UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(BIOLOGIA CELULAR E MOLECULAR)

# A glândula metapleural e suas secreções em formigas cultivadoras (Attini) e não cultivadoras de fungos (Myrmicini, Blepharidattini e Ectatommini) (Hymenoptera: Formicidae)

**Alexsandro Santana Vieira**

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Biologia Celular e Molecular).

**Rio Claro**
**Julho - 2012**

Alexsandro Santana Vieira

# A glândula metapleural e suas secreções em formigas cultivadoras (Attini) e não cultivadoras de fungos (Myrmicini, Blepharidattini e Ectatommini) (Hymenoptera: Formicidae)

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista Júlio de Mesquita Filho, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Biologia Celular e Molecular).

Orientadora: Profa. Dra. Maria Izabel Souza Camargo

Co-orientador: Prof. Dr. Odair Corrêa Bueno

Rio Claro
Julho - 2012

595.796 Vieira, Alexsandro Santana
V657g A glândula metapleural e suas secreções em formigas cultivadoras (Attini) e não cultivadoras de fungos (Myrmicini, Blepharidattini e Ectatommini) (Hymenoptera: Formicidae) / Alexsandro Santana Vieira. - Rio Claro : [s.n.], 2012
188 f. : il., figs., gráfs., tabs.

Tese (doutorado) - Universidade Estadual Paulista, Instituto de Biociências de Rio Claro
Orientador: Maria Izabel Souza Camargo
Co-Orientador: Odair Corrêa Bueno

1. Formiga. 2. Formigas cortadeiras. 3. Histoquímica. 4. Ultraestrutura. 5. Cromatografia em fase gasosa. 6. Compostos antibióticos. I. Título.

Ficha Catalográfica elaborada pela STATI - Biblioteca da UNESP
Campus de Rio Claro/SP

unesp **UNIVERSIDADE ESTADUAL PAULISTA**

**CAMPUS DE RIO CLARO**

**INSTITUTO DE BIOCIÊNCIAS DE RIO CLARO**

## CERTIFICADO DE APROVAÇÃO

**TÍTULO:** A glândula metapleural e suas secreções em formigas cultivadoras (Attini) e não cultivadoras de fungos (Myrmicini, Blepharidattini e Ectatommini) (Hymenoptera:Formicidae)

**AUTOR: ALEXSANDRO SANTANA VIEIRA**

**ORIENTADORA: Profa. Dra. MARIA IZABEL SOUZA CAMARGO**
**CO-ORIENTADOR: Prof. Dr. ODAIR CORREA BUENO**

Aprovado como parte das exigências para obtenção do Título de DOUTOR EM CIENCIAS BIOLÓGICAS (BIOLOGIA CELULAR E MOLECULAR) , pela Comissão Examinadora:

Profa. Dra. MARIA IZABEL SOUZA CAMARGO
Departamento de Biologia / Instituto de Biociências / UNESP - Rio Claro

Prof. Dr. JOSÉ EDUARDO SERRÃO
Universidade Federal de Viçosa

Prof. Dr. JOSÉ ROBERTO POSTALI PARRA
ESALQ / USP

Profa. Dra. RUSLEYD MARIA MAGALHÃES DE ABREU
Departamento de Ciências Da Natureza / Universidade Federal do Acre

Profa. Dra. GISLAINE CRISTINA ROMA
Departamento de Biologia / Instituto de Biociências / UNESP - Rio Claro (Jovem Pesquisadora)

Data da realização: 02 de julho de 2012.

*Aos meus pais Antonio Santana Vieira e Maria José Santana, que me deram a vida e me ensinaram a vivê-la com dignidade e dedicação para o trabalho. Aos meus irmãos Admilson Santana Vieira e André Santana Vieira pelo apoio moral e, à minha noiva Fernanda Carafa, que muitas vezes foi compreensiva aos meus atos.*

***Dedico esta tese.***

## *Agradecimentos*

*Agradecimento especial à FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), pelo apoio financeiro concedido, o qual possibilitou a realização deste trabalho (Processo nº 08/54301-3).*

*À minha orientadora, Bel (Maria Izabel Souza Camargo), a quem devo meu progresso científico, de quem vou guardar os seus ensinamentos pelo resto da vida. Agradeço de forma muito especial por me aceitar como orientado, desde o primeiro contato, e pela sua sabedoria, incentivo e exemplo de coragem e trabalho.*

*Ao meu co-orientador, Odair Corrêa Bueno, de quem vou guardar as suas discussões científicas, bem como sua sabedoria e incentivo de ousadia na pesquisa.*

*À todos os docentes do Departamento de Biologia da UNESP de Rio Claro por estarem sempre dispostos ao esclarecimento de dúvidas.*

*Aos técnicos de laboratório Antonio Teruyoshi Yabuki, Gerson Mello Souza, Mônica Iamonte pelos serviços técnicos prestados e amizade construída durante todos estes anos.*

*À todos os funcionários do Departamento de Biologia, Seção de Pós-Graduação e Biblioteca pela ajuda concedida.*

*À todos os meus amigos de pós-graduação, companheiros de todas as horas, pela grande amizade construída durante todos estes anos, Luís Flávio da Silva Costa, Pablo Henrique Nunes, Sandra Denardi, Gabriela Ortiz, Érika, Patrícia, Gislaine e Karim pela confiança, dedicação e atenção, pelo companheirismo, por dividirem comigo suas alegrias e tristezas.*

*E às pessoas fantásticas que passaram em meu caminho nestes anos, inclusive E. D. Morgan que foi meu supervisor do estágio realizado na Keele University (England-UK), auxiliou no estudo cromatográfico em fase gasosa da composição química da secreção das glândulas metapleurais. Meu muito obrigado!*

# SUMÁRIO

# *Resumo e Abstract*

## RESUMO

Vieira, Alexsandro Santana. **A glândula metapleural e suas secreções em formigas cultivadoras (Attini) e não cultivadoras de fungos (Myrmicini, Blepharidattini e Ectatommini) (Hymenoptera: Formicidae).** 2012. 188p. Tese (Doutorado em Ciências Biológicas – Biologia Celular e Molecular). Instituto de Biociências, Universidade Estadual Paulista "Júlio de Mesquita Filho", UNESP, Rio Claro, SP, 2012.

A glândula metapleural é um órgão exclusivo das formigas e sua principal função seria a de produzir secreções antibióticas. O objetivo foi de investigar e comparar as possíveis modificações na morfologia interna da glândula metapleural, assim como a composição química das suas secreções nas formigas cultivadoras (Attini) e não cultivadoras de fungos (Ectatommini, Blepharidattini e Myrmicini). Ficou demonstrado que a glândula metapleural nos grupos Ectatommini, Myrmicini, Blepharidatiini e Attini (basal e derivada), apresentam suas porções: secretora formada por várias células secretoras, e armazenadora (reservatório), conectadas entre si por meio de canalículos (intra e extracitoplasmáticos). As Attini derivadas e basais (cultivadoras de fungos) diferiram das não cultivadoras de fungos, por possuírem as células secretoras com forma oval. Ainda, as células secretoras da glândula metapleural das formigas cortadeiras apresentaram muitas mitocôndrias próximas as microvilosidades da porção intracitoplasmática do canalículo, ao contrário dos outros grupos, aqui estudado. A glândula metapleural das formigas não cultivadoras de fungos (Ectatommini, Mymicini e Blepharidattini) e a das atine basais possuem menor número de células secretoras do que aquelas das atines derivadas. Nas formigas cortadeiras, também, a glândula metapleural teve mais agrupamentos de células secretoras e placas perfuradas na câmara coletora. Morfometricamente o diâmetro do reservatório foi proporcional ao tamanho do corpo das operárias mínimas, médias e maiores de *Atta laevigata*, porém, o estudo ultraestrutural das glândulas metapleurais desta espécie indicou que as operárias mínimas seriam as responsáveis por produzirem maior variedade de secreções quando comparadas as médias e as maiores, dado este confirmado pela presença de retículo endoplasmático rugoso bem desenvolvido e da forte marcação lipídica e polissacarídica dos grânulos de secreção. A secreção produzida pelas glândulas metapleurais de todos os grupos de formigas, aqui estudados, é de natureza glicolipoprotéica. No entanto, as formigas cortadeiras apresentaram maiores teores de polissacarídeos e de lipídios ácidos do que as Mymicini, Blepharidattini e Attini (basal e derivado-*Trachymyrmex fuscus*), diferenças estas provavelmente advindas da atividade de cultivar fungos. O emprego da técnica variante de CEC mostrou que as células da porção secretora de *Acromyrmex coronatus* têm sincronia na atividade de produção de secreção, visto que todas apresentaram o mesmo padrão de marcação para os testes aplicados. No entanto, verificou-se que as operárias mínimas tem a secreção com caráter polissacarídico quando comparada aquela das operárias maiores. A porção intracitoplasmática dos canalículos, responsável por modificar a secreção, nos grupos Ectatommini e Myrmicini contornou o núcleo das células secretoras, e nos grupos Blepharidattini e Attini (basal e derivada) este apresentou-se sinuoso ao longo do citoplasma. O reservatório e a câmara coletora das glândulas metapleurais apresentaram-se em todos os grupos, aqui estudados, revestidos por epitélio simples pavimentoso e cutícula. As formigas cortadeiras, bem como *T. fuscus* apresentaram maiores quantidades de compostos voláteis quando comparadas com as outras atines e com as não cultivadoras de fungos, indicando maior capacidade das suas glândulas metapleurais em produzir compostos com provável papel antibiótico e antifúngico.

**Palavras-chave**: formigas cortadeiras, histoquímica, ultraestrutura, cromatografia em fase gasosa, compostos antibióticos.

## ABSTRACT

Vieira, Alexsandro Santana. **The metapleural gland and their secretion in fungus-growing (Attini) and non-fungus-growing ants (Myrmicini, Blepharidattini e Ectatommini) (Hymenoptera: Formicidae).** 2012. 188p. Thesis (Ph. D. in Biological Sciences – Cellular and Molecular Biology). Biosciences Institute, Universidade Estadual Paulista "Júlio de Mesquita Filho", UNESP, Rio Claro, SP, 2012.

The metapleural gland is an organ exclusive to ants. Its main role is to produce antimicrobial secretions. The aimed was investigating and comparing the possible changes in the internal morphology of metapleural gland, as well as the chemical composition of secretions in fungus-growing (Attini) and non-fungus-growing ants (Ectatommini, Blepharidattini and Myrmicini). The metapleural gland of the groups Ectatommini, Myrmicini, Blepharidatiini and Attini (basal and derived), exhibited secretory portion formed by many secretory cells, and the storage (reservoir) units connected by canaliculi (intra and extracytoplasmic). Derived and basal attines (fungus-growing ants) differed from non-fungus-growing ants by the presence of oval secretory cells. Also, the secretory cells of the metapleural gland of leaf-cutting ants exhibited many mitochondria near microvilli of the intracytoplasmic portion of the canaliculus, unlike other groups examined in this study. The metapleural gland of non-fungus-growing ants (Ectatommini, Mymicini, and Blepharidattini) and basal attines contained fewer secretory cells than derived attines. In leaf-cutting ants, the metapleural gland exhibited more clusters of secretory cells and sieve plates in the collecting chamber. The diameter of the reservoir was morphometrically proportional to the body size of minor, medium, and major workers of *Atta laevigata*. However, the ultrastructural analysis of the metapleural gland of this species revealed that minor workers may be responsible for producing a greater variety of secretions compared to medium and major workers, due to the presence of a well-developed rough endoplasmic reticulum and strongly stained secretion granules for lipids and polysaccharides. The secretion produced by the metapleural glands of all ant groups examined in this study consisted of glycoproteins. In attines (leaf-cutting ants), the levels of polysaccharides and acidic lipids were higher than in Mymicini, Blepharidattini, and Attini (basal and derived-*Trachymyrmex fuscus*). These differences might be due to the fungus-growing behavior. The variant technique of CEC revealed that the secretory cells of *Acromyrmex coronatus* were synchronized with secretory activity, as all cells exhibited the same staining patterns for the techniques used. The metapleural gland secretion of minor workers contained more polysaccharides than that of larger workers. The intracytoplasmic portion of canaliculi responsible for modifying the secretion in the groups Ectatommini and Myrmicini surrounded the nucleus of secretory cells, while in the Blepharidattini and Attini (basal and derived) groups, it meandered the cytoplasm. The reservoir and the collecting chamber of the metapleural glands of all groups examined were lined by a simple squamous epithelium and cuticle. Leaf-cutting ants as well as *T. fuscus* exhibited higher quantities of volatile compounds compared to other attines and non-fungus-growing ants, suggesting a higher capacity of metapleural glands to produce compounds with possible antibiotic and antifungal properties.

**Keywords**: leaf-cutting ants, histochemistry, ultrastructure, gas chromatography, antibiotic compounds.

# *Introdução*

## I. INTRODUÇÃO

As formigas são os insetos com o maior número de indivíduos do planeta, ocupando quase todos os ambientes terrestres, com exceção dos pólos (TERRA, 1991). A adaptação contra diversos tipos de patógenos é considerada há muito tempo um dos principais eventos na evolução da sociabilidade e na diversificação das formigas (WILSON, 1971). Muitos insetos sociais, principalmente as formigas, constroem seus ninhos e se alimentam no solo e em madeiras em decomposição, nos quais uma diversa e abundante comunidade microbiana patogênica prospera devido à umidade e temperatura constantes e, ausência de luz (WILSON, 1971; HÖLLDOBLER; WILSON, 1990; THORNE; TRANIELLO, 2003). A adaptação das formigas a conviver com diversos patógenos se dá devido à secreção de compostos secretados por diversas glândulas que teriam propriedades antibióticas (MASCHWITZ; KOOB; SCHILDKNECHT, 1970; MASCHWITZ, 1974; BEATTIE et al., 1985, 1986; DO NASCIMENTO et al., 1996). As formigas possuem muitos tipos de glândulas exócrinas que podem ter natureza morfológica, estrutural, química e complexidade funcionais diferentes (CAETANO; JAFFÉ; ZARA, 2002). Há evidências de que a glândula metapleural seja a responsável pela produção de secreções que inibam o desenvolvimento de patógenos (HÖLLDOBLER; WILSON, 1990). Elas são estruturas pares e localizam-se nas extremidades posterolaterais do metatórax. Cada glândula consiste de um grupo de células que secretam seu produto dentro de uma câmara coletora e daí seguindo diretamente para o reservatório (HÖLLDOBLER; WILSON, 1990).

