities in the shape and color of the spiders and their egg sacs, combined with the postures they often assume, could be a masquerade defense strategy against visually orientated predators, as the visual sign of spiders aligned with their egg sacs resembles dry and broken twigs found on their environment.

This study investigates the characteristics of maternal care in the spider *Miagrammopes unipus* (Chickering, 1968) (Uloboridae) and evaluated if spiders and their egg sacs are chromatically similar to each other and to their substrates. We describe females' behavior directed to their egg sacs, the number of egg sacs and eggs produced, and the occurrence of natural enemies attacking the egg sacs. We used a visual modeling analysis to investigate if *M. unipus* possible defensive masquerade strategy involves color matching with the substrates where they are found. We test if the colors of adult spiders, their egg sacs and the twigs they are associated with can be distinguished by birds and wasps, supposedly their potential predators.

METHODS

Study area

We conducted this study at the “Reserva Ecológica do Panga”, a protected area of 409 hectares close to Uberlândia, MG, Brazil (19°10'32"S 48°23'38"W). This reserve is property of the Universidade Federal de Uberlândia and contains typical Brazilian savanna vegetation (Cerrado), varying from open grasslands to forested areas. Observations and data collection were performed in the forest areas of the reserve. The climate in the region is characterized as Aw in Köppen's classification (Alvares et al. 2013), with rainy summers (October to April) and dry winters (May to September).

Study species

The spider *M. unipus* is a neotropical species that provides maternal care to its offspring. In the genus *Miagrammopes*, spiders construct reduced capture webs with just one or a few threads (Lubin et al. 1978). Webs used to capture flying insects appear to stop being produced by some species while they care for their egg sacs, which they may hold until the emergence of their offspring (Opell 1984). During the day, females remain aligned with their egg sacs, attaching them to the web thread used to remain in resting position. This makes spiders and their egg sacs being perceived as a single object, enhancing their cryptic appearance (Opell 2001). In addition, spiders and their egg sacs have similar colors (e.g. Opell 1989b), which is also similar to the twigs and may be important to the efficiency of the stick mimicry defense strategy (Opell 1984).

Data collection

We performed observations of the spiders in the field between April and July in 2019 and

2021, the period where adult females seem to be more abundant. In May and June of 2021, we collected 35 adult females of *M. unipus* with their egg sacs and the twigs used by the spiders to attach their webs, always at night. From the collected egg sacs, ten were allowed to hatch, looking for potential adult predators or parasitoids, while 25 were used for the spectrophotometric measurements. After the reflectance measurements were obtained, we opened all of them to count the number of eggs, spiders, predators and parasitoids within. All biological material was deposited in the collection from the Laboratory of Arachnology at the Universidade Federal de Uberlândia, MG, Brazil.

Visual modeling analysis

The visual modeling analysis was performed to access if the colors of *M. unipus* adult females, their egg sacs, and their substrates are similar enough to be indistinguishable or to be confused by potential predators (Cronin et al. 2014). The first information to perform these analyses is the reflectance curves of the objects. Therefore, we measured the percentage of spectral reflectance (wavelengths from 300 to 700 nm) from 25 triads of the subjects (spiders, their respective egg sacs, and twigs) using a spectrometer (Jaz; Ocean Optics Inc., Dunedin, FL, USA). We positioned the optical fiber probe perpendicularly and 2 mm from each sample and acquired 100 reflectance curves for each of them from a circular spot (1 mm diameter). Then, we averaged these curves to each sample with the SpectraSuit software (Ocean Optics Inc.).

In the models, reflectance curves were used as the basic stimuli to the observers' visual system. To estimate the observers' photoreceptors excitation and their capacity of discriminating the stimuli, we used the linear version of the receptor-noise limited model (RNLM) (Vorobyev & Osorio 1998). This method is considered suitable for discrimination questions (Kemp et al. 2015)

and produces consistent results in different situations if their basic assumptions are followed (Gawryszewski 2018).

Briefly, this type of visual modeling takes into account the neural opponency mechanism of color interpretation (Kelber et al. 2003; Cazetta et al. 2009; Kemp et al. 2015). The neural signals emitted by the classes of photoreceptors cells are simultaneously compared and opposed to each other. Each stimulus, then, assumably has a “perceptual coordinate” inside a color space. These coordinates are determined by the photoreceptors’ excitation values (E). Thus, we can quantify the “distance” between stimuli. The RNLM considers that noises (random variations) arising in photoreceptors excitation limits the color discrimination capacity (Vorobyev & Osorio 1998; Kemp et al. 2015). The extent of these noises is dependent on each class of photoreceptors and their abundance in the eye. The stimuli are distinguishable from each other if they trigger different enough photoreceptor excitations in relation to the produced noises. The unit of Just Noticeable Differences (JNDs) represents the distances between stimuli standardized by the produced noises (Vorobyev et al. 2001; Clark et al. 2017). Therefore, JND values lower than 1 are considered indistinguishable by a given viewer ($\frac{Signal}{Noise} < 1$), while values above this threshold represent stimuli that are perceived.

Thus, to calculate the photoreceptor’s excitation values (E) in response to the reflectance curves, we followed the formulae provided by Vorobyev & Osorio (1998). We modelled two types of observers. In the first one, we used the spectral sensitivities of *Apis mellifera* worker honeybee (Peitsch et al. 1992). It has a trichromatic visual system which includes photoreceptor cells that became excited in ultraviolet, blue, and green wavelengths (then, it produces E_{UV} , E_{BLUE} , and E_{GREEN} values). We also considered the achromatic channel of *A. mellifera*, which uses only E_{GREEN} values to detect objects at long distances and at small visual angles (Girufa et al. 1997; Giurfa &

Vorobyev 1998; Spaethe et al. 2001). In the second observer, we used the spectral sensitivities of *Cyanistes caeruleus* bird (Hart et al. 2000; Hart 2001) and its photoreceptors' oil droplets light filtering (Hart et al. 2000). It has a tetrachromatic visual system that includes photoreceptor cells that are excited in ultraviolet, blue, green, and red wavelengths (then, it produces E_{UV} , E_{BLUE} , E_{GREEN} , and E_{RED} values). We also considered the avian achromatic channel, which uses E_{DC} values (double-cones that are sensitive to green and red spectra) to detect objects at long distances (Hart et al. 2000; Hart 2001).

We chose these two representative species because they have enough information to conduct reliable modeling. They also have different chromatic sensitivities which provide a general idea about how the investigated subjects are perceived by other animals. Moreover, hymenopterans and birds are among the main potential predators of spiders (Gunnarson 2008). Finally, honeybee sensitivities are considerably superposed to those from hunter wasps (Peitsch et al. 1992) and blue tit sensitivities have a short wavelength that better represents Passeriformes and other groups (Hart 2001) composed of birds that predate spiders frequently (Gajdoš & Krištín 1997).

All these E values were calculated considering a D65 illumination (Commission internationale de l'éclairage – CIE) which is an intense light from mid-day. We chose this luminance since an illuminated environment is the original assumption of the RNLM. We repeated these analyses considering a standard forest shade luminance (Maia et al. 2019) and provided the analyses code in the Supplementary Material; these results led to similar conclusions to the current ones. Finally, we applied the *von Kries* correction in the formulae to consider the influence of a standard green forest background reflectance on the E values (Maia et al. 2019). This is a low background reflectance compared to the reflectance from the other objects. Then, it may not violate

the model assumptions and produce spurious results (Gawryszewski 2018).

We calculated the JNDs between all the subjects (spiders, egg sacs, and twigs). In the *A. mellifera* model, we calculated the JNDs based on their known photoreceptor noises (UV 0.13, Blue 0.06, Green 0.12; Vorobyev & Osorio 1998). In the *C. caeruleus* model, we calculated the JNDs based on the relative number of photoreceptors in its retina (UV 1, Blue 1.92, Green 2.68, Red 2.7; Hart et al., 2000). Finally, we conducted two sets of statistical approaches. In the first one, we evaluated if the objects in each triad were chromatically and achromatically indistinguishable (spiders, their respective egg sacs, and twigs). Then, we used one-sample t-tests to assess if the JNDs between objects in the same triads were greater than 1. If so, spiders were not producing egg sac colors similar to their own and selecting color-matched twigs. In the second statistical approach, we assessed if the entire set of objects, regardless of their triad, occupied the same chromatic and achromatic niche in the observers' brain. If so, spiders and their egg sacs are in an environment with available color-matched twigs. Therefore, irrespectively if spiders can directly identify these "optimal twigs" they have a great probability to occupy them throughout their lives. Thus, we performed the analytical approach proposed by Maia and White (2018). For each previous visual model, we calculated the centroids of the perceptual coordinates of object populations (spiders, egg sacs, and twigs). Given the ratio scale of color perception, these centroids were the geometrical means of the coordinates (Cardoso & Gomes 2015; Maia & White 2018). Then, we estimated bootstrap-based 95% confidence intervals with 1000 resamples to JNDs between perceptual centroids of objects populations. If these confidence intervals did not include and were above 1 JND, the respective populations of objects were occupying different chromatic or achromatic niches. We performed these visual modeling analyses using the 'pavo' package (Maia et al. 2019) and we designed graphics using the 'ggplot2' package (Wickham 2016) in the

R environment (R Development Core Team 2022, version 4.1.3).

RESULTS

Egg guarding behavior

Adult females of *M. unipus* were found associated mainly with small twigs. Females guard their egg sacs through all the period of egg development, only leaving them to reinforce their reduced web. They are still found with their egg sacs in the field after the emergence of their offspring (Fig. 1A), but this association is most likely for just a single instar. During the day females with egg sacs stay near the substrate that their webs are attached to, assuming a cryptic position in which females and egg sacs appear to be a continuous element (Fig. 1B). The egg sacs are often found further away from the substrates. Females, however, always stay in contact with the substrate (Fig. 1C). During the night females with egg sacs are usually found hanging on their webs, holding their egg sacs through a silk line connected to their webs (Fig. 1D). When disturbed during the day females also tend to hang from their webs with their egg sacs just as observed at night, moving away from their substrate.

The webs of *M. unipus* are reduced, consisting of a single resting thread and one or a few capture threads, that are laid at night when they start foraging activities. Adult females without egg sacs and juveniles were usually found during the night hunting with their capture threads (Fig. 2A). Females caring for egg sacs were never found in the field with capture threads, remaining hanging with their egg sacs on their resting threads. However, even without capture threads *M. unipus* females may still be capable of capturing prey on their resting threads (Fig. 2B).

Number of egg sacs, eggs, and parasitoids

All females found in the field had only one egg sac, with a single exception in which a female

produced two egg sacs connected to each other (Fig. 3A). Egg sacs of this spider are long and cylindrical, usually with just a single column of eggs. Still, egg sacs can also be found containing partially more than one column of eggs (Fig. 3B). The average number of eggs per egg sac was 18.2 ± 5.4 [4–30] ($\bar{X} \pm \text{SD}$ [min.–max.]) ($n = 34$). We also registered a few very small egg sacs (Fig. 3C) and at least one with a potential sign of partial predation (Fig. 3D). The parasitoid wasp *Baeus* sp. (Platygastridae: Scelioninae) was found outside multiple egg sacs of *M. unipus* during the first observations in the field (Fig. 4A). However, they were only found hatching from one egg sac out of the 34 egg sacs opened (2.9%). This parasitoid wasp appears to cause the complete mortality of eggs in most egg sacs attacked. However, egg sacs that both spiders and wasps hatched were also found in the field (Fig. 4B).

