



TESE DE DOUTORADO

História natural de *Aulacothrips* (Thysanoptera: Heterothripidae) e os efeitos do ectoparasitismo em cigarrinhas trofobiontes (Hemiptera: Auchenorrhyncha)

Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Doutor em Biologia Animal.

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História natural de *Aulacothrips* (Thysanoptera: Heterothripidae) e os efeitos do ectoparasitismo em cigarrinhas trofobiontes (Hemiptera: Auchenorrhyncha)

ADRIANO CAVALLERI

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Dra. Daniela Rodrigues

Dra. Aline Barcellos

Dra. Helena Piccoli Romanowski

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"Saber e não fazer é igual a não saber."

- Provérbio Zen

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Resumo

O registro do hábito ectoparasita em Thysanoptera estava limitado a *Aulacothrips dictyotus* (Heterothripidae). Esta espécie foi previamente registrada infestando ninfas e adultos de *Aetalion reticulatum* (Hemiptera: Aetalionidae), e acreditava-se que essa fosse uma associação única entre os tisanópteros. Entretanto, em recentes observações em áreas de Cerrado e floresta Amazônica, duas novas espécies de *Aulacothrips* foram encontradas, *Aulacothrips minor* e *Aulacothrips amazonicus*, respectivamente. Estes novos táxons apresentam histórias de vida distintas de *Au. dictyotus* e infestam diferentes hospedeiros. Ao mesmo tempo que não se conhecia a gama de hospedeiros destes tisanópteros, nada se sabia sobre a real interação deste tripses com as cigarrinhas e quais os efeitos da presença destes insetos para os Hemiptera. Nossos resultados indicam que *Au. minor* infesta várias espécies de Membracidae (Hemiptera), principalmente *Guayaquila xiphias* em áreas de Cerrado, enquanto que *Au. amazonicus* foi observada infestando cigarrinhas do gênero *Ramedia* (Membracidae) no Estado do Pará. Já *Au. dictyotus* ataca apenas *Ae. reticulatum*, uma cigarrinha de importância agrícola que possui uma ampla distribuição na América do Sul. Todas as espécies de *Aulacothrips* foram observadas sempre em hemípteros de hábito gregário atendidos por formigas, mas estas não molestam esses Thysanoptera. Estes tripses depositam seus ovos na planta, próximo à agregação de cigarrinhas. Este processo facilita as larvas de primeiro instar a encontrarem um hospedeiro. O hábito gregário destas cigarrinhas parece ser fundamental para estes tripses completarem seu ciclo de vida. Tal hábito permite que haja sempre hospedeiros disponíveis durante o processo de ecdise dos hemípteros, quando o tripses precisa abandonar seu hospedeiro e encontrar um novo indivíduo na mesma agregação. As três espécies de *Aulacothrips* apresentam diferenças marcantes nas áreas sensoriais dos antenômeros III–IV. Em *Au. amazonicus* estas áreas sensoriais são significativamente reduzidas enquanto que em *Au. dictyotus* estas são extremamente desenvolvidas. É provável que a diferença existente no tamanho destes órgãos entre as espécies esteja intimamente relacionada ao grau de especificidade parasitária e características do ambiente em que vivem. Observações da morfologia interna dos tripses e das cigarrinhas confirmaram o hábito ectoparasita de *Aulacothrips*. Estes parasitas foram observados sugando a hemolinfa das cigarrinhas, próximo aos corpos gordurosos. Avaliou-se o efeito da presença de *Au. dictyotus* no comportamento de *Ae. reticulatum* através da comparação de repertórios comportamentais de indivíduos infestados *versus* não infestados. Os resultados indicaram que

Au. dictyotus modifica o comportamento das cigarrinhas. Os indivíduos infectados apresentaram um grande número de atos comportamentais relacionados à limpeza corporal e executam estas atividades em frequências mais altas quando comparados às cigarrinhas sem tripes. O número de registros ligados à alimentação foi menor em cigarrinhas infestadas, e o número de registros de locomoção e dispersão para longe da agregação de origem foi maior. Os dados apresentados aqui constituem os primeiros passos para reconstruir o cenário evolutivo envolvido neste fascinante sistema multitrófico no qual *Aulacothrips* está presente.

Abstract

Ectoparasitism in Thysanoptera was recorded only from *Aulacothrips dictyotus* (Heterothripidae). This species was previously recorded infesting nymphs and adults of *Aetalion reticulatum* (Hemiptera: Aetalionidae) and this association was supposed to be singular amongst thrips. However, recent observations revealed two new *Aulacothrips* species in the Brazilian Cerrado and Amazon rainforest, *Aulacothrips minor* e *Aulacothrips amazonicus*, respectively. These new taxa exhibit distinct life-histories from *Au. dictyotus* and infest different hemipteran hosts. The host range of *Aulacothrips* was unknown, and it has not been demonstrated that the interaction with these insects is parasitic and what the effect of the thrips presence was to the Hemiptera. Our results showed that *Au. minor* infests several Membracidae (Hemiptera) species, especially *Guayaquila xiphias* in Cerrado areas, whereas *Au. amazonicus* was found infesting *Ramedia* treehoppers (Membracidae) in Pará state. In contrast, *Au. dictyotus* seems to attack only *Ae. reticulatum*, a widespread pest in South America. All *Aulacothrips* species were found attacking gregarious hemipterans tended by ants. However, the latter do not attack these Thysanoptera. *Aulacothrips* lay their eggs in the plant tissue, next to the Hemiptera aggregation. This behaviour allows first instar larvae to find available hosts upon eclosion. The gregarious behaviour exhibited by these hemipterans also seems to be crucial to the thrips life-cycle. This behaviour allows them to infest new individual hosts whilst the previously attacked Hemipteran host moults, then the thrips detaches from the host and infests another individual of the same aggregation. The three *Aulacothrips* species show remarkable differences on the sensorial areas on antennal segments III–IV. In *Au. amazonicus* these sensoria are significantly reduced while in *Au. dictyotus* they are extremely developed. This difference observed in sensoria length amongst *Aulacothrips* species might reflect the degree of specificity of these parasites and habitat characteristics. Observations on the internal morphology of the thysanopterans and their associated

hemipterans confirmed the ectoparasitic way of life of *Aulacothrips*. These parasites were observed sucking Hemiptera hemolymph, close to fat bodies. We analyzed the effect of *Au. dictyotus* presence on the behaviour of *Ae. reticulatum* through comparisons of behavioural repertoires of thrips-infested versus non-infested individuals. Our results indicated that *Au. dictyotus* alter host behaviour. Infested individuals displayed a large number of behavioural acts related to self-cleaning and they execute these activities in higher frequencies when compared to thrips-free hemipterans. The number of records related to feeding was lower in infested *Ae. reticulatum*. Moreover, thrips-infested aetalionids showed more locomotion and dispersal records. The records presented here are the first steps to reconstruct the evolutionary scenario behind this remarkable multitrophic system involving *Aulacothrips*.

1. INTRODUÇÃO GERAL

1.1. Interações multitróficas

Estudos abordando interações interespecíficas que envolvem três ou mais níveis tróficos (=multitróficas) em ambientes naturais têm recebido a atenção de muitos pesquisadores nas últimas décadas (ver Price *et al.* 1980, Tscharntke & Hawkins 2002). Os mecanismos e efeitos envolvidos nestas interações fornecem importantes informações sobre a composição da comunidade, bem como sobre os processos do ecossistema nos quais estão inseridas (Karban 1997, Schoonhoven *et al.* 1998, Del-Claro 2004, Oliveira & Del-Claro 2005). Segundo Price (2002), além da riqueza de espécies, a biodiversidade deve ser vista e avaliada de modo a contemplar a diversidade de interações entre os organismos, incluindo o papel ecológico das espécies, os tipos de interações e suas implicações. A conservação desta “biodiversidade das interações” deve ser tratada como uma parte fundamental em futuras estratégias para a manutenção da viabilidade das comunidades (Thompson 1997, Del-Claro 2004, Oliveira & Del-Claro 2005).

Dentro deste contexto, os insetos são componentes chave no funcionamento e conservação de diferentes tipos de sistemas, sendo o foco de muitos estudos referentes à dinâmica de interações multitróficas. Além disso, os insetos compreendem quase 70% do total de espécies animais conhecidas e estão presentes em quase todos os níveis tróficos (Janzen 1987, Daly *et al.* 1998, Walker 2001). Nos Neotrópicos, por exemplo, podemos destacar a grande abundância e diversidade de sistemas que envolvem plantas, formigas e insetos herbívoros (Rico-Gray & Oliveira 2007). Uma série de estudos destas interações tem sido desenvolvida no bioma Cerrado, fornecendo ferramentas para uma melhor compreensão do impacto de relações entre diferentes níveis tróficos sobre a diversidade de artrópodes na vegetação. As associações entre formigas e hemípteros trofobiontes (*i.e.*, que fornecem alimento açucarado às formigas atendentes) constituem um bom exemplo de sistemas multitróficos proeminentes na natureza (*e.g.* Del-Claro & Oliveira 1999, Oliveira & Freitas 2004, Moreira & Del-Claro 2005, Oliveira & Del-Claro 2005).

Entretanto, para determinados grupos de insetos, as informações sobre interações com outros organismos são escassas. Um claro exemplo ocorre com os Thysanoptera, popularmente chamados de tripes, cujos aspectos biológicos e ecológicos têm sido pouco estudados, particularmente na Região Neotropical.

1.2. Biologia e ecologia de Thysanoptera

Os tripses compreendem cerca de 6.000 espécies descritas, das quais mais de 2.000 estão registradas para a região Neotropical e 530 para o Brasil (Monteiro 2002, Mound 2002, Mound 2012). Os tisanópteros podem se alimentar de matéria de origem vegetal (fluidos vegetais), fúngica (esporos e hifas) e animal (principalmente outros artrópodes). Devido a essa plasticidade no seu hábito alimentar, estes insetos ocupam um número variado de habitats, tais como: flores e folhas de inúmeras espécies de plantas, cascas de árvores, galhas, folheto, entre outros (Buzzi & Miyazaki 1999). Cerca de 100 espécies de Thysanoptera são consideradas pragas em diversos tipos de plantas cultivadas (Lewis 1973, Mound & Teulon 1995, Mound & Marullo 1996). Os tripses promovem danos diretos, por destruírem os tecidos da planta ao succionar o fluido vegetal, e danos indiretos, pois através das lacerações tissulares, uma série de patógenos, como fungos, bactérias e vírus podem penetrar no vegetal. Certas espécies de tisanópteros, como algumas pertencentes aos gêneros *Frankliniella*, *Scirtothrips* e *Thrips* são transmissoras de viroses do gênero *Tospovirus*, que provocam, muitas vezes, prejuízos para a agricultura (Mound & Marullo 1996, Cavalleri & Mound 2012).

No Brasil, nos últimos 15 anos, menos de 5% dos trabalhos realizados com tripses abordam aspectos ecológicos em ambientes naturais (Cavalleri 2005). Oliveira & Del-Claro (2005) constataram o potencial dos tripses em trabalhos dessa natureza. Estes autores avaliaram o papel das formigas no controle da herbivoria pelo tripses *Pseudophilothrips didymopanicis* (Del-Claro & Mound) (Phlaeothripidae) em *Schefflera vinosa* (Araliaceae) no Cerrado. Este tisanóptero se alimenta principalmente nas folhas jovens e meristemas apicais, provocando deformações que mudam a arquitetura da planta, podendo inclusive causar a morte do vegetal. Ao mesmo tempo, nesta planta, agregações da cigarrinha *Guayaquila xiphias* (Fabricius) (Hemiptera: Membracidae) são comuns. Os indivíduos de *G. xiphias* produzem exsudações açucaradas (=honeydew) que atraem formigas, sendo conhecidos como trofobiontes (ver Hölldobler & Wilson 1990). Oliveira & Del-Claro (2005) verificaram que nos indivíduos de *S. vinosa* com formigas, os danos promovidos por *P. didymopanicis* foram significativamente menores. Ao mesmo tempo, a presença de determinadas espécies de formigas diminuíram os níveis de predação e parasitismo sobre *G. xiphias*, aumentando a sobrevivência e fecundidade das cigarrinhas (Del-Claro & Oliveira 2000, Oliveira & Del-Claro 2005).

Alguns estudos recentes vem apontando uma grande diversidade de tripes e interações envolvendo estes insetos no Brasil (Pinent *et al.* 2006, Cavalleri *et al.* 2006, Cavalleri & Kaminski 2007, Cavalleri & Mound 2012, Pereyra & Cavalleri 2012). Em relação a interações entre tripes e outros animais, a descoberta feita por Izzo *et al.* (2002) foi sem dúvida a mais marcante na região Neotropical nos últimos anos. Estes autores constataram pela primeira vez o hábito ectoparasita em Thysanoptera. Eles verificaram *Aulacothrips dictyotus* Hood (Heterothripidae) infestando cigarrinhas de hábito gregário da espécie *Aetalion reticulatum* L. (Hemiptera: Aetalionidae) no estado de São Paulo (SP) (Fig. 1a–c). Embora nenhum experimento tenha sido conduzido para verificar o hábito alimentar deste tripes, a sua biologia parecia estar intimamente associada à de seus hospedeiros. As larvas foram encontradas junto às tecas alares e asas de *Ae. reticulatum* e pupas foram encontradas debaixo das asas das cigarrinhas. Izzo *et al.* (2002) sugeriram que esta associação fosse específica, pois este tisanóptero não foi encontrado em uma outra espécie de *Aetalion*, também presente na mesma área de estudo. Além disso, foi observado que a presença do tripes parece interferir no comportamento dos indivíduos da agregação de cigarrinhas, que por sua vez apresentam associações mutualísticas com diversas espécies de formigas.

Pinent *et al.* (2002) levantam algumas hipóteses relacionadas à morfologia, biologia e ecologia de *Au. dictyotus*. Estes autores discutem que a distinta morfologia externa deste tisanóptero pode estar associada ao seu hábito de vida ectoparasita. Sugerem ainda que a oviposição ocorreria dentro do corpo da cigarrinha, o que seria um caso inédito dentro dos Thysanoptera. Esta hipótese é baseada a partir da presença de larvas recém emergidas de *Aulacothrips* presas ao corpo dos hospedeiros, assim como deformações no tegumento das cigarrinhas, o que poderia indicar lesões provocadas pelo ovipositor das fêmeas do tisanóptero. Após estes registros, nenhum trabalho foi conduzido com o objetivo de responder ou confirmar estes apontamentos.

Recentemente, em observações feitas em áreas de Cerrado e floresta Amazônica no Brasil, foi verificada a presença de outras espécies de *Aulacothrips* infestando várias espécies de cigarrinhas trofobiontes da família Membracidae. Estas espécies de cigarrinhas foram encontradas em inúmeras espécies de plantas pertencentes a famílias não proximamente relacionadas. Além disso, ovos de *Aulacothrips* foram encontrados em caules de *S. vinosa*, próximos às agregações de cigarrinhas, indicando que a oviposição ocorre na planta (endofiticamente), e não no inseto hospedeiro. Ainda assim, estes dados contrariam o padrão observado para a grande maioria das espécies da família Heterothripidae, que normalmente

estão associadas a uma ou poucas espécies de plantas hospedeiras (Mound & Marullo 1996, Del-Claro *et al.* 1997, Pereyra & Cavalleri 2012).

Isso reflete o quão pouco se sabia sobre aspectos da história de vida de *Aulacothrips* e sua real interação com as cigarrinhas. Também careciam de respostas outras questões fundamentais como: (i) qual a diversidade taxonômica de *Aulacothrips*? (ii) quais e quantas espécies de cigarrinhas são atacadas por estes tripes? (iii) quais as consequências da infestação de *Aulacothrips* para as cigarrinhas? Este trabalho se propõe a responder tais questionamentos e fornecer subsídios para melhor compreender a dinâmica e evolução desta única e intrigante interação entre tripes e cigarrinhas.

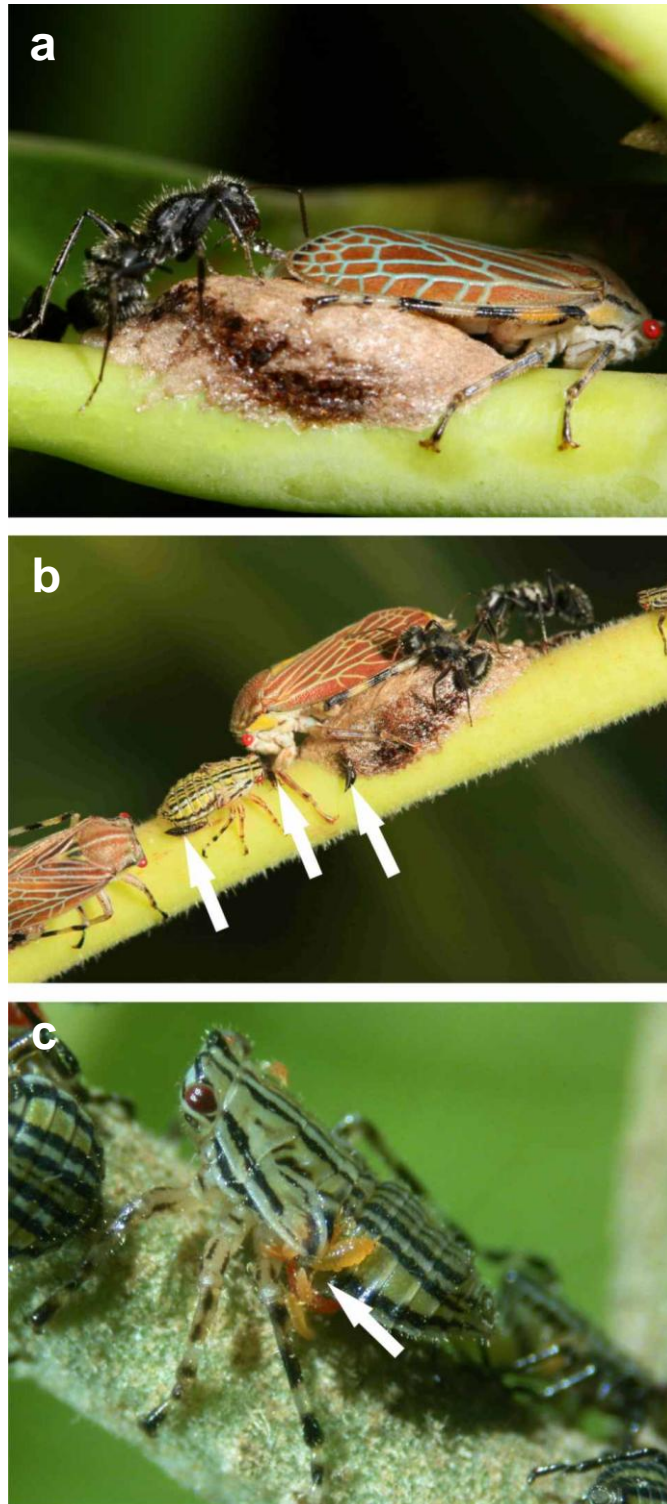


Figura 1a–c. Sistema multitrófico de interações envolvendo tripes-cigarrinhas-formigas. **(a)** Fêmea de *Aetalion reticulatum* sendo atendida por formiga do gênero *Camponotus*; **(b)** Agregação de *Ae. reticulatum* infestada por adultos de *Aulacothrips dictyotus* (setas) e atendida por formigas; **(c)** Ninfa de *Ae. reticulatum* infestada por larvas de *Au. dictyotus* (seta).

1.3. Objetivo geral

Conhecer aspectos da taxonomia, morfologia e biologia de *Aulacothrips*, inseridos em um sistema multitrófico de interações.

1.4. Objetivos específicos

I - Descrever a diversidade taxonômica presente no gênero *Aulacothrips*;

II - Fornecer informações que comprovem o hábito alimentar destes tripses;

III - Investigar a variação intra e interespecífica presente neste táxon;

IV - Descrever a morfologia externa e interna dos imaturos e adultos de *Aulacothrips*;

V - Avaliar o efeito da presença de *Aulacothrips dictyotus* no comportamento de *Aetalion reticulatum*.

1.5. Estrutura da tese

Este projeto visou abordar diferentes aspectos deste complexo sistema multitrófico de interações, conferindo uma visão multidisciplinar que envolveu estudos de taxonomia, morfologia e ecologia. A tese está dividida em 4 capítulos, cada um abordando diferentes aspectos da história natural de *Aulacothrips* e da interação tripes-cigarrinhas.

No primeiro capítulo é descrita a espécie *Aulacothrips minor*, que apresenta ampla distribuição no Cerrado brasileiro. A morfologia de *Aulacothrips* é detalhadamente analisada através de microscopia eletrônica de varredura e uma chave de identificação para adultos e larvas deste gênero é fornecida. Diferente de *Au. dictyotus*, este novo táxon possui um grande número de hospedeiros, quase todos pertencentes à família Membracidae. Ao que tudo indica, a presença do hábito gregário observado em todas as espécies hospedeiras é um ponto chave na biologia e evolução do ectoparasitismo em *Aulacothrips*. Além disso, todas as espécies de cigarrinhas atacadas por estes tripes possuem interações mutualísticas com formigas. As diferenças morfológicas e ecológicas entre as espécies de *Aulacothrips* e outros aspectos do ectoparasitismo são discutidos. Este capítulo encontra-se publicado no periódico *Zoologischer Anzeiger* (para referência completa veja Cavalleri *et al.* 2010).

O segundo capítulo apresenta a descrição de uma terceira espécie de *Aulacothrips*, encontrada na floresta Amazônica atacando cigarrinhas do gênero *Ramedia* (Membracidae). A descoberta desta nova espécie amplia a distribuição deste grupo assim como fornece informações importantes para compreensão da origem e evolução do hábito ectoparasita em Thysanoptera. *Aulacothrips amazonicus* apresenta características morfológicas muito distintas em relação às demais espécies do gênero, principalmente nas antenas. Em *Aulacothrips*, as áreas sensoriais dos antenômeros III–IV é bastante desenvolvida, formando uma série de sinuosidades que ocupam quase toda a área destes segmentos. No entanto, em *Au. amazonicus* estas áreas sensoriais são significativamente reduzidas. Neste capítulo, propomos que a diferença existente no tamanho destes órgãos entre as espécies do gênero está intimamente relacionada ao grau de especificidade parasitária e características do ambiente em que vivem. Este capítulo encontra-se publicado no periódico *Zootaxa* (para referência completa veja Cavalleri *et al.* 2012).

O terceiro capítulo investiga a morfologia interna de *Aulacothrips* e confirma a alimentação destes tripes na cigarrinha. Através de cortes histológicos, foi possível observar os estiletos maxilares inseridos no tecido dos hemípteros e provavelmente estes ectoparasitas se alimentem nos corpos gordurosos dos hospedeiros. Este é um tecido muito rico em energia

e a diminuição das reservas de lipídeos pode trazer consequências importantes para a fisiologia e comportamento dos hemípteros. Além disso, são apresentados e discutidos os principais aspectos da anatomia interna destes tripes. Este é o primeiro estudo sobre a morfologia interna de um representante da família Heterothripidae.

O quarto capítulo aborda o efeito da presença de *Aulacothrips* no comportamento das cigarrinhas hospedeiras. Estudos preliminares sugeriam que os tripes alteravam o comportamento dos hemípteros, tornando-os mais agitados. Para verificar tal hipótese, foram conduzidos experimentos *in situ* envolvendo *Au. dictyotus* e seu hospedeiro, *Ae. reticulatum*, em *Alchornea triplinervia* (Euphorbiaceae). Através da caracterização de repertórios comportamentais, o comportamento de cigarrinhas infestadas com tripes foi comparado àquele apresentado por cigarrinhas sem ectoparasitas. Os resultados indicam claramente que a presença de *Au. dictyotus* modifica o comportamento do hospedeiro. Os indivíduos infectados apresentam um grande número de atos comportamentais relacionados à limpeza corporal e executam estas atividades em frequências mais altas quando comparados às cigarrinhas sem tripes. O número de registros ligados à alimentação é menor em cigarrinhas infestadas. Além disso, cigarrinhas atacadas possuem mais registros de locomoção e de dispersão da agregação de origem. Os impactos e consequências dessas atividades na biologia e ecologia das cigarrinhas e dos tripes são discutidas.