A literatura registra a proposta de quatro funções para glândula metapleural (YEK; MUELLER, 2011): 1) reconhecimento de espécie e colônia, 2) marcação de território e ninho, 3) defesa química, e 4) anti-séptico. Em estudos anteriores autores postularam que essa

glândula seria o órgão responsável pela produção de feromônios de reconhecimento e de identificação de formigas companheiras de um mesmo ninho, bem como de indivíduos de outros ninhos (BROWN, 1968). A secreção da glândula metapleural provavelmente também seja usada para marcar a entrada de ninho ou o território em algumas espécies de formigas, incluindo *Tetramorium caespitum*, *T. impurum* (CAMMAERTS; CAMMAERTS, 2001), *Pheidole pallidula* (CAMMAERTS; CAMMAERTS, 1998), *Solenopsis geminata* (JAFFE; PUCHE, 1984) e *Pseudomyrmex triplarinus* (JAFFÉ; LOPEZ; ARAGORT, 1986). Jaffé e Puche (1984) mostraram que a glândula metapleural poderia contribuir na marcação de território (YEK; MUELLER, 2011). A função da secreção da glândula metapleural na defesa química foi confirmada em *Crematogaster* subgênero *Physocrema*, que possui esta glândula hipertrofiada (JANET, 1898; DONISTHORPE, 1941; HOSOISHI; OGATA, 2008), e sua secreção contém uma mistura de compostos fenólicos o nocivos a predadores (ATTYGALE et al., 1989; JONES et al., 2005). Quando operárias de *C.* (*Physocrema*) *inflata* são atacadas, elas expelem pela abertura da glândula metapleural uma gotícula de secreção esbranquiçada, viscosa e pegajosa a qual pode retornar novamente para o interior da glândula (MASCHWITZ, 1974; ITO et al., 2004).

De acordo com Wheeler (1910, APUD HÖLLDOBLER; WILSON, 1990) o comportamento de limpeza das formigas seria a principal forma de defesa contra fungos, uma vez que operárias realizariam, com as peças bucais e pernas anteriores, tanto a auto-limpeza quanto a limpeza de suas companheiras espalhando sobre o corpo substâncias antibióticas (WILSON, 1971). Maschwitz, Koob e Schildknecht (1970) e Maschwitz (1974) atribuíram função antibiótica ou antisséptica para as secreções da glândula metapleural de formigas. Alguns antibióticos produzidos por esta glândula foram descritos em *Atta sexdens*, como por exemplo, os ácidos fenilacético (MASCHWITZ; KOOB; SCHILDKNECHT, 1970), β-hidroxidecanoico (mirmecacina) e indoleacético (SCHILDKNECHT; KOOB, 1971). Esses autores sugeriram que esses componentes agiriam diferentemente na cultura de fungos simbiontes onde: o ácido fenilacético suprimiria o crescimento de bactérias, o ácido β-hidroxidecanoico inibiria a germinação de esporos de fungos parasitas e, o ácido indoleacético, um hormônio vegetal, estimularia o crescimento dos micélios. Em adição, as secreções da glândula metapleural de *A. sexdens* (MASCHWITZ; KOOB; SCHILDKNECHT, 1970) e de *A. octospinosus* (ORTIUS-LECHNER et al., 2000) seriam utilizadas para reduzir o pH do material vegetal afetando, assim, as cepas patogênicas.

Além desses compostos Bot et al. (2001) estudando *A. octospinosus*, encontraram na secreção proteínas e peptídeos, enquanto que Orthius-Lechener et al. (2000) identificaram 20 novos compostos. Attygalle et al. (1989) identificaram vários fenóis em *Crematogaster deformis* e Gusmão (2000) vários hidrocarbonetos insaturados em *Atta capiguara* e *A. sexdens rubropilosa*. Outras duas investigações a respeito da função das secreções da glândula metapleural foram realizadas por Poulsen et al. (2002) e Fernandez-Marin et al. (2006) que verificaram em operárias de *A. octospinosus* (FERNANDEZ-MARIN et al., 2006) que tiveram as glândulas metapleurais artificialmente fechadas ficaram significativamente mais suscetíveis ao ataque de parasitas do que aquelas do grupo controle. Os últimos autores observaram que formigas expostas a esporos de fungos limpariam suas glândulas metapleurais com mais frequência do que as formigas não expostas, confirmando, o papel relevante das secreções produzidas por essa glândula na higienização destes insetos.

Vários trabalhos têm demonstrado que as glândulas metapleurais das diferentes espécies de formigas seriam responsáveis pela produção de alguns componentes orgânicos eficientes contra o crescimento de bactérias (MASCHWITZ; KOOB; SCHILDKNECHT, 1970; IIZUKA; IWADARE; ORITO, 1979; VEAL; TRIMBLE; BEATTIE, 1992), de fungos e de esporos de fungos (MASCHWITZ; KOOB; SCHILDKNECHT, 1970; SCHILDKNECHT; KOOB, 1971; BEATTIE et al., 1985, 1986). Outros trabalhos, ainda, investigaram as propriedades das secreções por elas produzidas (DO NASCIMENTO et al., 1996; ORTIUS-LECHNER et al., 2000; POULSEN et al., 2002; THORNE; TRANIELLO, 2003; FERNANDEZ-MARIN et al., 2006), no entanto, poucos trabalhos trataram sobre a morfologia desta importante glândula (GUSMÃO, 2000; GUSMÃO; CAETANO; NAKANO, 2001; BOT et al., 2001, 2002; DE SOUZA et al., 2006).

A subfamília Myrmicinae constitui um grupo dominante entre as formigas com diversificados hábitos alimentares (FOWLER et al., 1991) e, com grande diversidade de espécies em regiões Neotropicais (LOPES; SANTOS, 1996). De acordo com Bolton (1994, 2003) essa subfamília é a maior, mais diversa e a mais próspera dentro dos Formicidae, estando dividida em várias tribos, algumas consideradas monofiléticas (grupos das tribos Dacetini, Cephalotini e Attini). Um estudo sobre a filogenia de formigas e baseado na morfologia e sequência de DNA (ASTRUC et al., 2004) mostrou a existência de três complexos: o Poneroide (Ponerinae, Cerapachyinae, Leptanillinae e as formigas legionárias), o Myrmecoide (Myrmicinae, Myrmeciinae, Pseudomyrmecinae, Nothomyrmeciinae), e o Formicoide (Dolichoderinae, Formicinae). Os mesmos autores relataram que a subfamília

Myrmicinae seria composta por três grupos: o primeiro formado pelas tribos Tetramoriini e Crematogastrini, o segundo por Myrmicini e Phedolini e o terceiro pela tribo Attini. Os autores descreveram, ainda, que mais progressos virão com um melhor conhecimento das estruturas internas das espécies de Ponerinae e Myrmicinae, uma vez que Bolton (2003) sugeriu uma possível sinapomorfia estrutural da glândula metapleural entre Ectatomminae e Myrmicinae.

De acordo com Mueller et al. (2001) sete hipóteses seriam propostas para a origem do cultivo de fungos pelas Attini, diferindo no critério substrato usado pelo ancestral das formigas atines. A mais antiga foi postulada por Von Ihering (1894, 1898 APUD MUELLER et al., 2001) que sugeriu que as atines evoluíram de formigas coletoras e armazenadoras de sementes, e o fungo simbionte teria surgido como um contaminante. Formigas Myrmicinae coletoras de sementes são especialmente abundantes em habitats secos, como por exemplo, a formiga *Pogonomyrmex*. A segunda hipótese (EMERY, 1899; SANTSCHI, 1910; FARQUHARSON, 1914 APUD MUELLER et al., 2001) postulou que o cultivo de fungos seria derivado do crescimento de fungos não desejados sobre as paredes dos ninhos, o que poderia ser exemplificado pelo fato de muitas das atines basais vivem no estreito substrato de folhas mortas no chão (serapilheira) (WEBER, 1972), além de outras como *Blepharidatta* (DINIZ; BRANDÃO; YAMAMOTO, 1998) e *Wasmania* (LONGINO; FERNANDEZ, 2007). A terceira hipótese, postulada por Forel em 1902 relatou que a origem deste comportamento seria a partir de ancestrais que viveram em madeira em decomposição, sugerindo, assim, que Dacetini e Attini seriam grupos-irmãos, visto que *Strumigenys* também possuiria este hábito aproximando-os comportamentalmente das atines. De acordo com Mueller et al. (2001) a hipótese de Forel se aplicaria ao ancestral de *Apterostigma* e não ao ancestral comum das atines. A quarta hipótese considerou que os fungos das atines teriam surgido a partir dos fungos que viviam em simbiose micorrizal, (raízes de plantas) (GARLING, 1979). A quinta hipótese foi postulada por Von Ihering (1894 APUD MUELLER et al., 2001), que sugeriu que o cultivo de fungos pelas atines teria derivado a partir de fungos que cresceriam sobre restos de artrópodes e de pilhas de refugo. Diniz, Brandão e Yamamoto (1998) observaram o crescimento de fungo sobre pilhas de refugo de *Blepharidata conops*, e os resultados demonstraram que os fungos lepiotaceos não se desenvolvem em exoesqueletos de artrópodes (WEBER, 1972; MUELLER et al., 2001). A sexta hipótese foi proposta por Wheeler (1907) e postulou que o cultivo de fungos teria surgido a partir da manipulação de fezes de insetos. *Apterostigma*, por exemplo, incorpora fezes de insetos como adubo em seus jardins de fungos.

A sétima e última postulou a ocorrência de um sistema de mirmecoforia de fungos especializados que utilizariam as formigas para sua dispersão a qual dar-se-ia por meio do transporte de "pellets infrabucais" (BAILEY, 1920) e, ainda, essas mesmas formigas dispersavam esses fungos para seus descendentes nidais, antecedendo o cultivo verdadeiro de fungo. No entanto, embora muitas hipóteses tenham sido postuladas a real origem e evolução das atines permanecem, ainda, não esclarecidas. Emery em 1895 mostrou que segundo a sua morfologia as Attini seriam filogeneticamente próximas de *Blepharidatta* (= *Ochetomyrmex*) e *Wasmannia* (HÖLLDOBLER; WILSON, 1990), evidências que não foram ainda confirmadas.

As formigas cultivadoras de fungos (Attini) são modelos particularmente interessantes em estudos comparativos por se tratar de uma tribo monofilética (NORTH; JACKSON; HOWSE, 1997). No entanto, existem transições evolutivas, sendo que uma delas está relacionada ao cultivo do fungo e a outra relacionada à mudança gradual do tamanho das colônias. Oito dos 12 gêneros (atines basais) possuem pequenas colônias (<100 indivíduos) que utilizam detritos, incluindo insetos mortos e excrementos, como substrato para alimentar seu fungo mutualístico (WEBER, 1972; MUELLER, 2002). Os outros quatro gêneros (atines derivadas) (milhares a milhões de indivíduos) exibem um mutualismo mais avançado no qual o cultivo de fungo produz corpos de alimentação especiais (gongilídeos), ricos em proteína (WEBER, 1972; MUELLER, 2002).

Um dos gêneros basais *Apterostigma* possui operárias monomórficas e cultivam seu fungo sobre insetos mortos ou sobre fezes e madeira (HÖLLDOBLER; WILSON, 1990). O gênero plesiomórfico (exemplo: característica considerada primitiva que foi modificada a outra mais recente dentro de uma linhagem) das atines derivadas, *Trachymyrmex*, e que possui colônias com operárias monomórficas, usam somente vegetação para alimentar seus fungos (WEBER, 1972; MUELLER, 2002). *Acromyrmex* e *Atta* consideradas apomórficas (exemplo: característica mais recente derivada de uma característica primitiva de uma espécie ancestral) dentro das atines derivadas são distintamente diferentes por possuirem operárias polimórficas, usando exclusivamente vegetação fresca como substrato para seus fungos e tendo colônias com dezenas de milhares (*Acromyrmex*) ou milhões (*Atta*) de operárias (WEBER, 1972; MULLER, 2002).

São poucos os estudos sobre a morfologia interna da glândula metapleural e nenhum deles aborda o assunto comparando grupos taxonômicos relacionados. Esses estudos têm mostrado um alto grau de similaridade entre as estruturas internas da glândula metapleural de

espécies próximas, por exemplo, *Atta* e *Acromyrmex* (BOT et al., 2001), *A. bisphaerica*, *A. capiguara* e *A. sexdens rubropilosa* (GUSMÃO; CAETANO; NAKANO, 2001), e duas subespécies de *A. subterraneus* (DE SOUZA et al., 2006). Além disso, a glândula metapleural também apresenta diferenças em espécies de formigas de outros grupos taxonômicos como em *Myrmecia gulosa*, *M. urens* (ANGUS; JONES; BEATTIE, 1993), em *Diacamma rugosum* e *D. vagans* (SCHOETERS; BILLEN, 1992) as células secretoras próximas do centro da glândula metapleural teriam a forma arredondada, enquanto que em *A. bisphaerica*, *A. capiguara* e *A. sexdens rubropilosa* (GUSMÃO; CAETANO; NAKANO, 2001) apresentaram forma ovalada.

Atualmente uma das discussões acerca da glândula metapleural envolve o trajeto da secreção da câmara coletora até o reservatório. Alguns autores sugeriram que este transporte seria inteiramente passivo (MARKL, 1966), mas Bot et al. (2001) demonstraram em operárias de *A. octospinosus* que a contração de fortes músculos poderia também comprimir a câmara coletora da glândula metapleural e, assim, melhoraria o fluxo da secreção para o reservatório. Os mesmos autores juntamente com Schoeters e Billen (1993) descreveram que o transporte da secreção do reservatório para a superfície do corpo ocorreria por capilaridade, uma vez que o reservatório apresentaria esclerotização dificultando as contrações dos músculos torácicos.

Outra característica morfológica da glândula metapleural seria sobre o tamanho do reservatório nos diferentes grupos taxonômicos. De acordo com De Souza et al. (2006) o tamanho da glândula metapleural seria diferente nas operárias mínimas, médias e maiores. Em operárias mínimas de *Atta* e *Acromyrmex* ela seria maior quando comparada a das operárias maiores, o que implicaria na maior produção de secreções antibióticas e antifúngicas, sugerindo ainda, que as operárias menores estariam mais envolvidas com a tarefa de cultivo do fungo. Hughes et al. (2008) utilizaram somente a bulla, visível externamente, como medição indireta para relacionar o tamanho da glândula metapelural com o cultivo de fungos na base das formigas cultivadoras de fungos. Além disso, o tamanho relativo do reservatório em relação ao tamanho do corpo da formiga estaria correlacionado com a resistência à parasitas entre castas de operárias de mesma espécie de formigas (BOT; BOOMSMA, 1996; HUGHES; EILENBERG; BOOMSMA, 2002; DE SOUZA et al., 2006; POULSEN; HUGHES; BOOMSMA, 2006). Sumner, Hughes e Boomsma (2003) demonstraram que formigas parasíticas sociais possuiriam as glândulas metapleurais maiores e seriam indivíduos mais susceptíveis a doenças.

# *Objetivos*

## II. OBJETIVOS

O objetivo geral deste trabalho foi o de investigar e comparar sob o ponto de vista evolutivo as possíveis modificações na morfologia interna da glândula metapleural, assim como a composição química das suas secreções nas formigas cultivadoras (Attini) e não cultivadoras de fungos (Ectatommini, Blepharidattini e Myrmicini).