Visual modeling analysis

The spectral reflectance curves of the studied subjects are summarized in Fig. 5. There was a high degree of superposition in the spectral reflectance emitted by the population of studied subjects. Nevertheless, egg sacs produced higher emissions in short and middle wavelengths, from 300 to 500 nm. The twigs, in turn, produced more reflective variation and a higher emission in long wavelengths, from 600 to 700 nm. In the visual modeling analyses (RNLM), honeybees and bluetits were assumably able to chromatically and achromatically distinguish the spiders from their respective egg sacs and occupied twigs (objects in the same triad). This is because all these visual contrasts were above the discrimination threshold of 1 JND (Fig. 6, Table 1, and Table 2). However, we verified that the perceptual centroids of each object population are prone to be indistinguishable. This is because the bootstrap-based confidence intervals to centroid distances include or are lower than the discrimination threshold of 1 JND in most comparisons (Fig. 7). In

the chromatic channel, only the sets of egg sacs and twigs are prone to be distinguished by the bird's vision. In the achromatic channel, the sets of spiders and egg sacs are prone to be distinguished by both observers (Fig. 7).

DISCUSSION

In the present study, we investigated the characteristics of maternal care and camouflage on the spider *M. unipus*, including its behavior, egg sac traits, and color matching. Considering this species' behavior, we found that during the day these spiders seem to be inactive and present a resting cryptic posture on their substrates. However, the adult females appear as a continuous object with their egg sacs in daylight, sometimes resembling broken twigs, which could be a masquerade defense strategy. This visual antipredator strategy relies on animals being inconspicuous and mistaken for inanimate objects from their habitat (Skelhorn et al. 2010; Robledo-Ospina & Rao 2022). During the night, the behavior of adult females caring for egg sacs is distinct from individuals without egg sacs, as they stop laying capture threads and are found hanging on their resting threads holding and guarding their egg sacs. The number of eggs on each egg sac in this species is small, up to 30, and the presence of more than a single egg sac was only observed once. However, it is possible that females can produce more egg sacs if they live long enough, but they may usually care for only one at a time. We registered for the first time the parasitoid wasp *Baeus* sp. attacking *M. unipus* eggs, generally causing the complete mortality of the eggs. We also found potential signs of partial predation in some egg sacs. To better understand camouflage in the studied species, we tested color matching between the spiders, their egg sacs and the twigs where they were found. Through visual modeling analysis we showed that potential visually orientated predators (i.e. birds and wasps) may distinguish the colors between these three

objects. However, the set of twigs available in the field may allow for color matching between them and the spiders (and the between them and the egg sacs in some situations), as these “objects” tend to be indistinguishable from each other. The frequent change of substrates in *M. unipus* also means that during some moments of their lives, these spiders are likely to be matching in color with their substrates. Adult females seem to be unable of always producing egg sacs in the same color as their own bodies, but the egg sacs may also change in color during their development. The variation in color of spider’s egg sacs evolved influenced by many distinct aspects of their ecology, such as environmental light, physical properties of the silk utilized, behavior and natural history of each species (Barrantes et al. 2013). Even so, the association of spiders with their egg sacs only occurs for a short period of their lives, and only for females, while color matching with their background is important throughout their whole lives.

Egg guarding behavior in *M. unipus* is a constant feature during the period of association between females and their egg sacs. These spiders present what is called transient sub-social behavior, staying with their offspring from the moment they are laid until a short period of time after they hatch (Yip & Rayor 2014). In *M. unipus*, the egg sacs are always connected to the spider and/or its web, and females only leave them briefly to reinforce their webs. During the day the egg sacs are usually found on the back of the spider, both looking like a continuous object close to its substrate. Sometimes they are both in contact with the substrate but often just the spider remains in contact, while the egg sac follows the direction of the web. This different cryptic resting posture could keep their egg sacs away from the substrate where potential predators could access them. At the same time, by assuming this position they may resemble a broken twig, potentially deceiving visually orientated predators through masquerade and crypsis, as probably happens with some twig-mimicking caterpillars (Skelhorn et al. 2010). At night, when the spiders are hanging from

their webs, adult females hold their egg sacs through a silk line connected to their web resting thread and do not lay capture threads. Adult females of *M. unipus* caring for egg sacs may not stop foraging completely, as we observed a female capturing prey on its resting thread, but the lack of capture threads likely limits prey capture during maternal care.

The egg sacs of *M. unipus* are elongated, containing usually around one to two dozen eggs stacked in a single column, with a single female found in the field with 2 egg sacs, all other females observed had a single one. Still, they could potentially lay more than one and care for them separately after the first one hatch. Most egg sacs have a similar shape but significant variation can be observed, from extremely reduced egg sacs to more than a single column in parts of some egg sacs. In others we found potential signs of predation, with parts of the egg sac appearing to have been removed by a predator like ants, which can be a major source of mortality for eggs of spiders and other arthropods (Santos et al. 2017). We registered the parasitoid wasp *Baeus* sp. (Platygastridae: Scelioninae) hatching from *M. unipus* egg sacs and apparently consuming all eggs within them in most of the cases observed. The females still seem to care for their egg sacs as usual even when wasps hatched instead of their own eggs. This wasp genus attacks a wide variety of spider species (e.g. Margaría et al. 2006; Triana et al. 2012; Gonzaga et al. 2015) and its incidence have potential to massively reduce host populations (Gonzaga et al. 2015). During first observations in the field, we observed many egg sacs attacked by the wasp, but in the collected egg sacs on a different year they were very rare, indicating the occurrence of intense fluctuation in the parasitoid population. The impact of this wasp on *M. unipus* egg survival could vary within a year, between years and between populations as is common in other host-parasitoid systems. Some wasps that attack spiders can rely on both visual and chemical cues to find their target (Uma & Weiss 2010), which may also contribute to the selective pressure on *M. unipus* color and

morphology. However, *Baeus* sp. could use chemical cues to locate the spiders or their egg sacs like other egg parasitoids (Fatouros et al. 2008), avoiding any visual anti-predator strategies present in the spider. Maternal care could also be inefficient against these specialized parasitoid wasps, like observed in other spiders (Moura et al. 2021; Santos & Gonzaga 2017). The impact of maternal care in *M. unipus* against the parasitoid *Baeus* sp. still needs to be evaluated.

Through the visual modeling analysis, we showed that spiders were not enough chromatically and achromatically matched to their respective egg sacs and twigs to be indistinguishable in many situations. However, in that environment, the color variation of twigs present allows for spiders, and their egg sacs, to frequently match in color with their twig background, as they are constantly moving and changing their substrate. We can also assume that *M. unipus* usually do not produce egg sacs with the same chromatic stimuli to their own, as observed in the only spider found in the field with two egg sacs, which vary in color. Moreover, these spiders may not search for specific twigs that make them and their egg sacs indistinguishable to predators. Many other spider species add special structures to their webs with defensive functions (Robledo-Ospina & Rao 2022). The most notable examples are *stabilimenta* added in orb-webs (e.g. Gonzaga & Vasconcellos-Neto 2012). In these cases, spiders build structures with silk, prey carcass and other environmental detritus. Many *Cyclosa* species, for example, stay on these structures and this positioning has been demonstrated to deceive wasp and bird attacks (Gonzaga & Vasconcellos-Neto 2005b; Ma et al. 2020). This attack deception is probably due to a refined color matching between spiders and their *stabilimenta* (Chou et al. 2005; Ma et al. 2020). However, this color matching may not be as attainable in species that cannot add environmental detritus in their webs. The spider *M. unipus* accounts only with its silk to envelop the eggs. Unlike the brownish color of *M. unipus*, spider silks tend to be more whitish. Thus, these spiders probably

darken their egg sac silk by adding pigments. However, given the highly reflective property of spider silks (Craig et al. 1996; Zschokke 2002), these pigments may be insufficient to make the threads as brownish as the spider body. Indeed, egg sacs were clearer than *M. unipus* spiders. A similar chromaticity difference between spiders and their egg sacs have been previously reported to uloborid species, in which adult females seems to hide on the tip of their cylindrical and yellowish egg sacs (Nascimento & Gonzaga 2016). However, the color matching between both subjects seems to be insufficient to make them indistinguishable (Nascimento et al. in prep.).

The environment of *M. unipus* had a set of twigs that match with colors of spiders and egg sacs. The frequency of such twigs was great enough to make the perceptual coordinates centroids do not surpass the discrimination threshold. Therefore, spiders may eventually move themselves and their egg sacs to these “optimal substrates” even not being able to directly identify them. We should note that *M. unipus* is a nocturnal species that may stop in different microhabitats every few days. Thus, these moments of color matching may benefit spiders with cryptic positions sometimes along their lives. Indeed, some spiders and egg sacs were below the discrimination threshold in their object triad. At the same time, irrespectively of the spider’s color, morphology and behavior (stretched cryptic resting position and slender shape) may have an important role in breaking its body contour in different places (e.g. Zhang et al. 2015). Therefore, it seems reasonable that these spiders rely on a general choice of vegetation types which encompass ideal substrates. In other cases, much better color matchings on vegetation branches by spiders have been achieved. The twig masquerading spider *Wixia abdominalis* (Araneidae), for example, is camouflaged via background matching in two different areas, a native and in *Eucalyptus* cultivated forests distant 130 km from each other (Xavier et al. 2018). One population of spiders and their twigs were clearer and this made them distinguishable from the other one in a colorimetric

assessment. This case, however, involves a simpler selection process, that does not act on egg sac color, but only on spiders.

In conclusion, we show that during maternal care *M. unipus* spiders adjust their usual behavior, focusing on guarding their egg sacs and incorporating them into their camouflage during the day. Because mothers are constantly with their egg sacs, repairing the web and moving its location frequently, it is likely that unattended egg sacs would be more exposed to potential biotic and abiotic agents of mortality. However, the impact of maternal care on offspring survival and on the incidence of egg predators or parasitoids like *Baeus* sp. in *M. unipus* still needs to be evaluated. With respect to camouflage and color matching, it seems that this spider is usually not capable of producing egg sacs or selecting substrates of the same color as their own, being distinguishable from each other by their potential visually orientated predators. Still, these spiders occupy a type of environment (the twigs used as substrates) where similar colors to their own and their egg sacs are frequent enough to color match and become indistinguishable. At the same time, their small size and posture (body and legs alignment with the twigs) may hamper their location. The adjustment of their usual cryptic defense strategy during the day into a frequent masquerade strategy when associated with their egg sacs also highlights the potential importance of behaviors, in this case maternal, for camouflage.

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Table 1. One-sample t tests. Chromatic and achromatic contrasts (in JNDs) between *M. unipes* spiders, their egg sacs and occupied twigs in a honeybee modelled vision (RNLM).

	Visual channel	Compared subjects	Contrasts in JNDs	Discrimination	<i>T</i>	<i>p</i> -value
			mean and 95% c.i. (lower limit, upper limit)	thresholds (JNDs)		
Bee vision	Chromatic	Spiders and egg sacs	1.93 (1.53, 2.33)	1	4.80	< 0.001
		Spiders and twigs	2.04 (1.59, 2.49)	1	4.79	< 0.001
		Egg sacs and twigs	2.19 (1.60, 2.79)	1	4.16	< 0.001
	Achromatic	Spiders and egg sacs	2.50 (1.81, 3.19)	1	4.51	< 0.001
		Spiders and twigs	2.20 (1.61, 2.80)	1	4.21	< 0.001
		Egg sacs and twigs	2.64 (1.87, 3.42)	1	4.39	< 0.001

Table 2. One-sample t tests. Chromatic and achromatic contrasts (in JNDs) between *M. unipes* spiders, their egg sacs and occupied twigs in a bluetit modelled vision (RNLM).