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2. CAPÍTULO I

**Ectoparasitism in *Aulacothrips* (Thysanoptera: Heterothripidae) revisited:
host diversity on honeydew-producing Hemiptera and description of a new
species***

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Ectoparasitism in *Aulacothrips* (Thysanoptera: Heterothripidae) revisited: host diversity on honeydew-producing Hemiptera and description of a new species

Adriano Cavalleri^{a, *}, Lucas A. Kaminski^b and Milton S. Mendonça Jr.^{a, c}

^a*PPG-Biologia Animal, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil*

^b*PPG-Ecologia, Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil*

^c*Departamento de Ecologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*

* *Corresponding author: Adriano Cavalleri*

Email addresses: cavalleri_adriano@yahoo.com.br (A. Cavalleri),

(lucaskaminski@yahoo.com.br) (L. A. Kaminski), milton.mendonca@ufrgs.br (M. S.

Mendonça Jr.).

Abstract

Until now, *Aulacothrips dictyotus* Hood (Heterothripidae) is the only known thrips to exhibit an ectoparasitic way of life, infesting nymphs and adults of the aetalionid treehopper *Aetalion reticulatum*. However, recent observations in Brazilian Cerrado showed another *Aulacothrips* species infecting several honeydew-producing hemipteran species, mainly membracid treehoppers. Both parasitic species are usually found within a complex multitrophic system, which involves ant-hemipteran mutualism, a host plant and associated insect herbivores. In this paper, we present new data about ectoparasitism in Thysanoptera, describe *Aulacothrips minor* sp. nov. as well as males of *A. dictyotus*, and provide identification keys for adults and larvae of both species. Records of the infected Hemiptera species are given, including their host plants and associated tending-ants. Our results suggest *A. dictyotus* to be a host specific thrips restricted to *A. reticulatum*. In contrast, *A. minor* has a wide range of hosts, attacking 15 hemipteran species, all of them showing a gregarious and myrmecophilous habit. Differences observed in morphology, host use and life history strategies between the *Aulacothrips* species are also discussed.

Keywords: Brazilian Cerrado; multitrophic interactions; myrmecophily; systematics; treehoppers.

1. Introduction

Members of the Order Thysanoptera are called thrips and are known to be relatively opportunistic in their way of life and feeding habits (Mound and Teulon 1995). The majority of the 5,800 described species are phytophagous, nearly 40% are fungivorous and few are facultative or obligate predators on other arthropods (Mound and Marullo 1996; Mound 2010). Moreover, some species use curious resources as food, like Lepidoptera exudations (Downey 1965) and human blood (Williams 1921).

Despite this great diversity of habits, *Aulacothrips dictyotus* Hood (Heterothripidae) is the only known thrips to exhibit an ectoparasitic life style (Fig. 1a, b). This remarkable way of life contrasts with the flower-living habit of the other heterothripid species (see Del-Claro et al. 1997). *A. dictyotus* was previously recorded by Izzo et al. (2002) feeding on nymphs and adults of *Aetalion reticulatum* L. (Hemiptera: Aetalionidae), a polyphagous and gregarious honeydew-producing hemipteran that exhibits symbiotic interactions with ants (Silva et al. 1968; Brown 1976; Almeida-Neto et al. 2003). Larvae of *A. dictyotus* were found in large numbers under the wings of *A. reticulatum*, and the second larval stage spins a pupal cocoon on the hemipteran body. Although *Aulacothrips* eggs were not recorded, Izzo et al. (2002) suggested that the deformations observed in the hind wings of infested bugs could indicate a scarring of the nymph wing buds by thrips oviposition. The presence of these thrips in *Aetalion* aggregations also affected host behaviour, which became agitated, possibly influencing host biology at several levels.

The external morphology of adult *A. dictyotus* is very distinctive in having, on the abdominal tergites, a dorsal furrow bearing large wing-retaining setae, and enlarged antennal segments III and IV, each one with a highly convoluted sensorium. All these differences in body structure are possibly linked to its parasitic life style (Pinent et al. 2002). These authors

also indicated that the association between these two insects was possibly specific, since *A. dictyotus* was not observed infecting any other *Aetalion* species present at the same study site or on the same plant species.

Until now, this was the only species in the genus, and the ectoparasitic behaviour in Thysanoptera was restricted to the association between these two insects. However, recent observations in the Brazilian Cerrado indicated a different *Aulacothrips* species associated with other honeydew-producing hemipteran species (Fig. 1c). Unlike its congener, this new taxon was found infesting a wide range of hemipteran hosts, showing significant differences in life strategies and host utilization. *Aulacothrips* parasitism probably has multiple consequences for Hemiptera hosts and to their interaction with ants. However, this singular relationship remains poorly studied and biological and ecological processes behind it still unknown.

In this paper, we describe a new *Aulacothrips* species and the as yet unrecorded *A. dictyotus* male. Identification keys for adults and larvae are also provided. Auchenorrhyncha species that constitute true hosts for these thrips were recorded, including their host plants and associated tending-ants when infected.

2. Material and methods

2.1 Study sites

Field work was carried out in several localities of southeastern Brazil, mainly in Cerrado areas, in Bahia (BA), Goiás (GO), Minas Gerais (MG) and São Paulo (SP) states. The Cerrado biome extends over approximately 25% of Brazilian territory and constitutes the predominant natural vegetation of central Brazil. About 90% of the rainfall is concentrated

from October to March, resulting in two well-defined seasons: dry and wet (Ribeiro and Walter 1998; Oliveira and Marquis 2002). Due to its high diversity and species endemism, which has been critically threatened by increasing deforestation, the Cerrado constitute a global biodiversity hotspot and an essential area for conservation (Silva and Bates 2002).

2.2 Sampling and observations

From January/2007 to February/2010, auchenorrhynchans on different plant species were examined to check the presence of *A. dictyotus* attached to their bodies. When larvae and/or adults of this thrips were found on a host, the infested branch was collected and transported to the laboratory. Tending-ants, when associated with infected hemipterans, were also collected and identified. Observations on thrips behaviour, including interactions with their hosts and ants, were made *ad libitum* (Altmann 1974). Almost all behavioural observations were made on *Guayaquila xiphias* (Membracidae) aggregations on *Schefflera vinosa* (Araliaceae). This ant-treehopper-plant system is widespread in Cerrado areas in southeastern Brazil, and has been studied on several ecological aspects under a multitrophic perspective (*e.g.* Del-Claro and Oliveira 1999, 2000; Oliveira and Del-Claro 2005). Scanning electron microscopy was conducted under JEOL[®] 5800 for more detailed analysis of thrips external morphology and damage on Hemiptera tegument.

2.3 Material identification and vouchers specimens

All thrips collected were prepared for species determination using the methodology proposed by Mound and Marullo (1996). The *A. dictyotus* holotype, deposited at the NMNH, Washington, USA, was also examined. Thrips and ant specimens are deposited in the

zoological collection of the Laboratório de Ecologia de Insetos (LEI), in the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul - UFRGS (Porto Alegre, Brazil).

Auchenorrhynchs were deposited in the collection cited above and also in the entomological collection “Padre Jesus Santiago Moure”, in the Departamento de Zoologia of the Universidade Federal do Paraná - UFPR (Curitiba, Brazil).

3. Results

3.1 Systematics of *Aulacothrips*

Family Heterothripidae Bagnall, 1912

Genus Aulacothrips Hood, 1952

Type species: A. dictyotus Hood, 1952

Diagnosis: Antennae 9-segmented, segments III and IV greatly elongated and slightly depressed dorsally; sensorial areas on these segments in the form of loops (Fig. 2a, b). Head longer than wide; three pairs of ocellar setae present, setae I and III small and pointed, setae II well developed and expanded at tips (Fig. 3a); postocular setae (PO) II long and capitate; mouth cone short and maxillary palps three segmented. Pronotum wider than long and with seven pairs of stout and long setae (Fig. 2e, f); metascutum triangular and strongly reticulated (Fig. 3b); tarsi 2-segmented (Fig. 3c); forewings very narrow but basal fourth greatly swollen. Abdominal tergites II-VIII with a deep furrow placed medially, margined with two pairs of long and conspicuous wing retaining setae; posterior margin of tergites with a toothed craspedum laterally, but with a fringe of independent microtrichia medially. Male with a minute glandular area on sternite VII, situated at the antecostal ridge. Larvae with extensive

red internal pigmentation.

3.1.1 Key to *Aulacothrips* species

Adults

1. Antennal segment III cylindrical dorsally, with slightly convex sides; sensorial areas extending largely in dorsal surface (Fig. 2a); female urosternite IX without a row of short and flattened setae, in few cases bearing only one or two minute and sparse setae (Fig. 2c); posterior margin of male abdominal tergite VIII with a group of 8-12 pairs of long and stout setae placed close together (Fig. 3e)..... *dictyotus*
– Antennal segment III conical dorsally, with basal third clearly narrower than apical third; sensorial areas reduced, with loops wide apart from each other in dorsal surface (Fig. 2b); female urosternite IX with 3-9 short, hyaline, flattened setae placed laterally in one or two rows (Fig. 2d); male abdominal tergite VIII without a group of long setae placed at posterior margin..... *minor* sp. nov.

Larva II

1. Body length about 2.0 mm or more; fore coxa with a long and finely acute seta, placed laterally, usually curved at apex..... *dictyotus*
– Body length about 1.5 mm or less; seta on fore coxa not finely acute and curved..... *minor* sp. nov.

3.1.2 Diagnosis of *Aulacothrips dictyotus* Hood, 1952 (Figs. 1a, 2a, c, e and 3e, f)

Hood (1952) provided a morphological description of this species based on two females, one collected in Santa Catarina and another one possibly in São Paulo state (referred by the author as S.P.). Excellent drafts of body structure of *A. dictyotus* holotype are illustrated in

Mound et al. (1980). However, males were unknown and body structure of immature stages was not detailed until now.

Male macroptera: About 1,660 μm long (distended) and similar to female in colour and structure; posterior margin of urosternite VI expanding about 40 μm medially toward VII and with a fringe of finely acute setae (Fig. 3f); antecostal ridge of sternite VII with a circular glandular of 7 μm diameter; tergite IX with about 20 long and acute setae; tergite X with two pairs of long, curved and weakly expanded setae.

Measurements of male, in microns (μm): Head length 195, greatest width across cheeks 182, PO II length 52, interval 40; ocellar setae II 48, interval 55; eye dorsal length 93; median length of pronotum 193, width 285; mesonotum width 202, metascutal triangle length 77, major width 80; tergite IX length 97, basal width 192; tergite X length 70, basal width 85; genitalia length 265, major width 63, aedeagus length 180; length and width (between parenthesis) of antennal segments III-IX (excluding pedicel): 85 (basal width 33 and apical width 45), 100 (45), 12 (22), 12 (15), 12 (12), 10 (7), 10 (5), respectively.

Larva II. Body with red colour and approximately 2,040 μm long (distended). All dorsal setae with capitate apices and ventral setae acute; one dorsal pair of long setae on head and four pairs on pronotum, prothorax narrower than subsequent segments; antennae 6-segmented; eyes with red pigmentation; body with weak transversal lines of sculpture (Fig. 1a). Length of antennal segments III 70, IV 87, V 20, VI 27.

3.1.3 Description of *Aulacothrips minor* sp. nov. (Figs. 1c, 2b, d, f and 3a-d)

Female macroptera. Body dark brown; fore tibiae and tarsi light brown, middle and hind tibiae largely brown; antennal segment I concolorous with head, II dark brown, III-IX light brown; forewings blackish brown, with a dark basal line; major setae light brown; urotergites I and II slightly paler than the others.

Head about 1.2 times as long as greatest width, cheeks straight and slightly constricted behind eyes; PO II expanded at tip and well developed (longer than the distance between their bases); ocellar setae II long and capitate; antennae 9-segmented, III and IV great enlarged and surrounded by a continuous sensoria, forming numerous loops; ocellar area with linear reticulation (Fig. 3a). Mouth-cone short and rounded at tip, extending about 77 μm beyond posterior dorsal margin of head.

Pronotum transverse and reticulated; seven pairs of long and capitate setae, subequal in length; about 30 small and finely acute discal setae; mesonotum with reticles elongated horizontally at anterior region; metascutum triangular, strongly reticulated and without internal markings, the remaining metanotal area also sculptured and covered by numerous microtrichia (Fig. 3b). Urotergites with strong polygonal reticulation and with a well developed toothed craspedum, I without long setae, II-VIII with a median furrow bearing a pair of long and pointed setae medially; two pairs of stout setae present at lateral margin of the dorsal furrow; II-VIII with three pairs of long discal setae and with a fringe of small microtrichia placed medially at posterior margin; IX weakly sculptured anteriorly and with several stout discal setae; long setae on X weakly expanded. Urosternite II-VII with two pairs of short and flattened setae placed laterally, VIII with four pairs, IX with a row of three of such setae on each side.

Measurements of female (holotype), in microns (μm): Length about 1,720; head length 197, greatest width across cheeks 160, PO II length 53, interval 42; ocellar setae II 43, interval 50; eye dorsal length 75; median length of pronotum 205, width 295; width of mesonotum 202; forewing length 940, median width 25; metascutal triangle length 90, major width 97; tergite IX length 172, basal width 202; tergite X length 105, basal width 87; major setae on X 112; length of antennal segments III-IX: 80 (basal width 30 and apical width 48), 112 (40), 8 (17), 10 (15), 10 (7), 10 (7), 10 (5), respectively.

Macropteros male. Similar to female in colouration and structure, though smaller; antecostal ridge of sternite VII with a small circular glandular, scarcely 3 µm of diameter; tergite IX with 12 long and acute discal setae; tergite X with two pairs of long, curved and weakly expanded setae (Fig. 3d).

Measurements of male (allotype), in microns (µm): Length about 1,320; head length 167, greatest width across cheeks 142; PO II length 40, interval 32; ocellar setae II 32, interval 60; eye dorsal length 67; median length of pronotum 195, width 247; width of mesonotum 175; metascutal triangle length 70, major width 78; tergite IX length 82, basal width 125; tergite X length 65, basal width 62; curved setae on X 100; genitalia length 185; major width 47, aedeagus length 137; length of antennal segments III-IX: 62 (basal width 27 and apical width 40), 100 (32), 8 (15), 8 (12), 8 (10), 8 (7), 7 (5), respectively.

Egg (Fig. 5d). Very small (175 µm long and about 80 µm wide) and uniformly yellowish in colour, sub-reniform shape.

Larva I. Body with extensive red pigmentation and about 1,080 µm long (distended). All dorsal setae with capitate apices and ventral setae acute; one dorsal pair of long setae on head and three on pronotum; antennae 6-segmented. Length of antennal segments III 35, IV 57, V 10, VI 12.

Larva II. Body with red colour and approximately 1,480 µm long (distended). One dorsal pair of long setae on head and four on pronotum; antennae 6-segmented; eyes with red pigmentation; body with weak and transverse lines of sculpture. Length of antennal segments III 55, IV 75, V 15, VI 20.

Type material. Holotype female, Brazil, Campinas, from *Guayaquila xiphias* aggregation in *Schefflera vinosa* branches, 01.VIII.2009 (Kaminski, L.A. col.), in the zoological collection of Laboratório de Ecologia de Insetos (LEI), Departamento de Zoologia, UFRGS (Brazil).

Paratypes: 15 females and 2 males collected with holotype, in the collection cited above; 1

female from Campinas, collected in same host and plant species, 23.VI.2008 (Cavalleri, A. col.); 1 female from Campinas, collected in Idiocerinae aggregations in *Eugenia bimarginata*, 9.IV.2009 (Kaminski, L.A. col.); 2 females from Campinas, collected in *G. xiphias* aggregations in *Luehea grandiflora*, 18.III.2010 (Cavalleri, A. col.); 2 females from Itirapina, collected in *G. xiphias* aggregations in *S. vinosa*, 14.III.2010 (Cavalleri, A. col.); 6 females and 6 males from Sumaré, collected in *Notogonioides* sp. aggregations in *Ocotea* sp., 5.VI.2010 (Kaminski, L.A. col.); 1 female from Mogi-Guaçu, collected in *G. xiphias* aggregations in *S. vinosa*, 4.III.2007 (Kaminski, L.A. col.). Paratypes will be available in CAS Entomology collection, California (USA) and CSIRO Entomology collection, Canberra (Australia).

3.2 Taxonomic comments

As pointed out by Mound and Morris (2007), *Aulacothrips* constitutes a remarkable group and its morphology contrasts with the remaining Heterothripidae. Beside this, all members of this family have antennae primarily 9-segmented, with sensorial areas on III and IV forming a continuous porous band. This group now comprises 75 species belonging to four genera, *Heterothrips* (67 spp.), *Scutothrips* (4 spp.), *Lenkothrips* (2 spp.) and *Aulacothrips* (2 spp.), all from the Americas (Mound 2010).

The convoluted sensoria found in *Aulacothrips* are also present in *Lenkothrips sensitivus* (De Santis and Sureda), but extend only to the mid-point on either side of antennal segments III and IV. With the exception of this character, *L. sensitivus* is structurally similar to *Heterothrips* species (Mound and Marullo 1996). Many *Heterothrips* have an abdominal tergal furrow with stout setae laterally, but their antennal and metanotum structures are remarkably different from *Aulacothrips* (Mound et al. 1980). The triangular metascutum is

also well developed and covered with strongly reticulation in *Scutothrips* and according to Mound and Marullo (1996) this genus is intermediate in structure, between *Heterothrips* and *Aulacothrips*.

Bhatti (2006) hypothesized a sister-group relationship between *Aulacothrips* with the rest of Heterothripidae. Based in differences on antennae, the author also proposed a separate family for *A. dictyotus*, called Aulacothripidae. However, this classification was not based in a phylogenetic context, lacked hypothesis testing and is treated here as a subjective decision. Further systematic studies are needed, together with molecular data, to elucidate phylogenetic relationships among Heterothripidae genera.

During our study, a remarkable difference in body length between *Aulacothrips* species was observed (Fig. 4). However, intraspecific variation recorded on this character is considerable and additional studies with different populations are needed to investigate its stability. Despite this, these two taxa are very similar in their external morphology, except by consistent differences in antennae and chaetotaxy of the last abdominal segments. Antennal segments III and IV in both species are dorsally depressed and the convoluted sensorial bands are arranged in loops around the segment. However, these loops are different in size and possibly in number. In all *A. dictyotus* females examined, the sensoria in segments III and IV are clearly more extensive dorsally than in *A. minor* (Fig. 2a, b). In contrast, the shape and position of these convolutions have considerable intraspecific variation and some *A. dictyotus* males showed reduced sensoria as in *A. minor*.

The number and position of PO and ocellar setae on head are the same in both species. The length of these setae also seems to have little taxonomic value since previous measurements indicated that they show allometric variation (see Cavalleri and Kaminski 2007). Despite this, there seem to be cases of differences approaching significance, but they all have overlapping ranges. A clear example could be observed in major PO setae length,

which varies from 50 μm to 68 in *A. dictyotus* and 37 to 53 μm in *A. minor*.

Although both species have extensive body sculpture, this is probably of limited use in their taxonomy. However, almost all *A. minor* studied have well defined reticles on the ocellar area, sometimes covered by ocellar pigmentation (Fig. 3a), while the sculpture lines in *A. dictyotus* are faintly indicated or absent. *A. minor* pronotum is always well reticulated (Fig. 1f), and this could be also observed in the *A. dictyotus* holotype, but several *A. dictyotus* individuals collected in São Paulo lack reticulation medially (Fig. 2e).

All *Aulacothrips* females have one or two small and flat setae placed laterally at each side of urosternites II-VII. On VIII they are usually grouped in 4 to 6 pairs of setae. The number of such structures on *A. minor* urotergite IX is variable but they are always present, characterized by 3 to 9 setae placed in one or two rows (Fig. 2d). In *A. dictyotus* these setae are frequently absent on IX, but sometimes one or two are present at each side (Fig. 1c).

A clear difference between these species could be also observed on the posterior margin of male abdominal tergite VIII. *A. dictyotus* exhibits numerous long setae placed close together near the wing-retaining setae (Fig. 3e). In *A. minor* such setae are completely absent. In addition, the abdominal sternite VI extends posteriorly in *A. dictyotus* males, covering the apical third of sternite VII and its glandular area. These differences might be associated with mating behaviour.

3.3 Ecological data

All thrips infesting *A. reticulatum* were identified as *A. dictyotus* and were collected in six unrelated plant species (Table 1). In contrast, *A. minor* was recorded in 15 Auchenorrhyncha species, where 14 of these belong to the Membracidae and one to the Cicadellidae (Table 2). These last hemipteran hosts were found in 20 plant species, distributed in 12 families. All

Aulacothrips hosts species showed gregarious and myrmecophilous habits and belonged to the superfamily Membracoidea (Figs. 5a-f and 6a-d). Tending-ants observed in symbiotic association with infected Membracoidea belong to *Camponotus* (8 spp.) (Formicinae), *Cephalotes* (2 spp.) (Myrmicinae) and *Ectatomma* (1 sp.) (Ectatomminae). *Camponotus rufipes* and *Camponotus crassus* were the most common tending species in infested hemipteran aggregations, found in 48% and 20% of all records, respectively. Tending ants were absent only from *Erechtia* sp. and *Guayaquila* sp. aggregations, although these genera are frequently associated with ants (Silva et al. 1968; Godoy et al. 2005). Aggressive behaviour or predation on *Aulacothrips* by ants was not observed in hemipteran aggregations.

All *A. minor* stages, with the exception of eggs and pupae, were observed on bodies of hemipteran nymphs and adults (Figs. 5b, e, f and 6c-d). Adult *Aulacothrips* species were commonly recorded on the dorsal part of the thorax and abdomen of immature and adult hemipteran hosts. Thrips larvae and adults of both species, including males, seem to have the same ectoparasitic feeding habit. However, in field observations *A. minor* females were often recorded walking along the branches of *S. vinosa* near floral buds. *A. dictyotus* larvae were frequently found attached to the leg and wing (or wing rudiment) articulations. In contrast, immature *A. minor* were usually recorded ventrally on the hemipteran thorax (Figs. 5f and 6d) and also underneath the adult pronotum. Oviposition behaviour was observed in *A. minor* and eggs were laid in a *S. vinosa* branch, inside plant tissue, near *G. xiphias* aggregations (Fig. 5c-d). Similarly, *A. minor* eggs were recorded inside the female abdomen in April, June and August, while in *A. dictyotus* they were present only in August.

A. dictyotus is now known from three Brazilian states: Bahia, Santa Catarina and São Paulo (Fig. 7), in latitudes ranging from 12°33' S (Lençóis, BA) to 27°09' S (Nova Teutônia, SC). *A. minor* was collected in Cerrado areas in Goiás, Minas Gerais and São Paulo (Fig. 6).

In Mogi-Guaçu (SP), both *Aulacothrips* species were found in sympatry, but infesting distinct hemipteran hosts.

4. Discussion

As suggested by Pinent et al. (2002), *A. dictyotus* seems to be a highly host-specific parasite, possibly restricted to *A. reticulatum*. In contrast, *A. minor* has a wide host range, infecting unrelated Membracoidea species. However, *A. minor* may have some degree of specificity to Membracidae treehoppers and *A. reticulatum* does not constitute a host to this thrips. Moreover, in environments with high treehopper diversity such as in the Brazilian Cerrado, *A. minor* might use additional species as hosts than present recorded. For example, Lopes (1995) collected more than 50 Membracidae species on 40 host-plant species in Mogi-Guaçu, in the same area where we recorded this thrips on Hemiptera. However, not all hemipteran species seem to be potential hosts of *A. minor*. All infected Membracoidea were found in aggregations, which usually were tended by ants. Solitary non-myrmecophilous species were also sampled and examined, but no *Aulacothrips* were present in such cases. As pointed out by Izzo et al. (2002), the gregarious habit of *A. reticulatum* may facilitate immature *A. dictyotus* transfer among host individuals when the hemipterans moult. The gregarious behaviour displayed by some Membracoidea is certainly an important and limiting factor for the completion of the *Aulacothrips* parasitic life cycle, allowing choice and changing of host individuals in the same aggregation.