Os objetivos específicos foram:

a) Estudar histologicamente e ultramorfologicamente as células, os canalículos, a câmara coletora e o reservatório das glândulas metapleurais das espécies *Ectatomma brunneum; Pogonomyrmex naegeli*; *Wasmannia auropunctata*; *Apterostigma pilosum*; *Mycetarotes parallelus*; *Trachymyrmex fuscus*; *Acromyrmex coronatus* e *Atta laevigata* comparando-as entre si.

b) Comparar os teores de proteínas, polissacarídeos e lipídios no citoplasma das células da glândula metapleural nos diferentes grupos taxonômicos supra citados.

c) Identificar quais os tipos de substâncias produzidas pela glândula metapleural dos indivíduos das diferentes espécies e analisá-los química e comparativamente.

# *Material e Métodos*

## III. MATERIAL E MÉTODOS

Para a realização deste estudo foram utilizados 410 indivíduos (300 para estudos morfofisiológicos e ultraestruturais; 110 para o estudo da composição química da secreção), incluindo operárias mínimas, médias e maiores de *A. laevigata* e *A. coronatus*, e respectivo tamanho da cabeça (mínimas: 0,773 ± 0,016 mm; médias: 2,048 ± 0,172 mm; maiores: 3,282 ± 0,335 mm) e (mínimas: 0,934 ± 0,004 mm; médias: 1,360 ± 0,127 mm; maiores: 1,716 ± 0,092 mm). Foram utilizados 48 indivíduos de cada espécie que tiveram também o parâmetro "medida da cabeça" tomado: em *E. brunneum* esta medida foi de 2,075 ± 0,132 mm; *P. naegeli* de 1,035 ± 0,13mm, *W. auropunctata* de 0,378 ± 0,038mm, *A. pilosum* de 0,716 ± 0,054mm, *M. parallelus* de 0,710 ± 0,017mm, *T. fuscus* de 1,149 ± 0,031mm.

A espécie *A. laevigata* foi obtida de colônias com aproximadamente dois anos de idade, oriundas da fazenda Corumbataí (22º17'S, 47º40'W), Rio Claro-SP, Brasil. As espécies *E. brunneum*, *P. naegeli*, *W. auropunctata*, *A. pilosum*, *M. parallelus*, *T. fuscus* e *A. coronatus* foram coletadas no campus da UNESP de Rio Claro-SP e, todas as colônias foram mantidas em condições naturais no Centro de Estudos de Insetos Sociais – CEIS/UNESP, campus de Rio Claro – SP, Brasil.

As formigas foram colocadas em placa de Petri, contendo solução fisiológica, onde tiveram suas pernas e cabeças removidas com auxílio de pinças de ponta fina e de microtesouras cirúrgicas sob estereomicroscópio ZEISS, restando depois disso o mesossoma, contendo as glândulas metapleurais. Na sequência as glândulas foram retiradas e fixadas em

substâncias específicas para o emprego das técnicas histológicas e histoquímicas a ser utilizada. O material contendo as glândulas foi desidratado em soluções crescentes de etanol a 70, 80, 90 e 95%, durante 15 minutos cada banho. Logo após, foi transferido para resina durante 24 horas e, finalmente, foi transferido para moldes plásticos, previamente preenchidos com resina mais catalisador, os quais foram selados com suportes de madeira. Depois de polimerizados, os blocos foram seccionados com 4 µm em micrótomo Leica RM 2145, hidratados e recolhidos em lâminas de vidro. Após a aplicação de cada técnica específica e depois da montagem das lâminas foi realizada a observação e documentação fotográfica em fotomicroscópio Leica acoplado a computador Intel Pentium 4.

### III.1. Análise Morfológica

#### III.1.1. Microscopia Eletrônica de Varredura (MEV)

Para a técnica de MEV seis glândulas metapleurais de *A. laevigata* foram fixadas em solução Karnovsky por 24 horas e desidratadas em série de acetona 50 a 95%, além de serem lavadas em dois banhos de acetona P.A., durante cinco minutos (cada). O material foi levado ao ponto crítico (Critical Point Drying), para completar a sua desidratação, e em seguida procedeu-se a metalização com carbon-paladium. O exame e a documentação fotográfica deram-se em microscópio eletrônico de varredura PHILIPS SEM 505.

#### III.1.2. Técnica da Hematoxilina de Harris – Eosina Aquosa (JUNQUEIRA; JUNQUEIRA, 1983)

Seis glândulas metapleurais, de cada espécie, foram fixadas em solução contendo paraformaldeído 4% durante 24 horas. As secções histológicas foram hidratadas por 1 minuto em água destilada e coradas com hematoxilina por 10 minutos e, em seguida foram mantidas em cubeta com água por 4 minutos e depois colocadas em água corrente para que a reação ocorresse. Logo, a seguir foram coradas com eosina por 5 minutos e lavadas em água corrente. Depois de secas as lâminas contendo as secções foram cobertas com bálsamo do Canadá e lamínula.

#### III.1.3. Morfometria

Anteriormente ao processo de dissecção da glândula metapleural das operárias mínimas, médias e grandes foram realizadas análises morfométricas da cabeça, pronoto e reservatório, de 17 operárias de *A. laevigata*, utilizando estereomicroscópio Motic BA 300. Os dados obtidos foram plotados no programa SPSS. Após a realização de teste de normalidade foram realizados testes de correlação de Person, nível de significância de 0,05,

entre comprimento da cabeça, comprimento do pronoto e diâmetro do reservatório, a fim de se verificar o tamanho do reservatório seria proporcional ao tamanho do corpo das operárias.

**III.2. Análise Histoquímica**

**III.2.1. Técnica do Azul de Bromofenol para detecção de proteínas totais, (PEARSE, 1985)**

Seis glândulas metapleurais, de cada espécie, foram fixadas em paraformaldeído 4% e NaCl 0,9% em tampão fosfato 10% (0,1M – pH 7,4) por 24 horas. Depois de seccionadas com 4 µm, os cortes foram corados com solução de azul de bromofenol durante 1 hora à temperatura ambiente. Em seguida as lâminas foram banhadas em solução aquosa de ácido acético 0,5%, durante 5 minutos e passadas no álcool butílico terciário por 5 minutos. Depois as lâminas foram diafanizadas em xilol e montadas em Bálsamo do Canadá.

**III.2.2. Técnica do PAS/Alcian Blue para detecção de polissacarídeos (glicoproteínas) com grupamentos 1-2 glicol e polissacarídeos ácidos, segundo Junqueira e Junqueira (1983)**

Seis glândulas metapleurais, de cada espécie, foram fixadas em Bouin aquoso por 24 horas. As secções com 4 µm foram coradas com alcian blue, pH 2,5 durante 30 minutos, e em seguida, lavadas em água destilada e passadas em ácido periódico 1%, durante 5 minutos, e lavadas em água destilada. Posteriormente, as secções foram submetidas ao reativo de Schiff por 30 minutos, lavadas em água sulfurosa durante 1 minuto e lavadas novamente em água corrente durante 10 minutos. Posteriormente, foram secas, diafanizadas em xilol e montadas em Bálsamo do Canadá.

**III.2.3. Reação pelo PAS (Periodic Acid Schiff) (McMANUS, 1946), para detecção de polissacarídeos, com contracoloração pelo Verde Metila para marcação de RNA.**

Seis glândulas metapleurais, de cada espécie, foram fixadas em Bouin aquoso por 24 horas. As secções histológicas foram hidratadas por 1 minuto em água destilada e, em seguida transferidas para solução de ácido periódico por 10 minutos. Novamente foram lavadas em água destilada por 1 minuto e na sequência colocadas, por 1 hora, no reagente de Schiff. A seguir foram lavadas, por 30 minutos, em água corrente e contracoradas por 20 segundos com verde de metila e lavadas novamente em água. Depois de secas as lâminas contendo as secções foram montadas em Bálsamo do Canadá e no prazo máximo de 48 horas foi realizada a documentação fotográfica.

**III.2.4. Técnica do azul de Nilo para detecção de lipídios ácidos, segundo Lison (1960)**

Seis glândulas metapleurais, de cada espécie, foram fixadas em formol cálcio por 24 horas. As lâminas contendo as secções com 4 µm de espessura foram coradas com azul de Nilo durante 5 minutos à 37º C. Em seguida foram lavadas em água corrente e passadas em solução de ácido acético 1%, durante 1 minuto. Depois de secas, as lâminas foram montadas com gelatina glicerinada e recobertas com lamínula.

**III.2.5. Técnica de Baker para detecção de lipídios totais, segundo Martoja e Martoja-Pierson, 1967.**

Seis glândulas metapleurais, de cada espécie, foram fixadas em formol cálcio por 24 horas. As lâminas contendo as secções com 4 µm foram coradas com bicromato-cálcio (5g de bicromato de potássio, 1g de cloreto de cálcio anidro, 100 mL de água destilada) durante 18 horas à temperatura ambiente. Posteriormente foram lavadas três vezes em água destilada e, em seguida reagidas pela hemateína-ácida (0,05g de hematoxilina cristalizada em 48 mL de água destilada, 1mL de solução aquosa de iodato de sódio a 1%, 1mL de ácido acético) durante 5 horas à temperatura ambiente. Em seguida procedeu-se a lavagem em água destilada (três vezes). Depois de secas as lâminas foram montadas com gelatina glicerinada e recobertas com lamínula.

**III.2.6. Variante de CEC (Critical Electrolyte Concentration) para detecção de DNA e RNA, segundo Mello et al. (1993) e Mello (1997)**

Seis glândulas metapleurais de *Acromyrmex coronatus* foram fixadas com Bouin aquoso por 24 horas. As secções histológicas foram coradas com azul de Toluidina (0,025%) em tampão McIlvane (pH 4,0) por 40 minutos à temperatura ambiente. O material foi transferido para solução aquosa de $MgCl_2$ (0,05M) por 4 minutos, e lavadas com água destilada. Depois de secas as lâminas contendo as secções foram montadas em Bálsamo do Canadá e recobertas com lamínulas.

**III.3. Análise Ultraestrutural**

**III.3.1. Microscopia Eletrônica de Transmissão (TEM)**

Seis glândulas metapleurais de cada espécie depois de retiradas foram fixadas em glutaraldeído a 2,5% em tampão cacodilato de sódio 0,1M (pH 7,2), durante 24 horas. Após a fixação o material foi lavado duas vezes em tampão cacodilato 0,1M por 15 minutos. Em seguida foi pós-fixado em tetróxido de ósmio a 1% em tampão cacodilato (0,1M, pH: 7,2) por

duas horas. O material então foi lavado por duas vezes em tampão cacodilato 0,1M por 15 minutos e passado em solução de álcool 10 % por 15 minutos.

A contrastação foi realizada com acetato de uranila a 2% em etanol a 10% durante 12 horas. O material foi desidratado em série crescente de acetona (50%, 60%, 70%, 80%, 90%, 95%), 5 minutos em cada uma e finalmente em acetona 100%, duas vezes por 5 minutos cada. Depois de desidratado, o material foi incluído em resina Epon Araldite e colocado em estufa a 60ºC durante 24 horas. Depois de polimerizados, os blocos foram seccionados em ultramicrótomo Sorvall-Porter Blum MT2-B. As secções ultrafinas foram obtidas e coletadas em grades de cobre, contrastadas com acetato de uranila 2% e citrato de chumbo 0.4%, durante 45 e 10 minutos, respectivamente. O material foi analisado e fotografado ao microscópio eletrônico de transmissão PHILIPS CM 100.

### III.4. Análise Química da Secreção

Dez glândulas metapleurais foram extraídas de cada espécie. A parte posterolateral do tórax, chamada metathorax, foi cortada com lâmina esterilizada (ORTIUS-LECHNER et al. 2000), e então, os tecidos da glândula metapleural foram colocados em um tubo capilar de vidro, o qual foi imediatamente selado para posterior análises em cromatógrafo em fase gasosa (MORGAN, 1990).

#### III.4.1. Cromatografia em fase Gasosa – Espectrometria de Massas

As analises químicas foram conduzidas na *Keele University* em equipamento Agilent Technologies 6890N Network GC com uma coluna capilar SGE HT5 (30m x 0,25 mm ID, 0,25 µm de espessura do filme) acoplado a um detector seletivo de massas Agilent 5973 Network. O cromatógrafo em fase gasosa foi acoplado a um computador e os dados foram processados com o software Agilent Chemstation. A eluição foi conduzida com gás hélio 1 mL/min.

As amostras foram injetadas em modo *splitless* (temperatura de injeção 250ºC) por quebra dos tubos capilares após 1 minuto dentro da porta do injetor após inserção (MAILE et al. 1998). A temperatura foi programada para ser iniciada em 40ºC por 5 minutos e então elevou-se (rampa) para 300ºC a 10ºC/min. O espectrômetro de massas foi operado em Ionização de Elétrons a 70eV, escaneamento de 40 m/z à 500 m/z em 1.5 scans $s^{-1}$.

Os compostos foram identificados por comparação de seus espectros de massa com aqueles na biblioteca NIST-Wiley (NIST08). Quando possível sua identificação foi confirmada por injeção de padrões sintéticos (Sigma-Aldrich, UK) e comparação de seu tempo de retenção. A quantificação dos compostos deu-se por aquisição da curva de

calibração de seis diferentes concentrações para cada composto nas mesmas condições, visto que compostos aromáticos como skatole tem respostas em massa espectral muito diferente daqueles dos compostos alifáticos como 2-nonanone, ambos os tipos tendo sido encontrados na glândula. A composição percentual registrada pelo íon total 'current' foi convertida em nanogramas (ng) para uma medida mais exata da proporção de peso para a composição percentual. Dois-nonanone foi usado como padrão para os compostos alifáticos simples, skatole para os aromáticos e methyl oleate para os ácidos e ésteres de cadeia longa.

# *Resultados*

## IV. RESULTADOS

Os resultados aqui obtidos foram apresentados na forma de artigos científicos, trazendo cada um deles os aspectos morfofiosiológicos, ultraestruturais e a composição química da secreção da glândula metapleural de diferentes espécies de formigas, com a finalidade de atingir os objetivos propostos.