	Visual channel	Compared subjects	Contrasts in JNDs	Discrimination	<i>T</i>	<i>p</i> -value
			mean and 95% c.i. (lower limit, upper limit)	thresholds (JNDs)		
Bird vision	Chromatic	Spiders and egg sacs	2.11 (1.68, 2.54)	1	5.36	< 0.001
		Spiders and twigs	1.93 (1.60, 2.27)	1	5.79	< 0.001
		Egg sacs and twigs	2.75 (2.23, 3.27)	1	6.99	< 0.001
	Achromatic	Spiders and egg sacs	2.22 (1.58, 2.86)	1	3.96	< 0.001
		Spiders and twigs	2.32 (1.68, 2.96)	1	4.26	< 0.001
		Egg sacs and twigs	2.38 (1.63, 3.13)	1	3.80	< 0.001

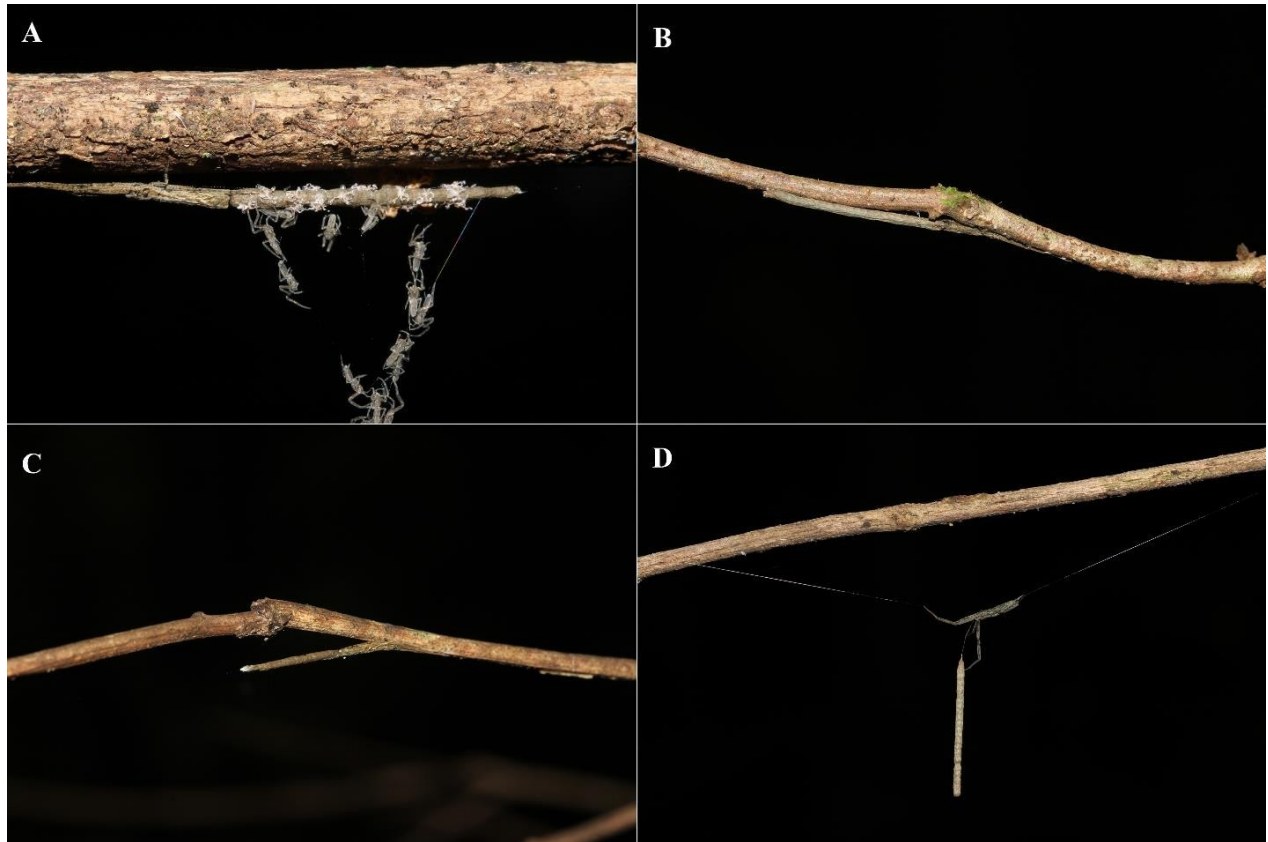
Figures

Fig. 1. (A). *M. unipus* female with egg sac and hatchlings. (B). *M. unipus* cryptic position during the day near the twig. (C). *M. unipus* cryptic position during the day with egg sac away from the twig. (D). *M. unipus* at night holding its egg sac.

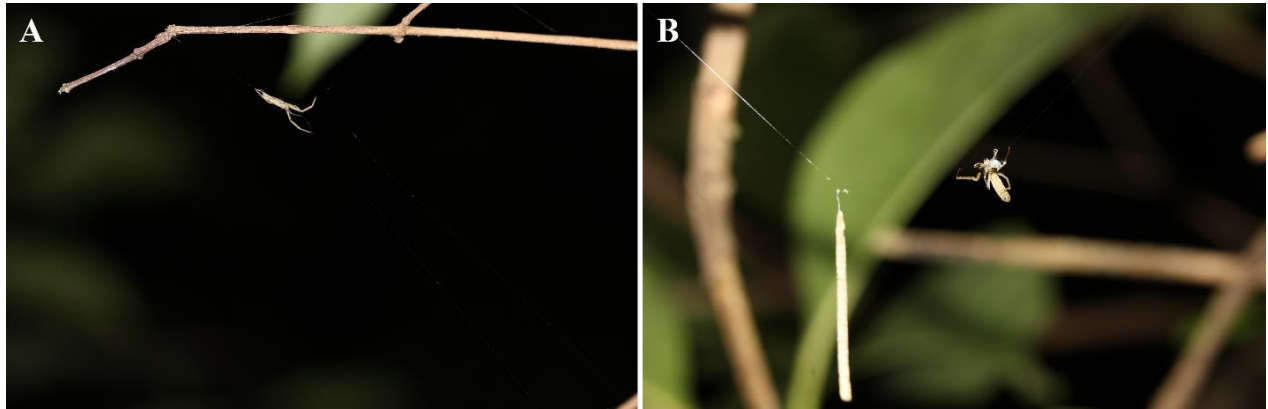


Fig. 2. (A). An adult *M. unipus* foraging with its capture threads. (B). Adult female with a prey item on its resting thread while caring for the egg sac.

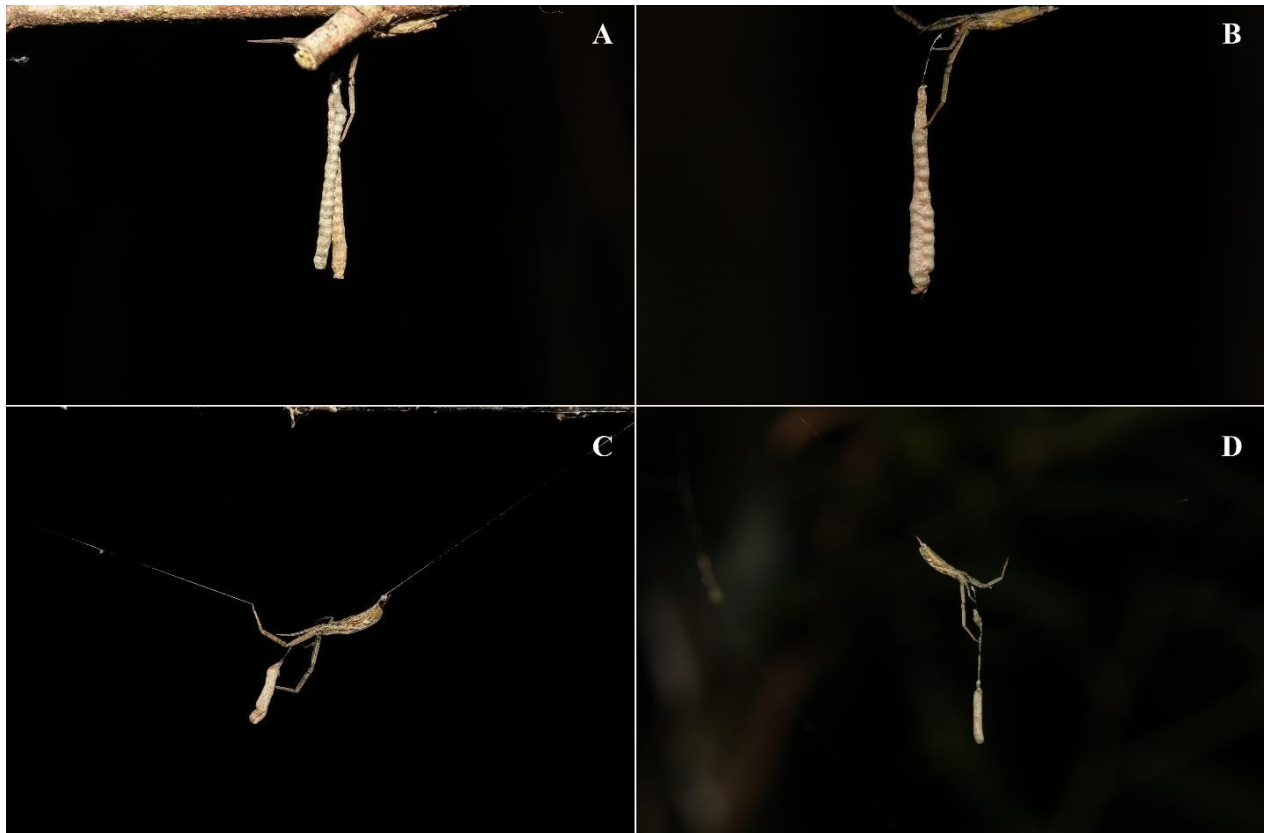


Fig. 3. (A). Adult female *M. unipus* with two egg sacs. (B). Egg sac not with a single column of eggs. (C). Extremely reduced egg sac. (D). Egg sac with potential signs of predation.



Fig. 4. (A). Parasitoid wasp *Baeus* sp. recently emerged from the egg sac of *M. unipus* while mother still holds it. (B). An egg sac that both wasps and spiders hatched from it.