The ant genera recorded are widely associated with trophobiont insects (DeVries 1991; Fiedler 2001), and could attack their natural enemies and other herbivorous insects feeding on the same host-plants (Oliveira and Del-Claro 2005). The presence of tending ants in almost all records and personal observations here indicate that they do not attack *Aulacothrips*

individuals. According to Lohman et al. (2006), in some multitrophic interactions involving ants and partners, natural enemies could use chemical cues to avoid ant predation. Frequently, this kind of strategy involves cuticular hydrocarbons, making these natural enemies undetectable by tending-ants (Liepert and Dettner 1993, 1996). In the multitrophic association comprised by host plant, honeydew-producing hemipterans, ectoparasitic thrips and ants, it is possible that *Aulacothrips* species could prevent predation by using chemical mimicry or camouflage mechanisms (see Silveira et al. 2010). In this context, the presence of tending-ants on Membracoidea aggregations could be beneficial to these thrips, creating an enemy-free space for *Aulacothrips* species.

A. minor females were found walking on the host plant, and so plant feeding by these adults cannot at present be excluded. Based on extensive collecting and field observations, both *Aulacothrips* species populations are undoubtedly female-biased and males are also associated with Auchenorrhyncha. As dissections of infected *A. reticulatum* did not reveal any thrips eggs on or in the host body, we believe that both *Aulacothrips* species lay their eggs in the plant tissue, near hemipteran aggregations. This behaviour might possibly facilitate finding a hemipteran host for the first instar thrips larva. There is also no reason to believe that all plant species associated with these hemipterans also host *A. minor*, although further studies may record new host-plants.

The absence of *A. minor* pupal cocoons on treehoppers might indicate the existence of different strategies and behaviours for this stage, perhaps with different hosts involved. The small length of *A. minor*, its distinct biology and host use pattern are possibly linked with the remarkable Membracidae morphology. For instance, the minute larvae size allows the infestation of small Membracoidea species, as *Bolbonota* sp.1 and *Erecthia* sp., with adults smaller than 3.0 mm long. In contrast to its congener, *A. minor* seems to have a reduced

suitable area to pupate, because most of the adult treehopper body is covered and protected by a hard and well developed pronotum (Fig. 5b, e).

A. dictyotus geographical distribution is not restricted to the Brazilian Cerrado (Fig. 7). The holotype was collected in Southern Brazil, in an area with Atlantic Rainforest influence and located approximately 350 Km from a savanna area. Similarly, *A. minor* was found in Cerrado and adjacent areas, and might also have a larger latitudinal distribution than presently recorded. Indeed, since many infected Membracoidea species are widespread in Brazilian territory, these parasitic thrips have a potentially wide distribution about the country. Field observations suggest that both *Aulacothrips* species are very abundant in Cerrado, especially by the end of the wet summer season. As in many parasitic insects, populations of *Aulacothrips* are probably density dependent, influenced by hemipteran host availability and abiotic factors, which could affect their spatial and temporal distribution (Godfray 1994; Poulin 1998).

Based on the life history of the remaining Heterothripidae species (all of them presently recorded as flower-living insects), we believe that this newly discovered parasitic behaviour may have derived from a flower-living ancestor that opportunistically started feeding on gregarious hemipterans, commonly found in flowering branches (Liu 2006). The mechanisms involved in *Aulacothrips* speciation are not easy to define. Given the high treehopper diversity in study areas, the idea of *Aulacothrips* divergence based in the isolation of parasite populations after colonization of new host species seems plausible. The active behaviour observed in *Aulacothrips* adults might have played an important role in the evolution of this group. As in several ectoparasites, *Aulacothrips* females are able to choose their host actively and possibly lay their eggs in plants with available Membracoidea aggregations. This behavioural trait also increases the probability of colonization of new hosts, facilitates individual contact within a population, and the distance between hosts may not constitute an

extrinsic barrier (McCoy 2003). Undoubtedly, both *Aulacothrips* species actually coexist in several Cerrado areas and the marked difference in male copulatory organ length is a strong indicative of reproductive isolation, acting as a mechanical barrier against hybridization.

The records presented here are the first steps to reconstruct the evolutionary scenario behind this remarkable system involving *Aulacothrips*. Indeed, these thrips are only a piece of an intricate multitrophic system and the evolution of this singular lifestyle amongst Thysanoptera can be studied at several hierarchical levels. Further ecological, genetical and chemical approaches will undoubtedly allow understanding the implications of thrips ectoparasitism to all the organisms involved.

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Table 1. Hemipteran host species for *Aulacothrips dictyotus*, with their respective host-plant, tending-ant species and locality. State abbreviations: Bahia (BA) and São Paulo (SP).

Hemipteran host	Associated plant	Tending-ants	Locality (State)
Family Aetalionidae			
<i>Aetalion reticulatum</i>	<i>Alchornea triplinervia</i> (Euphorbiaceae)	<i>Camponotus crassus</i> , <i>Camponotus renggeri</i> , <i>Camponotus rufipes</i>	Mogi-Guaçu (SP)
	<i>Bauhinia variegata</i> (Fabaceae)	<i>C. crassus</i>	São José do Rio Preto (SP)*
	Fabaceae	<i>C. rufipes</i>	Mogi-Guaçu (SP)
	<i>Nectandra</i> sp. (Lauraceae)	<i>C. rufipes</i>	Mogi-Guaçu (SP)
	<i>Schinus terebinthifolius</i> (Anacardiaceae)	<i>C. rufipes</i>	Lençóis (BA)
	<i>Siparuna guianensis</i> (Siparunaceae)	<i>C. rufipes</i>	Mogi-Guaçu (SP)
	<i>Viola sebifera</i> (Myristicaceae)	Not recorded	Mogi-Guaçu (SP)
	<i>Xylopia aromatica</i> (Annonaceae)	<i>C. rufipes</i>	Mogi-Guaçu (SP)

* Izzo et al. (2002)

Table 2. Hemipteran host species for *Aulacothrips minor* sp. nov., with their respective host-plant, tending-ant species and locality. State abbreviations: Goiás (GO), Minas Gerais (MG) and São Paulo (SP).

Hemipteran host	Associated plant	Tending-ants	Locality (State)
Family Cicadellidae			
Idiocerinae sp.	<i>Eugenia bimarginata</i> (Myrtaceae)	<i>Camponotus crassus</i>	Campinas (SP)
Family Membracidae			
Membracidae sp.1	Loranthaceae	<i>Camponotus sericeiventris</i>	Rio Piracicaba (MG)
<i>Amastris</i> sp.	<i>Byrsonima intermedia</i> (Malpighiaceae)	<i>Camponotus</i> sp.1	Campinas (SP)
	<i>Byrsonima coccolobifolia</i> (Malpighiaceae)	<i>Camponotus</i> sp.1	Campinas (SP)
<i>Bolbonota</i> sp. 1	<i>Solanum lycocarpum</i> (Solanaceae)	<i>Camponotus rufipes</i>	Mogi-Guaçu (SP)
<i>Bolbonota</i> sp. 2	<i>Vernonia</i> sp. (Asteraceae)	<i>C. rufipes</i>	Itirapina (SP)
<i>Calloconophora pungionata</i>	<i>Alchornea triplinervia</i> (Euphorbiaceae)	<i>Camponotus</i> sp.1	Mogi-Guaçu (SP)
	<i>Bauhinia rufa</i> (Fabaceae)	<i>C. rufipes</i>	Mogi-Guaçu (SP)
<i>Enchenopa brasiliensis</i>	<i>S. lycocarpum</i>	<i>C. rufipes</i>	Itirapina (SP)
<i>Enchenopa gracilis</i>	<i>Banisteriopsis stellaris</i> (Malpighiaceae)	<i>C. rufipes</i>	Itirapina (SP)
	<i>Bauhinia variegata</i> (Fabaceae)	<i>Camponotus leydigii</i> , <i>Ectatomma ruidum</i>	Campinas (SP)
	<i>Luehea grandiflora</i> (Malvaceae)	<i>Camponotus blandus</i> , <i>C. crassus</i> , <i>Camponotus renggeri</i> , <i>C. rufipes</i>	Campinas (SP)
	<i>Banisteriopsis argyrophylla</i>	<i>C. rufipes</i>	Mogi-Guaçu (SP)
<i>Enchenopa</i> sp.	(Malpighiaceae)	<i>C. rufipes</i>	Mogi-Guaçu (SP)

<i>Erecthia</i> sp.	<i>Nectandra</i> sp. (Lauraceae)	Not recorded	Mogi-Guaçu (SP)
<i>Guayaquila xiphias</i>	<i>Aegiphila</i> sp. (Verbenaceae)	<i>C. rufipes</i>	Campinas (SP)
	Lauraceae	Not recorded	Alto Paraíso de Goiás (GO)
	<i>L. grandiflora</i> (Malvaceae)	<i>C. rufipes</i>	Campinas (SP)
	<i>Piptocarpha</i> cf. <i>rotundifolia</i> (Asteraceae)	<i>Camponotus</i> sp.2	Campinas (SP)
	<i>Schefflera macrocarpa</i> (Araliaceae)	<i>C. crassus</i>	Mogi-Guaçu (SP)
	<i>Schefflera vinosa</i> (Araliaceae)	<i>C. blandus</i> , <i>C. crassus</i> , <i>C. renggeri</i> , <i>C. rufipes</i> , <i>C. sericeiventris</i>	Campinas, Mogi-Guaçu (SP)
<i>Guayaquila</i> sp.	<i>S. lycocarpum</i>	<i>C. rufipes</i>	Mogi-Guaçu (SP)
	<i>Psittacanthus robustus</i> (Loranthaceae)	<i>C. rufipes</i>	Conceição do Mato Dentro (MG)
<i>Notogonioides erythropus</i>	<i>Ocotea</i> sp. (Lauraceae)	<i>C. crassus</i>	Sumaré (SP)
<i>Ramedia pauperata</i>	<i>L. grandiflora</i>	<i>C. blandus</i>	Campinas (SP)
<i>Tragopa albimacula</i>	<i>S. vinosa</i>	<i>Cephalotes atratus</i>	Mogi-Guaçu (SP)

Figure captions

Fig. 1. Ectoparasitic *Aulacothrips* larvae. (a) *A. dictyotus* larva II; (b) damage on *Aetalion reticulatum* abdomen caused by *A. dictyotus* mouth parts; (c) *Aulacothrips minor* sp. nov. larva II (arrow) attached underneath the pronotum of a Membracidae. Scale bars = 200, 1 and 100 μm , respectively.

Fig. 2. External morphology of *Aulacothrips* females. (a) dorsal view of *A. dictyotus* antennal segments III-IV; (b) dorsal view of *Aulacothrips minor* sp. nov. antennal segments III-IV; (c) *A. dictyotus* sternite IX; (d) *A. minor* sternite IX (arrows indicate the flattened setae); (e) *A. dictyotus* head and pronotum; (f) *A. minor* head and pronotum. Scale bars = 20 μm ; except (e) and (f) = 50 μm .

Fig. 3. External morphology of *Aulacothrips*. a-d, *A. minor*. (a) ocellar region; (b) metascutum; (c) male fore tarsus; (d) male terminalia in lateral view; (e) and (f) *A. dictyotus* male: (e) urotergite VIII (arrows indicate the group of long setae); (f) urosternites VI-VIII (arrow indicates the fringe of setae). Scale bars = 20 μm .

Fig. 4. Body length of *Aulacothrips dictyotus* (n=16) and *Aulacothrips minor* sp. nov. (n=19) females.

Fig. 5. Natural history of *Aulacothrips minor* sp. nov. infecting *Guayaquila xiphias* on shrubs of *Schefflera vinosa* (Araliaceae) in Brazilian Cerrado. (a) general aspect of *G. xiphias* aggregation tended by *Camponotus rengerii*; (b) two thrips (arrow) attacking an adult treehopper; (c) female thrips laying egg next to *G. xiphias* aggregation (arrow); (d) *A. minor* egg after dissection of plant tissue (arrow); e, larvae (white arrows) and adult thrips (black

arrow) infecting the aggregation; (f) *G. xiphias* nymphs infected by *A. minor* larvae (arrow). Scale bars = 3 mm; except (d) = 0.2 mm.

Fig. 6. *Aulacothrips* species infecting different hemipteran species. (a) *A. dictyotus* adult (black arrow) and larvae (white arrow) infesting *Aetalion reticulatum* on *Alchornea triplinervia* (Euphorbiaceae); (b) adult thrips on *A. reticulatum* nymph; (c) *A. minor* (arrow) attacking an Idiocerinae leafhopper aggregation tended by *Camponotus crassus* ants; (d) *Enchenopa gracilis* nymph infected by *A. minor* larvae (arrow) on *Bauhinia variegata* (Fabaceae). Scale bars = 0.3 cm.

Fig. 7. Political map of Brazil showing *Aulacothrips* species distribution and cerrado biome distribution; literature records are also included.