Dentre os artigos apresentados alguns já foram publicados, outros estão submetidos e encontram-se organizados da seguinte forma:

**CAPÍTULO 1**:

The functional morphology of the metapleural gland of the leaf-cutting ant *Atta laevigata* (Formicidae: Attini)

**Autores**: Alexsandro Santana Vieira, Odair Corrêa Bueno e Maria Izabel Camargo-Mathias

**Periódico**: Micron, v. 41, p.149–157, 2010.

**CAPÍTULO 2**:

Ultrastructural profile of metapleural gland cells of the ant *Atta laevigata* (F. Smith, 1858) (Formicidae: Attini)

**Autores**: Alexsandro Santana Vieira, Odair Corrêa Bueno e Maria Izabel Camargo-Mathias

**Periódico**: Animal Biology, v. 62, p. 1–11, 2012.

**CAPÍTULO 3**:

Secretory profile of metapleural gland cells of the leaf-cutting ant *Acromyrmex coronatus* (Formicidae: Attini)

**Autores**: Alexsandro Santana Vieira, Odair Corrêa Bueno e Maria Izabel Camargo-Mathias

**Periódico**: Microscopy Research and Technique, v. 74, p. 76–83, 2011.

**CAPÍTULO 4**:

Morphophysiological differences between the metapleural glands of fungus-growing and non-fungus-growing ants (Hymenoptera, Formicidae)

**Autores**: Alexsandro Santana Vieira, Odair Corrêa Bueno e Maria Izabel Camargo-Mathias

**Periódico**: Submetido ao periódico Plos one (2012)

**Situação**: corrigido e resubmetido

**CAPÍTULO 5**:

Comparative ultrastructure of the metapleural glands of fungus-growing (Attini) and non-fungus-growing ants (Blepharidattini and Ectatommini) (Hymenoptera: Formicidae)

**Autores**: Alexsandro Santana Vieira, Odair Corrêa Bueno e Maria Izabel Camargo-Mathias

**Periódico**: Submetido ao periódico Insectes Sociaux (2012)

**CAPÍTULO 6**:

Chemical composition of metapleural glands secretions of fungus-growing and non–fungus-growing ants (Hymenoptera, Formicidae)

**Autores**: Alexsandro Santana Vieira, E. David Morgan, Falko P. Drijfhout e Maria Izabel Camargo-Mathias

**Periódico**: Submetido ao periódico Journal of Chemical Ecology (2012)

# *Capítulo 1*

**A morfologia funcional da glândula metapleural da formiga cortadeira *Atta laevigata* (Formicidae: Attini)**

**Autores**: Alexsandro Santana Vieira, Odair Corrêa Bueno e Maria Izabel Camargo-Mathias
**Periódico**: Micron, v. 41, p.149–157, 2010.

## RESUMO

Dada a importância da glândula metapleural nas formigas o presente estudo descreveu-as nas operárias mínimas, médias e maiores de *Atta laevigata*, com ênfase na sua morfofisiologia utilizando técnicas de microscopia eletrônica de varredura (MEV), de histologia e de histoquímica. Os resultados revelaram que nesta espécie a glândula metapleural é um órgão par localizado na região do metatórax e que possui duas porções: a secretora e a armazenadora, ambas conectadas entre si por canalículos extracitoplasmáticos que partem de cada célula secretora e desembocam em grupos em orifícios na placa perfurada, localizada na parede da câmara coletora. As células secretoras são ovaladas e alongadas e formam grupamentos de aproximadamente 20 células, unidas por um frouxo tecido conectivo. Cada célula secretora se comunica com o reservatório através de um canalículo, que se divide em porção intra e a extracitoplasmática. A câmara coletora e o reservatório estão revestidos internamente por um epitélio simples pavimentoso que está recoberto por íntima cuticular, pregueada na região da câmara coletora e lisa no restante do reservatório. Externamente o reservatório está revestido por camadas musculares, as quais provavelmente auxiliem a glândula na expulsão da secreção para o exterior via abertura glandular localizada no exoesqueleto (uma de cada lado) próxima às coxas posteriores. As células secretoras reagiram com forte positividade aos testes para detecção de lipídios ácidos e totais, proteínas e polissacarídeos ácidos, indicando, assim a natureza glicolipoprotéica da secreção final que é liberada externamente. Morfometricamente o diâmetro do reservatório foi proporcional ao tamanho do corpo das operárias mínimas, médias e maiores, sinalizando na maior capacidade para produção de secreção, no caso substâncias antibióticas, nos indivíduos que desempenham tarefas específicas dentro da colônia.

**Palavras-chave**: *Atta laevigata*, Attini, glândula metapleural, células secretoras.

Micron 41 (2010) 149–157

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

# The functional morphology of the metapleural gland of the leaf-cutting ant *Atta laevigata* (Formicidae: Attini)

Alexsandro Santana Vieira [a], Odair Correa Bueno [a,b], Maria Izabel Camargo-Mathias [a,*]

[a] Departamento de Biologia, Instituto de Biociências, UNESP. Av. 24A, no. 1515, Cx. Postal 199, CEP: 13506-900, Rio Claro, S.P., Brazil
[b] Centro de Estudos de Insetos Sociais (CEIS), Instituto de Biociências, UNESP. Av. 24A, no. 1515, Cx. Postal 199, CEP: 13506-900, Rio Claro, S.P., Brazil

ARTICLE INFO

*Article history:*
Received 5 August 2009
Received in revised form 21 August 2009
Accepted 21 August 2009

*Keywords:*
*Atta laevigata*
Attini
Metapleural gland
Secretory cells

ABSTRACT

Given the importance of the metapleural gland in ants, the present study aimed at describing it in minor, media, and major workers of *Atta laevigata*, focusing their physiology using scanning electron microscopy (SEM), histologic, and histochemical techniques. The results revealed that this gland is paired and located in the metathoracic region, consisted of two portions: secretory and the storage ones. Both portions are connected by extracytoplasmic canaliculi that drain the secretion of each secretory cell they form groups that open into the perforated plate located in the wall of the collecting chamber. The oval-shaped and elongated secretory cells form clusters of approximately 20 cells, bundled together by connective tissue. Each secretory cell connects to the reservoir through a canaliculus divided into an intra- and extracytoplasmic portion. The collecting chamber and the reservoir are internally lined by a single squamous epithelium with a cuticular intima, with folds in the collecting chamber and smooth in the remaining of the reservoir. External muscle layers surrounding the reservoir are observed which aid the release of secretion by the gland opening (one on each side) located in the exoskeleton near coxae of hind legs. Secretory cells were strongly positive for acidic and total lipids, proteins, and acidic polysaccharides, suggesting the glycolipoproteins nature of the final secretion. Morphometrically, the diameter of the reservoir is proportional to body size of minor, media, and major workers. This implies a larger capacity to produce secretion, including antibiotic substances, by the individuals that perform specific tasks within the colony.



## 1. Introduction

Social insects, including ants, built their nests and obtain their food from the soil and decomposing wood, moist habitats in which a diverse and abundant pathogenic microbial community thrives (Wilson, 1971; Hölldobler and Wilson, 1990; Thorne and Traniello, 2003). The metapleural gland, also called metastermal gland, is a paired structure located at the postolateral end of the alitrunk and is only found in ants. It produces secretions that are released on the body surface (Poulsen et al., 2002) and inhibit infections, as well as the growth of different pathogens (Hölldobler and Wilson, 1990; Bot et al., 2002).

The roles of the metapleural gland secretions in leaf-cutting ants are more evident than in other ones, as they suppress pathogens and microorganisms that may compete in the fungus garden (Hölldobler and Wilson, 1990; Bot et al., 2001, 2002). One important characteristic that possesses a functional role in the metapleural gland is its size. In minor workers of *Atta* and *Acromyrmex*, this structure is larger than that of major workers (Bot et al., 2001, 2002). This has also been observed in two subspecies of *Acromyrmex subterraneus*, in which the gland reservoir of minor workers is larger than that of major ones, indicating in these castes a higher production of antibiotic and antifungal substances (De Souza et al., 2006), and also suggesting that minor workers might be more closely involved in fungus growing.

Among the substances that compose the secretion produced by the metapleural gland is: phenylacetic acid (Schildknecht and Koob, 1971), β-hydroxydecanoic (myrmicacin), indoleacetic acid, in addition to two other β-hydroxy acid (Maschwitz et al., 1970), in *Atta sexdens* (Do Nascimento et al., 1996) and *Acromyrmex octospinosus* (Bot et al., 2002). Attygalle et al. (1989) found several phenols in the metapleural gland of *Crematogaster deformis*, and Gusmão (2000), using gas chromatography and mass spectrometry found, in addition to phenylacetic acid and hydroxy acids, unsaturated hydrocarbons.

Recent studies on the metapleural gland focused on analyzing the chemical composition of its secretions, but studies on the morphology of the gland structure (Do Nascimento et al., 1996;

* Corresponding author. Tel.: +55 19 3526 4135.
*E-mail address:* micm@rc.unesp.br (M.I. Camargo-Mathias).

0968-4328/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micron.2009.08.012

Ortius-Lechner et al., 2000; Bot et al., 2001, 2002; Poulsen et al., 2002; Fernandez-Marin et al., 2006), and internal parts (Gusmão, 2000; Gusmão et al., 2001; De Souza et al., 2006) are scarce.

Thus, the present study aimed at examining the morpho-histology, physiology and histochemistry of gland components (cells, ducts, collecting chamber, reservoir, and secretion) of the metapleural gland of workers of three castes of *Atta laevigata* (F. Smith, 1858). Some studies have reported that the secretions produced by attines have antibiotic properties (Maschwitz et al., 1970; Maschwitz, 1974; Beattie et al., 1986; Do Nascimento et al., 1996), which could have the potential of suppressing the growth of fungi, bacteria, and other organisms that might interfere in the development and maintenance of the fungus garden of the colony. The leaf-cutting ant *A. laevigata*, known in Brazil as glass-head leaf-cutting ant, uses leaf fragments collected from dicotyledons, as well as some grasses (Paiva Castro et al., 1961), and occurs basically in all Brazilian territories, being a very important pest insect.

## 2. Materials and methods

### 2.1. Materials

For this study, were used 36 workers of *A. laevigata* of three classes with corresponding head sizes (11 minors: 0.773 ± 0.016 mm; 11—media: 2.048 ± 0.172 mm; 11—majors: 3.282 ± 0.335 mm) from colonies with approximately two years of age collected from the farm Corumbataí (22°17′45″S, 47°40′81″W), Rio Claro-SP, Brazil. After, the colony was kept under natural conditions at the Center for the Study of Social Insects – CEIS/UNESP, Rio Claro campus – SP, Brazil. The legs and heads of ants were removed in Petri dishes under a ZEISS stereomicroscope with the aid of dissecting forceps and microscissors, remaining only the mesossoma containing the metapleural glands. Each gland was immersed in specific fixative solutions according to each technique.

### 2.2. Morphological analysis

#### 2.2.1. Scanning electron microscopy (SEM)

For SEM, six metapleural glands were fixed in Karnovsky's solution for 24 h, dehydrated in a series of acetone solutions from 50 to 95%, and washed in two baths of acetone 100%, during 5 min each. The material was critical point dried, sputter-coated with carbon-palladium, and examined and photographed under scanning electron microscope PHILIPS SEM 505.

#### 2.2.2. Harris hematoxylin—aqueous eosin staining (according to Junqueira and Junqueira, 1983)

After being removed, six glands were fixed in 4% paraformaldehyde for 24 h and dehydrated in increasing series of ethanol 70, 80, 90, and 95%, during 15 min each. The material was then immersed in resin during 24 h and transferred to plastic molds previously filled with resin and catalyzer, which were sealed with metal supports. After polymerization, blocs were sectioned at 4 μm with a Leica RM 2145 microtom, and sections were hydrated and placed on glass slides. After drying, slides were subjected to stain with hematoxylin and eosin (HE), mounted in Canada balsam and slipcovered according histological routine processing, for later observation and photographic documentation under photomicroscope Motic BA 300 linked to an Intel Pentium 4 computer.

#### 2.2.3. Morphometry

Prior to the extraction of the metapleural gland of minor, media, and major workers, measurements of the head, pronotum, and reservoir of 17 workers were taken under stereomicroscope Motic BA 300. Using the program SPSS, measurements of head length, pronotum length, and diameter of reservoir were analyzed with a Pearson correlation test, significance level of 0.05, to examine whether the size of the reservoir is proportional to the body size of workers.

### 2.3. Histochemical analysis

#### 2.3.1. Bromophenol blue staining to detect total proteins, according to Pearse (1985)

Six metapleural glands were removed and fixed with 4% paraformaldehyde and 0.9% NaCl in 10% phosphate buffer (0.1 M—pH 7.4) for 24 h. The material was sectioned at 4 μm, stained with bromophenol blue for 1 h at room temperature. Slides were then immersed in aqueous solution of 0.5% acetic acid during 5 min and transferred to tertiary butyl alcohol for 5 min. Slides were cleared in xylol and mounted in Canada balsam for later examination and photographic documentation under photomicroscope Motic BA 300 linked to an Intel Pentium 4 computer.

#### 2.3.2. PAS/alcian blue staining to detect polysaccharides (glycoproteins) with groups 1–2 glycol and acidic polysaccharides, according to Junqueira and Junqueira (1983)

Six metapleural glands were removed and fixed in aqueous Bouin solution for 24 h. The 4-μm sections were stained with alcian blue, pH 2.5 for 30 min, rinsed in distilled water, immersed in 1% periodic acid for 5 min, and rinsed again in distilled water. Sections were subsequently subjected to the Schiff's reagent for 30 min, washed in sulfur water for 1 min, and washed with tap water for 10 min. The material was then counterstained with Harris hematoxylin for 2 min and washed with tap water. Slides were dried, cleared in xylol, and mounted in Canada balsam for later examination and photographic documentation under photomicroscope Motic BA 300 linked to an Intel Pentium 4 computer.

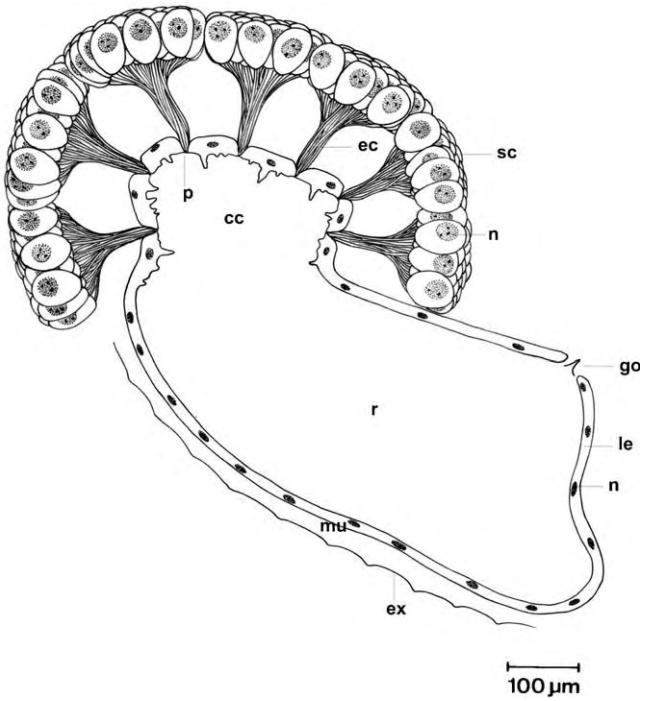



**Fig. 1.** Schematic representation of the metapleural gland of workers of *Atta laevigata*, where they are observed the secretory cells (sc) and their nuclei (n), the extracytoplasmic portions of the canaliculi (ec), the collecting chamber (cc) covered by the perforated plates (p), the reservoir (r) lined by single epithelium (le) with nuclei (n) of cells. External region evolved by fine muscular layer (mu). Note the presence of the glandular opening (go) and exoskeleton (ex).