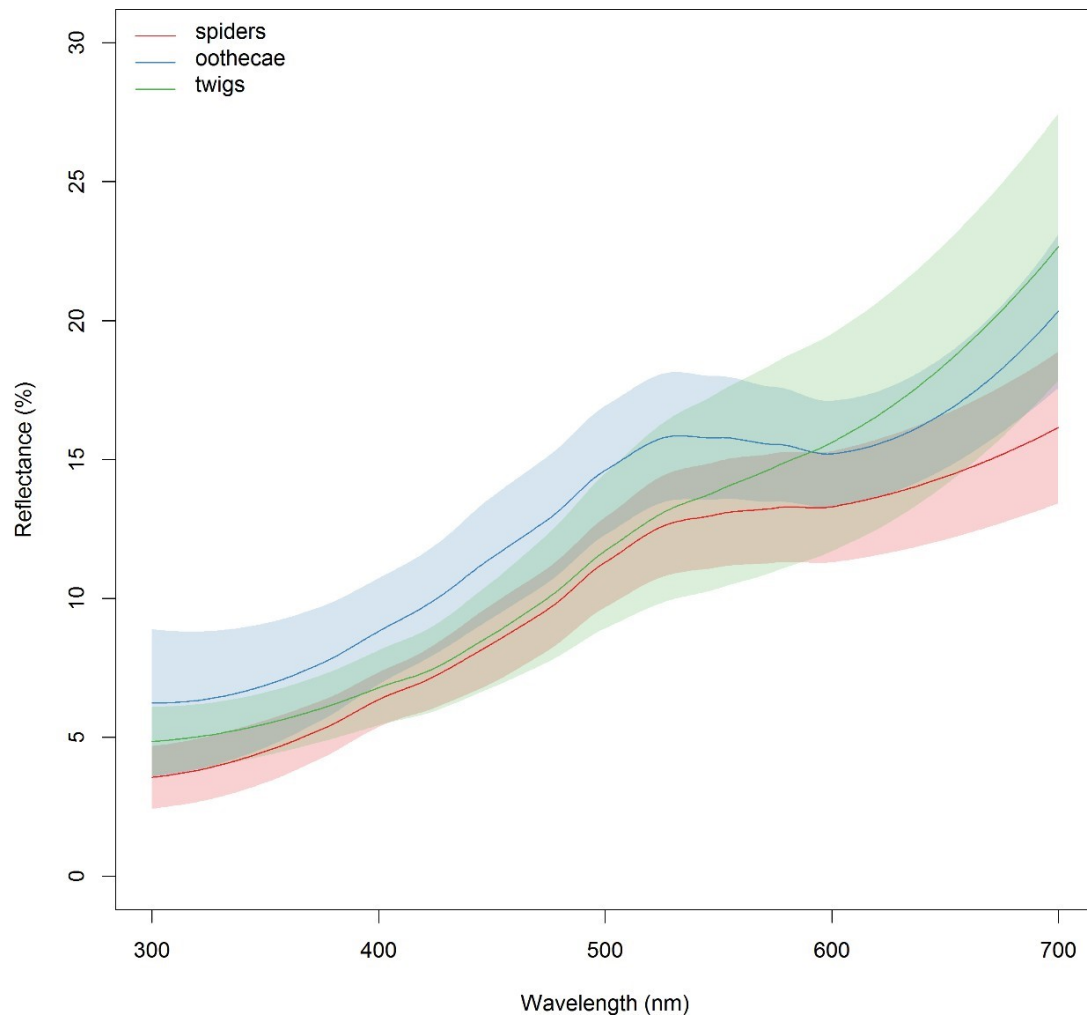


Fig. 5. Spectral reflectance (%) (mean $\pm$ SD) of wavelengths covering the range from 300 to 700 nm for *M. unipes* spiders, their egg sacs (oothecae) and twigs.

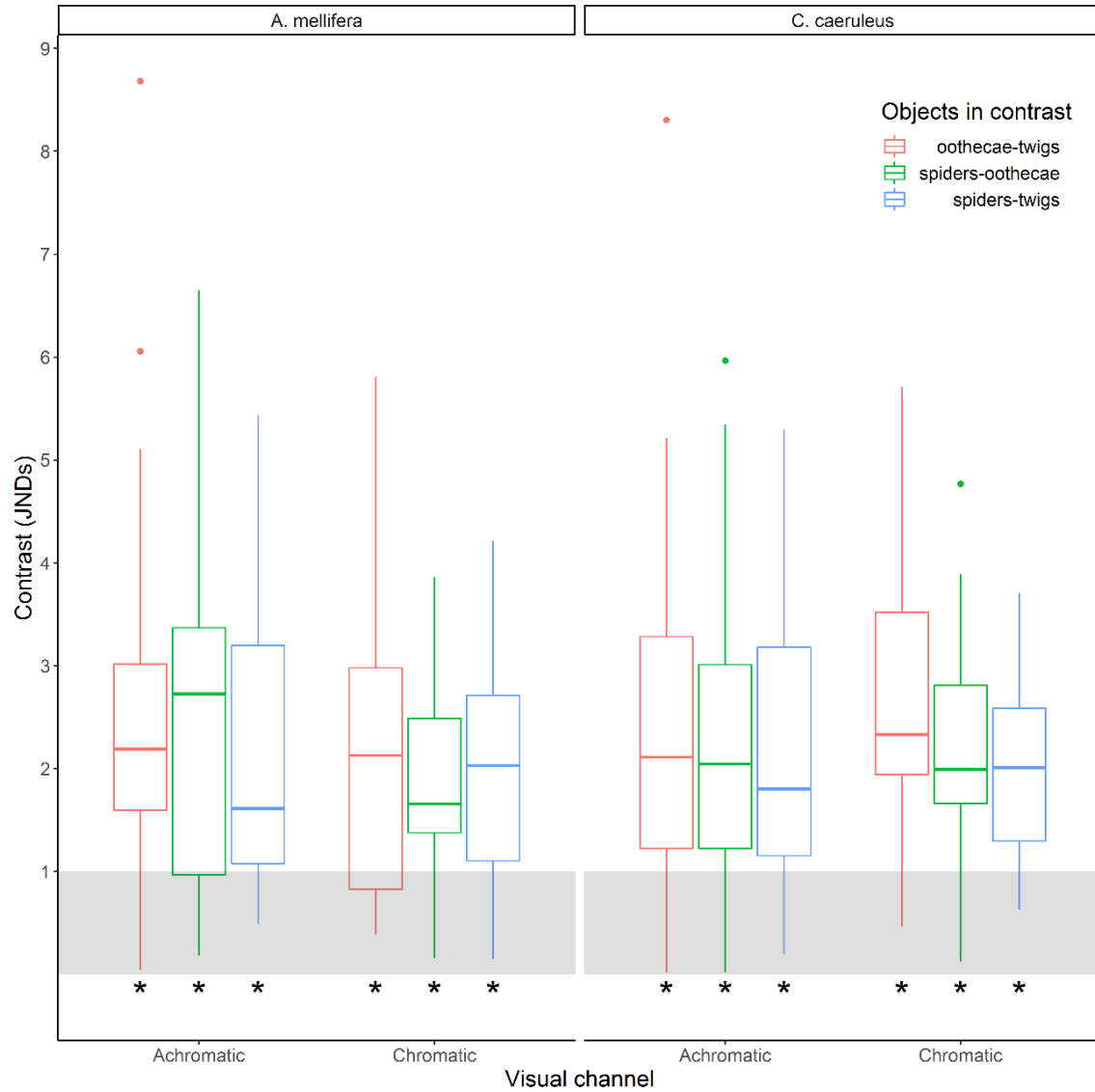


Fig. 6. Chromatic and achromatic visual contrasts (JNDs) between objects in each triad: spiders, their respective egg sacs (oothecae) and occupied twigs. The grey area detaches JNDs ≤ 1 and * marks pairs of objects which significantly produced values above this discrimination threshold.

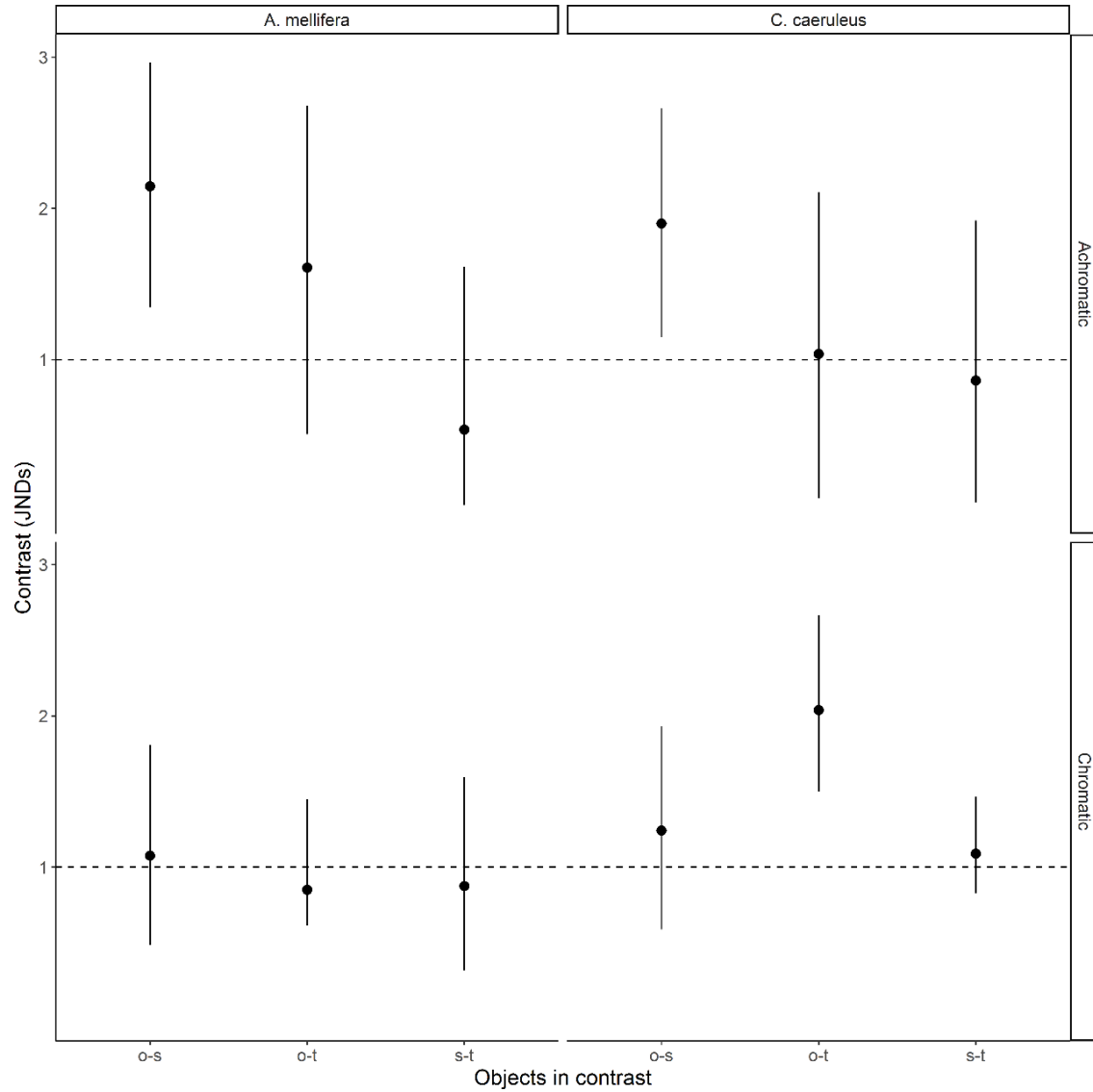
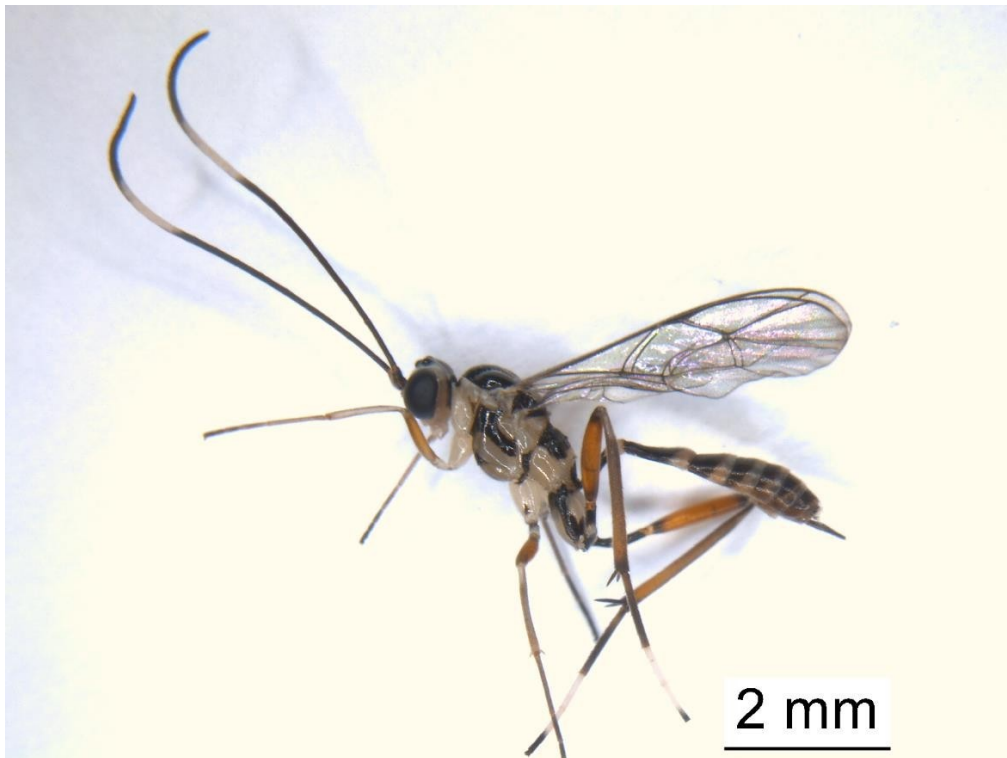