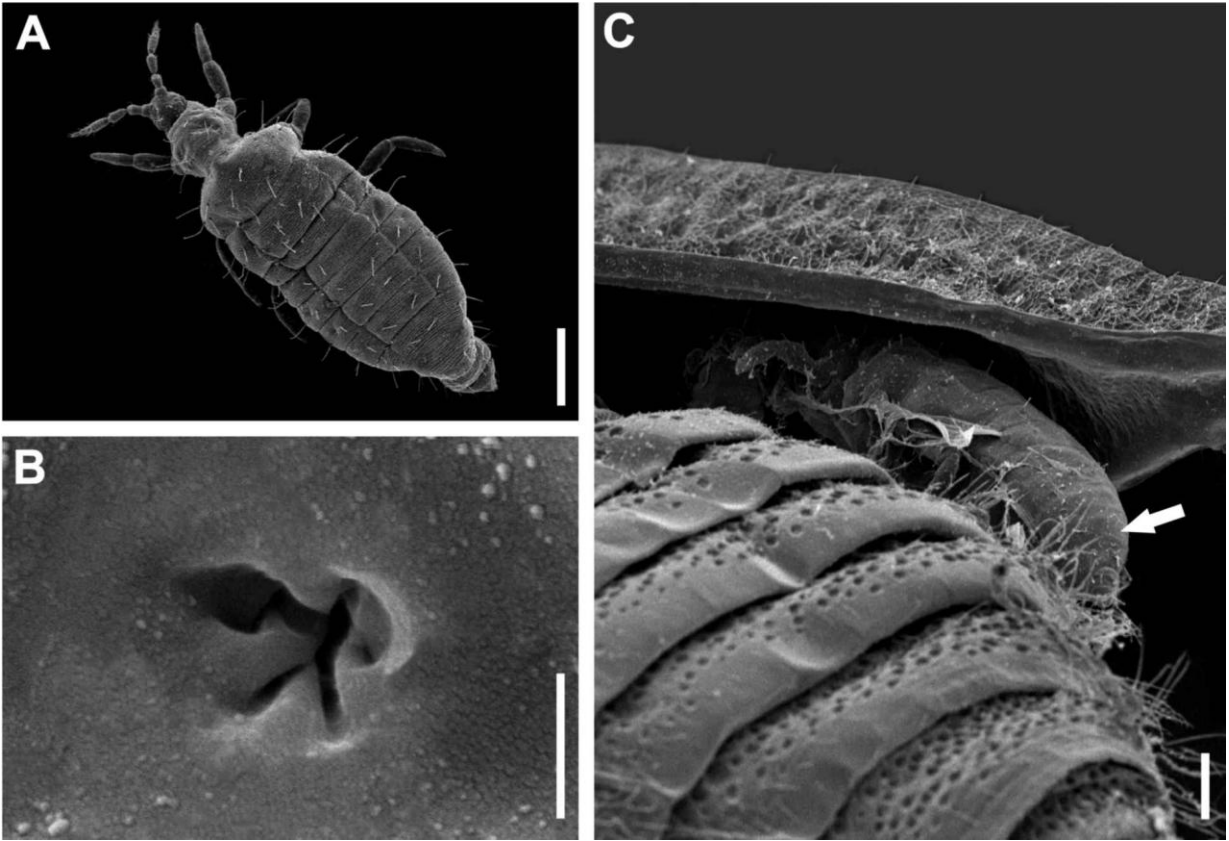


Fig. 1

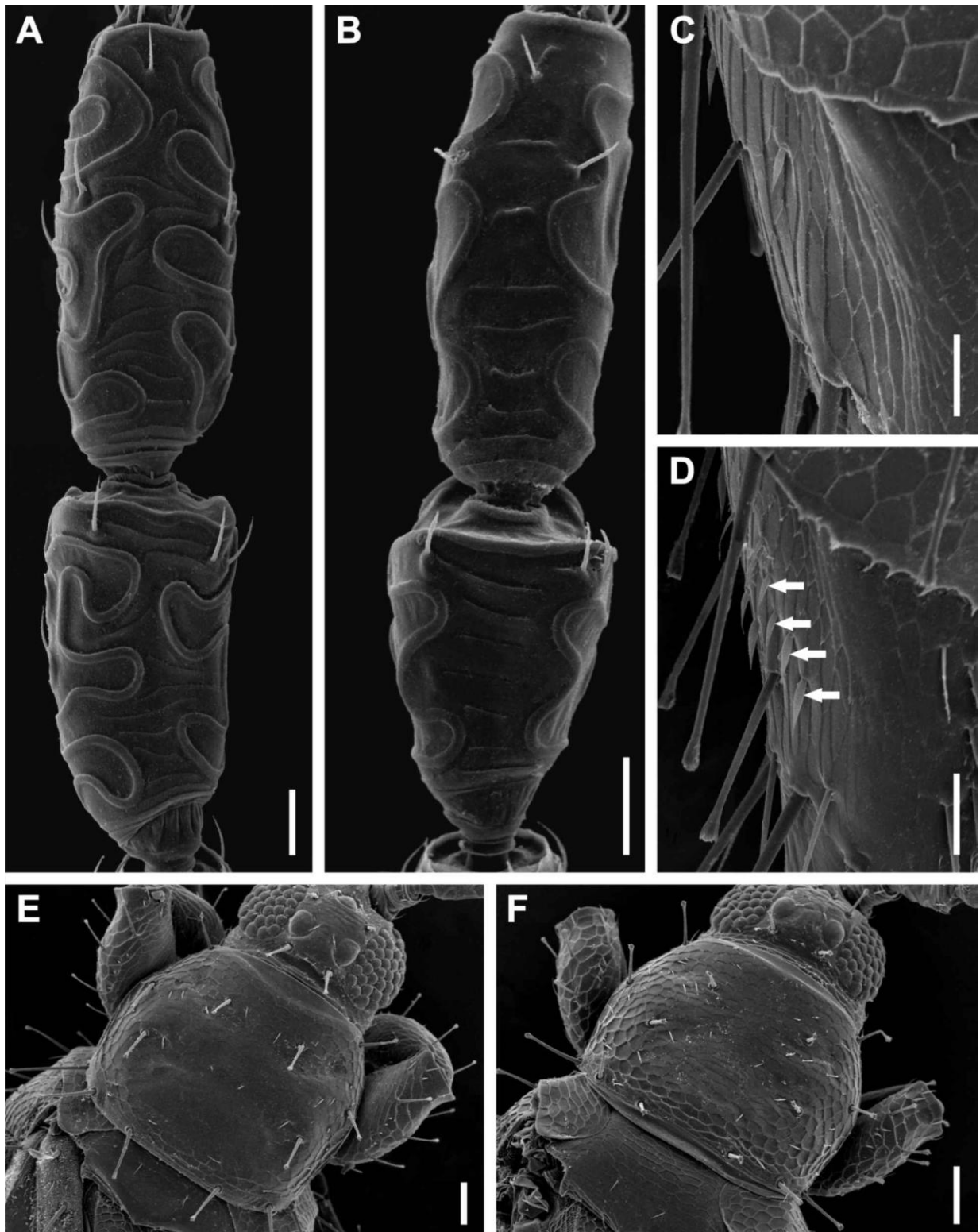


Fig. 2

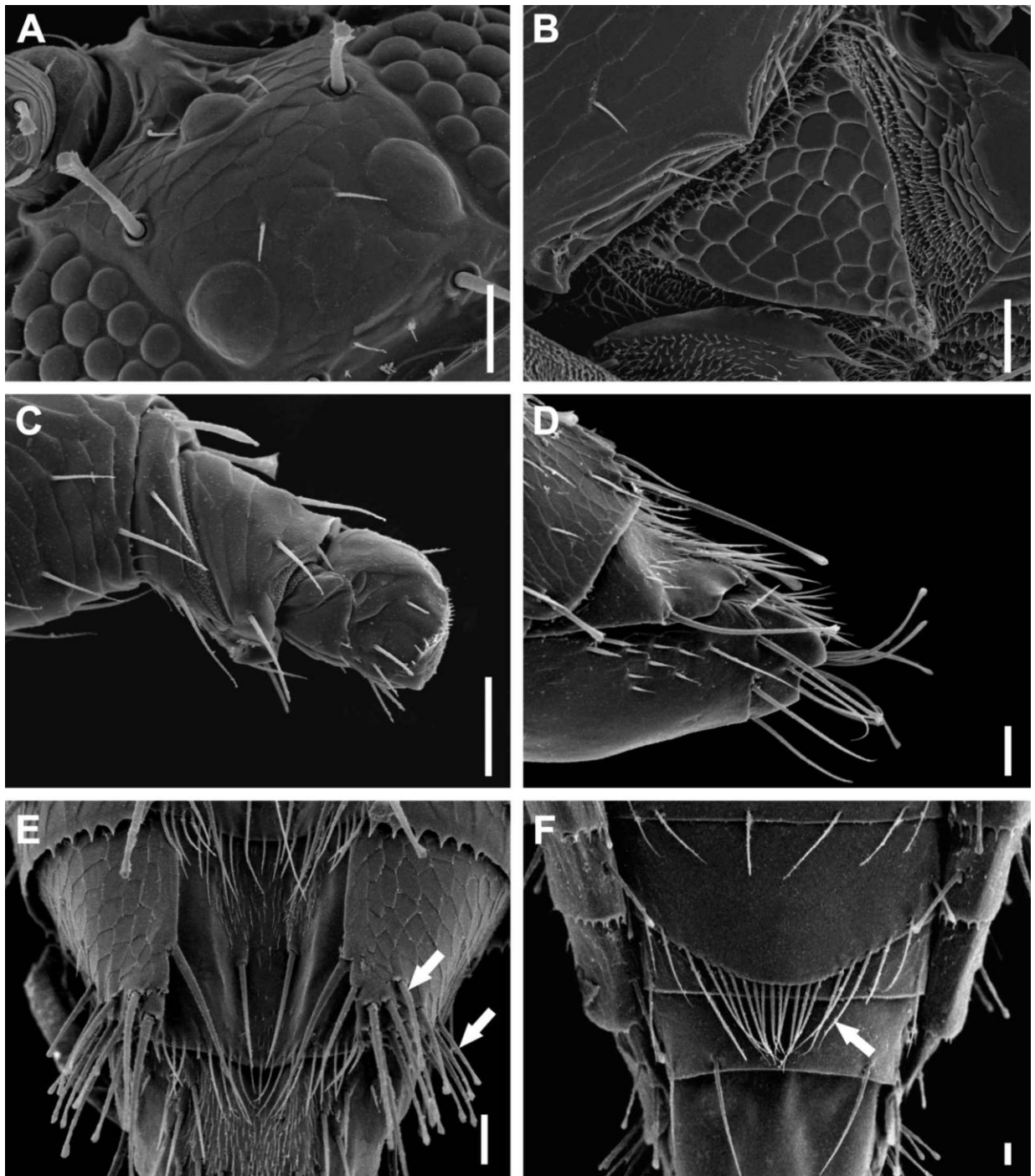


Fig. 3

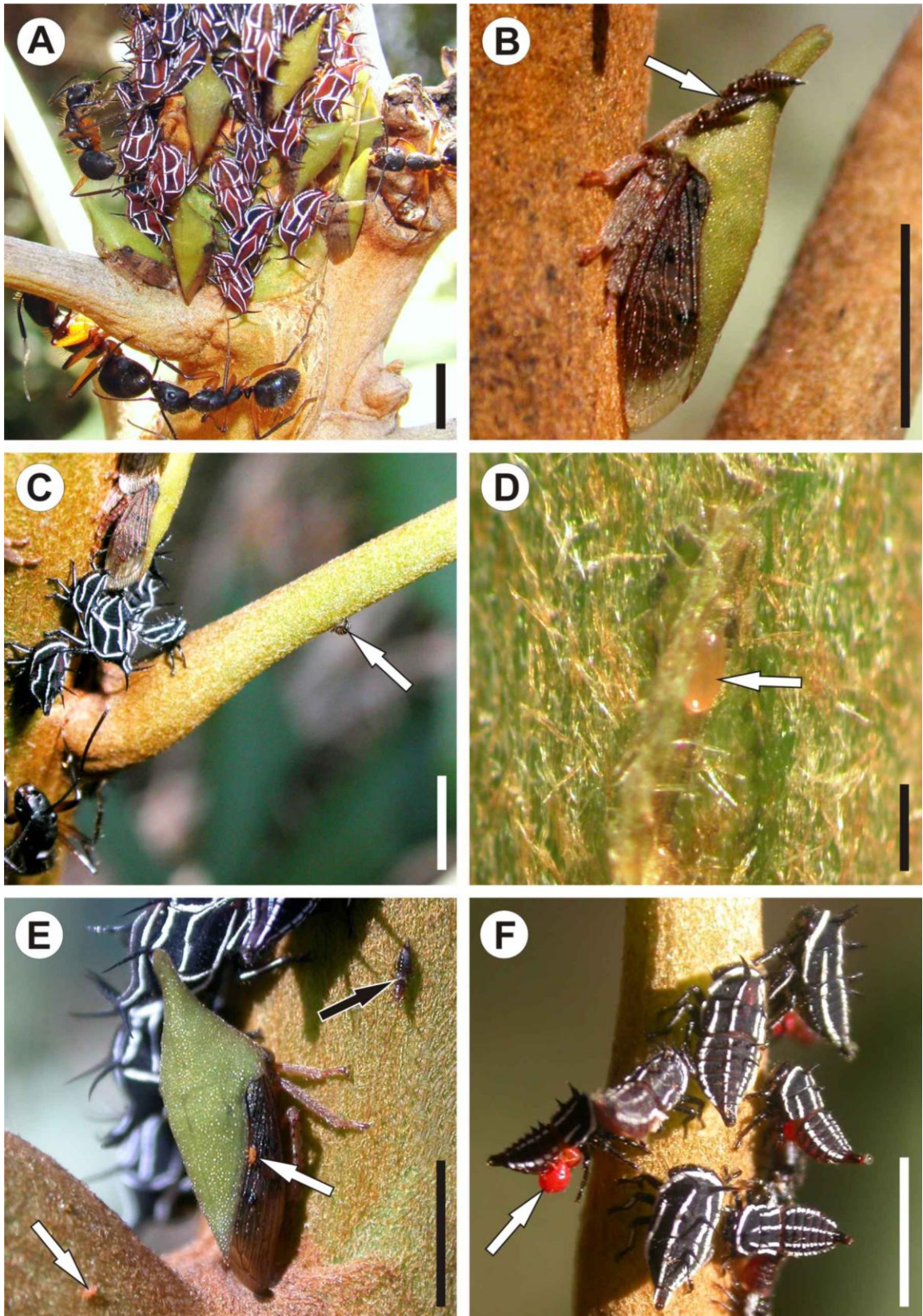


Fig. 5

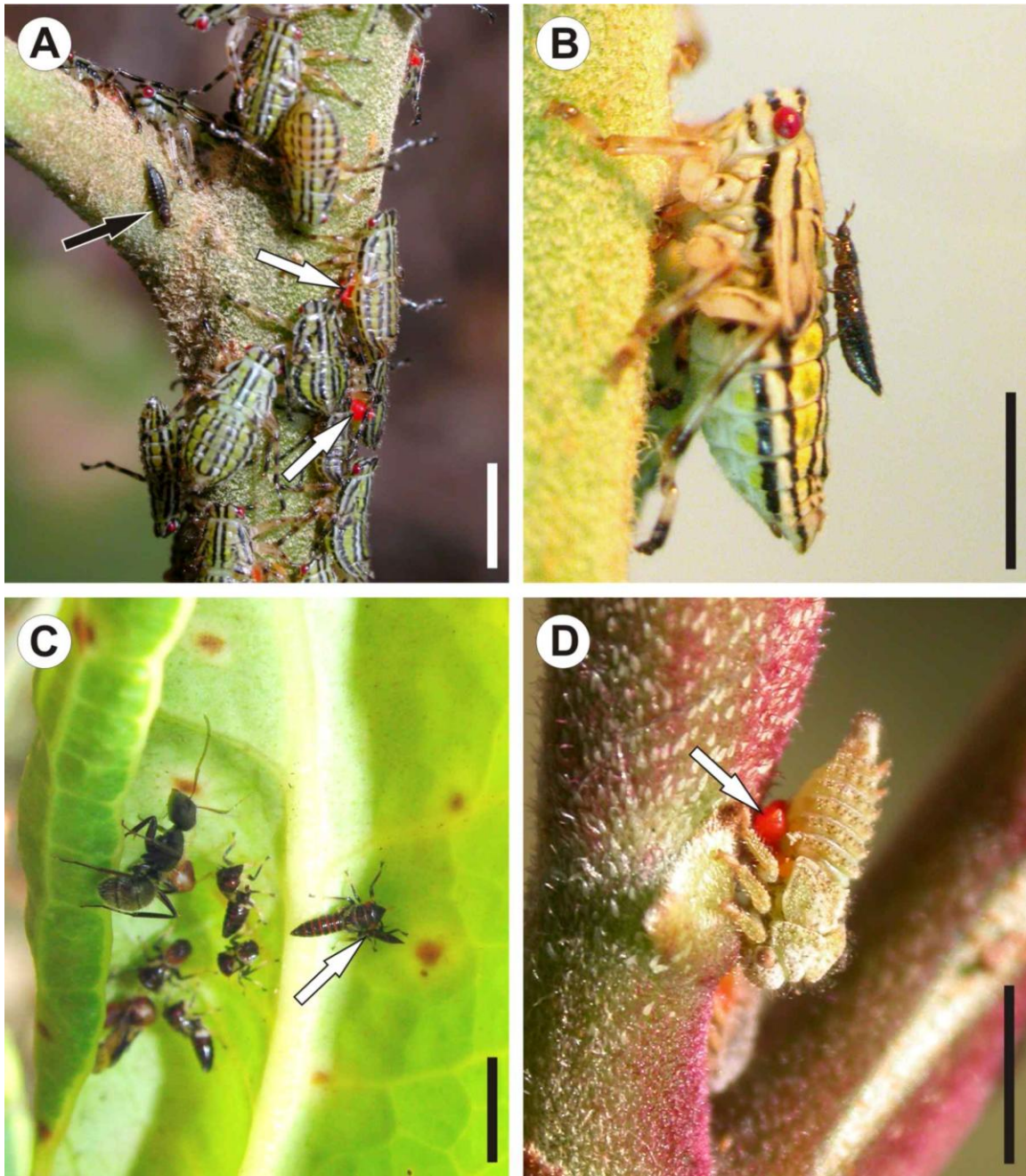


Fig. 6

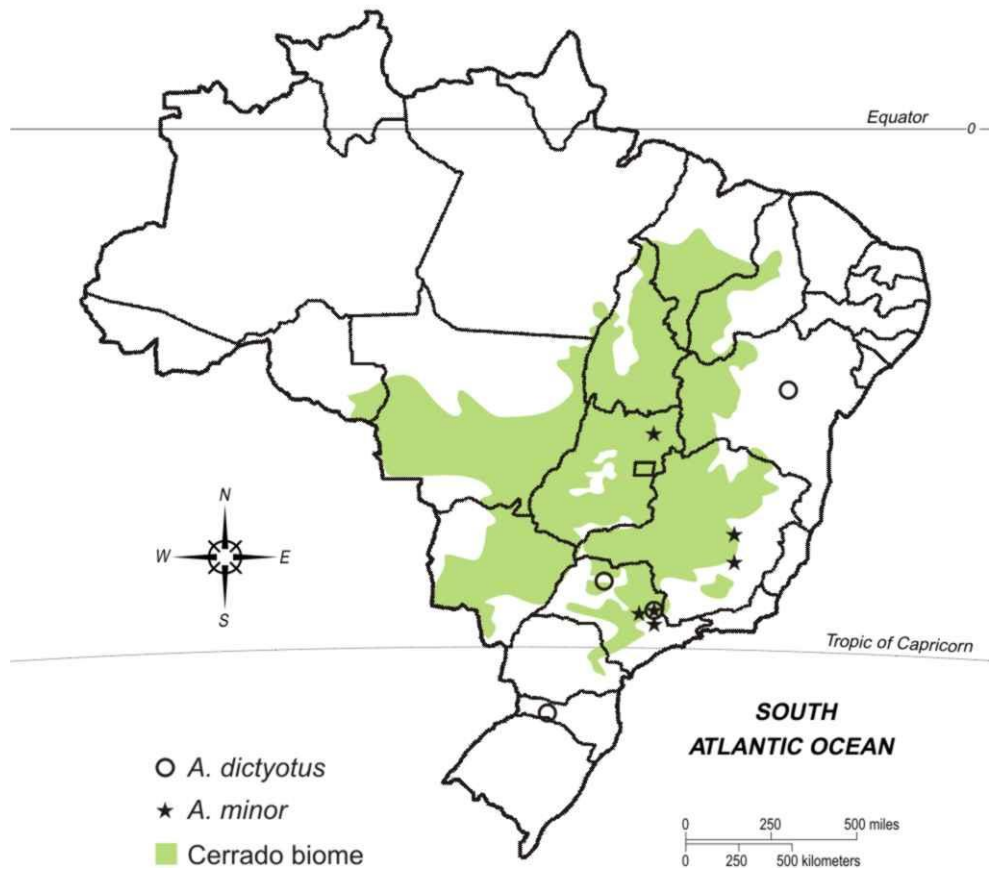


Fig. 7

3. CAPÍTULO II

A new ectoparasitic *Aulacothrips* (Thysanoptera: Heterothripidae) from Amazon rainforest and the significance of variation in antennal sensoria*

* Este manuscrito está publicado em *Zootaxa*, 3438: 62–68, 2012.

A new ectoparasitic *Aulacothrips* (Thysanoptera: Heterothripidae) from Amazon rainforest and the significance of variation in antennal sensoria

ADRIANO CAVALLERI¹, LUCAS A. KAMINSKI² & MILTON S. MENDONÇA JR.^{1,3}

¹*PPG-Biologia Animal, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. E-mail: cavalleri_adriano@yahoo.com.br*

²*Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil. E-mail: lucaskaminski@yahoo.com.br*

³*Departamento de Ecologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. E-mail: milton.mendonca@ufrgs.br*

Abstract

Aulacothrips amazonicus **sp.n.** is described from Northern Brazil, with larvae and adults ectoparasitic on ant-tended Membracidae (Hemiptera) on *Solanum* shrubs. This new taxon differs from its congeners by (i) body distinctively paler; (ii) sensoria on antennal segments III & IV much less convoluted; (iii) male tergite IX posterior margin straight and with several long and stout setae. The possible biological significance of interspecific differences in antennal sensoria among *Aulacothrips* species is discussed.

Key words: ectoparasitism, *Dolichoderus bispinosus*, Membracidae, Neotropics, treehoppers

Introduction

By exhibiting an ectoparasitic way of life, the species of *Aulacothrips* are amongst the most remarkable taxa within the order Thysanoptera. They belong to the Heterothripidae, a family that is restricted to the Americas and all remaining members are considered to be

phytophagous, feeding and breeding in flowers (Mound & Marullo 1996; Izzo *et al.* 2002; Cavalleri *et al.* 2010; Pereyra & Cavalleri 2012). Larvae and adults of *Aulacothrips* infest aggregations of several ant-tended hemipterans, and females are known to lay their eggs in the plant tissue, very close to the host individuals (Cavalleri *et al.* 2010).

This genus is known only from Brazil, and includes two species, *Aulacothrips dictyotus* Hood and *Au. minor* Cavalleri, Kaminski & Mendonça. These are very similar in their external morphology but differ significantly in life strategies and host use. The first is a highly specific ectoparasite of *Aetalion reticulatum* (Aetalionidae) while the second attacks several hemipteran species, almost all of them ant-tended treehoppers of the Membracidae family (Cavalleri *et al.* 2010). Silva and Del-Claro (2011) recently recorded *Au. dictyotus* infesting *Enchenopa brasiliensis* (Membracidae) in Minas Gerais, but further examination of this material revealed that those specimens belong to *Au. minor*.

These ectoparasites were previously recorded from several savannah areas in Central and Southeast Brazil, sometimes living in sympatry but always infesting distinct hemipteran hosts (Cavalleri *et al.* 2010). However, recent samples showed a third *Aulacothrips* species infesting Membracidae in the Brazilian Amazon rainforest, about 1,500 km distant from the currently known distribution of its two congeners. Interesting morphological differences were observed amongst these three species, particularly the sensoria on antennal segments III and IV. All *Aulacothrips* have a remarkable antennal morphology, with segments III and IV enlarged and with highly convoluted porous sensoria, but this new species has clearly smaller sensorial bands.

Here, we describe this new taxon, and consider the possible biological significance of interspecific variation in these antennal sensoria to these ectoparasitic thrips.

Material and methods

All thrips specimens were fixed in 70% ethanol and prepared on microscope slides using a standard procedure (Mound and Marullo 1996). The holotype was deposited in the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil and paratypes are also available in Australian National Insect Collection (ANIC), Canberra, Australia.

The software ImageTool 3.00 was used to calculate the length of the sensoria on the dorsal surface of antennal segment III. These measurements were taken using photographs of antennae of females from the three *Aulacothrips* species, and the ratio of sensoria length/antennal segment III apical width was calculated.

Key to *Aulacothrips* species (adapted from Cavalleri *et al.* 2010)

1. Sensoria on antennal segments III–IV curved laterally toward base but without loops on dorsal and ventral surface of these two segments (Fig. 5); two pairs of long midlateral setae aligned to pronotal lateral margin (Fig. 6); male largely yellowish brown, except for median portion of abdominal tergites II–VIII and abdominal segments IX–X, which are darker (Fig. 3) ***amazonicus* sp.n.**
- . Sensoria on III–IV longer and forming several loops over the entire surfaces of these two segments (Figs 12–13); only one pair of long midlateral setae aligned to pronotal lateral margin (second pair arising almost medially); male uniformly brown 2
2. Antennal segment III conical dorsally, with basal third clearly narrower than apical third; sensorial areas with loops wide apart from each other in dorsal surface (Fig. 13); female abdominal segment IX with 3–9 short, hyaline, flattened setae placed laterally in one or two rows; male abdominal tergite VIII without a group of long setae placed at posterior margin; associated to gregarious membracids (Hemiptera) *minor*

- Antennal segment III cylindrical dorsally, with slightly convex sides; sensorial areas extending largely in both surfaces, almost touching each other medially (Fig. 12); female abdominal segment IX without a row of short and flattened setae, in few cases bearing only one or two minute and sparse setae; posterior margin of male abdominal tergite VIII with a group of 8–12 pairs of long and stout setae placed close together; associated to *Aetalion reticulatum* (Hemiptera)*dictyotus*

***Aulacothrips amazonicus* sp.n. (Figs 2–11)**

Female macroptera. Body brown (Fig. 2); all legs yellow; antennae extensively brown, segment II darker and III paler in basal third; fore wings blackish brown with a dark basal line; major setae light brown; abdominal tergites VII–VIII somewhat paler in lateral thirds.

Head about 1.2 times wider than long (Fig. 4); three pairs of ocellar setae, pair II long and capitate, arising close to eye inner margin; postocular setal pair II expanded at tip and well developed; pair III small and within ocellar triangle; ocellar area reticulate; antennae 9-segmented, III & IV enlarged and with continuous porous sensoria, curved laterally toward base and extending to segment midpoint (Fig. 5). Mouth-cone short and rounded at tip, extending 53 microns beyond posterior dorsal margin of head.

Pronotum transverse and reticulated, except a small area medially (Fig. 6); six pairs of long and capitate setae (two specimens with seven pairs on only one side) and one pair arising well behind the anterior margin; about 30 small and finely acute discal setae; mesonotum reticulate; metascutum triangular, polygonally reticulate, faintly sculptured medially and without internal markings, remaining metanotal area also sculptured and covered by numerous microtrichia; paired metanotal campaniform sensillae placed at posterior third. Abdominal tergites with strong polygonal reticulation (Fig. 8), with well-developed craspedum bearing stout teeth; tergite I without long setae, II–VIII with median furrow bearing pair of long and pointed setae

medially, lateral margin of furrow with two pairs of stout setae, posterior margin with fringe of small microtrichia medially and three pairs of long discal setae, median pair arising in line with wing-retaining setae; IX with numerous long and pointed discal setae (Fig. 11); long setae on X weakly expanded at apex. Abdominal sternites II–VIII with about five pairs of stout and flattened setae placed laterally, IX with two rows of five such setae each side (Fig. 10).

Measurements of female holotype, in microns: Length about 1,520 (distended); head length 150, greatest width across cheeks 167; ocellar setae II length 43, interval 55; postocular setae pair II 52, interval 55; eye dorsal length 70; pronotum median length 175, width 250; mesonotum width 190; fore wing length 780, median width 20; metascutal triangle length 75, major width 92; tergite IX length 130, basal width 167; tergite X length 73, basal width 68; major setae on X 98; antennal segments III–IX length (width): 80 (basal width 30, apical width 41), 82 (35), 8 (18), 8 (15), 10 (8), 10 (8), 12 (5).

Male macroptera. Smaller and paler than female (Fig. 3); body largely yellowish brown, anterior margin of mesonotum and abdominal tergites IX–X darker; lateral thirds and furrow on abdominal tergites II–VIII also darkened; posterior margin of abdominal tergite VIII with only one pair of long and capitate setae; tergite IX posterior margin not extending toward X; tergite X with two pairs of long, curved and weakly expanded setae; sternite VII antecostal ridge with small circular pore plate, diameter about 11 microns, covered by a posterior extension of sternite VI bearing several long and pointed setae (Fig. 9); IX with several stout and hyaline setae laterally.

Measurements of paratype male, in microns: Length about 1,240; head length 105, greatest width across cheeks 140; pronotum median length 165, width 213; mesonotum width 158; metascutal triangle length 65, maximum width 82; tergite IX length 70, basal width 122; tergite X length 55, basal width 55; curved setae on X 93; genitalia length 178; major width 47,

aedeagus length 145; antennal segments III–IX length (width): 65 (basal width 22, apical width 33), 70 (33), 8 (15), 11 (12), 11 (8), 10 (7), 12 (5).

Material examined. Holotype female, **Brazil:** Pará, Ilha de Jurupari (01°36'S, 52°49'W), 04.vii.2010, associated with *Ramedia* sp. (Membracidae) aggregation on *Solanum* sp. branches (L.A. Kaminski). Paratypes: 10 males and 11 females collected with holotype.

Comments

This new species was found infesting *Ramedia* (Membracidae) treehoppers on *Solanum* shrubs in Amazon rainforest. Larvae, 17 females and 12 males were found living together, most of them attached to the bodies of the hemipterans. These bugs were tended by ants of the species *Dolichoderus bispinosus* (Formicidae: Dolichoderinae). Although very distinct on its lighter colouration, *Au. amazonicus* is possibly closely related to *A. minor* due to its host preference and general morphology. Both species have abdominal sternite IX with several small flattened setae laterally in one or two rows, and the conical format of antennal segment III is also similar. Despite this, the sensorial areas on antennal segments III and IV are remarkably reduced in *Au. amazonicus*, and are similar to those of *Lenkothrips* species (Fig. 14). However, as in *Au. dictyotus*, males of this new species also have the posterior margin of abdominal sternite VI prolonged, and bearing several setae, thus covering the minute pore plate on the antecostal ridge of sternite VII.

Adaptive significance of *Aulacothrips* antennal sensoria

The shape and morphology of sensoria on antennal segments III and IV are relatively diverse in Thysanoptera, but despite this, the olfactory sensillae are the most characteristic and easily visible using light microscopy (De Facci *et al.* 2011). The form of these structures is useful in family recognition and is a strong indication of relationships amongst different thrips lineages

(Mound *et al.* 1980; Mound & Marullo 1996). In the two largest families of the Order, Thripidae and Phlaeothripidae, olfactory sensoria are emergent sensillae (= sense cones) that vary in shape and number among species (De Facci *et al.* 2011). These emergent sensoria are possibly derived from the plesiotypic condition found in Merothripidae, where antennal segments III and IV each bear one transverse sensorial area apically (Mound *et al.* 1980). In contrast, Stenurothripidae species exhibit an intermediate condition, with a stout conical sensorium on III and IV, a character state that is found otherwise only in a few fossil Thysanoptera (Moritz *et al.* 2001). The single extant member of Uzelothripidae shows an interesting and unique antennal structure, with IV bearing an emergent trichome but III with a ventral circular sensorium near the apex. In the two families Aeolothripidae and Heterothripidae, the species have linear sensoria on III and IV, frequently transverse at apex but sometimes curling slightly around the segment. In Heterothripidae and a very few Melanthripidae the sensorial areas form a continuous band that circles the apex of the segment, but is convoluted in *Aulacothrips* and *Lenkothrips* species (Mound *et al.* 1980). These bands bear several equidistant pores with presumably chemosensory function. Intriguingly, the antennal sensoria of Heterothripidae larvae are remarkably distinct from those found in adults, with emergent apical sense cones (A. Cavalleri pers. obs.).

The length of the porous bands on antennal segments III and IV differs significantly among the three *Aulacothrips* species (Fig. 15). In *Au. amazonicus* these structures have only one lateral loop which extends to the midpoint of each segment (Fig. 5). In contrast, *Au. minor* and particularly *Au. dictyotus* have more extensive porous bands (Figs 12, 13), with additional loops along the segments. *Au. dictyotus* is remarkable in having sensorial areas extending largely on the dorsal surface, almost touching medially and occupying a great portion of antennal segment III. Moreover, in *Aulacothrips* females the sensoria are larger than in males,

suggesting that these structures may be important in finding hemipteran aggregations during the colonization process.

This variation among *Aulacothrips* species (Fig. 15) might reflect different life-history strategies evolving in different scenarios. We suggest these morphological differences may have adaptive and biological significance. *Au. amazonicus*, which is expected to be associated with many treehopper species as in *Au. minor*, is known only from an area very close to the Equator, in the middle of the Amazon Rainforest. Together with Colombia, Ecuador, Guyanas and Peru, this area has the richest fauna of Membracidae, in terms of subfamilies, tribes, genera and species (Wood & Olmstead 1984). Moreover, the climate stability at these low latitudes probably extends the availability of hemipteran hosts for *Au. amazonicus* throughout the year. Under these circumstances, the relatively small sensoria on antennal segments III and IV might reflect reduced effort necessary to find an available host. *Au. minor* is known for infesting many membracid species, but is currently known only from higher latitudes, almost exclusively in Brazilian Cerrado formations. This extensive and diverse woodland-savannah biome has over 90% of the rainfall concentrated from October to March, resulting in two well-defined seasons (Ratter *et al.* 1997). This climate directly affects the abundance of treehoppers throughout the year, such as *Guayaquila xiphias* (Membracidae), one of the most thrips-infected hemipterans (Del-Claro 1995; Del-Claro & Oliveira 1999; Cavalleri *et al.* 2010). Thus, the more developed sensoria on III and IV observed in the generalist *Au. minor* might be essential in searching for available hosts, particularly during seasons where these become less abundant. Conversely, *Au. dictyotus* is a highly-specific ectoparasite that infests only the polyphagous *Ae. reticulatum*, and its extraordinarily large porous bands are possibly associated with the localization of one particular host species, requiring an increased sensorial area to detect aetalionid aggregations. The highly specialized sensoria of *Au. dictyotus* are presumably the most highly derived.