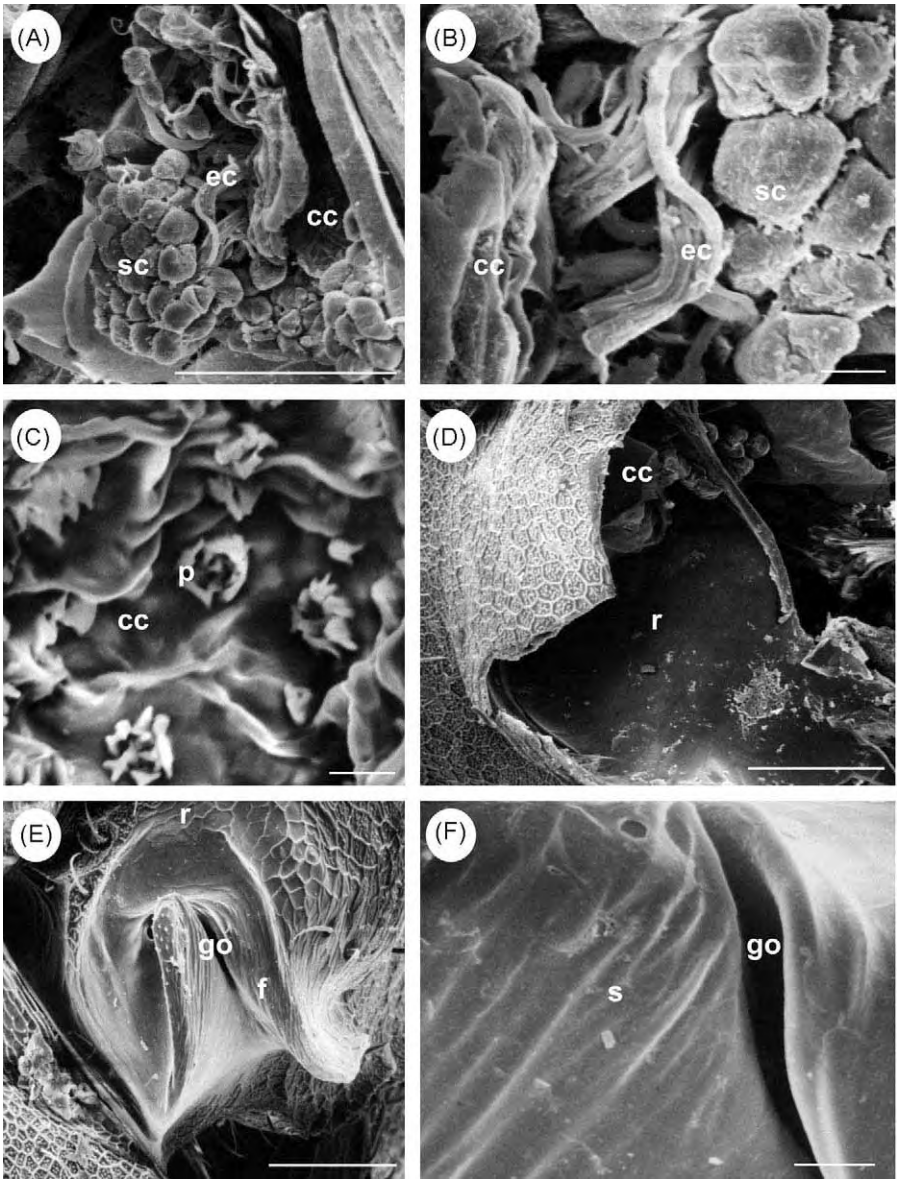



**Fig. 2.** Scanning electron microscopy (SEM) of the metapleural gland of *Atta laevigata*. (A) General view of secretory cells (sc), extracytoplasmic canaliculi (ec), and the collecting chamber (cc) in major workers. Note the cluster formed by secretory cells, which are not fused. Scale bar = 0.1 mm. (B) Details of secretory cells (sc), extracytoplasmic canaliculi (ec), collecting chamber (cc) in major workers. Scale bar = 10 μm. (C) Details of the collecting chamber (cc) and the perforated plates (p) in media workers. Note that the extracytoplasmic canaliculi arise individually from secretory cells and attach to the collecting chamber through the perforated plate. Scale bar = 10 μm. (D) General view of the reservoir (r) and the collecting chamber (cc) in major workers. Scale bar = 0.1 mm. (E) Details of the reservoir (r) showing the semicircular slit-shaped gland opening (go), and furrow (f) that surrounds the gland opening in media workers. Scale bar = 0.1 mm. (F) Details of the semicircular slit-shaped gland opening (go) and the surface (s) near the gland opening in major workers. Note that the surface is smooth and free of sculptures. Scale bar = 10 μm.

*2.3.3. Nile blue staining to detect acidic lipids, according to Lison (1960)*

Six metapleural glands were removed and fixed with calcium formol for 24 h. Sections with 4 μm were stained with Nile blue for 5 min at 37 °C, rinsed in tap water, and immersed in 1% acetic acid for 1 min. After drying, slides containing the glands sections were mounted in glycerinated gelatin and slipcovered for observation and photographic documentation under photomicroscope Motic BA 300 linked to an Intel Pentium 4 computer.

*2.3.4. Baker staining to detect total lipids according to Martoja and Martoja-Pierson (1967), modified by Giovannetti (2009, personal information)*

Six metapleural glands were removed and fixed in formol calcium for 24 h. Slides with 4 μm sections were stained with calcium dichromate (5 g of calcium dichromate, 1 g of anhydrous calcium chloride, 100 mL of distilled water) for 18 h at room temperature, washed three times with distilled water and treated with acid hematein (0.05 g of crystallized hematoxylin in 48 mL of distilled water, 1 mL of 1% aqueous solution of sodium iodate, 1 mL of acetic acid) for 5 h at room temperature, and washed with distilled water (three times). After drying, slides were mounted in glycerinated gelatin and slipcovered for observation and photographic documentation under photomicroscope Motic BA 300 linked to an Intel Pentium 4 computer.

## 3. Results

### *3.1. Scanning electron microscopy*

Scanning electron microscopy confirmed that the metapleural glands of *A. laevigata* ants of all castes are paired structures

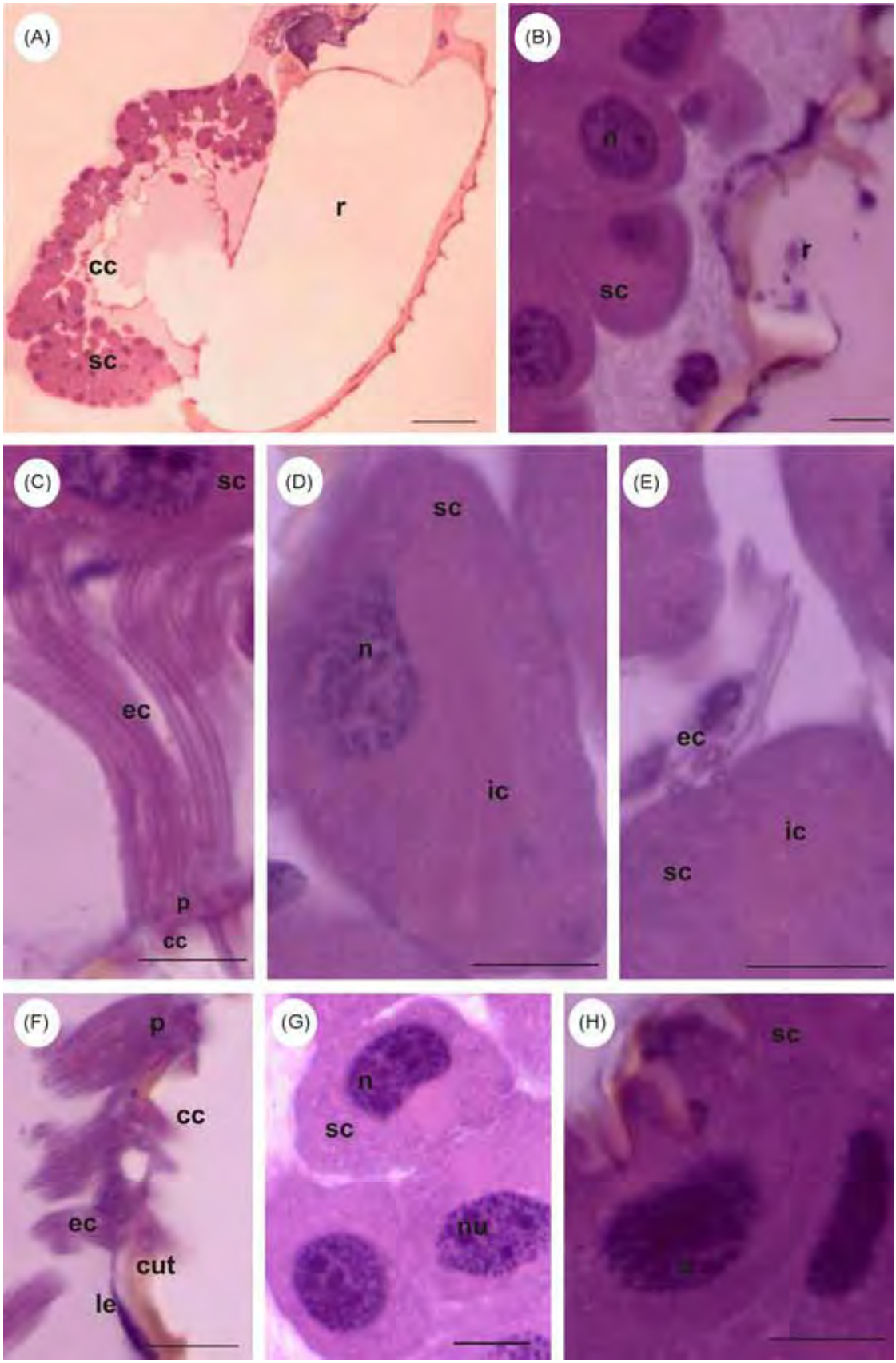



**Fig. 3.** Histological sections of the metapleural gland of *Atta laevigata* workers stained with hematoxylin and eosin. (A) General view of the metapleural gland with secretory cells (sc), collecting chamber (cc) and the reservoir (r) in major workers. Scale bar = 100 μm. (B) Details of secretory cells (sc), nuclei (n) and the reservoir (r) in major workers. Scale bar = 10 μm. (C) Details of the extracytoplasmic portions of canaliculi (ec) and perforated plates (p) in major workers. Note canaliculi arising individually from secretory cells (sc) and opening in the perforated plate of the collecting chamber (cc). Scale bar = 10 μm. (D) Longitudinal section of an intracytoplasmic portion of canaliculi (ic) and its corresponding nucleus (n) of secretory cells (sc). Scale bar = 10 μm. (E) Cross-sections of intra- (ic) and extracytoplasmic (ec) portion of canaliculi and secretory cells (sc) in

composed of: (a) *secretory portion* consisted of secretory cells, and (b) *storage portion* consisted of a collecting chamber and a reservoir. These portions are connected by individual extracytoplasmic canaliculi that drain the secretion of each secretory cell they form groups that open into the collecting chamber (Figs. 1 and 2A–F).

Secretory cells form clusters (Figs. 1 and 2A and B); each cell possesses its own extracytoplasmic canaliculus that, along with others, opens into the collecting chamber (Figs. 1 and 2A and B). The latter is a modified area of the gland with an irregular surface at the site of attachment of extracytoplasmic canaliculi (Fig. 2C), similar to the perforated plate, named perforated plate (several perforated plates) with 6–8 perforations.

The reservoir is internally lined by a cuticular intima, which also lines the epithelium. The area of the reservoir near the integument is vaulted, with hexagonal sculpture similar to that found in the exoskeleton (Fig. 2D and E).

The semicircular slit-shaped gland openings (Fig. 2E and F) in the exoskeleton are surrounded by a furrow (Fig. 2E), near coxae of hind legs, one on each side of the body. The external surface of the exoskeleton surrounding the gland opening is smooth, thus free of sculpture patterns and devoid of hairs (Fig. 2E and F).

### 3.2. Histology

The metapleural glands of *A. laevigata* workers consist of clusters of secretory cells and each one is drained by a canaliculus composed of two distinct portions: (a) *intracytoplasmic* and (b) *extracytoplasmic* one. Canaliculi form groups of up to 20 that attach to the perforated plate of the collecting chamber, from which the secretion flows toward the reservoir (Fig. 3A–E). The secretory portion is located more internally into the ant's body, while part of the reservoir wall is in contact with the integument, thus more external compared with the secretory portion (Fig. 3A). Secretory cells are oval or elongated with a single nucleus, and from the cytoplasm, collecting canaliculi arise (Figs. 1 and 3B, C and F).

The intracytoplasmic portion exhibits a smaller diameter than that of the extracytoplasmic portion (Fig. 3D and E), and is internally lined by a thin cuticle. The extracytoplasmic portions have larger diameter, although the internal cuticular lining is thicker, and open into the collecting chamber (Fig. 3D and E) through the perforated plate, which distinguishes this portion from the secretory gland portion (Fig. 3D).

The collecting chamber is a narrowing of the reservoir, where canaliculi attached, lined by a single squamous epithelium with cells with flattened nuclei, and with a cuticular intima that is folded (Figs. 1 and 3G). Behind the collecting chamber is the reservoir, a sac consisted internally by an epithelium and externally by muscles. Similarly to the collecting chamber, the reservoir is also lined by cuticular intima (Figs. 1 and 3A), but devoid of folds.

Secretory cells of individuals of all castes are mononucleated and have predominantly uncondensed chromatin (Fig. 3G and H). In minor workers, however, chromatin is more condensed (Fig. 3H). Nucleoli were frequently observed in secretory cells of the metapleural gland. In media and major workers, the number of nucleoli ranged from 2 to 4, while in minors, from 1 to 2 (Fig. 3).

### 3.3. Morphometry

The morphometric analysis of the metapleural gland of *A. laevigata* workers revealed that in the three castes the diameter of the reservoir is significantly correlated ($r = 0.982$; $p = 0.001$) with head length (Fig. 4). The length of the pronotum was also significantly correlated ($r = 0.914$; $p = 0.001$) with the diameter of the reservoir (Fig. 5), indicating that the size of the reservoir is proportional to body size. Since majors are larger than minor and media workers, the reservoir of their metapleural gland is also larger.

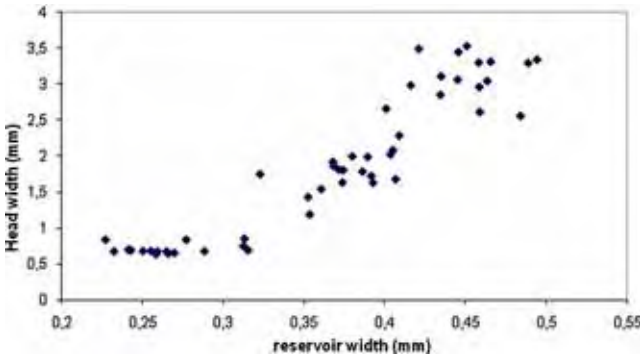



**Fig. 4.** Dispersion graphic showing the correlation between head length and diameter of the reservoir for the three size classes of workers of *Atta laevigata*.