Fig. 7. Chromatic and achromatic visual contrasts (JNDs) (mean $\pm$ 95% CI) between centroids of perceptual coordinates of object populations: spiders, egg sacs (oothecae), and twigs. Confidence intervals that are above and that do not include 1 JND represent distinguishable populations.

Capítulo 3

Predators and parasitoids associated with egg sacs of web-building spiders (Araneidae and Uloboridae) from Minas Gerais, Brazil



Abstract

The variation on spider's egg sacs structure and coloration, combined with adult behaviors such as their suspension in webs and egg sac guarding may be important barriers against generalist egg predators. Specialized predators and parasitoids, however, are likely to access them even in the presence of multiple additional lines of defense. Spider eggs are attacked by specialized predators like mantispids, dipterans and wasps, which are also often parasitoids. We investigate in this study the frequencies of attacked egg sacs in 10 species of orb web spider species (Araneidae and Uloboridae) and between two samples for three of those 10 species. We also describe the diversity of insect families associated with these egg sacs. The most attacked spider was *P. bistriata* with 66.7% of their egg sacs attacked, while the only species not attacked by any egg predator or parasitoid was *C. citricola*, an invasive species in Brazil. We found differences in the frequencies of attack for two spider species between samples in different years and months. Wasps were the most diverse and frequent group of natural enemy, with six families, followed by Diptera with 2 families and a mantispid neuropteran, which are a single family of Neuroptera. The spider *P. bistriata* was the only species where all three main groups of insects were found. The variation in the frequencies of attacked egg sacs observed between the spider species is significant, but even within species we can expect variation in both time and space. The specificity of the interactions between egg predators and parasitoids and their hosts, and their impact on egg survival is still poorly understood as most species interactions are unknown. Still, they may have influenced the evolution of spider's behavior and egg sacs traits to mitigate their impact on offspring survival when this impact is high enough.

Keywords: egg predators, egg parasitoids, parasitoid wasps, mantispid, hyperparasitoids

INTRODUCTION

The egg sacs of spiders are valuable resources for many different natural enemies. For several arthropods, the main source of egg mortality is predation by ants, while cannibalism and the action of parasitoids may also be important in some cases (Santos et al. 2017). Opportunistic predators, such as ants and birds, are capable of causing great mortality, but they need to locate and have access to the spider's egg sacs to do so. The complexity of the structure of the egg sacs walls built by the spiders indicates that they can be a barrier against some natural enemies (Austin 1985). However, many other strategies may have evolved to further improve protection against predators and parasitoids. Some spider species suspend their egg sacs in silk, for example, which can be an effective barrier against ants, but not against birds (Hieber 1992). Other strategies may also be involved in reducing the susceptibility of the offspring to agents of mortality against visually oriented predators, such as birds and some wasps. Spider egg sacs vary significantly in coloration, in many cases showing color patterns that are similar to those of the background or are placed in low light environments, which may help to camouflage them in their surroundings (Barrantes et al. 2013). However, specialized predators and parasitoids, even when multiple lines of defense are present, may still have abilities of locating and attacking spider egg sacs.

Many insect groups, as hymenopterans, dipterans and neuropterans contain species specialized in attacking spider eggs. Mantispids (Neuroptera) of the subfamily Mantispinae, for example, are frequent natural enemies of spiders (e.g. Redbord & Redborg 2000), concluding their larval development inside the egg sacs (Redborg 1998). However, the impact of this predator at the egg sac level may still be low, especially when spiders produce hundreds of eggs (e.g. Berti-Filho et al. 2002). Their larvae can penetrate the egg sacs directly or board females and live associated with them until the production of their egg sac (Redbord 1998). Some species of this

insect, such as *Zeugomantispa virescens*, can be widespread, (Machado & Rafael 2010), but the information on the diversity of hosts for this and other mantispid species is still limited. Diptera is another common insect group of predators and parasitoids of eggs, including species in multiple families acting as natural enemies of spiders (Feener & Brown 1997; Gillung & Borkent 2017). However, due to the lack of data on the ecology of most species and their hosts, it is still unclear which dipteran species are predators or parasitoids of spiders' eggs (Gillung & Borkent 2017).

Wasps are by far the most diverse and studied group of arthropods natural enemies of spider eggs, including organisms of diverse groups (e.g. Ichneumonidae, Eulophidae and Platygasteridae) that act as parasitoids or predators (e.g. Nascimento & Gonzaga 2016; Sobczak 2015; Gonzaga et al. 2015). Parasitoid wasps that attack spider's eggs can be very small and lay multiple eggs (e.g. Chuang et al. 2019). At the same time, some wasps are hyperparasitoids, attacking flies or other wasps that are natural enemies of spiders or their eggs (e.g. Miranda et al. 2020; Tavares et al. 2019). Therefore, the egg sacs of some spiders may have multiple species of wasps, from distinct families, associated with them (e.g. Miranda et al. 2020). However, the ecology of most spider species, including their egg predators and parasitoids, is still to be described in detail, and the potential diversity of wasps to be found associated with their egg sacs is likely to be higher than indicated by the known interactions reported to date.

The occurrence and the impact of predators and parasitoids in the web-building families Araneidae and Uloboridae associated with their egg sacs are still unknown for most species. For the few species where some agents of egg mortality were described, the potential variation in the frequency of predators and parasitoids between populations and between different years or seasons is yet to be explored in detail. In this context, the present study aims to investigate the diversity and frequency of insects associated with the egg sacs of multiple Araneidae and Uloboridae spider

species from the state of Minas Gerais, Brazil. We described the families of insects found associated with the spider's egg sacs, and compared the frequency of egg sacs attacked between species and between samples for the species that were sampled twice.

METHODS

Studied areas and species

We collected egg sacs from web-building spiders in three locations in the state of Minas Gerais (MG), southeastern Brazil. The first location is a protected area of Cerrado (Brazilian Savanna), the “Reserva Ecológica do Panga” (REP, 19°10'32"S 48°23'38"W), a private property of the Universidade Federal de Uberlândia near the city of Uberlândia, MG, Brazil. The second location was within the city of Uberlândia, MG (UDI, 18°55'08"S 48°16'37"W), in parks and other green areas. The third location is another protected area of Cerrado, the “Estação Ecológica de Pirapitinga” (EEP, 18°21'18"S 45°19'19"W), located close to the city of Três Marias, MG, and managed by “Instituto Chico Mendes de Conservação da Biodiversidade” (ICMBio).

The 10 species of spiders studied are from the families Araneidae (N = 7) and Uloboridae (N = 3). Araneidae species were: *Araneus guttatus*, *Araneus* sp.; *Argiope argentata*, *Cyrtophora citricola*, *Manogea porracea*, *Parawixia bistrata* and *Trichonephila clavipes*. All of these spiders construct some kind of orb-web, with variable additional complexity, some being exposed throughout the day and night, and others being constructed and reabsorbed daily. Uloboridae species included *Miagrammopes unipus*, *Philoponella* sp. 1 and *Philoponella* sp. 2. The webs of *M. unipus* are extremely reduced, consisting of just one or a few threads, while both *Philoponella* species construct orb webs associated with the complex three-dimensional funnel webs of the spider *Aglaoctenus lagotis* (Lycosidae).

Data collection

We collected egg sacs between 2019 and 2022. The period when egg sacs can be found vary among the ten spider species included in the study, but most of them were found mainly during a few weeks each year. The egg sacs of five species (*P. bistrata*, *Araneus* sp., *M. unipus*, *Philoponella* spp. 1 and 2) were collected in the first location (REP). The egg sacs of three species (*A. argentata*, *C. citricola* and *T. clavipes*) were collected in the second location (UDI). The egg sacs of two species (*A. guttatus* and *M. porracea*) were collected in the third location (EEP). We sampled the egg sacs from seven out of the 10 spider species only once. The other three species were sampled twice, each in a distinct location and on different years. The spider species sampled twice were *M. porracea* (EEP 2019 and 2021), *M. unipus* (REP 2019 and 2021) and *C. citricola* (UDI 2019 and 2022). The adult spiders from these species are usually found in the field with their egg sacs so we only used egg sacs from webs when parents were still present.

We used a total of 314 egg sacs that were collected before their hatching. The number of egg sacs obtained per species was 111 for *M. porracea* (54 in the first sample and 57 in the second), 77 for *C. citricola* (36 in the first sample, 41 in the second), 41 for *M. unipus* (seven in the first sample and 34 in the second), 25 for *A. guttatus*, 16 for *Araneus* sp., 15 for *P. bistrata*, 14 for *A. argentata*, six for *T. clavipes*, six for *Philoponella* sp. 1 and four for *Philoponella* sp. 2. After the collection of the egg sacs we kept them under laboratory conditions in individual vials for one to two months allowing the spiders and their potential predators and parasitoids to emerge. The only exceptions to this procedure were with the second sample of *C. citricola* and most of the second sample of *M. unipus*, which were frozen before all of egg sacs could hatch to obtain eggs and egg sacs measurements. Once predators or parasitoids emerged from the egg sacs we separated and counted them after their death, preserving them in alcohol. They were later sent to specialists for

identification and deposited in institutes or universities zoological collections, such as “Universidade Federal do Paraná” (UFPR) and “Instituto Nacional de Pesquisas da Amazônia” (INPA) zoological collections. We obtained the overall frequency of attacked egg sacs for species with two samples by combining the two. However, the frequencies per sample are also described separately.