Das *et al.* (2011) also observed differences in antennal sensoria in two endoparasitic wasps species with different degree of host specificity. The chemosensillae on the antenna of the specialist parasitoid, *Microplitis croceipes*, were more abundant than in the generalist wasp, *Cotesia marginiventris*. In addition, these authors observed that females of both wasp species bear a greater number of chemosensilla putatively involved in the detection of host-related/host-specific volatiles than conspecific males.

The similarity in form of the porous bands between *Au. amazonicus* and *Lenkothrips* species is interesting, as the species have completely different life histories, and do not appear to be closely related. Moreover, antennal sensoria vary in structure amongst *Heterothrips* species, with species such as *H. peixotoa* and *H. watsoni* having two or more large porous bands on III and IV, instead of one row of minute pores as in many species. This range of forms suggests that antennal sensoria in Heterothripidae are subject to strong selective pressures, and the differences probably play an important role in the evolution and ecology of these species.

Acknowledgments

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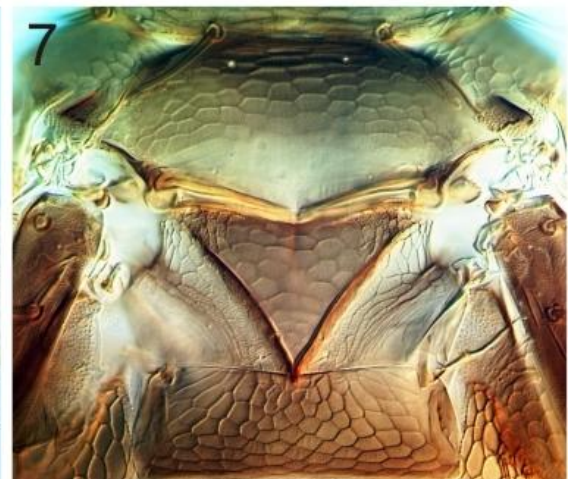
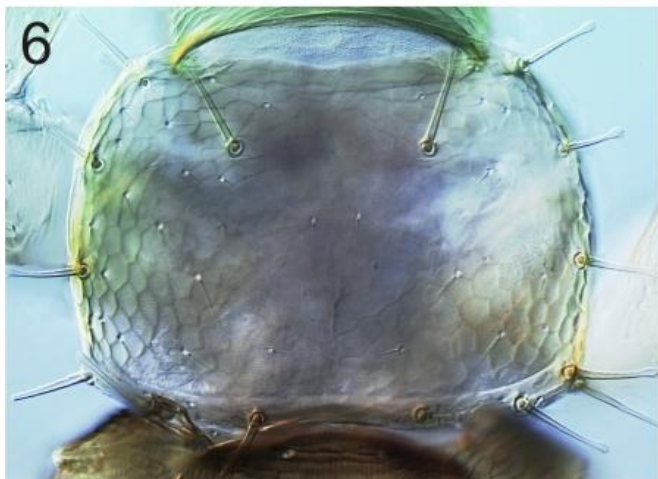
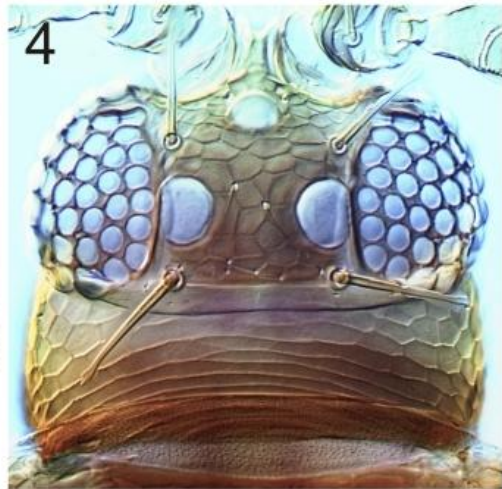
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FIGURES 1–7. *Aulacothrips amazonicus* sp.n. and its host. (1) *Ramedia* treehoppers tended by *Dolichoderus bispinosus* ants on *Solanum* shrub; (2) Female; (3) Male; (4) Head; (5) Antenna in dorsal view; (6) Pronotum; (7) Mesonotum and Metanotum.

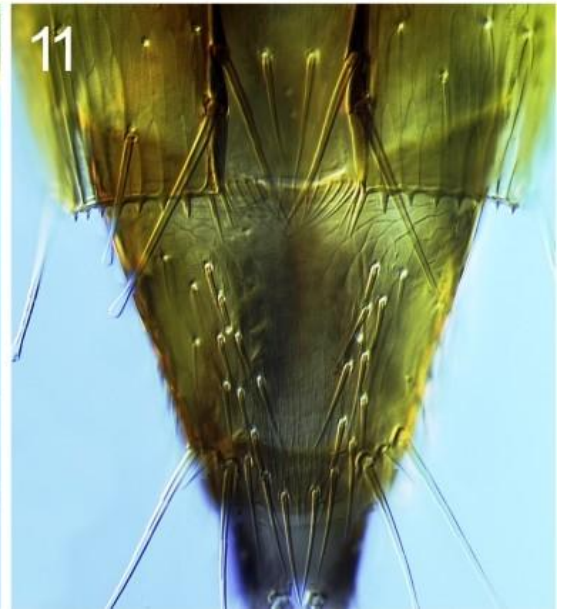
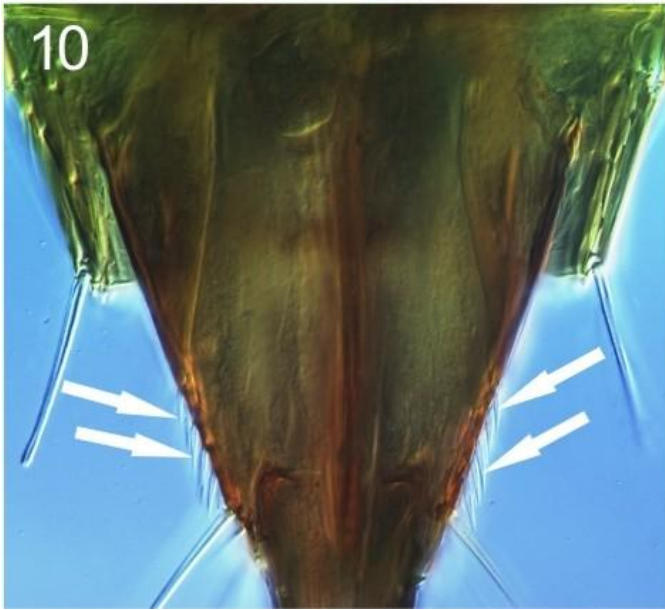
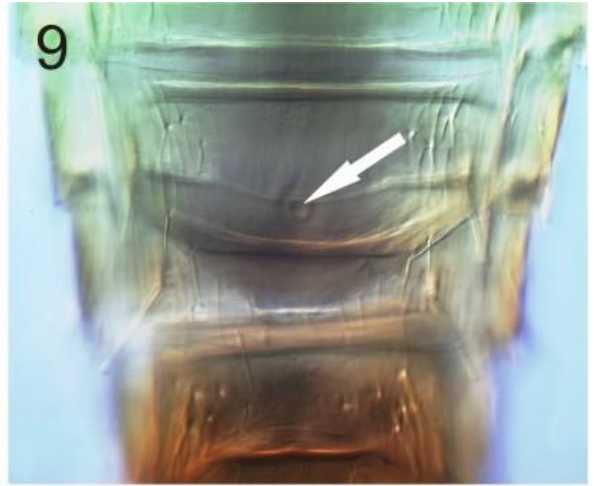
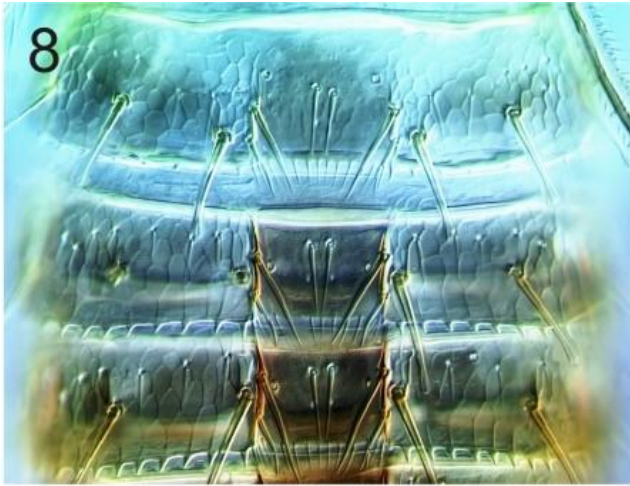
FIGURES 8–11. *Aulacothrips amazonicus* sp.n.; (8) Male abdominal tergites II–IV; (9) Male abdominal sternites VI–VIII (arrow indicates the minute pore plate); (10) Female abdominal sternites VIII–IX (arrows indicate flattened and hyaline lateral setae on IX); (11) Female abdominal tergites VIII–X.

FIGURES 12–14. Dorsal view of antennae in Heterothripidae species. (12) *Aulacothrips dictyotus*; (13) *Aulacothrips minor*; (14) *Lenkothrips* sp.

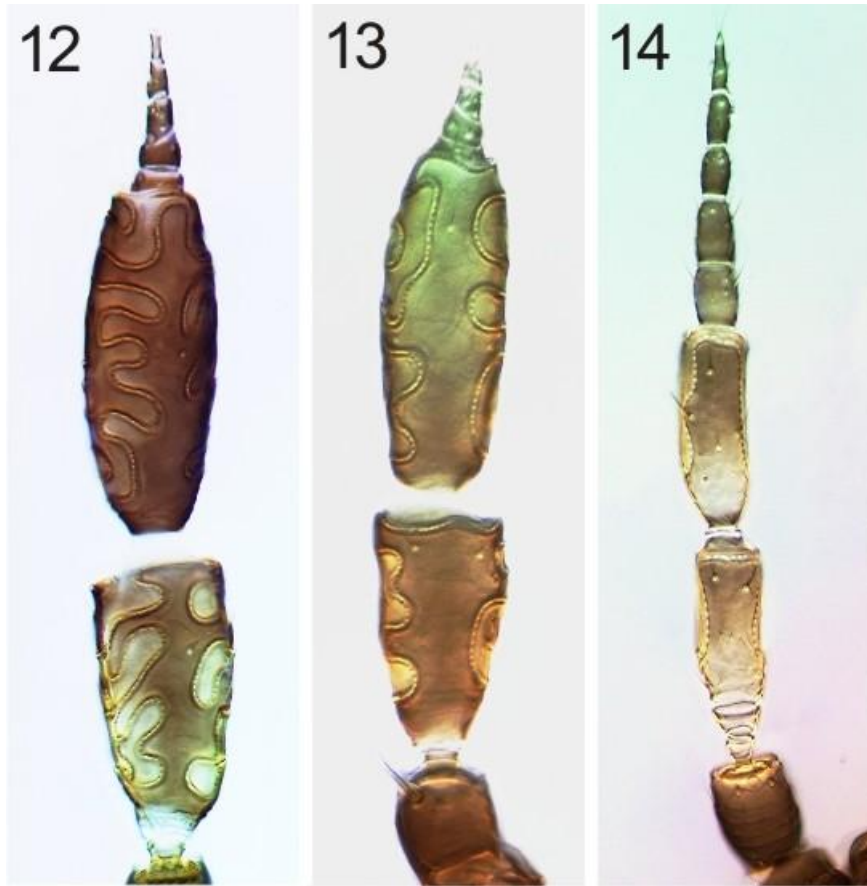
FIGURE 15. Relative length between female antennal sensorium on segment III and antennal segment III apical width in three *Aulacothrips* species. Error bars represent 95% confidence intervals.



Figs 1-7



Figs 8–11



Figs 12–14

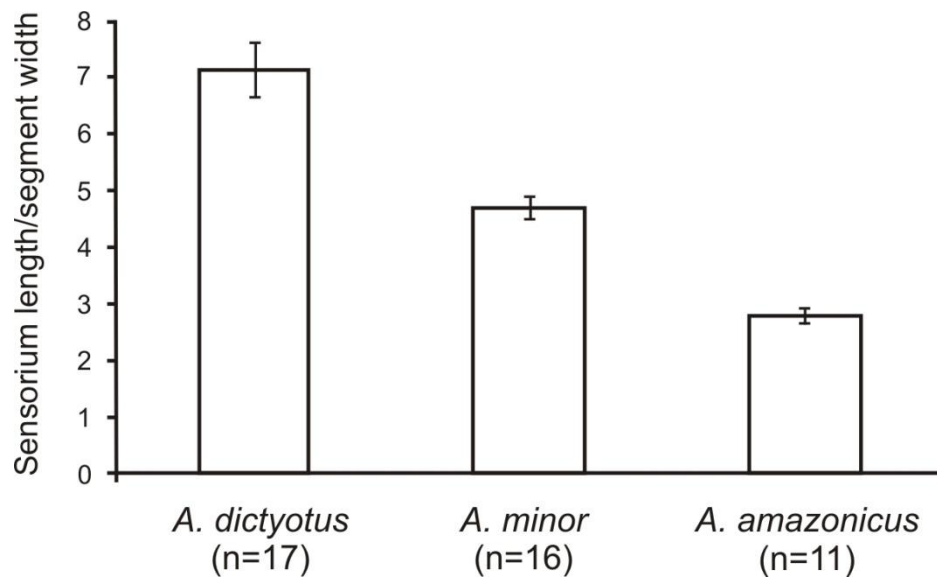


Fig. 15

4. CAPÍTULO III

**Internal morphology of *Aulacothrips* (Heterothripidae: Thysanoptera) with
reference to their ectoparasitic feeding habit***

* Manuscrito a ser submetido para **Arthropod Structure & Development**.

Internal morphology of *Aulacothrips* (Heterothripidae: Thysanoptera) with reference to their ectoparasitic feeding habit

Adriano Cavalleri ^{a,*}; Milton de S. Mendonça Jr. ^{a,b}; Stephanie Schneider ^c, Gerald Moritz ^c

^a*PPG-Biologia Animal, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil*

^b*Departamento de Ecologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*

^c*Martin-Luther-University Halle-Wittenberg, Faculty of Biosciences I, Developmental Biology, Domplatz 4, 06108 Halle/Saale, Germany*

* Corresponding author. Tel.: +55 51 33087660

E-mail address: cavalleri_adriano@yahoo.com.br (A. Cavalleri).

Abstract

This paper investigates the internal morphology of two remarkable species of ectoparasitic thrips, *Aulacothrips dictyotus* and *Aulacothrips minor*. Both larvae and adults of these insects are found attached to ant-tended Hemiptera (Auchenorrhyncha) and they have been considered as the only known ectoparasites among thysanopterans. However, almost nothing is known about their biology and association with hemipterans. The internal anatomy of *Aulacothrips* and thrips feeding habits were studied using semi-thin section technique and further examination under light microscopy. Our results did not reveal any obvious differences between the two *Aulacothrips* species. The feeding strategy found in *Aulacothrips* seems to be the same used by predatory thrips which usually feed on small arthropods such as mites and other thrips species. The mandible is used to puncture the host cuticle and maxillary stylets are then extruded about 20 µm in hemipteran tissues, usually fat body cells. There are three groups

of salivary glands in *Aulacothrips*, one within the head, a large and ovoid pair in the mesothorax, and a pair of tubular glands which extends from the mesothorax to the abdomen. The first abdominal ganglia are completely fused with the metathoracic ganglion. *Aulacothrips* males bear an unusual sac-like sternal gland which differs from all previous glands described in Thysanoptera. We observed that silk is expelled through the anus by larvae I and II and the role of such substance in *Aulacothrips* life-history is discussed. This is the first approach to examine the internal morphology of members of the Heterothripidae family and the information provided here might help clarifying the evolution of the ectoparasitism in Thysanoptera.

Keywords

Aetalion reticulatum; ectoparasite; Hemiptera; Membracidae; silk; sternal glands

1. Introduction

Thrips are amongst the most minute and opportunistic insects in nature, displaying a large number of feeding habits and behaviours (Lewis, 1973; Ananthkrishnan, 1979; 1993; Crespi et al., 2004). About 60% of the species in the suborder Tubulifera are fungivorous, whereas more than 95% of the suborder Terebrantia are associated with green plants (Mound, 2005). Some phytophagous thrips have been notorious for causing extensive crop damage, vectoring viral diseases, and permanently destabilizing IPM systems (Mound and Teulon, 1995; Morse and Hoddle, 2006). Obligate and facultative predatory species are also common in both suborders and they have been recorded feeding on a wide range of minute arthropods (Lewis, 1973; Ananthkrishnan, 1979).

Interestingly, one lineage evolved a very particular strategy by infesting comparatively large ant-tended hemipterans. These thrips belong to the genus *Aulacothrips* (Heterothripidae)

and their closest relatives are all flower-feeding insects restricted to Americas (Bailey and Cott, 1954; Mound and Marullo, 1996; Cavalleri et al., 2010). These thrips have been considered as ectoparasites and their life-cycle seems to be highly dependent on hemipteran hosts (Izzo et al., 2002; Pinent et al., 2002; Cavalleri et al., 2010). However, their foraging behaviour and metabolic dependence on the hemipteran host are still a mystery. There are three species in this genus, exhibiting distinct life histories. *Aulacothrips dictyotus* Hood is associated only to a single host, *Aetalion reticulatum* L., a cosmopolitan aetalionid which is considered a pest of citrus in the Americas (Fig. 1A). By contrast, *Aulacothrips minor* Cavalleri, Kaminski & Mendonça has a wide range of hosts, attacking more than 15 treehoppers species in the Membracidae and Cicadellidae in Brazilian savannah. More recently, *Aulacothrips amazonicus* Cavalleri, Kaminski & Mendonça was described from the Amazon rainforest, also infesting ant-tended membracids (Cavalleri et al., 2012).

The knowledge of the internal morphology of these thrips and their associated hemipterans is critical for understanding the interaction involved in this remarkable association. Despite the crucial importance of such comparative studies, surprisingly few papers have examined the internal anatomy of Thysanoptera. General information on this was detailed by Sharga (1933) and Pesson (1951) where numerous thrips species were examined under light microscopy. Subsequent studies provided a better understanding of such aspects, including the structure and function of their mouth-parts relative to plant injury (*e.g.* Mound, 1971; Chisholm and Lewis, 1984), and embryonic and post-embryonic development, where deep internal change and rearrangement occurs (*e.g.* Heming, 1973; Ullman et al., 1989; Moritz et al., 2004). Most of these approaches deal with the more advanced families Thripidae and Phlaeothripidae, whereas there is absolutely no information regarding the internal morphology of intermediate families such as Heterothripidae.

Moreover, the traditional external morphology was revealed insufficient to provide a clear understanding of the complex relationships within thrips family-groups. A more critical concern may be the lack of informative data at the base of the thysanopteran clade (Mound and Morris, 2007). Studies on the internal morphology could provide important information helping to construct a clearer and more robust phylogeny of Thysanoptera families.

The goal of this paper is to examine the internal anatomy of two *Aulacothrips* species and test their feeding on hemipterans. Moreover, the similarities, differences and characteristics of various internal structures are presented and compared to other previously studied Thysanoptera families.

2. Material and methods

2.1. Studied species

The internal morphology of *Au. dictyotus* and *Au. minor* were investigated, as well as the thrips-infested *Ae. reticulatum* and *Enchenopa gracilis* (Membracidae) nymphs and adults. Thrips and hemipterans were collected from 2008 to 2011 from several localities of São Paulo state, Brazil. All insects were killed in 70% ethanol and voucher specimens are available at Halle-Wittenberg Martin-Luther (Germany) and Departamento de Zoologia, UFRGS (Brazil).

2.2. Section technique

Semi-thin section technique was used to study the internal anatomy of *Aulacothrips* and to detect thrips mouth-parts on hemipterans tegument. All specimens were fixed in Carnoy's solution for 1h and dehydrated in ascending ethanol series (70%, 80%, 90% and 100%) and finally stored in isopropanol for several hours. Specimens were subsequently embedded in paraffin following Kumm (2002) protocol and a microtome Leica SM2010R was used to cut 8µm-thick tissue sections. Serial sections were cut in three different directions (frontal, sagittal,

transversal) which were placed on microscope slides. For paraffin removal, slides were embedded in xylene (5 min) and subsequently transferred to isopropanol (3 min) and rehydrated in 3-minute series of ethanol and water (96%, 80%, 60% and distilled H₂O). All tissues were stained using Haematoxylin and Eosin solutions and later mounted in Canada balsam (for more information see Romeis, 1989; Moritz, 2006). Microphotographs and measurements were taken with a Leica DM 6000 microscope.

3. Results

3.1. Mouth-parts and feeding mechanisms

Aulacothrips larvae and adults have the typical asymmetrical piercing and sucking mouth-parts found in all Thysanoptera. In these thrips such structures are housed in a relatively short ventral mouth-cone, and are comprised by only one complete left mandible, two well-developed maxillary stylets, an elongate hypopharynx, the labrum in front and the labium behind (Fig. 2). The mandible is fused to the exoskeleton, slightly curved and 100 µm long in *Au. dictyotus* females. In immature, this length is positively correlated with total body length ($r=0.82$, $gl=15$, $P<0.0001$) ranging from about 53 µm in larva I to 60 µm in larva II. The maxillary stylets are interlocked at the tip and can be moved independently in a tongue and groove fashion. Moreover, the stylets are asymmetrical, and the right stylet is slightly broader than the left one, particularly at the tip. In *Au. dictyotus*, these structures measure about 40 µm in larva I, 50 µm in larva II and 115 µm in females. *Aulacothrips* larva I and II were found feeding on hemipterans (Fig. 3A). The mandible is used to puncture the host tegument and the paired maxillary stylets are then extruded forming a feeding tube which is protruded into the host by thrips muscular control. During feeding, thrips stand with the tip of the mouth-cone pressed on the host surface with an angle of about 140° in relation to their longitudinal body axis. Thrips maxillary stylets were observed right above the Hemiptera tegument, where there

is a broad layer of fat body tissue with cells showing several irregular nuclei. Some of the sections of *Au. dictyotus* larvae revealed that stylets were inserted about 20 μm into host tissues (Fig. 3A).

3.2. Alimentary tract

The digestive system of *Aulacothrips* consists of a tubular alimentary canal, and as in all thysanopterans it is divisible on a structural basis into three parts: the foregut, the midgut and the hindgut (Fig. 3). The oesophagus forms a slender and straight tube, about 95 μm in length and 25 μm in width in *Au. dictyotus* females (Fig. 3B). The cardiac valve is located in the junction between the foregut and midgut within the mesothorax region (Fig. 3C). The latter is a large and convoluted tube, and constrictions in various parts were also observed in adults and immature (Fig. 3D). The anterior portion of the midgut is the widest and measures about 70 μm in breadth in *Au. dictyotus* larva II and 80 μm in adults. The midgut is formed by a single layer of columnar epithelial cells that are lined with many microvilli. The hindgut in *Aulacothrips* is about twice as long as the foregut and one third as long as the midgut. The pyloric valve is situated in the junction between the midgut and the hindgut and this is also the place at which the Malpighian tubules arise.

3.3. Salivary glands

There are three groups of salivary glands in *Aulacothrips*, a small unpaired one within the head, a large and ovoid pair of salivary glands located within the mesothorax, and a pair of tubular glands which is extended from the mesothorax to the hind part of the abdomen (Fig. 2). Each ovoid salivary gland is about 150 μm x 70 μm in adults, formed by loosely-aggregated cells with relatively prominent nuclei and large vacuoles (Fig. 3B). The number of salivary glands found in immature and adults is the same. In larvae, the ovoid glands are pushed against

the midgut (Fig. 2A), but there is virtually no contact between them and the muscles of the digestive tube in adults (Fig. 2B). The second pair of glands is a long, narrow, clear, thin-walled tube, situated laterally to the alimentary canal. They have about 5 μm in diameter in adults and are adhered to the first portion of the midgut but without any sort of fusion between these two organs.