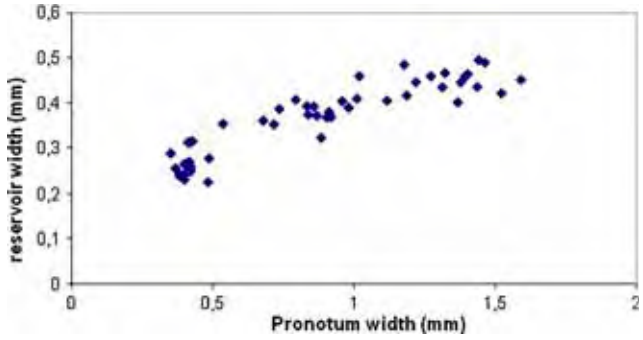



**Fig. 5.** Dispersion graphic showing correlation between pronotum length and diameter of the reservoir for the three size classes of workers of *Atta laevigata*.

## 4. Histochemistry

### 4.1. Detection of polysaccharides

PAS/alcian blue staining of the metapleural glands of the three castes of *A. laevigata* workers revealed that secretory cells of individuals present polysaccharides (Fig. 6A–C), as the cytoplasm of secretory cells was weakly positive for glycoproteins and moderately to strongly positive for acidic polysaccharides (Fig. 6A–C). Most granules of acidic polysaccharides were found in the periphery of cells, near the cell boundaries, mainly in media and major workers (Fig. 6A–C).

The periphery of intracytoplasmic canaliculi of individuals of the three castes was not stained (Fig. 6A–C). However, extracytoplasmic canaliculi was weakly positive for glycoproteins and moderately positive for acidic polysaccharides in the three castes examined (Fig. 6B and C).

The lumen of intra- and extracytoplasmic canaliculi were negative for glycoproteins. However, it was moderately to strongly positive for acidic polysaccharides in all worker castes (Fig. 6A–C). Cells of the epithelium lining the collecting chamber and the

media worker. Scale bar = 10 μm. (F) Details of the extracytoplasmic portion of canaliculi (ec) and perforated plate (p) lined by cuticular intima (cut) of the collecting chamber (cc) and, lining epithelium (le) in major worker. Scale bar = 10 μm. (G) Cluster of secretory cells (sc) showing large nuclei (n), and nucleoli (nu) in major worker. Scale bar = 10 μm. (H) Secretory cells (sc) with large nuclei (n), and very condensed chromatin in minor workers. Scale bar = 10 μm.

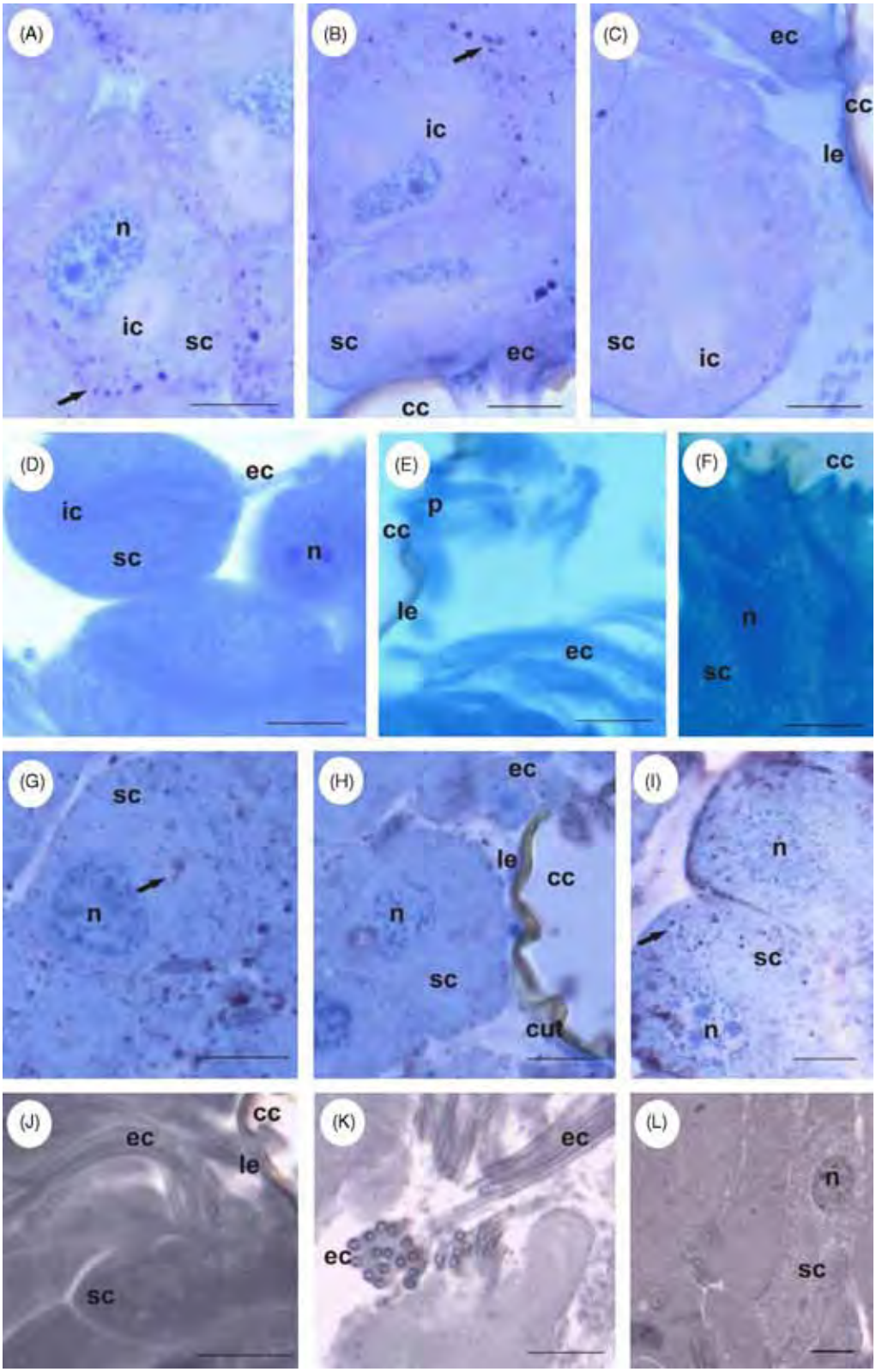



**Fig. 6.** Histological sections of the metapleural gland of *Atta laevigata* workers stained with PAS/alcian blue (A–C), bromophenol blue (D–F), Nile blue (G–I), and Baker method (J–L). (A-C) Details of secretory cells (sc) showing the cytoplasm, nucleus (n), intracytoplasmic canaliculi (ic) in cross-section, extracytoplasmic canaliculi (ec) in longitudinal section, collecting chamber (cc) lining epithelium (le) of the collecting chamber (cc) in major worker. Note the presence of granules (arrow) of acidic polysaccharides in the cytoplasm, mainly in the region near the cell boundaries. Scale bar = 10 μm. (D–F) Details of secretory cells (sc) showing cytoplasm, nucleus (n), intracytoplasmic (ic) and extracytoplasmic (ec) portions of canaliculi in longitudinal sections, perforated plate (p), collecting chamber (cc) and its lining epithelium (le): (D and E) media workers and (F) minor workers. Scale bar = 10 μm. (G–I) Details of secretory cells (sc) showing cytoplasm, nucleus (n), extracytoplasmic canaliculi (ec) in longitudinal sections, collecting chamber (cc), lining epithelium (le) and cuticular intima (cut): (G and H) media workers and (I) major worker. Note the presence of granules of lipids (arrow) in the cytoplasm of secretory cells (G–I). Scale bar = 10 μm. (J–L) Details of secretory cells (sc) showing cytoplasm, nucleus (n), extracytoplasmic canaliculi (ec) in longitudinal and cross-sections, collecting chamber (cc) and lining epithelium (le): (J) minor workers and, (K and L) media workers. Scale bar = 10 μm.

**Table 1**
Histochemical tests of the metapleural glands of workers of three castes of *Atta laevigata*.

| Portions of the metapleural gland | Histochemical tests | | | | |
|---|---|---|---|---|---|
| | PAS | Alcian blue | Bromophenol blue | Nile blue | Baker |
| Cytoplasm of secretory cells | Ma+ | Ma+++ | Ma+++ | Ma+++ | Ma+++ |
| | Me+ | Me+++ | Me+++ | Me+++ | Me+++ |
| | Mi+ | Mi+++ | Mi+++ | Mi+++ | Mi+++ |
| Periphery of the intracytoplasmic canaliculus | Ma− | Ma− | Ma+++ | Ma− | Ma− |
| | Me− | Me− | Me+++ | Me− | Me− |
| | Mi− | Mi− | Mi+++ | Mi− | Mi− |
| Extracytoplasmic canaliculus | Ma+ | Ma++ | Ma+++ | Ma++ | Ma+++ |
| | Me+ | Me++ | Me+++ | Me++ | Me+++ |
| | Mi+ | Mi++ | Mi+++ | Mi++ | Mi+++ |
| Lumen of intra- and extracytoplasmic canaliculus | Ma− | Ma+++ | Ma+ | Ma+++ | Ma− |
| | Me− | Me+++ | Me+ | Me+++ | Me− |
| | Mi− | Mi+++ | Mi+ | Mi+++ | Mi− |
| Epithelial cells lining the collecting chamber | Ma+ | Ma++ | Ma+++ | Ma++ | Ma++ |
| | Me+ | Me++ | Me+++ | Me++ | Me++ |
| | Mi+ | Mi++ | Mi+++ | Mi++ | Mi++ |
| Epithelial cells lining the reservoir | Ma+ | Ma++ | Ma+++ | Ma++ | Ma++ |
| | Me+ | Me++ | Me+++ | Me++ | Me++ |
| | Mi+ | Mi++ | Mi+++ | Mi++ | Mi++ |

Ma: major worker; Me: media worker; Mi: minor worker. (+) Weak staining; (++) moderate staining; (+++) strong staining; (−) negative.

reservoir were weakly positive for glycoproteins and moderately positive for acidic polysaccharides (Fig. 6A–C).

### 4.2. Detection of proteins

Bromophenol blue staining of the metapleural glands of minor, media, and major workers of *A. laevigata* was positive for proteins (Fig. 6D–F). The cytoplasm of secretory cells, the periphery of intracytoplasmic canaliculi, the extracytoplasmic canaliculi, and epithelial cells lining the collecting chamber and the reservoir were strongly positive in all castes. The lumen of intra- and extracytoplasmic canaliculi were weakly positive (Fig. 6D and E) in the three castes.

### 4.3. Detection of acidic lipids

Nile blue staining of the metapleural glands of minor, media, and major workers of *A. laevigata* is positive to acidic lipids (Fig. 6G–I). The cytoplasm of secretory cells and the lumen of intra- and extracytoplasmic canaliculi were strongly positive (Fig. 6G–I) in the three castes examined, indicating the presence of lipids in the secretory cells, mostly located in the periphery, near the cell boundaries (Fig. 6G and I).

The periphery of intracytoplasmic canaliculi were not stained, however, extracytoplasmic canaliculi, and epithelial cells lining the collecting chamber and the reservoir were moderately stained in the three worker castes (Fig. 6G–I).

### 4.4. Detection of total lipids

Total lipids were detected in the metapleural glands of *A. laevigata* workers (Fig. 6J–L). The cytoplasm of secretory cells and the extracytoplasmic canaliculi were strongly positive in the three worker castes.

The periphery of intracytoplasmic canaliculi, the lumen of intra- and extracytoplasmic canaliculi, and the cuticular intima of the collecting chamber of the reservoir were not stained. The epithelial cells lining the chamber and the reservoir were moderately positive in the three worker castes (Fig. 6J–L).

For better visualization, results of histochemical tests are summarized in Table 1.

## 5. Discussion

The morphological results obtained in the present study on the metapleural gland of minor, media, and major of *A. laevigata* workers confirm the reported by Hölldobler and Wilson (1990) for *A. cephalotes*, in which glands are located in the ventral-posterior portion of the metathorax, on a elevated area of the exoskeleton that can be seen from the outside of the insect and that houses the reservoir of the metapleural gland, and is present on both sides of the alitrunk.

The metapleural gland of workers of *A. laevigata* presents oval and/or elongated secretory cells forming clusters (20 cells on average), as also observed in other attines, such as *Ac. subterraneus* (De Souza et al., 2006), *Ac. octospinosus* (Bot et al., 2001), and *A. bisphaerica* and *A. sexdens rubropilosa* (Gusmão, 2000). In *Diacamma rugosum* and *D. vagans* (Schoeters and Billen, 1992), secretory cells located in the center of the metapleural gland are round-shaped, while peripheral ones are more elongated or even cylindrical.

Secretory cells of the metapleural gland of *A. laevigata* in all castes are very close to each other, but without fusion of cytoplasm, surrounded by a connective tissue. The same organization was observed in *A. laevigata*, *A. bisphaerica*, *A. sexdens sexdens* and *A. sexdens rubropilosa* by Schoeters and Billen (1993). The presence of connective tissue is expected, as each cell works as an independent unit with its own collecting canaliculus with the extracytoplasmic portion arriving at the reservoir along with others forming groups of approximately 20, and opening into the collecting chamber. This same arrangement was observed in the mandibular glands of the bee *Trigona* (*Oxytrigona*) *tataria* (Cruz-Landim, 1967) and of the *A. sexdens* (Pavon and Camargo-Mathias, 2001).

In secretory cells in general, the presence of a large nucleus with mostly uncondensed chromatin, especially in media and major workers of *A. laevigata*, indicate that cells are actively synthesizing proteins (Alberts et al., 2004). However, the condensed chromatin observed under light microscope in gland cells of minor workers suggests an unexpected lower production of secretion, since minor workers are responsible for maintaining the fungus garden inside the colony.

Histochemical techniques demonstrated that secretory cells of the metapleural gland of *A. laevigata* are strongly positive for total

and acidic lipids, suggesting that these cells participate in the production of pheromones and volatile substances, as lipids are the molecular base of these compounds. Gusmão (2000), studying the metapleural gland of *A. bisphaerica* and *A. sexdens rubropilosa* by transmission electron microscopy, observed a significant amount of smooth endoplasmic reticulum, corroborating its role in lipid production. Its function in the production of antibiotic substances also suggests intense lipids synthesis in these glands in *A. laevigata*.

The cytoplasm of secretory cells of the metapleural glands were also strongly positive for proteins, glycoproteins, and acidic polysaccharides, indicating that these cells produce these compounds in large amounts and that they probably play an important role in the composition of the secretion of the metapleural gland.