RESULTS

The frequency of egg sacs that hatched with any kind of insect varied in the 10 species of spider from 66% in *P. bistrata* to 0% in *C. citricola* (Table 1). In the nine species where predators/parasitoids hatched from their egg sacs, four of them had a single insect species and the other five had from two to five different species (Table 1). Some of the wasps that hatched from the spider’s egg sacs are likely hyperparasitoids, such as *Conura* species (Chalcididae), possibly attacking spiders egg predators/parasitoids. From the three species sampled twice, one species (*C. citricola*) was never attacked by any predator or parasitoid, while the other two had significant variation in the frequencies of egg sacs attacked between samples (Table 2).

All predators and parasitoids found hatching from egg sacs of the 10 species of spiders were insects that can be separated into three main groups: wasps (Fig. 1A-D), flies (Fig. 1E) and mantispids (Fig. 1F). Wasps were the most frequent and diverse type of predator/parasitoid, hatching from seven of the 10 spider species, represented by six distinct families (Ichneumonidae, Eulophidae, Chalcididae, Platygasteridae, Signiphoridae and Pteromalidae). Flies were represented by two families of Diptera (Chloropidae and Phoridae), hatching from three of the 10 spider species. The mantispids (Mantispidae) are just a single family of the Neuroptera order, hatching from three out of the 10 spider species. The only spider that all three different groups of insects

hatched from their egg sacs was *P. bistrata*, while the only spider where no predator/parasitoid hatched was *C. citricola*, an invasive species found in Brazil recently.

DISCUSSION

All but one species of spider had their egg sacs attacked by at least one of the three specialized agents of mortality: wasps, diptera or mantispids. The invasive spider *C. citricola* was the only species with no natural enemies associated with the eggs. It is possible that insects capable of attacking these spider's eggs have not yet started to use them as hosts, or at least not in the population studied, as they have just recently been found in Brazil (Álvares & De Maria 2004). In the areas included in the original distribution of this species, the eggs are attacked by some of these specialized insects, but with significant variation in their frequencies between different populations (Chuang et al. 2019). Therefore, the collection of more egg sacs of *C. citricola* in other regions of Brazil is important to confirm the absence of native insects attacking the eggs of this invasive species. The lack of insects or lower levels of incidence in the egg sacs of *C. citricola* could also be due in part to a higher preference for native egg sacs by their potential predators and parasitoids, as observed for two *Latrodectus* spiders (Mowery et al. 2022).

The frequency of attacked egg sacs in the studied spiders varied significantly. The variation observed for the Araneidae and Uloboridae species was similar to what was found in some Theridiidae spiders (Triana et al. 2012). The most frequently attacked species we found was *P. bistrata*. Two thirds of their egg sacs had insects associated, from all three major taxonomic groups of natural enemies (three different wasp families, dipterans Chloropidae and one Mantispidae). The spider with the lowest incidence of predators or parasitoids, besides the absence in *C. citricola*, was *M. porracea* with an average of less than 10% of egg sacs attacked by the

mantispid *Zeugomantispa virescens* (Neuroptera: Mantispidae). This egg predator also attacked *P. bistrata* and *T. clavipes* egg sacs, but their impact on egg survival per egg sac appears to be much higher for *M. porracea* due to their reduced number of eggs. However, females of this spider produce multiple egg sacs (Moura et al. 2017), so even if some are totally lost the others may still survive. The significant variation in size and coloration reported for the insect *Z. virescens* (Machado & Rafael 2010) could be influenced by the different hosts utilized for their development as observed in our study.

For the three spiders sampled twice, *C. citricola* egg sacs were never attacked, while *M. porracea* egg sacs were attacked rarely in both samples, but around three times more often in the first sample. However, the frequencies of egg sacs attacked in *Miagrammopes unipus* varied significantly between samples. The wasp *Baeus* sp. was almost 30 times more frequent in the first sample, being present in more than half of the egg sacs collected. The high incidence in this sample may be influenced by the smaller number of egg sacs, but the second sample had many more egg sacs and only one of them was attacked. Therefore, this variation could occur in part due to differences between the years and/or months sampled. Some predators and parasitoids may be more frequent in some spider's egg sacs during the dry or rainy season, for example (Triana et al. 2012). More studies are required to understand the high variation between samples in the occurrence of the wasp *Baeus* sp. on the egg sacs of *M. unipus*. The frequency of attacked egg sacs may also differ between locations, as observed in other wasps that attack the eggs of spiders (Chuang et al. 2019). Studies comparing populations of this spider are also important.

The high diversity of insects associated with spider egg sacs in this study illustrates some of the most common agents of mortality for spiders and other arthropod eggs. The six wasp families that we found vary significantly in their morphology and behavior. Ichneumonidae wasps,

for example, are common predators and parasitoids of spider eggs (e.g. Sobczak et al. 2012; Nascimento & Gonzaga 2016). Meanwhile, in the family Chalcididae wasps like *Conura* sp. 1 which emerged from some egg sacs are likely hyperparasitoids, attacking other insects like Ichneumonidae wasps that attack the eggs of spiders (e.g. Miranda et al. 2020). It is still unclear for most of the wasps found emerging from the spider's egg sacs whether they act as egg predators or parasitoids, or even hyperparasitoids like *Conura* species. The same occurs in Diptera, with just a few species confirmed as true predators or true parasitoids (Gillung & Borkent 2017). The family Chloropidae was represented by 4 species in our study, with some being very abundant and others very rare. In the genus *Pseudogaurax*, for example, some species are found associated with the egg sacs of multiple spider families (e.g. Barnes et al. 1992; Vetter et al. 2012; Riccardi & Pádua 2021), but little is known about these insect's life histories (Gillung & Borkent 2017). Finally, the mantispids are all part of a single insect family (Mantispidae) and are likely true predators of the eggs of spiders. They have also been found associated with the egg sacs of multiple families of spiders (Fink 1987; Rittschof 2011; Triana et al. 2012). The species found in this study *Z. virescens* is widespread in Brazil (Machado & Rafael 2010) and use at least three different spider species as hosts, which likely has influence in the variation in size found in these insects. For these egg predators, we still lack information on which strategy they use to attack the egg sacs. Some penetrate them directly while others board the spiders before entering the egg sacs during their construction. However, they may possibly act as egg sac penetrators of web building spiders (Redborg 1998).

In conclusion, we were able to show that the egg sacs of Araneidae and Uloboridae spiders are frequently attacked by specialized egg predators and parasitoids and that the incidence of these agents of mortality seems to vary significantly between species, but also between years or months

within a year and between populations. For most of the wasps and Diptera species found associated with spiders, it is still unclear how specialized is the association with their hosts and if they are egg predators or egg parasitoids. In the case of the mantispids, the specificity with hosts and the type of strategy used to attack the egg sacs are also poorly known. Describing these interactions will improve our understanding on the frequency and impact of these insects on the egg sacs of spiders. The impact of lines of defense against these insects is yet to be explored, but they are likely more impactful against generalist predators. Even so, for specialized predators and parasitoids that have a big impact on offspring survival, some strategies may have evolved to mitigate their access to the egg sacs or their impact on egg survival.

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Table 1. Number of sampled egg sacs and frequency of hatched insects per spider species. (Hy = Hymenoptera, Ne = Neuroptera, Di = Diptera).

Spider Species	Egg Sacs	% Attacked	Hatched insects
<i>Manogea porracea</i> *	111	7.2	<i>Zeugomantispa virescens</i> (Ne: Mantispidae)
<i>Cyrtophora citricola</i> *	77	0	
<i>Miagrammopes unipus</i> *	41	12.2	<i>Baeus</i> sp. (Hy: Platygasteridae, Scelioninae)
<i>Araneus guttatus</i>	25	16.0	Chloropidae sp. 1 and sp. 2 (Di), Phoridae sp. (Di)
<i>Araneus</i> sp.	15	46.7	<i>Aprostocetus</i> sp. (Hy: Eulophidae), Cryptinae sp. 1** (Hy: Ichneumonidae), Orthocentrinae sp.** (Hy: Ichneumonidae), Chloropidae sp. 3 (Di), Pimplinae sp.*** (Hy: Ichneumonidae)
<i>Parawixia bistrata</i>	15	66.7	<i>Zeugomantispa virescens</i> (Ne: Mantispidae), <i>Conura</i> sp.1** (Hy: Chalcididae), <i>Tachinobia</i> sp.** (Hy: Eulophidae), Signiphoridae sp.** (Hy), Chloropidae sp. 4 (Di)
<i>Argiope argentata</i>	14	21.4	<i>Conura</i> sp.2** (Hy: Chalcididae), Cryptinae sp. 2** (Hy: Ichneumonidae)
<i>Trichonephila clavipes</i>	6	33.3	<i>Zeugomantispa virescens</i> (Ne: Mantispidae), <i>Horismenus</i> sp.** (Hy: Eulophidae)
<i>Philoponella</i> sp.1 (small)	6	16.7	<i>Bathyzonus</i> sp. (Hy: Ichneumonidae)
<i>Philoponella</i> sp.2 (big)	4	25.0	Pteromalidae sp.** (Hy)

* Species of spider sampled twice. Number of egg sacs and % attacked obtained from both samples combined.

** Potential Hyperparasitoid wasp species that attack insects which prey on spider eggs.

*** Species found in the field inspecting an egg sac but did not hatch from any egg sac.

Table 2. Variation in the frequency of attacked egg sacs between samples for the species of spiders sampled twice. (Number of egg sacs sampled).

Spider Species	1 st Sample	2 nd Sample	Total
<i>Manogea porracea</i>	11.1 (54)	3.5 (57)	7.2 (111)
<i>Cyrtophora citricola</i>	0 (36)	0 (41)	0 (77)
<i>Miagrammopes unipus</i>	57.1 (7)	2.9 (34)	12.2 (41)