3.4. Malpighian tubules and rectal papillae

Aulacothrips larvae and adults have four Malpighian tubules, each of 0.5 mm in length and similar in diameter (about 10 μm) (Fig. 3D and 3F). Two are directed anteriorly and are free in the hoemocoel beyond their junction at the pyloric region. The other two are directed posteriorly, also free of the intestine and are tightly adhering distally, for a short region, to the hindgut wall. The Malpighian tubules are poorly differentiated and a single cell type is recognizable along each tubule. The rectum bears a thickened layer of cells which form four rectal papillae.

3.5. Nervous system

The relative small head capsule in *Aulacothrips* larvae contains large groups of cibarial muscles that displace the brain through the thorax (Fig. 2A). During the immature stage the brain is formed by two large lobes which are separated by a median suture. In *Au. dictyotus* larva II, the ventral lobe measures about 130 μm in length and extends until the mesothorax, whereas the dorsal lobe is distinctively shorter, scarcely 90 μm long. In adults, the large brain is concentrated into the head and the suboesophageal ganglion is broadly fused to the prothoracic ganglion (Fig. 2B). The latter is connected to the mesothoracic ganglion by a relatively short and thick commissure and subsequently linked to a more elongated

metathoracic ganglion. The first three abdominal ganglia are completely fused with the metathoracic ganglion.

3.6. Reproductive system

3.6.1. Females

The reproductive system consists of a pair of panoistic ovaries which open into a lateral and paired oviduct, forming a common duct posteriorly. This duct is continuous distally with a wider passage or vagina, and measures about 60 μm in length. Associated with the vagina is the single spherical and blind-ended spermatheca. This organ is scarcely 30 μm in diameter and lies at the base of the ovipositor. The ovaries are situated underneath the midgut and in the oviposition phase they reach the abdominal segment I. Each ovary contains four ovarioles grouped at either side of the abdomen. The ovarioles are long tubes in which the oocytes lie in a single chain, becoming more progressively mature towards the posterior end (Fig. 3C and 3D). The single accessory gland is a relatively large organ with an apical bulb and a long duct opening into the vagina separately from the spermatheca.

3.6.2. Males

The male reproductive system consists in paired testes, vasa differentia, seminal vesicles and paired accessory glands. The testes are pear-shaped, short, scarcely 50 μm long in the middle in *Au. minor* (Fig. 3E and 3F). Sperm develop in testes and are stored in the seminal vesicles until mating occurs. Each seminal vesicle opens posteriorly into the lumen of a median ejaculatory bulb together with the accessory gland. These glands are more or less spherical and measure about 60 μm long, lying almost dorsal to hindgut (Fig. 3E). An ejaculatory duct exits the bulb posteriorly, enters the base of the phallus within the IX abdominal segment, and extends through the short vasa differentia to the apex of the endotheca, where it opens in the gonopore.

The testes, accessory glands and vasa deferentia are covered with an orange-yellow pigment (Fig. 3F). The antecostal region of abdominal sternite VII bears a minute pore plate in both species. Above this opening there is a relatively small and sac-like gland, which is epidermic in origin and is formed by an epidermal invagination of the tegument. In *Au. minor*, this structure extends about 90 µm along the internal cavity, almost touching the midgut (Fig. 3F).

3.7. Fat body

Fat bodies were found both in larval and adult stages. These structures arise primarily in the form of two long layers on both dorsal and ventral side of the body and secondarily form many lobes packed round the various internal structures (Figs. 2 and 3). It is particularly well developed dorsally and consists of large globular cells loosely connected together. Some cells present large and round nuclei but these are absent in others. All *Aulacothrips* larvae have a thin layer of red hypodermic pigment just between the fat body and the cuticle (Fig. 3A).

3.8. Silk secretion

The *Aulacothrips* larva II spins a silk cocoon which is loosely-woven and apparently has more than one layer (Fig. 1D). Silk was also observed on the bodies of thrips-infested hemipterans, expelled by *Aulacothrips* larvae I and II, allowing them to remain firmly attached to host tegument (Fig. 1C). The analysis of our microscopic sections suggests that silk secretion is released directly into the rectum rather than through a duct. Larva I has seta pair I and II on abdominal tergite IX modified into stout spines in both *Aulacothrips* species.

4. Discussion

4.1. *Aulacothrips* feeding habit

These findings are the first support to the previous assumption of ectoparasitism in *Aulacothrips* made by Izzo et al. (2002) and Cavalleri et al. (2010). Our sections revealed immature thrips puncturing the hard chitinized tegument of their hosts instead of the softer membranous tissues (Fig. 3A). This external tegument measures about 5 μm in *Ae. reticulatum* nymphs, and given the small length of *Aulacothrips* maxillary stylets, it is unlikely that these structures reach vital organs of the host. Heming (1978) indicated that a maximum protraction of the thrips mandible would be about one-third of its length. The maximum depth of maxillary penetration by *Aulacothrips* is thus very limited, particularly for minute first instar larvae.

Our results also suggest that *Aulacothrips* feeds on host fat bodies. According to Arrese and Soulages (2010), the fat body is a dynamic tissue involved in multiple metabolic functions, playing major roles in the life of insects. One of these functions is to store and release energy in response to the demands of the insect (Chapman, 1998). Loss of nutrients stored in depots such as the fat body is frequently also reported consequences of parasitism in invertebrates (Polak, 1996). Moreover, disturbances to hemolymph by external parasites might alter insect water balance and pressure gradients essential for blood circulation, as well as reduce net nutrient availability to metabolic processes (Roeder, 1953; Chapman, 1998). If reduction of energy reserves occurs in infested hemipterans, resultant impairment of nutrient mobilization could restrict performance in some high-endurance activities such as long-distance dispersal by adults.

Thrips larvae and adults occupy similar niches, sometimes infesting the same host individual at the same time (Cavalleri et al., 2010). However, our field and laboratory observations suggest they use different body parts to attach and to feed. Immature are often firmly fixed in tiny spaces such as under nymphs wing buds and adults wings, whereas adults

feed on exposed areas on the host dorsal thorax and abdomen. These microhabitats used by soft-bodied larvae might provide less exposure to predation, extreme temperatures and water loss.

4.2. *Aulacothrips internal morphology*

We did not find any strong differences between *Au. minor* and *Au. dictyotus*, and the organization of the internal anatomy of these thrips has much in common with what is observed in other Terebrantia (Sharga, 1933; Pesson, 1951).

The mouth-cone is remarkably short in *Aulacothrips* when compared with other thrips families, but all known heterothripids also have this structure not extending the fore coxae in slide mounted individuals. Nevertheless, the presence of a short mouth-cone in these ectoparasites might be crucial in feeding in closed spaces such as under host wings. Similarly, the maxillary stylets in *Aulacothrips* are relatively short when compared to the other previously studied Terebrantia. In *Limothrips cerealium* (Haliday) for example, the stylets can be extended about 60 µm through plant tissue during feeding (Chisholm and Lewis, 1984).

The length of the oesophagus is usually longer in Terebrantia than in Tubulifera (Sharga, 1933; Pesson, 1951). However, this structure seems to vary greatly within species. In some phytophagous species, such as *Heliothrips hemmorrhiodalis* (Bouché), the oesophagus is also extremely slender but extends well into the abdomen before joining the midgut (Pesson, 1951). In contrast, the oesophagus of the *Frankliniella occidentalis* (Pergande) is shorter and stouter and joins the midgut within the mesothoracic segment (Ulmann et al., 1989). The relatively narrow size of the oesophagus in *Aulacothrips* might indicate they feed primarily on host fluids, and the numerous nuclei found in hemipteran fat body cells are also likely to be ingested by these thrips. As observed in *Parthenothrips dracaenae* (Heeger) by Müller (1927),

the shape of the foregut in *Aulacothrips* also differs between immature and adults, being short in larvae and extending through the metathorax in adults.

The shape and structure of the Malpighian tubules of *Aulacothrips* is similar to that shown by *F. occidentalis* (Ullman et al., 1989; Dallai et al., 1991). The number of four Malpighian tubules has also been reported for other members of the thysanopteran group (Sharga, 1933; Dallai et al., 1991) and this finding suggests that this is the typical condition for this insect group. The presence of four rectal papillae is also characteristic in Thysanoptera, although *Melanthrips* species however, differs in exhibiting five (Moritz, 1997). These structures are presumably associated with the reabsorption of water and the movement of ions for osmoregulation (Bode, 1977; Dallai et al., 1991b).

According to Del Bene et al. (1998), the tubular salivary glands seem to produce a watery secretion and the large lobed glands a viscous one, although the chemical composition and function of the saliva in Thysanoptera remains not well known. However, the shape of the lobed salivary glands is variable amongst taxa (Sharga, 1933). In Terebrantia, some are long and thin, while in other species they are oval and thick. The ovoid shape of these glands in *Aulacothrips* resembles what is observed in some phytophagous genera such as *Heliothrips*, *Frankliniella* and *Thrips* (Sharga, 1933; Del Bene et al., 1991a; 1999). The position of such organs is more or less the same amongst thysanopterans, being usually situated within the pro- and mesothorax (Moritz, 1997). In *Aptinothrips rufus*, however, they lie partly in the metathorax and partly in the first abdominal segment (Sharga, 1933).

The nervous system in *Aulacothrips* is represented by a strongly developed brain and a greatly concentrated chain of large ganglia lying in the thorax and abdomen. In some unrelated thrips genera such as *Aeolothrips*, *Haplothrips* and *Thrips*, the first three pairs of abdominal ganglia are fused with the metathoracic ganglion as found in *Aulacothrips* (Moritz, 1997). By

contrast, the abdominal ganglia are joined by long connectives in many Thripidae (*e.g.* *Frankliniella*, *Limothrips*, *Parthenothrips*) and in merothripids (Moritz, 1984).

The internal reproductive system of *Aulacothrips* does not differ from the other previously studied thrips species (see Sharga, 1933; Heming, 2003; Moritz, 2006). Presumably all thrips exhibits panoistic ovarioles (Pesson, 1951; Heming, 1970), although these are possibly secondarily derived from polytrophic ovariole-type of some psocopteroid ancestor. This is supported by clonal proliferation of oogonia after hatching and by persistence of intercellular bridges between them until they separate to form primary oocytes (Heming, 1995). The ovaries are similar to those found in most Terebrantia, which differ from Tubulifera and some Panchaetothripinae in having the terminal filaments of the ovarioles not connected to the salivary glands (Sharga, 1933; Lewis, 1973). In males of Aeolothripidae, Stenurothripidae, Heterothripidae and Thripidae, and possibly in other Terebrantia, each testis contains a single cyst of synchronously developing germ cells (Mound et al., 1980; Heming, 1995). The form of testis is variable amongst Thysanoptera. In some Tubulifera they are long and wide, while in Terebrantia they are usually club-shaped (*e.g.* *Aptinothrips* and *Limothrips*) or pyriform (*e.g.* *Aeolothrips* and *Thrips*) (Sharga, 1933). As in all terebrantians, *Aulacothrips* have only one pair of accessory glands, whereas tubuliferans have two pairs of such structures.

The male sternal glands found in Thysanoptera are presumably associated to pheromone secretion (Moritz, 1997; Kirk and Hamilton, 2004). Its external opening has been named as pore plates and its shape and number is particularly diverse amongst terebrantians (Mound, 2009). The male pore plates found in Heterothripidae, Adiheterothripidae and Fauriellidae are situated always anterior to the sternal antecostal ridge, whereas in the large and diverse family Thripidae these structures are usually on the discal area of the sternites (Mound et al., 1980; Mound, 2009). However, details of the internal structure of pore plates have been investigated in very few species and the knowledge on the homologies for such organs is thus

very limited. The single minute and circular pore plate found in *Aulacothrips* is amongst the smallest within Thysanoptera and the sac-like shape of their sternal glands is very different from those referred for other terebrantians such as *Thrips* and *Frankliniella* species and tubuliferans (Bode, 1978; Sudo and Tsutsumi, 2002). In sagittal sections of Thripidae, these glands are always roughly semi-circular in shape, often slightly flattened, with a radius of about 12–19 μm (El-Ghariani and Kirk, 2008). As in other Thysanoptera, the gland cells of *Aulacothrips* males are simply covered by cuticle, without ducts formed by other types of cell. As a result, the secretion has to pass directly through the cuticle without passing through any other cells, so they can be classified as class 1, according to the scheme of Noirot and Quennedy (1974, 1991).

Silk production commonly occurs in immature Terebrantia and few Phlaeothripidae (Phlaeothripinae) which construct tent-like shelters for protection (Lewis, 1973; Crespi et al., 2004). Heming (1973) refers that second instar larvae of Aeolothripidae, Adiheterothripidae and Merothripidae also spin cocoons prior to pupation. According to Sutherland et al. (2010), thrips secrete silk from the anal region, likely from Malpighian tubules. The silk found in Hemiptera tegument provide an appropriate fixing point and substrate for immature thrips, which usually remain firmly attached to the host by the anal region. This fixation strategy is widely found in phoretic mites (Bajerlein and Witaliński, 2012) and possibly allows an effective dispersal for immature *Aulacothrips* by adult hemipterans during flight (see Alves-Silva and Del-Claro, 2011). The stout spines found in the abdominal tergite IX in *Aulacothrips* larvae are similar to those observed in aeolothripids and other heterothripids (Speyer and Parr, 1941; Heming, 1991), and although their function is not known, they might be related to silk manipulation by larvae. Interestingly, these structures are present only in larva II in aeolothripids and other heterothripids, while in *Aulacothrips*, only larva I bears these spines.

4.3. Final remarks

This is the first approach to examine the internal morphology of members of the Heterothripidae family. All information provided here might be useful in future discussions regarding Thysanoptera phylogeny. The few available analysis of morphological and molecular data indicated that Heterothripidae might be closely related to the flower-feeding *Holarthrothrips* in the Stenurothripidae family (=Adiheterothripidae) (Mound and Marullo, 1996; Mound and Morris, 2007). According to Mound and Morris (2007) however, the phylogenetic position of the clade comprised by these two families remains unclear. One of the phylograms resulted from Parsimony analysis of 18S rDNA suggested that this clade is the sister-group of Aeolothripidae, whereas another phylogenetic tree resulting from maximum likelihood analysis of the same data indicated a polyphyletic Panchaetothripinae (Thripidae) as their closest relatives.

In this study we confirmed that *Aulacothrips* species feed by puncturing their hosts with the single developed mandible, sucking out the body contents with a pair of maxillary stylets. This is the same strategy used by predatory thrips which usually feed on small arthropods such as mites and other thrips species (Mound, 1971; Chisholm and Lewis, 1984). Further studies are necessary to detect the physiological consequences of thrips feeding to Hemiptera hosts, which might provide a basis to use these thrips in biological control programs against *Ae. reticulatum*.

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Figure captions

Fig. 1. A: *Aetalion reticulatum* nymph infested with *Au. dictyotus* larvae (arrow); B: *Au. dictyotus* larvae in detail; C: *Aulacothrips* larval silk attached to *Ae. reticulatum* tegument; D: *Au. dictyotus* second instar larva spinning a silk cocoon.

Fig. 2. Sagittal cross section of *Aulacothrips* spp. A: *Au. dictyotus* first instar larva; B: *Au. minor* female. acl – anteclypeal membrane; ant – antenna; cb – cibarium; cib. mus – cibarial muscles; eye – compound eye; fat bd – fat body; fgut – foregut; hst – host tegument (*Aetalion reticulatum*); mgut – midgut; msggl – mesothoracic ganglion; m.stnl – mesothoracic sternonotalis muscle; m.stnc – mesothoracic sterno-noticus muscle; mtggl – metathoracic ganglion; oe – oesophagus; ppgl – prothoracic ganglion; pst – prosternum; sal. gl – salivary gland; sg – subesophageal ganglion; ste – stemmata; the star indicates the large vacuoles in the salivary glands; the arrow head indicates the maxillary stylets; scale bars = 50 μ m.

Fig. 3. Sagittal cross section of *Aulacothrips* spp. A: *Au. dictyotus* larva feeding on *Aetalion reticulatum*; B – C: thoxax of adult female of *Au. dictyotus*; D: abdomen of adult female of *Au. dictyotus*; E – F: abdomen of adult male of *Au. minor*. acs. gl – accessory gland; cib. mus – cibarial muscles; fat bd – fat body; fgut – foregut; fw – fore wing; hgut – hindgut; hpm – hypodermal pigment; hst – host tegument (*Aetalion reticulatum*); mgut1 – first portion of the midgut; mgut2 – second portion of the midgut; mgut3 – third portion of the midgut; msggl – mesothoracic ganglion; msst – mesosternum; mtggl – metathoracic ganglion; mtub – Malpighian tubules; mxl – maxillary stylets; oe – oesophagus; ppgl – prothoracic ganglion; ppt – pore plate; pst – prosternum; sal. gl – salivary gland; sg – subesophageal ganglion; stgl – sternal gland; stn7 – abdominal sternite VII; ova – ovaries; tes – testicle; vl. cd – valvula cardiaca; the stars indicate the large vacuoles in the salivary glands; the arrow head indicates the constriction in the midgut; scale bars = 50 μ m.

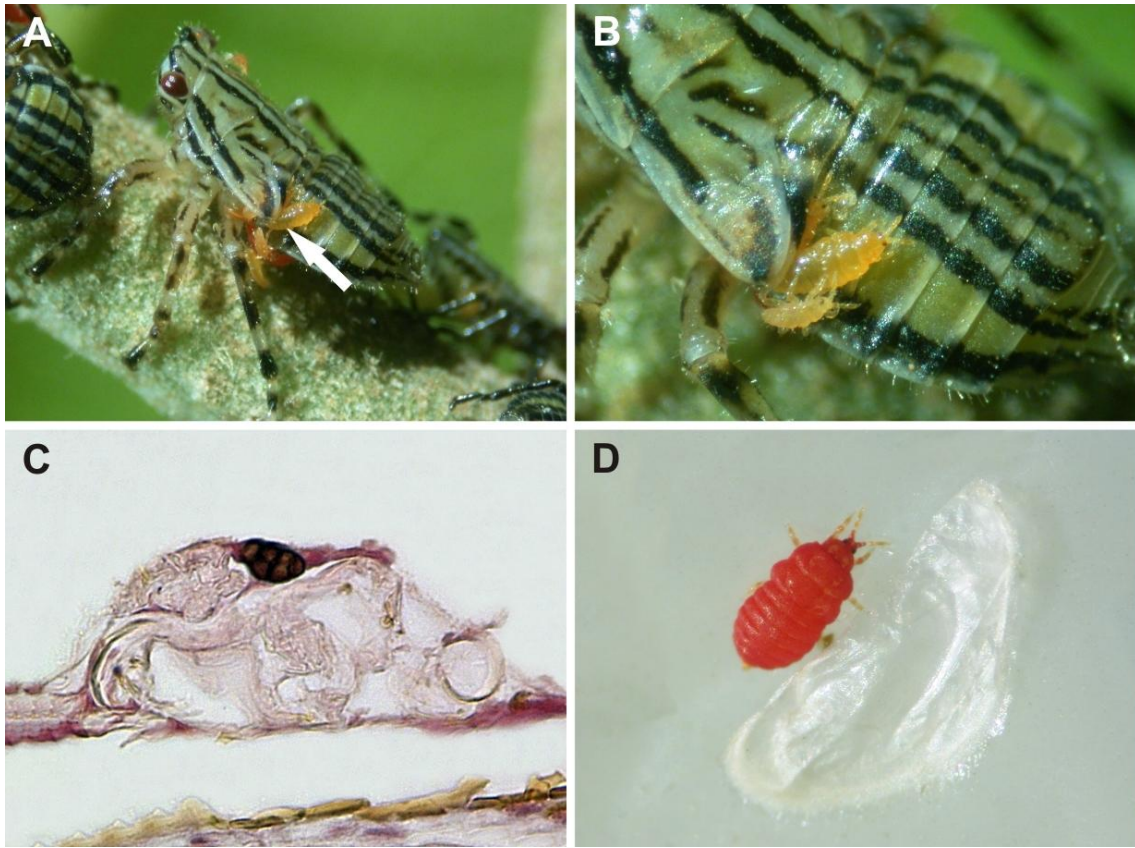


Fig. 1

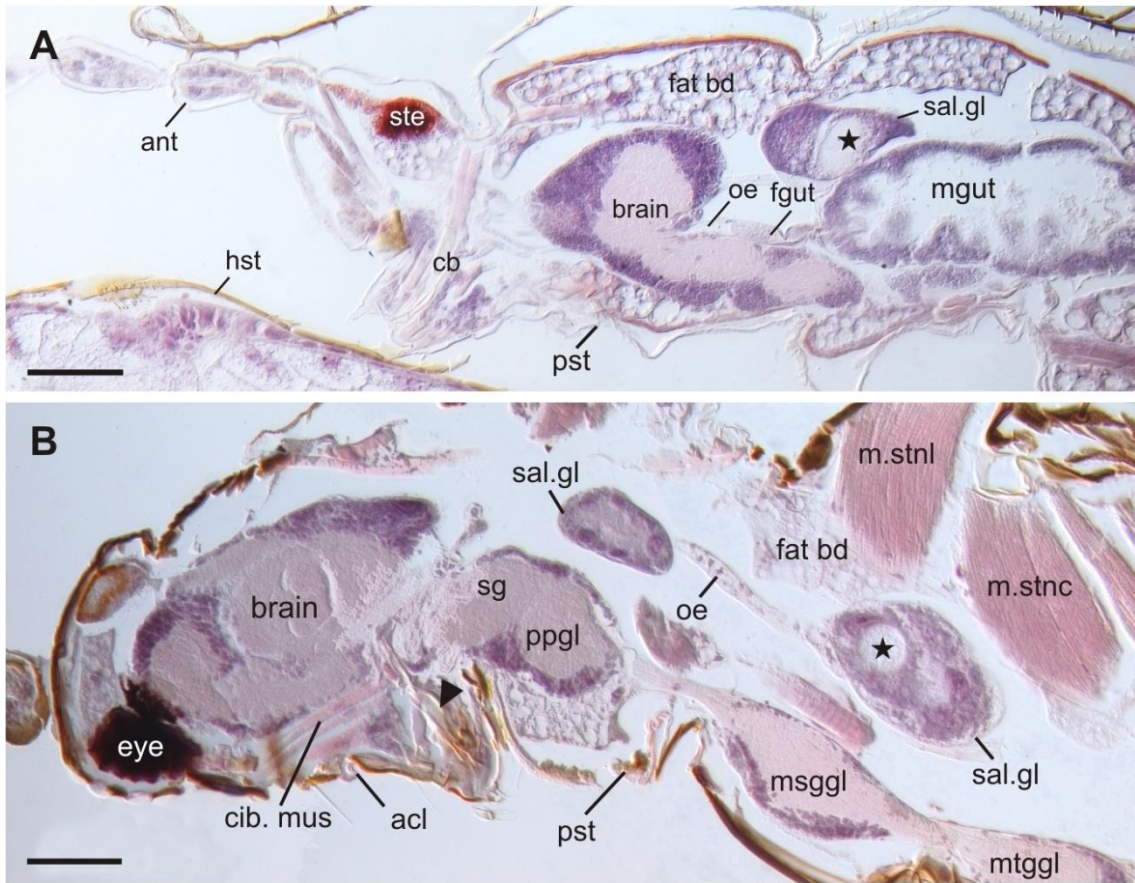


Fig. 2

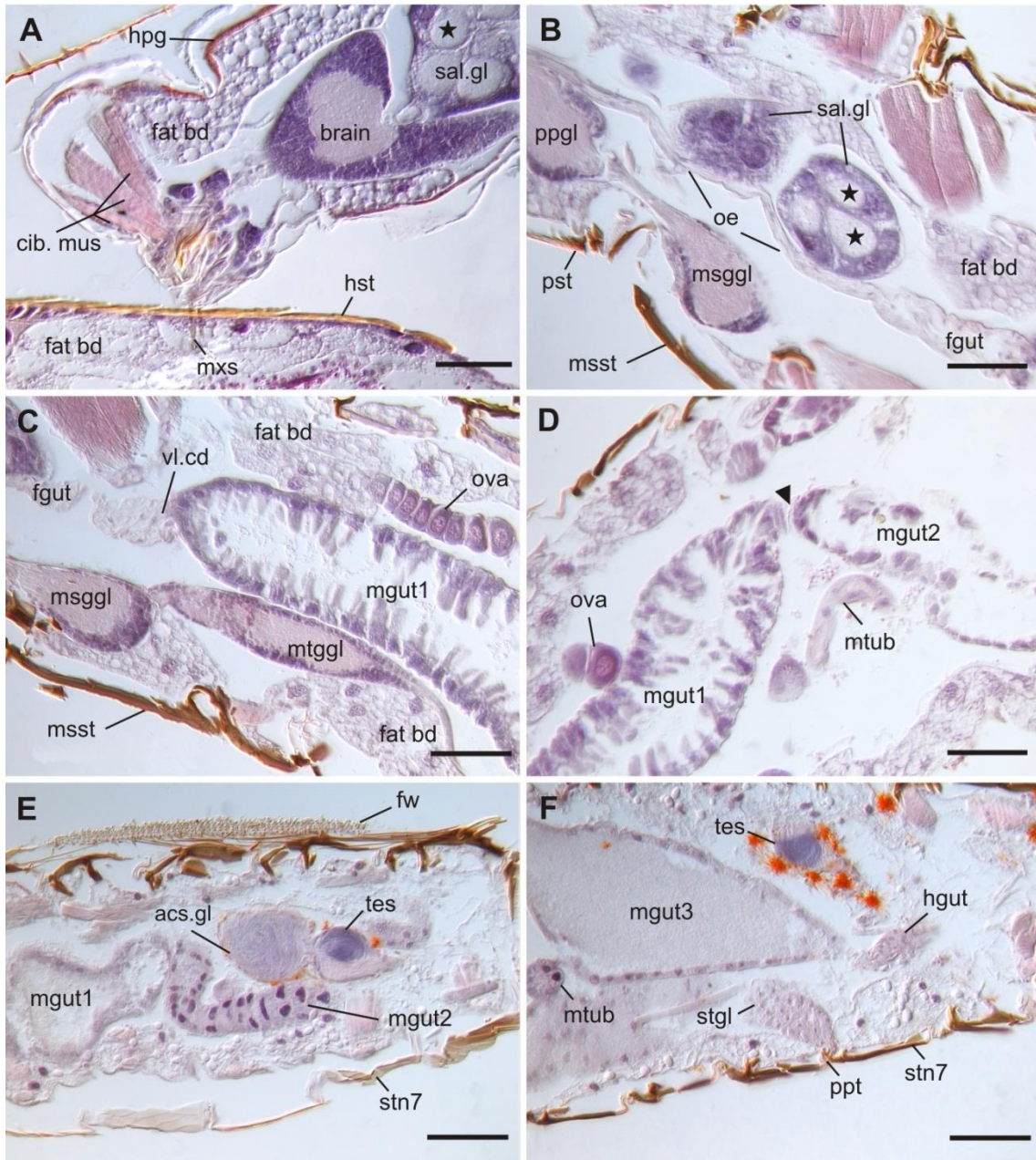


Fig. 3

5. CAPÍTULO IV

**Does the presence of an ectoparasitic thrips affect the behavior of its
aetalionid treehopper host?***

* Manuscrito a ser submetido para **Behavioral Ecology and Sociobiology**.