The secretion of the metapleural gland of *A. laevigata* after being produced by secretory cells is transported to the reservoir through canaliculi, which have intra- and extracytoplasmic portions. The presence of canaliculi collecting the secretion in the extracytoplasmic portion was also reported for other attines such as *A. laevigata*, *A. bisphaerica*, *A. sexdens sexdens*, and *A. sexdens rubropilosa* (Hölldobler and Engel-Siegel, 1984; Schoeters and Billen, 1993; Bot et al. 2001; De Souza et al., 2006). According to the classification of Noirot and Quennedey (1991), secretory cells of metapleural glands of *A. laevigata* are class III cells, as they have cuticular canaliculi divided into two intracellular portions (or intracytoplasmic) and extracellular (or extracytoplasmic). The intracytoplasmic portion of canaliculi immediately drains the secretion produced by the cell. This portion might be also responsible for modifying the secretion, as class III secretory cells often have microvilli surrounding the intracytoplasmic portion of canaliculi. The extracytoplasmic portion conducts the secretion of the cell until the collecting chamber, the first part of the reservoir. They lack microvilli and probably only conducts the secretion, corroborating the reported in *A. bisphaerica* and *A. sexdens rubropilosa* (Gusmão, 2000).

The periphery of intracytoplasmic canaliculi was strongly positive for proteins, confirming their role in, besides collecting and transporting, modifying the composition of the secretion, reabsorbing elements that can be reused by the individual. This is similar to the role played by cells of the striated duct of salivary glands of mammals, where the salivary secretion is drastically modified before being discharged to the outside (Junqueira and Carneiro, 2008).

The extracytoplasmic portion of canaliculi of metapleural glands of *A. laevigata* was moderately positive for proteins, weakly positive for glycoproteins, moderately to strongly positive for acidic and total lipids, and strongly positive for acidic polysaccharides, supporting the hypothesis that the secretion produced by secretory cells does not have the same composition than that found in the extracytoplasmic portion of canaliculi.

Similarly to the reported for *A. bisphaerica* and *A. sexdens rubropilosa* (Gusmão, 2000), in *A. laevigata*, the internal area of the collecting chamber of the metapleural gland, which is the region of the reservoir in contact with the secretory portion, is irregularly shaped and lined in the contact region with the chitinous perforated plate with various perforations. Groups of several canaliculi are formed, but there are not fused, as they attach individually to these perforations to release the secretion produced by each cell into the reservoir. The shape of these perforations in the plates is the same already described by Tulloch et al. (1962) in *Myrmecia nigrocincta*. In *D. vagans*, a single large plate is present in the wall of the collecting chamber (Schoeters and Billen, 1992).

The reservoir and the collecting chamber, parts of the metapleural gland responsible for storing the secretion, are lined by a single squamous epithelium in *A. laevigata*. In addition to lining these structures, epithelial cells also secrets the cuticular intima that protects the epithelial wall from acids of the secretion and/or protects the secretion from contaminants that could decrease or inactivate its action.

The opening of the metapleural gland of the three castes *A. laevigata* are semicircular slits located near the coxae of hind legs in individuals, confirming the observed by Gusmão (2000), for gardeners, foragers, and soldiers of *A. bisphaerica* and *A. sexdens rubropilosa*. The region surrounding the gland opening, which allows the secretion to move freely (Schoeters and Billen, 1993; Gusmão, 2000), in *A. laevigata* is smooth and free from sculptures, following the typical hexagonal pattern of the conspicuous reticulation found in the rest of the body of individuals of the genus *Atta*.

Around the opening of the metapleural gland of *A. laevigata* is a furrow, which conducts the secretion to coxae of the hind legs. This facilitates the spreading of secretion (with movements of coxae) to the ant's body protecting for example, intersegmental areas, which are more susceptible to the attack and penetration of entomopathogens. Similar results were obtained by Gusmão (2000) for *A. bisphaerica* and *A. sexdens rubropilosa*.

In workers of *A. laevigata*, as well as of *A. bisphaerica* and *A. sexdens rubropilosa* (Gusmão, 2000), *Camponotus gigas*, *Catalacus intrudens*, *Crematogaster* sp., *Podomyrma pulchra* (Hölldobler and Engel-Siegel, 1984), *C. striatula* (Fanfini and Dazzini, 1991), *D. rugosum* and *D. vagans* (Schoeters and Billen, 1992), the metapleural gland opening is devoid of hairs. According those authors the hairs might in part prevent the flow of secretion toward coxae of hind leg.

An important aspect that should be considered is the path which the secretion goes through since its production until its release into the reservoir and then to the gland opening. This transport may be entirely passive, and have the participation of thoracic muscles (Markl, 1966), through contraction and relaxing movements. Bot et al. (2001), studying *Ac. octospinosus*, observed that these muscles contract and compress the reservoir, increasing the flow of chemical substances toward the glandular opening. In the present study, thin muscle layers externally surrounding the collecting chamber and the reservoir were observed, confirming that they can be distended when the chamber and reservoir are filled with secretion or contracted, through nervous stimulus during the release of secretion to the exterior.

Other morphological studies on the metapleural gland carried out by Bot and Boomsma (1996); Bot et al. (2001), have shown that the diameter of the reservoir can be used as a parameter to estimate the size of the metapleural gland. Minor workers of *Ac. octospinosus* (Bot et al., 2001) had approximately 200–300 secretory cells, media workers 350–450 secretory cells, and major workers, 500–600 secretory cells, indicating that the more cells, the larger the size of the reservoir or vice versa, thus, positively correlated with the size of the reservoir. In *A. laevigata*, majors worker have larger reservoir than minor and media ones, indicating that the metapleural gland of the former is larger than those of latter. This could also imply a larger capacity of secretion production, including antibiotic substances. It should be pointed out that the production of secretion might be reduced under stressful conditions or due to age (Maschwitz et al., 1970; Bot et al., 2001). In contrast, Bot and Boomsma (1996) observed that the reservoir of major workers of some species of *Acromyrmex* was relatively small compared with the size of the individuals and correlated the large size of the metapleural gland of minor workers of leaf-cutting ants: (a) with a larger capacity of production of antibiotics by these individuals due to their role in the protection of the fungus garden against microorganisms, and/or (b) due to more contact with microorganisms, as minors tend the fungus garden and thus require more protection than other castes individuals. The results here presented indicate that individuals of all caste workers actively participate in the control of

microorganisms, thus minor workers or gardeners perform tasks associated with fungus growing and tending the brood, media workers or generalists that perform within nests tasks, including refuse disposal and tending the queen, and major workers or foragers that cut and transport plant material to the fungus garden. This last role might be one of the most important, as major workers represent the frontline in the control of microorganisms.

Based on the presented findings, the metapleural gland of minor, media, and major workers of *A. laevigata* is similar to that of other attines. The product synthesized by secretory cells consists of lipids, proteins, acidic polysaccharides, and glycoproteins, indicating that the final secretion of the metapleural gland of *A. laevigata* is composed of glycolipoproteins. The high level of similarity in the morphology of the metapleural gland among species of *Atta* and *Acromyrmex* suggest that this gland is responsible for the defense of the colony against pathogenic microorganisms, although other roles are possible. Thus studies on the metapleural gland of primitive attines and other basal tribes are needed to examine whether the structure and role of this gland remained the same throughout evolution, as well as its role in the different castes of individuals within the same species.

## Acknowledgements

We thank the Center for the Study of Social Insects – CEIS – UNESP (São Paulo State University) for the support in the collection and identification of specimens used in this study and to FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for financial support (grant 2008/54301-3).

## References

Alberts, B., Johnson, A., Walter, P., 2004. Biologia Molecular da Célula, 4 ed. Artmed, Porto Alegre, 1549 pp.

Attygalle, A.B., Siegel, B., Vostowsky, O., Bestmann, H.J., Maschwitz, U., 1989. Chemical composition and function of metapleural gland secretion of the ant, *Crematogaster deformis* Smith (Hymenoptera: Myrmicinae). J. Chem. Ecol. 15, 317–328.

Beattie, A.J., Turnbull, C., Hough, T., Knox, R.B., 1986. Antibiotic production: a possible function for the metapleural glands of ants (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 79, 448–450.

Bot, A.N.M., Boomsma, J.J., 1996. Variable metapleural gland size-allometries in *Acromyrmex* leafcutter ants (Hymenoptera: Formicidae). J. Kans. Entomol. Soc. 69, 375–383.

Bot, A.N.M., Obermayer, M.L., Hölldobler, B., Boomsma, J.J., 2001. Functional morphology of the metapleural gland in the leaf-cutting ant *Acromyrmex octospinosus*. Insectes Soc. 48, 63–66.

Bot, A.N.M., Ortius-Lechner, D., Finster, K., Maile, R., Boomsma, J.J., 2002. Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. Insectes Soc. 49, 363–370.

Cruz-Landim, C., 1967. Cell reorganization and cell death during the secretory cycle of the hypopharyngeal gland in Meliponinae bee workers (Hymenoptera: Apidae), Acta Microscopia, 6. Suppl. B.In: Proc. XVI Meeting of the Brazilian Society for Electron Microscopy. pp. 75–78.

De Souza, A.L.B., Soares, I.M.F., Cyrino, L.T., Serrão, J.E., 2006. The metapleural gland in two subspecies of *Acromyrmex subterraneus* (Hymenoptera: Formicidae). Sociobiology 47, 19–25.

Do Nascimento, R.R.D., Schoeters, E., Morgan, E.D., Billen, J., Stradling, D.J., 1996. Chemistry of metapleural gland secretions of three attine ants, *Atta sexdens rubropilosa*, *Atta cephalotes*, and *Acromyrmex octospinosus* (Hymenoptera: Formicidae). J. Chem. Ecol. 22, 987–1000.

Fanfini, A., Dazzini, M.V., 1991. Le glandole metatoraciche di *Crematogaster striatula* (Hymenoptera, Formicidae, Myrmicinae). Fragm. Entomol. 23, 191–200.

Fernandez-Marin, H., Zimmerman, J., Rehner, S., Wcislo, W., 2006. Active use of the metapleural glands by ants in controlling fungal infection. Proc. Roy. Soc. Lond. B: Biol. Sci. 273, 1689–1695.

Gusmão, L.G., 2000. Estudo comparado da glândula metapleural e sua secreção em diferentes castas de três espécies de *Atta* (Hymenoptera: Formicidae). Tese (Doutorado) - ESALQ-USP, Piracicaba-SP, 60 pp.

Gusmão, L.G., Caetano, F.H., Nakano, O., 2001. Ultramorphology of the metapleural gland in three species of *Atta* (Hymenoptera, Formicidae). Iheringia Zool. 91, 33–36.

Hölldobler, B., Engel-Siegel, H., 1984. On the metapleural gland in ants. Psyche 91, 201–224.

Hölldobler, B., Wilson, E.O., 1990. The Ants. Harvard Univ. Press, Cambridge.

Junqueira, L.C.U., Junqueira, L.M.M.S., 1983. Técnicas básicas de citologia e histologia. Livraria Editora Santos 48–81.

Junqueira, L.C., Carneiro, J., 2008. Histologia básica, 11 ed. Guanabara Koogan, Rio de Janeiro.

Lison, L., 1960. Histochimie et Cytochimie Animales. Gauthier-Villars, Éditeur, Paris.

Markl, H., 1966. Peripheres Nervensystem und Muskulatur im Thorax der Arbeiterin von *Apis mellifera* L., *Formica polyctena* Foerster und *Vespa vulgaris* L. und der Grundplan der Innervierung des Insektenthorax. Zool. Jb. Anat. 83, 107–184.

Martoja, R., Martoja-Pierson, M., 1967. Initiation aux techneques de l'histologie animale. Masson et c[ie], éditeurs, bd, St-Germain, Paris IV.

Maschwitz, U., Koob, K., Schildknecht, H., 1970. Ein Beitrag zur Funktion der Metathoracaldrüse der Ameisen. J. Insect Physiol. 16, 387–404.

Maschwitz, U., 1974. Vergleichende Untersuchungen zur Funktion der Ameisen-metathorakaldrüse. Oecologia 16, 303–310.

Noirot, C., Quennedey, A., 1991. Glands, gland cells, glandular units: some comments on terminology and classification. Ann. Soc. Entomol. Fr. 27, 123–128.

Ortius-Lechner, D., Maile, R., Morgan, E.D., Boomsma, J.J., 2000. Metapleural gland secretion of the leaf-cutter ant *Acromyrmex octospinosus*: new compounds and their functional significance. J. Chem. Ecol. 26, 1667–1683.

Paiva Castro, U., Zamith, A.P.L., Mariconi, F.A.M., 1961. Contribuição para o conhecimento da "saúva de vidro" *Atta laevigata* Fred. Smith, 1858. Anais da ESALQ 18, 313–325.

Pavon, L.F., Camargo-Mathias, M.I., 2001. A morpho-histological and ultrastructural studies of the mandibular glands of *Atta sexdens* worker ants (Hymenoptera: Formicidae). Sociobiology 38, 449–464.

Pearse, A.G.E., 1985. Histochemistry. Theoretical and Applied, 4th ed. J & A Churchill, London.

Poulsen, M., Bot, A.N.M., Nielsen, M.G., Boomsma, J.J., 2002. Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. Behav. Ecol. Sociobiol. 52, 151–157.

Schildknecht, H., Koob, K., 1971. Myrmicacin, the first insect herbicide. Angew. Chem. Int. Edit. 10, 124–125.

Schoeters, E., Billen, J., 1992. Morphological and ultrastructural study of metapleural gland in *Diacamma* (Hymenoptera, Formicidae). In: European Congress of Social Insects, 1o, Leuven, 1991, Proceedings Leuven, Leuven University, pp. 239–247.

Schoeters, E., Billen, J., 1993. Anatomy and fine structure of the metapleural gland in *Atta*. Belg. J. Zool. 123, 67–75.

Thorne, B.L., Traniello, J.F.A., 2003. Comparative social biology of basal taxa of ants and termites. Annu. Rev. Entomol. 48, 283–306.

Tulloch, G.S., Shapiro, J.E., Hershenov, B., 1962. The ultrastructure of the metasternal glands of ants. Bull. Brooklyn Entomol. Soc. 57, 91–101.

Wilson, E.O., 1971. The Insect Societies. Harvard Univ. Press, Cambridge, MA.

# *Capítulo 2*

**Perfil ultraestrutural das células da glândula metapleural da formiga *Atta laevigata* (F. Smith, 1858) (Formicidae: Attini)**

**Autores**: Alexsandro Santana Vieira, Odair Corrêa Bueno e Maria Izabel Camargo-Mathias
**Periódico**: Animal Biology, v. 62, p. 1–11, 2012.