Figures

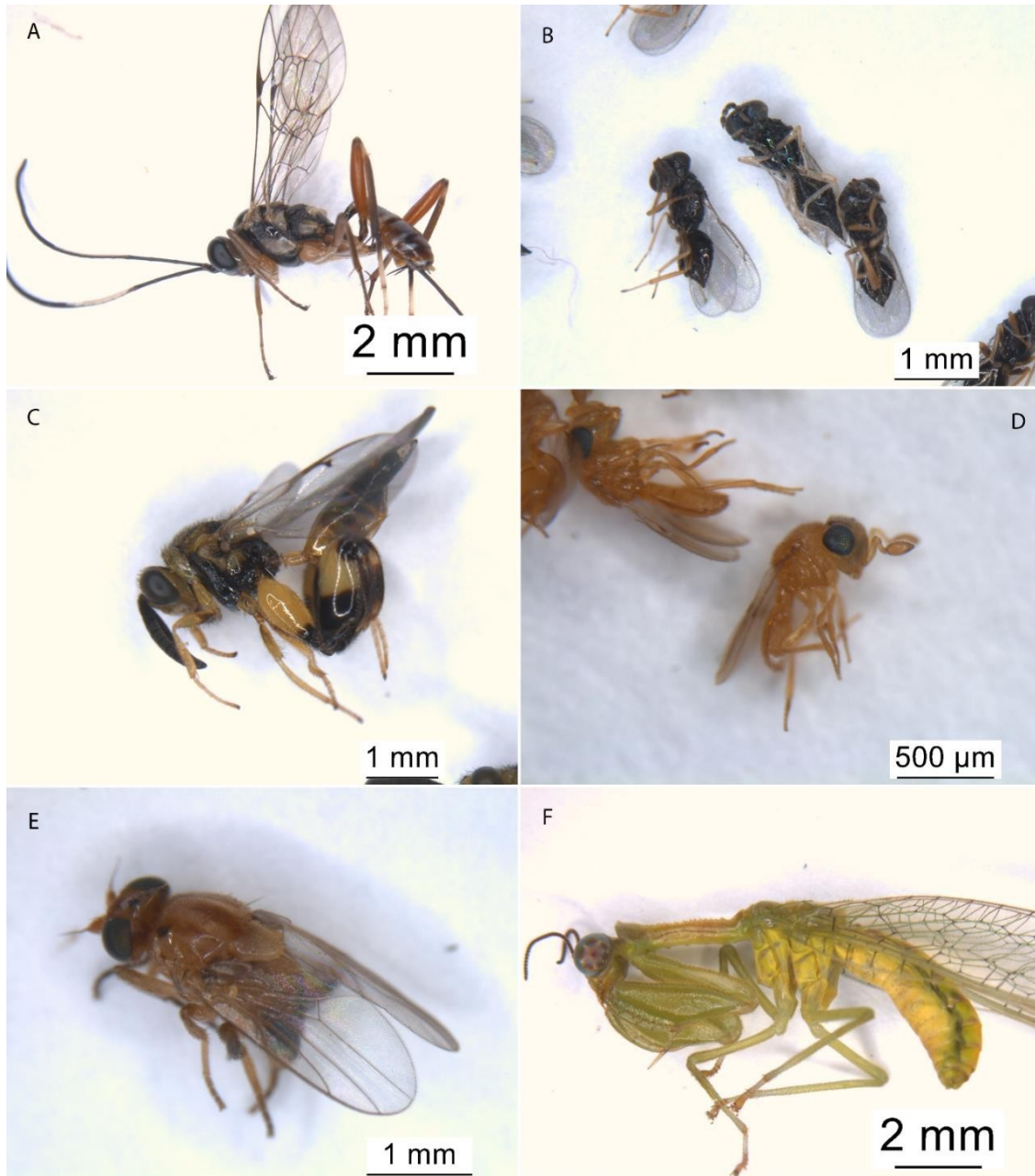


Fig. 1. Some of the insects that hatched from the egg sacs of the 10 studied species of spiders. (A). Cryptinae sp. 2 (Hymenoptera: Ichneumonidae); (B). *Aprostocetus* sp. (Hymenoptera: Eulophidae); (C). *Conura* sp. (Hymenoptera: Chalcididae); (D). *Baeus* sp. (Hymenoptera: Platygasteridae); (E). Chloropidae sp. 4 (Diptera: Chloropidae); (F). *Zeugomantispa virescens* (Neuroptera: Mantispidae).

Capítulo 4

Inclusion of an alien species on the host range of the Neotropical parasitoid *Hymenoepimecis bicolor* (Hymenoptera, Ichneumonidae)



Abstract

In this study we report the first case of an introduced alien host spider species being parasitized and manipulated by an ichneumonid wasp. *Hymenoepimecis bicolor*, previously described parasitizing exclusively *Trichonephila clavipes* (Araneidae), was observed parasitizing the European species *Cyrtophora citricola* (Araneidae) in southeastern Brazil. The cocoon web built by the parasitized spider is composed of a reduced horizontal sheet, which maintains the radial structure. The reduced number of radii and spirals probably reduce the chances of insect interception by these modified structures. In addition, the density of supporting threads is apparently very different between normal and modified webs. The cocoon web spun by *C. citricola* lacks the protective barrier structure usually observed in cocoon webs spun by parasitized females of *T. clavipes*. Our observations are in agreement with several predictions of the ecdysteroid hypothesis and represent an interesting opportunity for further investigation of interactions between these parasitoids and their spider hosts.

Keywords: Host behavioral manipulation, host-parasitoid interactions, introduced species

INTRODUCTION

Hymenoepimecis Viereck, 1912 is a genus of parasitoid wasps included in the *Polysphincta* group of genera, members of which are usually referred to as ‘polysphinctines’ (Hymenoptera, Ichneumonidae, Pimplinae) (Matsumoto 2016). The genus currently comprises 27 species, all of which are restricted to the Neotropical region (Yu et al. 2016; Pádua et al. 2020). Most species were previously reported to parasitize only one host species, but three species have been found to attack two host spiders each: *H. heidyae* Gauld, 1991: *Kapogea cyrtophoroides* (F. O. Pickard-Cambridge, 1904) and *K. sexnotata* (Simon, 1895) (Araneidae); *H. japi* Sobczak et al., 2009: *Leucauge roseosignata* Mello-Leitão, 1943 (Tetragnathidae) and *Mecynogea bigibba* Simon, 1903 (Araneidae); *H. veranii* Loffredo & Penteado-Dias, 2009: *Araneus omnicolor* (Keyserling, 1893) and *A. orgaos* Levi, 1991 (Araneidae) (Gonzaga and Sobczak 2007; Sobczak et al. 2014; Barrantes et al. 2018; Eberhard and Gonzaga 2019). The apparent specificity in the host-parasitoid interactions in the other cases, however, may be a consequence of a lack of information, since parasitoid behaviour has usually been described based on few observations. Conversely, host immobilization and manipulation during the egg laying process of polysphinctines usually involve specific approaching behaviors (see Gonzaga and Sobczak 2007; Takasuka et al. 2009; Kloss et al. 2016) and wasps seems to be very selective on host size (Gonzaga and Sobczak 2007). These factors may reduce the availability of the potential hosts. In addition, most reports of the interactions between *Hymenoepimecis* and their hosts indicate that spiders change their web-building behaviors because of the action of late instar wasp larvae, possibly through the inoculation of the hormone ecdysone or some precursor of this hormone (see Kloss et al. 2017; Eberhard and Gonzaga 2019). These changes result in modified cocoon webs with particular designs, and the survival of pupae of each parasitoid species may depend on specific designs obtained from their

hosts. This may be another factor driving host specificity.

To date, all spiders reported as hosts of *Hymenoepimecis* are species native to the geographic range of the parasitoids. In this study we report the first case of a recently introduced host species (*Cyrtophora citricola* (Forsskål, 1775), Araneidae) that was attacked and had its web building behavior altered by a parasitoid previously reared only from a native host spider. We compared the structure of the cocoon web built by the parasitized host with that of normal webs. Additionally, we searched for egg parasitoids of *C. citricola* in two localities in Brazil, namely Uberlândia (MG) and Volta Redonda (RJ), in order to describe possible new interactions between other native parasitoids and this potential new host.

Hymenoepimecis bicolor has a wide geographical distribution, occurring from the Amazon basin (Pádua et al. 2015; Sobczak et al. 2018) to the southern region of Brazil (pers. obs.). This species was previously observed to attack *Trichonephila clavipes* (Linnaeus, 1767) (Araneidae) in Serra do Japi, state of São Paulo (Gonzaga et al. 2010), as well as in several other Brazilian localities, such as Uberlândia (MG), Palmas (TO) and Cascavel (RS) (pers. obs.). The wasp performs direct attacks (Sobczak 2013) after hovering around the potential host, grasping the spider and introducing the ovipositor into the mouth of the host. The host is then paralyzed, while the wasp searches for and damages eggs previously attached by other individuals, finally depositing her own. The cocoon web spun by the spider shortly before being killed by the parasitoid larva is reduced, lacks spirals, and often presents barrier threads in a position close to that later occupied by the wasp cocoon.

The original distribution of *Cyrtophora citricola* includes Southern Europe, Africa, the Middle East, Pakistan, India, China, and Japan (World Spider Catalog 2022). Levi (1997) reported the first record of individuals collected in South America, in Valle del Cauca, Colombia. The first

well-documented record of this species in Brazil was made by Álvares and De Maria (2004), who reported specimens collected in the municipalities of Belo Horizonte (19°52'S, 43°58'W) and Prudente de Moraes (19°30'S, 43°07'W), both in the state of Minas Gerais. The specimens examined by these authors were collected in 2000 and 2001. Another possible previous record was made by Alves-Costa and Gonzaga (2001) during a study conducted in August 1998, in an area 80 km north of Manaus (2°30'S, 60°00'W), Amazonas State. These authors identified one of the species included in the study as *Cyrtophora* sp. (possibly *C. citricola*) based on juveniles and the shape of the webs. Later, Álvares and De Maria (2004) mentioned that they searched unsuccessfully for these specimens in the collection of the Instituto Nacional de Pesquisas da Amazônia (INPA) to confirm the identification.

METHODS

We found three cocoon webs of *C. citricola* in Volta Redonda, RJ. The first one was attached to the branches of a tree (*Anadenanthera colubrina*, Fabaceae), at a height of approximately 2.5 m from the ground, close to a small river within the urban area (22°31'24.17"S, 44°05'42.62"W). The other two were located within the area of the city zoo (Zoológico Municipal de Volta Redonda) (22°31'56.18"S, 44°06'12.74"W), attached to perennial shrubs (*Euphorbia milii*, Euphorbiaceae), both at heights of about 1 m. The first cocoon web was discovered on August 8, 2019 and the second on July 16, 2021. We located eight other webs of unparasitized individuals of *C. citricola* in a transect of 5 × 200 m from the site where we found the first cocoon web, on the same day. All egg sacs of these spiders were collected. The cocoon web was photographed and the cocoon was carefully removed and maintained in the laboratory until the emergence of the adult wasp. The second cocoon was collected from a damaged web and we kept

the cocoon in the laboratory until the emergence of the adult, but no information was collected on the web structure. The third cocoon web had a larva in its last instar attached by its dorsal tubercles on a web thread. We observed the beginning of cocoon construction by the larva and collected the cocoon 24 hours later to rear the adult wasp in the laboratory.

The egg sacs of another 35 spiders were collected in Uberlândia (18°57'11.37"S, 48°17'15.70"W), MG. This additional sampling was conducted two weeks after collecting the first cocoon (in August 2019), searching for parasitized individuals in another population of *C. citricola* (located about 1000 km from the first locality). All the egg sacs (77 from Uberlândia and eight from Volta Redonda) were opened in the laboratory and the egg masses were dissected to locate and identify possible egg parasitoids. We also counted the numbers of eggs inside 36 of those 77 egg sacs from Uberlândia (120.4 ± 79.9 [39 – 366] (mean $\pm$ sd [min – max])).

Photographs of normal and cocoon webs spun by *Trichonephila clavipes*, for comparison with webs spun by *C. citricola*, were previously obtained during studies conducted in 2010 in Serra do Japi, Jundiaí, SP, Brazil. The *H. bicolor* images were taken using a Leica DMC4500 digital camera attached to a Leica M205A stereomicroscope and multiple layers were stacked by using the software Helicon Focus 5.3 Pro.

RESULTS

The general structure of the cocoon webs of *C. citricola* differs from those of normal webs, including the reduction of the fine meshed sheet of dry silk, which is always present in webs of unparasitized individuals (Figs 1, 2). The larva consumed the spider at the same location occupied by the host while in its resting position, just below the center of the horizontal radial structure. Its dorsal tubercles then attached to the supporting threads located just above this position (Fig. 3).

The cocoon was constructed and remained connected to several radii (Figs 4–6). The *H. bicolor* collected just after cocoon construction emerged as an adult female 15 days later (Fig. 7). Females of *C. citricola* produced 120.4 ± 79.9 eggs (mean $\pm$ sd), but we did not find any egg parasitoids or egg predators within these egg masses.