Does the presence of an ectoparasitic thrips affect the behavior of its aetalionid treehopper host?

Adriano Cavalleri^{a,*} and Milton de S. Mendonça Jr.^{a,b}

^a*PPG-Biologia Animal, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil*

^b*Departamento de Ecologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*

* *Corresponding author: Adriano Cavalleri*

Email addresses: cavalleri_adriano@yahoo.com.br (A. Cavalleri), milton.mendonca@ufrgs.br (M. de S. Mendonça Jr.).

Abstract

Aulacothrips dictyotus (Insecta: Thysanoptera) is a highly specific ectoparasite which infests nymphs and adults of *Aetalion reticulatum* (Insecta: Hemiptera) in South America. However, nothing is known about the consequences of this remarkable interaction to the hemipterans. In this study, we test the hypothesis that *Au. dictyotus* directly affects *Ae. reticulatum* behavior by comparing ethograms of thrips-infested and uninfested aetalionids. An analysis of 18 behavioral components showed significant differences in the behavior of *Ae. reticulatum* between these two experimental groups. Hemipterans were clearly more agitated under *Au. dictyotus* presence and infested nymphs showed more than twice as many behavioral act records than non-infested individuals. The frequencies of some behaviors related to locomotion such as walking rapidly and dispersing from aggregation were statistically higher in thrips-infested Hemiptera, as well as kicking movements using the hind legs. Moreover, infected individuals showed significantly lower frequencies in feeding behavior. We discuss the

strategies used by aetalionds to avoid thrips infestation as well as the possible consequences of ectoparasitism to host life-history.

Keywords

Aggregation, ethogram, external parasites, grooming behavior, Neotropics

Introduction

Ectoparasites are semi-independent organisms living on the surface of their hosts but possessing the ability to live free from their hosts for short periods or to move from one individual to another (Nelson et al. 1975). Several studies had shown that they negatively affect host population dynamics by reducing survivorship, mating success, fitness and altering activity patterns (Price 1980; Hart 1992; Moore 2002). Traditionally, external parasites of vertebrates have received attention because they are economically important pests and disease vectors (Poulin 2000). Conversely, little emphasis has been devoted to ectoparasites affecting invertebrate wildlife (Hurd 1990; Polak 1996; Libersat et al. 2009).

Amongst invertebrate ectoparasites, mites are the most numerically dominant organisms known, usually associated to other arthropods (Smith 1988; Downes 1990). In contrast, despite the enormous diversity of insects, it is surprising that relatively few groups in this class have evolved as external parasites of other invertebrates. More intriguingly, only a very small proportion of insects are known as external parasites of other insects. This life habit has nevertheless evolved independently in some particular groups such as Diptera (Steffan 1967), Hymenoptera (Coudron 1991; Rivers et al. 2002) and recorded recently in Thysanoptera (Izzo et al. 2002; Cavalleri et al. 2010).

The Thysanoptera, or thrips, are highly diverse in their feeding habits but *Aulacothrips* is the only known group to exhibit an ectoparasitic way of life. This genus includes three

species which attacks nymphs and adults of gregarious honeydew-producing hemipterans in Brazil (Izzo et al. 2002; Cavalleri et al. 2010). Despite the similarity in their external morphology, these taxa show distinct life histories. *Aulacothrips minor* Cavalleri, Kaminski & Mendonça has a wide range of hosts, infesting at least 15 Membracidae treehopper species in the Brazilian savannah (Cavalleri et al. 2010). *Aulacothrips amazonicus* Cavalleri, Kaminski & Mendonça was found attacking *Ramedia* treehoppers (Membracidae) in the Amazon rainforest (Cavalleri et al. 2012). In contrast, *Aulacothrips dicytotus* Hood is a highly specific ectoparasite that attacks only the aetalionid *Aetalion reticulatum* L. (Izzo et al. 2002; Cavalleri et al. 2010) (Figs. 1–2). Both adults and immatures feed on hemipterans and the damage produced by the thrips piercing and sucking mouthparts measure only a few microns (Cavalleri et al. 2010). *Aulacothrips* females lay their eggs in plant tissue and freshly emerged larvae subsequently find and attach themselves to the host tegument. Moreover, *Au. dictyotus* juveniles were found pupating in cocoons under the wings of *Ae. reticulatum* (Izzo et al. 2002; Pinent et al. 2002). Preliminary observations suggest that the presence of these thrips in *Aetalion* aggregations affect host behavior, which become agitated, although the mechanisms and consequences of this remarkable interaction are still unknown.

Indeed, parasites alter host behaviors such as phototaxis, locomotion, foraging, reproduction, and a variety of social interactions, also inducing changes such as behavioral fevers (Moore 2002; Libersat et al 2009). A study of host-parasite interactions can provide new insights into the biochemistry, physiology, behavior and ecology of the respective partners (Hurd 1990). However, the significance of ectoparasitism to invertebrates is poorly understood and research on these interactions is often complex because parasites are minute and hosts are mobile, resulting in field observations being difficult unless specialized techniques are used (Lehmann 1993). In this context, the *Aulacothrips*-Hemiptera system is ideal for investigations

on host-parasite interactions amongst insects for its ubiquity and relative motionless host behavior.

This paper presents the first quantitative study of the behavioral acts displayed by a host of an ectoparasitic thrips. *Ae. reticulatum* behavior is explored and here we test the hypothesis that *Au. dictyotus* affects host behavioral repertory. We compare hemipteran ethograms under *Aulacothrips* presence and absence and predict that thrips-infected aetalionds will behave differently in response to ectoparasite presence, displaying parasite avoidance behaviors. We also discuss the impact of ectoparasitism on the life history of *Ae. reticulatum*.

Material and methods

Study area

Experiments were conducted between May and June, 2010, at Estação Experimental de Mogi-Guaçu (EEMG) in São Paulo State (Brazil), 20°15-16'S, 47°08-12'W. This area belongs to the Cerrado biome, an important biodiversity global hotspot which represents approximately 25% of Brazilian territory (Ratter et al. 1997; Myers et al. 2000). The Cerrado are woody savannahs, which show great variation in physiognomy, ranging from dense forest areas with tree height up to 15 m to nearly treeless grassland areas with only few or no shrubs. Its climate is tropical, with a well-defined seasonality, characterized by rainy summers and dry winters (Ribeiro and Walter 1998). EEMG is also largely occupied by *Eucalyptus* and *Pinus* trees, from which wood and resin are economically exploited.

Study organisms and experimentation

Ae. reticulatum is a polyphagous sap-sucking insect widely distributed in the Neotropics and considered a serious pest that causes yield losses to citrus in South America (Azevedo Marques

1925; 1928). Females lay their eggs inside plant tissue and exhibit parental care, guarding the egg mass until the nymphs hatch. Five nymphal instars are present in *Ae. reticulatum*, and lifespan lasts for about 110 days (Gallo et al. 2002). Brown (1976) found that first-instars forage with the female on the stem near the egg mass, while later-instars are more vagile, but always congregating to feed. This hemipteran species is usually associated with several ant species, which feed on its sugar-rich excretion, called honeydew (Almeida-Neto et al. 2003).

Cavalleri et al. (2010) recorded *Au. dictyotus* attacking *Ae. reticulatum* aggregations in six unrelated plant species in Brazil (Figs. 1–2). Personal observation of the authors indicated that this interaction frequently occurs on *Alchornea triplinervia* (Euphorbiaceae), a native tree of 2 to 20 m in height.

To investigate *Ae. reticulatum* behavior, observations were made in 13 *A. triplinervia* individuals, each one containing two distinct (on different shoots) aetalionid aggregations, assigned to treatment and control. The first one was naturally infected with *Au. dictyotus* adults whereas the latter included only non-infested individuals. As a result, 26 aggregations were studied, with an average number of *Ae. reticulatum* of 30.54 (SE±5.02) in infested aggregations and 25.38 (SE±4.72) in non-infested aggregations. As *Au. dictyotus* adults were not observed attacking early *Ae. reticulatum* nymphs, only adults and third to fifth-instar juveniles were studied. Tending-ants and other arthropods were manually excluded and their presence was avoided using tanglefoot resin on plants (Tanglefoot Co., Grand Rapids, MI, USA).

All behaviors of *Ae. reticulatum* were defined after 20 hours of qualitative observations made on individuals on both treatment and control groups, resulting in an ethogram. Subsequently, 86 hours of quantitative observations of nonsequential parameters were made, in a total of 43 hours of sampling for each experimental group. Observations on each aggregation were made in 60-minute sessions with five minutes of sampling interspersed with two minute

intervals. During each 5-minute observation, all occurrences were recorded in thrips-bearing individuals from the treatment group and in thrips-free individuals from the control group. Each behavioral act was recorded only once during the 5-minute sessions and a stopwatch was used to time behaviors. Two observers recorded different experimental groups after random selection and observations were made concomitantly on a given plant individual. The number of individuals examined in the same 5-minute sessions ranged from one to five in each experimental group. Some plant individuals were observed more than once, but with a minimum of 24 h of interval between observations.

Given the small size of the thrips, it was not possible to observe the exact moment in which *Au. dictyotus* begins to feed on aetalionids. As a result, observations in the treatment group were conducted in those individuals who had at least one adult thrips in contact with their body and/or appendages. Voucher specimens are deposited in the zoological collection of Laboratório de Ecologia de Interações, Departamento de Ecologia, Universidade Federal do Rio Grande do Sul - UFRGS (Porto Alegre, Brazil).

Statistical analysis

A matrix containing the records made in all plant individuals and behavioral acts was constructed. To detect differences between experimental groups, all behavioral frequencies were calculated and paired comparisons between (i) treatment and control group and (ii) adults and immature within groups were made using randomization tests (behavioral frequencies as dependent variables). The same test was performed to detect differences in frequencies of each behavioral act between the infested and non-infested aetalionids. Behavioral events recorded less than four times overall were excluded from the paired comparisons. All statistical analyses were performed with Multiv software (Pillar 2006) using $N=1,000$ random permutations and $\alpha = 0.05$.

Results

After 86 hours of sampling, 224 *Ae. reticulatum* individuals were observed and a total of 5,086 behavioral events were recorded (see below). Most of the examined aetalionids were fifth instar nymphs (45%), followed by fourth instar (30%), adults (18%) and third instar (7%). The majority of the adults were females, all exhibiting parental care toward their brood. A total of 18 discrete behavioral acts were used to describe *Ae. reticulatum* behavior with thrips either present or absent. A short description of these behaviors is given below:

1. *Feeding*: sap-sucking activity, with stylets inserted in plant tissue.
2. *Resting*: sedentary phase, during which individuals remain motionless. Stylets are retracted to thorax ventral surface and no locomotion occurs.
3. *Walking slowly*: locomotion over short distances characterized by moving along the plant by slow movements of the legs, always next to aggregation.
4. *Walking rapidly*: typical escaping behavior, characterized by quick and vigorous movement of the legs. Occurs along the plant and next to aggregation but lacks orientation and is associated with covering larger distances than walking slowly behavior.
5. *Dispersing from aggregation*: walking away from the aggregation without specific orientation, but usually in the direction of shoot extremities. Individuals also frequently observed “deserting” to other shoot of the same plant.
6. *Walking over conspecifics*: crawling over another individual in the same aggregation.
7. *Slow movement of the legs*: raising one or more legs slowly, during more than 2 s, followed by lowering to their original resting position for 5 s. This movement occurs without locomotion.
8. *Rapid movement of the legs*: raising one or more legs quickly, resembling a kick movement.

Occurs faster than 2 s and usually without locomotion.

9. *Suspending posterior legs*: raising one or both posterior legs for more than 6 s. Occurs without locomotion on the plant and often involves delicate up and down body movements.
10. *Suspending anterior and/or median legs*: raising one or more legs for more than 6 s. This movement occurs without locomotion and usually does not involve up and down movements of the legs.
11. *Raising the body from the plant*: suspending abdomen and/or thorax from the plant surface in a fast movement. This movement occurs without locomotion and this raising position may last for several minutes.
12. *Lateral movement of the abdomen*: repeated lateral movements, not touching the plant with abdomen. Usually a quick movement that occurs with or without locomotion.
13. *Grooming on dorsal surface*: self-preening abdominal tergites or wings using posterior legs.
14. *Grooming on ventral surface*: self-preening abdominal sternites using posterior legs.
15. *Rubbing onto a conspecific body*: frenetic and repeated movements, touching the body of another individual in the same aggregation.
16. *Rubbing on the plant*: frenetic and repeated movements, touching any plant organ. Usually observed toward branches and petioles.
17. *Rubbing posterior legs*: repeated movements of the legs, touching each other when suspended from the plant. This movement occurs without locomotion.
18. *Honeydew excretion*: occurs by releasing one or more droplets from anus, which is generally preceded by a slow elevation of the abdomen.

Ectoparasitic infestation

Although *Ae. reticulatum* infested with *Au. dictyotus* are quite common in the study area, the number of adult thrips per aggregation ranged considerably, from one to more than 30.

Ectoparasite approach toward an aetalionid is usually accompanied by antennating on the *Ae. reticulatum* body and climbing on the host through its legs or head (Online Resources 1–2).

Thrips were usually found attached to the aetalionids dorsal surface and the number of *Au. dictyotus* per *Ae. reticulatum* individual ranged from one to nine (Figs. 1–2). When aetalionids moult, thrips possibly desert the host and reattach on to soft-bodied teneral individuals.

Immediately after thrips attack, the new host usually indulged in a frenzy of self-cleaning movements (Online Resources 1–2). After infestation, thrips individuals usually do not move to another host for a long period of time. Successful removal of attached *Aulacothrips* from the hemipteran body was observed in a few cases, usually after self-cleaning behavior.

Behavioral records

An analysis of the 18 behavioral components showed significant differences in the behavior of *Ae. reticulatum* when infested and not infested by thrips ($P=0.002$) (Table 1). The same result was observed when nymphs ($P=0.001$) (Table 2) and adults ($P=0.02$) (Table 3) are analyzed separately. The behavioral pattern between infected nymphs and infected adults was also significantly different ($P=0.04$). Interestingly, statistical analysis revealed no significant differences between non-infested adults and non-infested nymphs. All behavioral components were displayed by both immature and adult hemipterans, except rubbing posterior legs, which was present only in adults, and grooming on ventral surface and rubbing onto a conspecific body, which were observed only in nymphs. The thrips presence did not affect some frequent *Ae. reticulatum* behaviors, such as resting, walking slowly or over conspecifics, suspending legs and honeydew excretion (Table 1).

Aetalionids were clearly more agitated under *Au. dictyotus* presence. This was especially true for *Ae. reticulatum* immature stages, which showed more than twice as many behavioral act records than non-infested individuals (Table 2). The majority of the records displayed by infested *Ae. reticulatum* are comprised by rapid movement of the legs (23.4%), feeding (18.9%), and abdominal movements (lateral and raising movements) (22.7%). The most common acts displayed by non-infested individuals were feeding behavior, which comprised nearly 55% of the records, followed by slow (10.9%) and rapid (7.8%) movements of the legs. The frequencies of some behaviors related to locomotion such as walking rapidly ($P=0.003$) and dispersing from aggregation ($P=0.007$), were significantly higher in infected Hemiptera, as well as rapid movements of the legs ($P=0.001$). Except by rubbing posterior legs, which was observed only four times, all behaviors related to self-cleaning activities were significantly more frequent in thrips-bearing individuals (Table 1). Frequencies of 11 behaviors differed statically between infected and non-infested nymphs (Table 3). These differences were observed for feeding, locomotion, slow and rapid appendage movements, abdominal movements and self-cleaning behaviors. In contrast, only feeding ($P=0.01$), lateral movement of the abdomen ($P=0.01$) and rapid movements of the legs ($P=0.019$) differed between infested and non-infested adults. The behavior of raising the body from the plant showed a marginally significant value between these two groups (Table 3).

Discussion

Aulacothrips has a large impact on *Ae. reticulatum* behavior, which shows signs of apparent discomfort under thrips contact. Our experiment was not designed to unravel the time spent by *Ae. reticulatum* on each behavioral component. Our results do, however, demonstrate remarkable differences in the frequencies of approximately 60% of the behavioral acts between

infested and non-infested aetalionids (Table 1). The significant differences observed in feeding records under thrips presence and absence is closely related to high frequencies of rapid movement of the legs and abdomen in infected hosts. These self-cleaning behaviors were commonly displayed under *Au. dictyotus* contact, usually just after thrips fixation (Online Resource 1).

The differences observed between infested *Ae. reticulatum* immature and adults probably resulted from parental care exhibited by the females. Unlike nymphs, adults stay in the same place for long periods feeding on the plant, always near their brood. They also avoid distancing themselves from their brood, even when disturbed by other organisms. This behavior was also referred by Brown (1976) on *Piper umbellatum* in Costa Rica, where *Ae. reticulatum* females were relatively non-mobile and hesitant to leave either a feeding site or an egg mass. Our findings suggest that evasive behaviors are frequent strategies used by immature to escape from *Au. dictyotus* attacks while adults tend to continue in the same position on the plant, trying to dislodge thrips by grooming themselves. In a few situations, this mechanism was efficient to remove firmly attached *Aulacothrips* adults and it appears that *Ae. reticulatum* uses grooming as a first line of defense against thrips. Indeed, grooming is one of the most frequently and regularly performed behavioral patterns for animals, constituting an important deterrent against parasites in both vertebrates and invertebrates (Hart 1992; Léonard et al. 1999).

The behavior of suspending posterior legs was equally present in the treatment and control groups, but its biological significance is unknown. Brown (1976) stated that artificial disturbances in the form of movement of large bodies near aggregations or loud sound also resulted in similar movement responses. On the other hand, kick movements using hind legs were significantly higher in infested individuals and they were clearly induced by thrips fixation. Although this seems to be little effective against thrips, some egg parasitoids were

observed several times being knocked off the egg masses by female leg spurring in Costa Rica (Brown 1976). In general, all movements were faster in infested hemipterans and were also closely related to thrips attacks.

It was impossible to maintain an equal number of thrips per host within the treatment individuals, but we suggest that the number of ectoparasites also has an important role in *Ae. reticulatum* fitness. Similarly, as thrips larvae presence was not studied, the effect of ectoparasitism might be stronger on the hosts than recorded here. The number of juvenile thrips attached to aetalonids varies greatly, but considering fifth instar nymphs only, this number ranged from 1 to 15, with an average of 2.47 larvae per infected bug (SE \pm 2.91, n=105) (Cavalleri, A. unpublished data). Moreover, about 30% of *Ae. reticulatum* immature and adults observed by Izzo et al. (2002) were infected with *Aulacothrips* larvae in *Bauhinia variegata* (Fabaceae).

According to Hart (1992), almost any parasite control behavior represents some cost in fitness to the animal from loss of feeding time, energy utilized, or distraction from predator vigilance. Our results suggest that *Au. dictyotus* infestation plays an important role on *Ae. reticulatum* development, by perturbing host feeding behavior and leading to a number of unusual behavioral acts. Maintaining defenses against parasites can be costly for hosts, forcing them to allocate limited resources to defense rather than other life history components (Sheldon and Verhulst 1996; Norris and Evans 2000). This phenomenon is also common in other ectoparasites as mites feeding on other insects, causing reduction of food consumed and also draining substantial quantities of hemolymph from their host (Lehmann 1993). However, the energy costs involved in active and repeated behaviors such as those used to avoid and/or remove *Aulacothrips* are difficult to quantify. Some studies have shown that external parasites of invertebrates could also alter host physiology. For example, LaMunyon and Eisner (1990) reported that ectoparasitic erythraeid mites have a negative effect on wax production by flatid

planthoppers (Hemiptera). However, our results did not reveal any effect of the thrips presence on the frequency with which the honeydew was expelled, for example. Nevertheless, in two cases, infested nymphs, visibly disturbed by the thrips attached to their tegument, expelled an unusually larger amount of droplets. Further studies are necessary to investigate the effect of the ectoparasitism on the quantity and quality of hemipteran exudation.

Thrips ectoparasitism may have an indirect effect on aetalionid survivorship as well. It was not uncommon to observe infested nymphs isolated from their aggregation, inhabiting a distinct branch of the same host plant. Some studies had demonstrated that the ants alertness and hostility decreased with distance from the ant-tended Hemiptera aggregations (Smith and Armitage 1931; Way 1954). In this context, the protection provided by the ants against hemipterans natural enemies is possibly reduced when *Ae. reticulatum* individuals desert their original aggregation. LaMunyon and Eisner (1990) verified that infestation by ectoparasitic mites can render planthoppers less prone to leap away when molested and more susceptible to predatory capture by chrysopids.

As indicated by Cavalleri et al. (2010), the gregarious and sedentary behavior exhibited by the known hemipteran hosts is certainly a key factor influencing the infestation dynamics in *Aulacothrips*. This behavior contrasts with those of other potential Hemiptera hosts such as planthoppers and leafhoppers, which usually exhibit more agile movements and saltatorial escaping. Interestingly, deserting the aggregation and other evasive behaviors possibly reduce the abundance of ectoparasites by keeping *Au. dictyotus* away from the rest of the brood at minimum expense to the host population. But as stated above, such evasive behaviors might inadvertently increase the vulnerability to predation of the infected individual by leaving the aggregation. In addition, the thrips effect on host behavior may also increase the risk of ectoparasitic transmission. In particular, *Au. dictyotus* dislodgement by rubbing on another individual or host-plant could lighten the ectoparasite load for an infested individual but might

increase thrips infestation to a greater number of individuals as well. In this context, host behavior can influence both parasite transmission rates and parasite/host survival (Moore 2002). Conversely, personal observations on *Guayaquila xiphias* treehoppers, which is commonly attacked by *Au. minor*, suggest a more passive behavior under thrips presence when compared to *Ae. reticulatum* (Cavalleri, A. unpublished data). Alves-Silva and Del-Claro (2011) also did not observe any evident change in *Enchenopa brasiliensis* behavior under *Au. minor* infestation.

Various aspects of social, feeding, and reproductive behavior of animals are shaped by the forces of predation and resource limitation that animals must address to survive to reproductive age and successfully rear young. A relatively unappreciated force shaping behavior is the existence of external and internal parasites (Hart 1992; Libersat et al. 2009). The available information on the effects of ectoparasitic infection in invertebrates is scarce, which limits our ability to predict parasite abundance and distribution and to detect ecological patterns of host-parasite relationship in arthropods. In this study, we examined a remarkable interaction between hemipterans and thrips and its effects on host behavior. Characterizing the behavioral strategies used by aetalionids against *Aulacothrips* might be useful for understanding the role of such interaction on the ecology and evolution of both insects. Further monitoring is needed to assess the long-term impact of *Aulacothrips* on the population dynamics of host hemipterans and on their interaction with tending ants.

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Tables and figures captions

Table 1 Behavioral repertory of *Aetalion reticulatum* nymphs and adults under *Aulacothrips dictyotus* presence (treatment) and absence (control) on *Alchornea triplinervia* shoots. The number of records of each behavior (N) and its relative frequency (RF) is provided

Table 2 Behavioral repertory of *Aetalion reticulatum* nymphs under *Aulacothrips dictyotus* presence (treatment) and absence (control) on *Alchornea triplinervia* shoots. The number of records of each behavior (N) and its relative frequency (RF) is provided

Table 3 Behavioral repertory of *Aetalion reticulatum* adults under *Aulacothrips dictyotus* presence (treatment) and absence (control) on *Alchornea triplinervia* shoots. The number of records of each behavior (N) and its relative frequency (RF) is provided

Fig. 1 *Aetalion reticulatum* aggregation infested with *Aulacothrips dictyotus* (small arrows). Large arrow indicates a typical rapid movement of posterior legs, which is closely related to thrips infection (see Table 1)

Fig. 2 *Au. dictyotus* adult attacking *Ae. reticulatum* nymph

Online Resource 1 *Ae. reticulatum* nymph infested with *Au. dictyotus*. Note the agitated behavior due to thrips attack and the diversity of movements displayed by infested hosts

Online Resource 2 *Ae. reticulatum* aggregation infested on the dorsal surface with several *Au. dictyotus*. Note the cleaning behaviors exhibited by hosts and the thrips process of climbing on aetalionids

TABLE 1

Behavioral act	Treatment (n=106)		Control (n=118)		
	N	RF	N	RF	<i>P</i>
Feeding	651	18.90%	903	54.99%	0.001
Resting	181	5.26%	38	2.31%	<i>ns</i>
Walking slowly	102	2.96%	63	3.84%	<i>ns</i>
Walking rapidly	107	3.11%	1	0.06%	0.003
Dispersing from aggregation	88	2.56%	1	0.06%	0.007
Walking over conspecifics	68	1.97%	31	1.89%	<i>ns</i>
Slow movement of the legs	95	2.76%	180	10.96%	0.001
Rapid movement of the legs	806	23.40%	128	7.80%	0.001
Suspending posterior legs	116	3.37%	98	5.97%	<i>ns</i>
Suspending anterior and/or median legs	13	0.38%	22	1.34%	<i>ns</i>
Raising the body from the plant	419	12.17%	92	5.60%	0.001
Lateral movement of the abdomen	363	10.54%	65	3.96%	0.002
Grooming on dorsal surface	111	3.22%	3	0.18%	0.009
Grooming on ventral surface	12	0.35%	1	0.06%	0.010
Rubbing onto a conspecific body	59	1.71%	0	0.00%	0.008
Rubbing on the plant	225	6.53%	4	0.24%	0.001
Rubbing posterior legs	3	0.09%	1	0.06%	<i>ns</i>
Honeydew excretion	25	0.73%	11	0.67%	<i>ns</i>
Total	3444	100%	1642	100%	0.002

TABLE 2

Behavioral act	Treatment (n=94)		Control (n=87)		<i>P</i>
	N	RF	N	RF	
Feeding	563	17.67%	670	52.55%	0.001
Resting	179	5.62%	30	2.35%	<i>ns</i>
Walking slowly	100	3.14%	54	4.24%	<i>ns</i>
Walking rapidly	106	3.33%	1	0.08%	0.003
Dispersing from aggregation	87	2.73%	1	0.08%	0.007
Walking over conspecifics	68	2.13%	28	2.20%	<i>ns</i>
Slow movement of the legs	84	2.64%	145	11.37%	0.001
Rapid movement of the legs	746	23.41%	97	7.61%	0.001
Suspending posterior legs	93	2.92%	73	5.73%	<i>ns</i>
Suspending anterior and/or median legs	12	0.38%	18	1.41%	<i>ns</i>
Raising the body from the plant	392	12.30%	83	6.51%	0.002
Lateral movement of the abdomen	339	10.64%	57	4.47%	0.002
Grooming on dorsal surface	104	3.26%	1	0.08%	0.01
Grooming on ventral surface	12	0.38%	1	0.08%	0.01
Rubbing onto a conspecific body	59	1.85%	0	0.00%	0.008
Rubbing on the plant	219	6.87%	4	0.31%	0.001
Rubbing posterior legs	0	0.00%	1	0.08%	<i>ns</i>
Honeydew excretion	23	0.72%	11	0.86%	<i>ns</i>
Total	3186	100%	1275	100%	0.001

TABLE 3

Behavioral act	Treatment (n=11)		Control (n=30)		
	N	RF	N	RF	<i>P</i>
Feeding	88	34.11%	233	63.49%	0.01
Resting	2	0.78%	8	2.18%	<i>ns</i>
Walking slowly	2	0.78%	9	2.45%	<i>ns</i>
Walking rapidly	1	0.39%	0	0.00%	<i>ns</i>
Dispersing from aggregation	1	0.39%	0	0.00%	<i>ns</i>
Walking over conspecifics	0	0.00%	3	0.82%	<i>ns</i>
Slow movement of the legs	11	4.26%	35	9.54%	<i>ns</i>
Rapid movement of the legs	60	23.26%	31	8.45%	0.02
Suspending posterior legs	23	8.91%	25	6.81%	<i>ns</i>
Suspending anterior and/or median legs	1	0.39%	4	1.09%	<i>ns</i>
Raising the body from the plant	27	10.47%	9	2.45%	0.051
Lateral movement of the abdomen	24	9.30%	8	2.18%	0.02
Grooming on dorsal surface	7	2.71%	2	0.54%	<i>ns</i>
Grooming on ventral surface	0	0.00%	0	0.00%	<i>ns</i>
Rubbing onto a conspecific body	0	0.00%	0	0.00%	<i>ns</i>
Rubbing on the plant	6	2.33%	0	0.00%	<i>ns</i>
Rubbing posterior legs	3	1.16%	0	0.00%	<i>ns</i>
Honeydew excretion	2	0.78%	0	0.00%	<i>ns</i>
Total	258	100%	367	100%	0.02



Figs 1 & 2

6. APÊNDICES

6.1. Camuflagem química em *Aulacothrips*

Alguns estudos apontam que a interação entre formigas e herbívoros trofobiontes pode ser benéfica a ambos os insetos e inclusive para a planta. Por exemplo, Del-Claro & Oliveira (2000) demonstraram que a presença de formigas atendentes reduz a abundância de inimigos naturais de *Guayaquila xiphias* e de outros herbívoros na planta hospedeira, aumentando a sobrevivência dos membracídeos e diminuindo a taxa de herbivoria do vegetal. Essa defesa indireta produzida pela presença de formigas é condicional, pois varia ao longo do tempo, de acordo com as mudanças bióticas e abióticas do meio (Oliveira & Del-Claro 2005).

Entretanto, a capacidade fisiológica de tornar a composição do exsudato mais adequado para as formigas, pode também representar uma defesa primária de hemípteros contra ataques pelas formigas que os atendem. Estudos vêm demonstrando que a predação de insetos herbívoros por formigas pode ser diminuída pela semelhança de seus hidrocarbonetos cuticulares (HCCs) com aqueles encontrados na sua planta hospedeira. Segundo Silveira *et al.* (2010), isto ocorre inclusive em hemípteros trofobiontes como *G. xiphias*. Estes autores verificaram que a semelhança química entre ninfas e adultos desta cigarrinha e sua planta hospedeira confere a esse herbívoro uma camuflagem química contra ataques por formigas, resultando em uma defesa adicional à produção de exsudato.

Observações nas agregações de cigarrinhas infestadas por *Aulacothrips* indicaram que as formigas atendentes não atacam os tripes, mesmo aqueles que foram observados se locomovendo na planta. Visando testar a hipótese de que os tripes também evitam a predação por formigas através de camuflagem química, estamos verificando a similaridade química entre os compostos de *Aulacothrips*, das cigarrinhas hospedeiras e das plantas. Os extratos químicos da cutícula dos tripes, seus hospedeiros e plantas estão sendo analisados através de cromatografia gasosa-espectrometria de massas e para avaliar a similaridade química destas substâncias será utilizado o índice de Morisita. Os procedimentos para a extração e análise dos HCCs de *Aulacothrips* spp. e dos demais organismos são os mesmos utilizados por Portugal (2001).

Até o momento, cerca de 10 amostras foram analisadas (tripes, respectivo hemíptero hospedeiro e planta), e os resultados preliminares indicam que os compostos da cutícula de *Aulacothrips* possuem uma alta similaridade com aqueles presentes em seus hospedeiros, que por sua vez são similares aos da planta (Fig. 2a–e). Isso sugere que, assim como as cigarrinhas, os tripes também utilizam mecanismos de camuflagem química para evitar a predação pelas

formigas atendedoras. Este resultado é particularmente interessante, pois há um mesmo padrão de HHCs em três níveis tróficos: plantas, herbívoros e ectoparasitas. É possível ainda que estes trips estejam se beneficiando da presença das formigas atendedoras, pois elas podem gerar um espaço livre de inimigos naturais para estes ectoparasitas. Este estudo está sendo desenvolvido com a colaboração do Dr. José R. Trigo (Unicamp) e resultará em um artigo intitulado “*Cuticular hydrocarbons across three trophic levels: from plants to ectoparasitic thrips*”.

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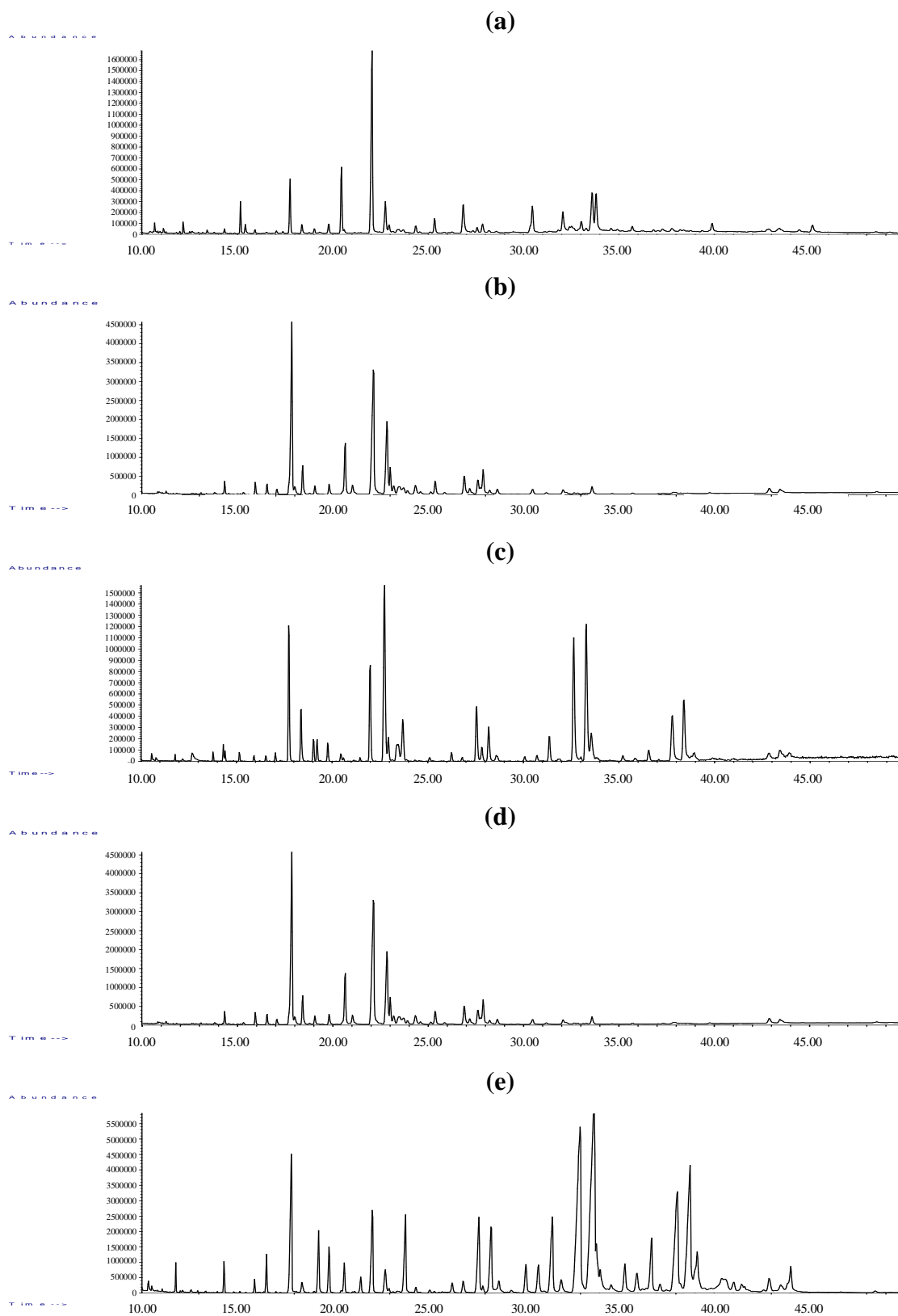


Figura 2a–e. Cromatogramas dos extratos da cutícula da planta hospedeira e organismos associados. (a) Ramos de *Aegyphila* sp. (Verbenaceae); (b) Ninfas de *Guayaquila xiphias* (Membracidae); (c) Adultos de *G. xiphias*; (d) Adultos de *Aulacothrips minor*

(Heterothripidae); (e) Adultos de *Camponotus rufipes* (Formicidae). Material coletado em 23/05/2010, Campinas, SP. Eixo y representa a abundância dos compostos da cutícula enquanto o eixo x representa o tempo em minutos.

7. CONCLUSÕES E CONSIDERAÇÕES FINAIS

CONCLUSÕES

→ As larvas e adultos do gênero *Aulacothrips* são ectoparasitas de Hemiptera. Os tripes perfuram o tegumento das cigarrinhas usando a mandíbula e inserem os estiletos maxilares na região dos corpos gordurosos do hospedeiro.

→ Existem pelo menos três espécies de tripes ectoparasitas, todas atacando cigarrinhas de hábito gregário e com associações mutualísticas com formigas.

→ Larvas e adultos de *Aulacothrips* não foram encontrados em plantas sem a presença de cigarrinhas. Os ovos dos tripes são depositados na planta, geralmente próximos à agregação de cigarrinhas, facilitando as larvas recém eclodidas a encontrarem um hospedeiro.

→ O hábito gregário destas cigarrinhas parece ser fundamental para que estes ectoparasitas completem seu ciclo de vida, pois permite que haja sempre hospedeiros disponíveis durante o processo de ecdise dos hemípteros, durante o qual o tripe precisa deixar o hospedeiro mesmo que temporariamente.

→ *Aulacothrips dictyotus* é um parasita específico de *Aetalion reticulatum*. *Aulacothrips minor* possui um amplo espectro de hospedeiros, atacando pelo menos 15 espécies de Membracidae no Cerrado brasileiro. *Aulacothrips amazonicus* ocorre na Amazônia e infesta pelo menos uma espécie de membracídeo.

→ As três espécies de *Aulacothrips* apresentam diferenças marcantes nas áreas sensoriais dos antenômeros III–IV. Em *Au. amazonicus* estas áreas sensoriais são significativamente reduzidas enquanto que em *Au. dictyotus* estas são extremamente desenvolvidas. É provável que a diferença existente no tamanho destes órgãos entre as espécies do gênero esteja intimamente relacionada ao grau de especificidade parasitária e características do ambiente em que vivem.

→ Os resultados indicam que a presença de *Au. dictyotus* modifica o comportamento de *Ae. reticulatum*. Os indivíduos infectados apresentam um grande número de atos comportamentais relacionados à limpeza corporal e executam estas atividades em frequências significativamente mais altas quando comparados às cigarrinhas sem tripes. O efeito da presença dos tripes afeta principalmente o comportamento das ninfas de *Ae. reticulatum*.

→ Adultos e larvas destes tripes não são atacados pelas formigas associadas aos hemípteros hospedeiros. Resultados preliminares indicam que existe uma elevada semelhança química entre os compostos da cutícula de *Aulacothrips* e suas respectivas cigarrinhas hospedeiras, que por sua vez apresentam alta similaridade química com suas plantas hospedeiras. Este

mecanismo utilizado pelos tripses contra formigas coletoras de exsudatos pode ser definido como camuflagem química, e possivelmente protege *Aulacothrips* contra a predação.

CONSIDERAÇÕES FINAIS

As pressões ecológicas que levaram à evolução do hábito ectoparasita em *Aulacothrips* ainda são difíceis de compreender. Os seus parentes mais próximos são todos fitófagos em flores e possuem ampla distribuição nas Américas, especialmente Central e do Sul. O fato da maioria das cigarrinhas hospedeiras utilizarem os ramos com flores para sua alimentação pode ter sido um fator chave para a invasão dos ancestrais destes tripses nas agregações de hemípteros. Plantas da família Malpighiaceae, por exemplo, suportam uma grande abundância e diversidade de cigarrinhas em seus ramos e de Heterothripidae fitófagos em suas flores, particularmente no Cerrado. Provavelmente beneficiados pela proteção indireta fornecida pelas formigas atendentes e pelo alimento de elevado valor energético, estes tripses encontraram um nicho bastante favorável para se estabelecer.

Os tripses podem ser considerados pré-adaptados ao hábito ectoparasita, pois seu aparelho bucal perfurador-sugador, assim como aquele observado em outros parasitas externos, facilita tal estilo de vida. O corpo das larvas e adultos de *Aulacothrips* é coberto por longas cerdas de ápice expandido que parecem desempenhar um papel importante na abrasão que ocorre devido aos comportamentos de limpeza corporal realizados pelo hospedeiro. As asas firmemente presas ao corpo através de uma depressão dorsal no abdômen e cerdas robustas também conferem uma maior proteção a estes delicados órgãos contra eventuais danos mecânicos. As antenas talvez sejam as adaptações mais evidentes destes tripses ao estilo de vida parasitário e contrastam com os demais membros da família Heterothripidae. As áreas sensoriais extremamente longas dos antenômeros III & IV são supostamente importantes na localização de cigarrinhas hospedeiras e a variação interespecífica observada nestes órgãos se mostrou muito útil no reconhecimento das espécies de *Aulacothrips*.

Até o presente momento, os tripses ectoparasitas estão restritos ao Brasil, mas dada a ampla distribuição dos hospedeiros, estes insetos devem estar presentes em uma extensa área na América do Sul. Nesse sentido, é provável que a diversidade destes tripses esteja ainda subestimada e futuras expedições possivelmente detectarão a presença de *Aulacothrips* em biomas ainda não investigados, tais como o Pantanal, a Caatinga e regiões andinas.

Não podemos afirmar quais foram os primeiros hospedeiros a serem utilizados por estes tripes. Considerando a morfologia mais especializada de *Au. dictyotus* e o comportamento agitado de *Ae. reticulatum* na presença dos tisanópteros, é possível que esta associação seja relativamente recente. Observações em campo sugerem que os membracídeos estejam mais adaptados à presença dos tripes, e comportamentos de limpeza corporal por parte dos hemípteros foram raramente observados.

A capacidade dos tripes de se infiltrarem e se estabelecerem em sistemas ecológicos complexos (p.ex. galhas, vespeiros, cupinzeiros) é realmente notável. O seu tamanho diminuto, a diversidade de hábitos alimentares e o oportunismo certamente são fundamentais na colonização de novos ambientes. Ao longo deste estudo averiguamos que estes ectoparasitas se utilizam ainda de um disfarce químico para passarem despercebidos por outros organismos. Isto provavelmente lhes confere também uma proteção por parte das formigas atendentes contra possíveis inimigos naturais. É possível ainda que o ectoparasitismo afete a quantidade e qualidade de *honeydew* excretado pelas cigarrinhas, e estudos futuros serão conduzidos para avaliar se esta alteração afeta a proteção dos hemípteros (e da planta). Isso poderia se dar através da diminuição da atratividade do *honeydew* levando a um menor recrutamento das formigas para este recurso. Desta forma, estes tisanópteros podem ser considerados parasitas também da relação mutualística existente entre cigarrinhas e formigas. As consequências ecológicas e evolutivas desse “parasitismo sobre um mutualismo” são um campo fértil para entender a dinâmica dessa relação multitrófica – os tripes podem desestruturar a interação entre cigarrinhas e formigas, ou o dano causado à relação é suficientemente pequeno para que a mesma se mantenha inalterada? Estudos mais aprofundados da potencial cascata de efeitos do parasitismo pelos tripes nessa malha de relações (cigarrinhas, formigas, plantas) são necessários para revelar qual cenário é mais provável.

Destaca-se também o potencial destes tripes como agentes no controle biológico de cigarrinhas em sistemas de manejo integrado de pragas. Por exemplo, *Ae. reticulatum* é considerada uma praga de citros, alimentando-se nos pedúnculos dos frutos e atrasando o seu desenvolvimento, podendo ocasionar sua queda. O controle deste inseto é geralmente feito através de pulverizações com inseticidas fosforados, clorofosforados e carbamatos. Estudos futuros avaliarão o efeito do tripes na sobrevivência assim como no número de ovos produzidos por *Ae. reticulatum*. Se for comprovada a redução nesses aspectos demográficos da cigarrinha, se poderá pensar em formas de usar esses tripes efetivamente, por exemplo através de liberações nas culturas dos citros, advindos de criação massal dos tripes.

Estudos abordando aspectos ecológicos de espécies nativas de tripes ainda são raros na América do Sul e grande parte da atenção é dedicada àquelas poucas espécies consideradas pragas agrícolas. Isso é insuficiente, pois sabemos que para entender os sistemas alterados pelo homem, como os agrícolas, precisamos de termos de comparação, que encontramos nos sistemas naturais. Dada a relevância do intrigante hábito alimentar observado em *Aulacothrips*, o único registrado para os Thysanoptera, e suas consequências nas interações com outros organismos, torna-se importante a busca por informações que permitam o entendimento dos mecanismos envolvidos neste processo. Após responder questões fundamentais envolvidas nesta interação multitrófica, um leque de outras perguntas interessantes foi aberto, fazendo deste sistema um excelente modelo para estudos de ecologia e evolução da interação parasita-hospedeiro. Através deste trabalho, esperamos encorajar outros pesquisadores a investigarem as associações envolvendo tripes e outros organismos, diversificando os estudos com este fascinante grupo de insetos.