DISCUSSION

The observation reported here is, as far as we know, the first record of a recently introduced alien host being parasitized by a species of *Hymenoepimecis* (Eberhard and Gonzaga 2019). One idiosyncrasy of the interaction between *H. bicolor* and its native host, *T. clavipes*, is that adult and sometimes subadult host females are much larger than the juveniles usually attacked (Sobczak 2013). Consequently, when most spiders within a population reach body sizes above the ideal, there is a pressure for wasps to attack unsuitable individuals (specifically large and potentially dangerous females or small adult males). In fact, Sobczak (2013) observed *T. clavipes* males carrying two larvae of *H. bicolor*. The same pressure may have directed the first attempts of *H. bicolor* to parasitize *C. citricola*, a new potential host within the size range usually selected for oviposition. In addition, individuals of both host species do not construct any form of shelter in their webs, remaining exposed during the day.

The selection of a new host species is an interesting occurrence because it has implications for our understanding of the plasticity of attacking and subduing behaviors of these wasps and in the mechanism of host behavior manipulation. New interactions between this introduced species and native egg parasitoids or predators, however, were not observed in the studied populations. Currently, records of egg parasitism of *C. citricola* are restricted to Europe (by *Philolema palanichamyi* Narendran, 1994 – Eurytomidae, and *Pediobius pyrgo* (Walker, 1839) – Eulophidae)

(Chuang et al. 2019) and to Yemen (by *Eurytoma cyrtophora* Zerova et al., 2008 – Eurytomidae) (Zerova et al. 2008, but see Chuang et al. 2019 on this identification). Further sampling of Brazilian populations will be conducted to investigate the possibility of the establishment of such interactions.

Manipulation of host *T. clavipes* behavior by *H. bicolor* usually results in cocoon webs presenting barrier threads surrounding the position originally occupied by the spider (Gonzaga et al. 2010) (Figs 8, 9). In the two-dimensional normal orb webs of unparasitized individuals, this position is usually exposed (Fig. 8). The increased complexity of this region may be important to reduce cocoon susceptibility to predators and/or hyperparasitoids, and also to increase stability of the web during the wasp's development, as the cocoon is suspended from the web. This new protective component is absent from cocoon webs spun by *C. citricola*. In this case, however, the position occupied by the cocoon is already surrounded by several supporting threads in the normal webs and the connection of the cocoon to the radial threads ensures its stability. As in cocoon webs of *T. clavipes*, the reduction of web components associated with prey interception and retention is the main characteristic of cocoon webs spun by *C. citricola*. This alteration may reduce the risks of web destruction by struggling insects and is often observed in cocoon webs spun by several hosts of polysphinctines (e.g., Eberhard 2000; Gonzaga and Sobczak 2007, 2011; Korenko et al. 2015; Gonzaga et al. 2016).

Eberhard and Gonzaga (2019) listed eight theoretical predictions derived from the ecdysteroid hypothesis (i.e., modifications in web structure induced by parasitoids included in the *Polysphincta* genus group occur as an effect of the injection of ecdysteroids or some precursor of this substance). The case described here corroborates at least four of these predictions: i) strict host species specificity in the wasps should be rare; ii) the phylogenies of the wasps and the spiders

that they parasitize should differ; iii) wasps of a single species should induce cocoon web designs that differ widely when they parasitize spiders with different natural histories; and iv) cocoon web design should be adjusted appropriately to the natural history of the spider species to provide protection. A fifth prediction is that cocoon webs should provide increased stability or protection for the cocoon of the wasp. We cannot be sure about this prediction based on a few observations, but the apparently stable structure of *C. citricola* webs likely requires little modification to offer protection to the cocoon (unlike the bidimensional design of *T. clavipes* webs). As previously mentioned, however, the cocoon remains suspended by several threads of the radial structure and probably is subject to a relatively reduced risk of damage or falling due to the action of intercepted insects. Further observation and experimental studies on this new system will clarify the generality of this finding and provide additional relevant information on the extension and limits of manipulation in this particular case.

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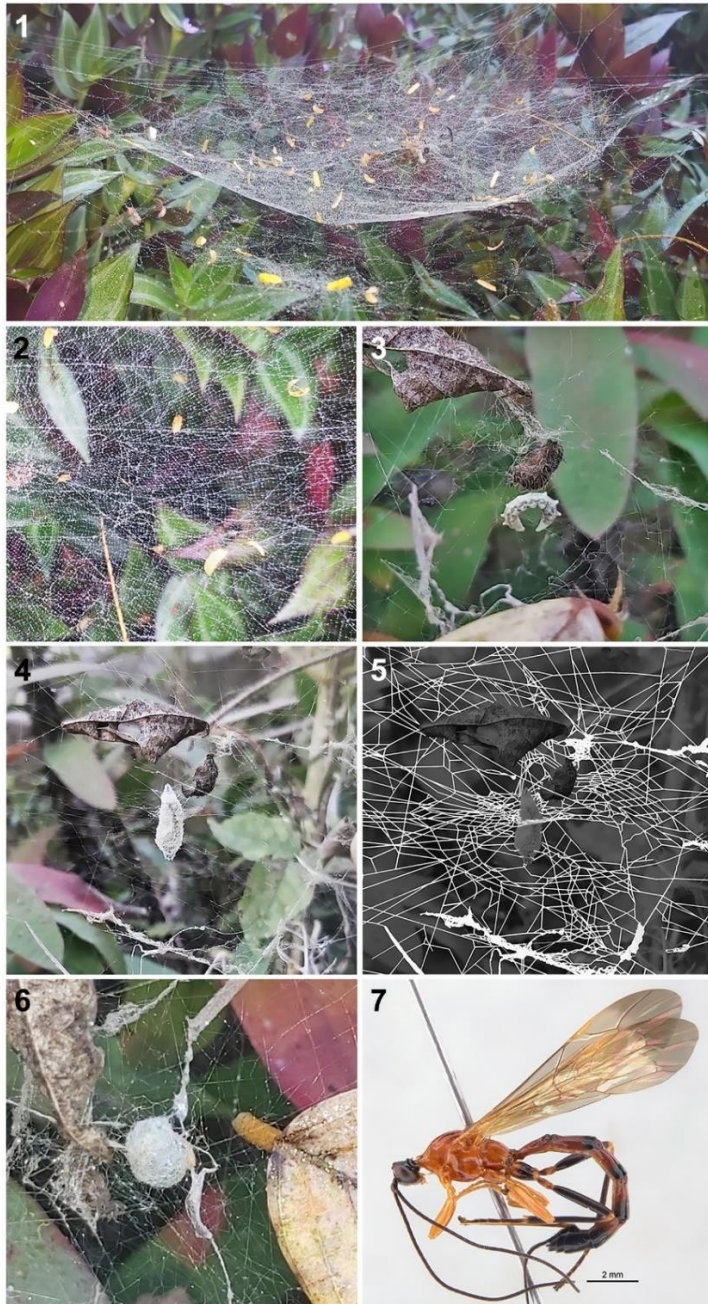
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Figures



Figures 1–7. 1 normal web structure of *Cyrtophora citricola* 2 detail of the fine meshed sheet of dry silk of a normal web. 3 larva in its last instar, attached to the web by the dorsal hooks 4, 5 frontal view of a cocoon web with a cocoon attached (in 5 the web threads are highlighted) 6 upper view of the same cocoon web 7 female of *Hymenoepimecis bicolor*.



Figures 8–9. 8 normal web of *Trichonephila clavipes* 9 cocoon of *H. bicolor* attached to a cocoon web spun by *Trichonephila clavipes* (photographs previously obtained in Serra do Japi, Jundiaí, SP).

CONSIDERAÇÕES FINAIS

Com este estudo que investigou questões a respeito da ecologia do cuidado parental em aranhas construtoras de teias, foi possível obter informações sobre quais fatores podem ter sido importantes para favorecer a evolução e a manutenção do cuidado parental nas espécies de aranhas estudadas. Diversos fatores bióticos e abióticos devem influenciar na sobrevivência da prole de aranhas e de outros animais, e o papel dos pais no cuidado parental pode estar relacionado às maiores pressões seletivas presentes em cada espécie e/ou população. Vimos neste trabalho que a guarda de ootecas pelos pais pode beneficiar as suas proles de diversas formas, as protegendo contra fatores ambientais e biológicos de mortalidade. Os comportamentos parentais como reparo de teias, controle de humidade das ootecas, mediação da camuflagem (como escolha de plano de fundo, orientação e posição corporal) e defesa contra inimigos naturais foram importantes para as espécies estudadas. No entanto, o cuidado parental pode não ser eficiente contra certos possíveis fatores de mortalidade. O impacto na sobrevivência dos ovos causado por eventos climáticos extremos como tempestades, secas e incêndios pode ser grande, e o efeito positivo do cuidado parental nessas situações deve ser limitado ou ineficiente. Ao mesmo tempo, o benefício do cuidado parental na sobrevivência da prole contra inimigos naturais pode ser menor contra predadores especializados do que generalistas, já que os mesmos comumente ultrapassam linhas de defesas existentes. Ainda assim, pouco é conhecido sobre a ocorrência de predadores e parasitoides de aranhas e de seus ovos, sobre a biologia desses inimigos naturais, sobre o impacto de cada espécie e sobre a especificidade das interações. Neste estudo investigamos a associação de vários predadores, parasitoides e hiperparasitoides com ootecas de aranhas construtoras de teia. Algumas interações foram registradas pela primeira vez entre as espécies estudadas e seus inimigos naturais.

A partir dos resultados obtidos nos capítulos desta tese, diversas novas questões a respeito da ecologia e evolução do cuidado parental surgiram e poderão ser investigadas no futuro. Primeiramente, as diferenças entre populações de uma mesma espécie com ampla distribuição geográfica podem ser importantes fatores que influenciam a ecologia do cuidado parental em animais. A composição e a diversidade de predadores e parasitoides pode ser bastante distinta, levando a diferentes balanços nas pressões seletivas em cada população. Ao mesmo tempo, as características abióticas como precipitação e temperatura variam entre populações amplamente distribuídas, podendo afetar diretamente e indiretamente a sobrevivência da prole de animais como as aranhas. Portanto, os papéis e os benefícios do cuidado parental podem variar de forma significativa mesmo dentro de uma mesma espécie. Outra questão a ser investigada é como os pais podem ser capazes de garantir condições favoráveis de temperatura e umidade para o desenvolvimento de seus ovos. Nesse aspecto, pouco ainda se sabe sobre a importância do cuidado parental para aranhas, principalmente quando comparadas com outros animais como anfíbios. As estratégias anti-predadores e parasitoides, tanto comportamentais quanto visuais, e a sua eficiência no aumento da sobrevivência de aranhas e de suas proles também é um aspecto pouco conhecido, já que a maioria das espécies de aranhas não foi estudada em relação a seus inimigos naturais e suas estratégias de defesa. A especificidade de cada inimigo natural e o uso de espécies invasoras como novos hospedeiros para parasitoides são aspectos a serem explorados em mais detalhe, já que no contexto atual algumas espécies de aranhas se tornaram amplamente distribuídas por várias regiões do planeta. Por fim, a grande diversidade de predadores e parasitoides de aranhas e de seus ovos, e o efeito do cuidado parental sobre cada potencial agente de mortalidade de suas proles são questões interessantes para serem investigadas, já que essas interações podem ter sido importantes para a evolução e manutenção do cuidado parental nesse grupo de animais.