## RESUMO

A glândula metapleural é considerada uma sinapomorfia das formigas, e apresenta-se como estrutura par localizada nas duas extremidades posterolaterais do tórax tendo como função a de secretar substâncias capazes de inibir e controlar a proliferação de fungos e bactérias no jardim de fungo e no interior do ninho. O objetivo deste trabalho foi o de investigar quais são as diferenças ultraestruturais que ocorrem nas células da glândula metapleural das operárias (três castas) de *Atta laevigata* utilizando-se microscopia eletrônica de transmissão (TEM). Os resultados mostraram que no citoplasma das células secretoras há a presença de regiões de Golgi, retículo endoplasmático rugoso (lamelar e vesicular), retículo endoplasmático liso, mitocôndrias (alongadas, arredondadas), vacúolos, grânulos de secreção com diferentes eletrondensidades, além de figuras mielínicas, indicando que esta glândula é ativa na produção de substâncias de natureza protéica, lipídica e, polissacarídica (glicogênio nas operárias maiores). Gotas lipídicas e grânulos de secreção foram encontrados muito próximos às microvilosidades, principalmente nas operárias mínimas. A porção intracelular dos canalículos apresentou numerosas invaginações responsáveis por aumentar a superfície de contato entre o citoplasma da célula secretora e por modificar a secreção produzida pela célula secretora. Nas três castas estudadas, a glândula apresentou um reservatório precedido de câmara coletora, ambos revestidos por epitélio simples pavimentoso e recobertos pela intima cuticular. Sugere-se que operárias das três castas de *A. laevigata* estariam envolvidas na produção de secreção, principalmente de natureza protéica com função antibiótica e, que as mínimas, possivelmente seriam responsáveis por produzirem maior variedade de secreção no interior da colônia em relação às médias e maiores.

**Palavras-chave**: Attini, *Atta laevigata*, glândula metapleural.

BRILL

*Animal Biology 62 (2012) 1–11*

Animal Biology
brill.nl/ab

# Ultrastructural profile of metapleural gland cells of the ant *Atta laevigata* (F. Smith, 1858) (Formicidae: Attini)

**Alexsandro Santana Vieira**[1], **Odair Correa Bueno**[1,2] **and Maria Izabel Camargo-Mathias**[1,*]

[1] Departamento de Biologia; Universidade Estadual Paulista (UNESP), Av. 24A, 1515, Bela Vista, CEP 13506-900, Rio Claro, São Paulo, Brazil

[2] Centro de Estudos de Insetos Sociais; Universidade Estadual Paulista (UNESP), Av. 24A, 1515, Bela Vista, CEP 13506-900, Rio Claro, São Paulo, Brazil

**Abstract**

The metapleural gland is considered a synapomorphy of ants, and is characterized as a paired structure located at the two postlateral ends of the thorax and secretes substances capable of inhibiting and controlling fungi and bacteria in the fungus garden and inside the nest. This study was aimed at investigating if and which are the ultrastructural differences in the metapleural gland cells of workers (three castes) of *Atta laevigata* using transmission electron microscopy (TEM). This study revealed the presence of Golgi regions, rough endoplasmic reticulum (lamellar and vesicular shapes), smooth endoplasmic reticulum, mitochondria (elongated, round-shaped), vacuoles, secretion granules with different electron densities, and myelin figures in the cytoplasm of secretory cells, indicating that this gland produces substances composed of proteins, lipids, and polysaccharides (glycogen in major workers). Lipid droplets and secretion granules were found very near to the microvilli, especially in minor workers. The intracellular portion of canaliculi exhibited invaginations that increased the surface area and modified the secretion produced by the secretory cells. In the three castes examined, the gland exhibited a reservoir preceded by a collecting chamber, both lined by a simple squamous epithelium with a cuticular intima. Workers of the three castes of *A. laevigata* might be involved in the production of secretion mainly composed of proteins with antibiotic properties and, minor workers, may be responsible for producing a wider variety of secretions compared to median and major workers in the colony.



**Keywords**

Attini; *Atta laevigata*; metapleural gland

*) Corresponding author; e-mail: micm@rc.unesp.br



DOI 10.1163/157075511X597575

## Introduction

The adaptation against several types of pathogens has been considered one of the main events in the evolution of sociability and diversification of ants (Wilson, 1971). This occurs through the action of compounds with antibiotic properties secreted mainly by the metapleural gland that suppress the development of pathogens (Maschwitz et al., 1970, 1974; Beattie et al., 1985, 1986; Do Nascimento et al., 1996).

The metapleural glands are synapomorphic paired structures in ants (Bolton, 2003), located at the postlateral ends of the thorax of ants (Hölldobler and Wilson, 1990). Morphohistological studies conducted by Vieira et al. (2010) with workers of *Atta laevigata* and *Acromyrmex coronatus* have shown that the metapleural gland consists of two portions: a) a secretory and, b) a storage portion connected by extracytoplasmic canaliculi that drain each secretory cell and form groups that open into perforations of the sieve plate located in the collecting chamber. The secretory cells are round or oval-shaped, bundled together by connective tissue (Tulloch et al., 1962; Brown, 1968).

Gusmão (2000) analyzed the morphophysiology of the metapleural glands of *A. bisphaerica* and *A. sexdens rubropilosa* and described the presence of secretory cells closely associated with collecting cells. According to Tulloch et al. (1962) and Shoeters and Billen (1992), the winding intracellular canaliculus comes in contact with the secretory cell, similar to microvilli.

Other studies also revealed that in the ants *Diacamma rugosum*, *D. vagans*, *A. bisphaerica*, and *A. sexdens rubropilosa*, the cytoplasm of secretory cells of the metapleural gland exhibits large quantities of mitochondria of various shapes and sizes that can be elongated, thinner, and more or less electron dense (Shoeters and Billen, 1992; Gusmão, 2000). In addition to mitochondria, Golgi regions are also common, although they were not frequently observed in soldiers of *A. bisphaerica*, and queens of *A. laevigata* (Shoeters and Billen, 1993). Smooth endoplasmic reticulum was observed in the secretory cells of *D. rugosum* and *D. vagans* (Shoeters and Billen, 1992), while in workers of *A. sexdens rubropilosa* (Shoeters and Billen, 1993), only lamellar endoplasmic reticulum was found.

Based on this information, the present study was aimed at investigating if and which are the structural differences that occur in the metapleural gland cells in three worker castes of the leaf-cutting ant *Atta laevigata*, locally known as "saúva cabeça de vidro", which uses leaves from different dicotyledons and some grasses in their fungus garden (Paiva Castro et al., 1961). This species is considered a pest insect found in all Brazilian states, and therefore it is of economic importance (Paiva Castro et al., 1961), due to its ability to defoliate crops.

## Materials and methods

For this study, 21 workers (7/caste) of *A. laevigata* were used. Ants were separated by caste based on head size, $0.773 \pm 0.016$ mm for minor; $2.048 \pm 0.172$ mm for

median, and $3.282 \pm 0.335$ mm for major workers. Workers were obtained from two-year-old colonies found at the Corumbataí Farm (22°17′45″S, 47°40′81″W). Colonies were collected and maintained in the laboratory (Center for the Study of Social Insects), UNESP, Rio Claro, SP, Brazil.

*Ultrastructural analysis: transmission electron microscopy (TEM)*

To obtain metapleural glands, the legs and heads of the individuals were removed and placed in Petri dishes with colored wax and physiological solution (NaCl 7.5 g/L, $Na_2HPO_4$ 2.38 g/L and $KH_2PO_2$ 2.72 g/L) under a ZEISS stereomicroscope with the aid of dissecting forceps and microscissors. Metapleural glands (mesossoma) were carefully removed and fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 24 h. After fixation, the material was rinsed twice with 0.1 M sodium cacodylate buffer for 15 minutes, and post-fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.2) for two hours. The material was rinsed twice with 0.1 M sodium cacodylate buffer for 15 min and then with 10% ethanol solution for 15 minutes.

Contrast enhancement was carried out with 2% uranyl acetate in 10% ethanol for 12 hours. The material was dehydrated in a series of acetone solutions (50%, 60%, 70%, 80%, 90%, and 95%), five minutes each and finally 100% acetone, twice for five minutes each. After dehydration, the material was included in Epon Araldite and oven-dried at 60°C for 24 hours. After polymerization, blocs were sectioned with a Sorvall-Porter Blum MT2-B ultramicrotome. Ultrathin sections were collected with a copper screen, contrasted with 2% uranyl acetate and 0.4% lead citrate for 45 and 10 minutes, respectively. The material was examined and photographed under a transmission electron microscope PHILIPS CM 100.

## Results

The metapleural gland of *A. laevigata* is divided in two portions: a secretory and a storage portion connected by canaliculi.

*Secretory portion*

The results obtained for the metapleural gland of the three castes of *A. laevigata* are similar. The secretory cells are very close to each other, cells are oval or elongated (apical region wider than basal one), but plasma membranes are not fused (fig. 1a). Secretory cells are clustered by the presence of connective tissue. However, the plasma membrane has small infoldings (fig. 1). In some adjacent cells, the basal lamina is fused, and a single structure is observed (fig. 1a). The main ultrastructural characteristic of secretory cells is the presence of several mitochondria located mainly around the intracellular portion of canaliculi (fig. 1d, i). Most of the mitochondria are small with various shapes, sizes, and internal arrangements of cristae (fig. 1d-i).

Around the nuclei of secretory cells, especially in minor workers, rough endoplasmic reticulum (RER), mainly lamellar (fig. 1g), is well developed, although it may also be vesicular (fig. 1b). Smooth endoplasmic reticulum was not commonly observed (fig. 1g). The shape of Golgi regions are characterized by flat overlapping cisternae, with dilated ends (fig. 1e). Vacuoles with different contents are also observed (fig. 1d, i).

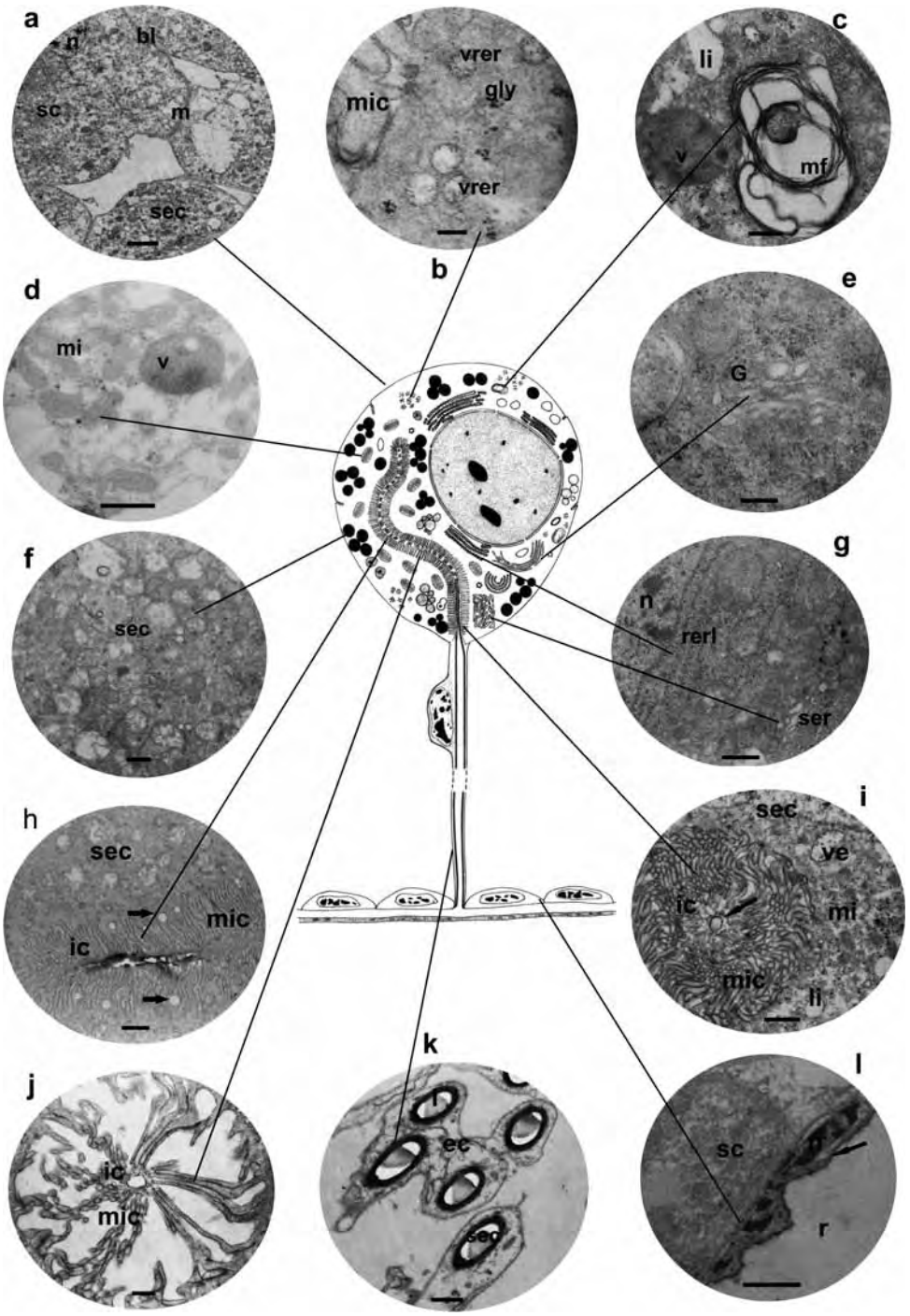
In the cytoplasm of secretory cells, a large quantity of secretion granules of various electron densities is observed (fig. 1f, h). The largest quantities were observed in minor workers. The cytoplasm of major workers contains large amounts of glycogen (fig. 1b) and myelin figures (fig. 1c) with lamellar arrangement.

The nuclei of secretory cells of workers of the three castes are large, with heterochromatin not evident, euchromatin predominating as well as pores in the nuclear envelope (fig. 1). Some cells exhibit two nucleoli (fig. 1).

*Intracytoplasmic canaliculus*

A canaliculus originates from each secretory cell of the metapleural gland and is divided into intra and extracytoplasmic portion. The canaliculus collects and modifies the secretion, moving it to the gland reservoir. A large quantity of microvilli (pericanalicular space) is observed in the intracellular portion of canaliculi (fig. 1h-j) in the three castes of *A. laevigata*. Canaliculi are internally lined by a cuticular intima

**Figure 1.** Schematic representation of the secretory cell of the metapleural gland of *Atta laevigata* and electron micrographs of its different portions. **a** – Major workers. General view of secretory cells (sc), of the nucleus (n), plasma membranes (m) not fused but with fused basal laminae (bl), contact areas between two secretory cells (sc) and secretion (sec). Scale bar = 10 $\mu$m. **b** – Major workers. Vesicular rough endoplasmic reticulum (vrer) near microvilli (mic) of intracellular microvilli. Note the presence of glycogen (gly). Scale bar = 0.5 $\mu$m. **c** – Major workers. Detail of myelin figure (mf) with lamellas, lipids (li) and vacuoles (v). Scale bar = 1 $\mu$m. **d** – Minor workers. Detail of mitochondria (mi) of various shapes: elongated and round-shaped, and vacuole (v). Scale bar = 1 $\mu$m. **e** – Median workers. Golgi regions (G) with typical features, flat cisternae overlapping with flat ends. Scale bar = 1 $\mu$m. **f** – Minor workers. Secretion (sec) of secretory cells composed of granules of different electron densities and electron lucent. Note larger amounts and wider variety of secretion. Scale bar = 2 $\mu$m. **g** – Minor workers. Lamellar rough endoplasmic reticulum (rerl) in contact with the nucleus (n) and smooth endoplasmic reticulum (ser) in the secretory cell. Scale bar = 1 $\mu$m. **h** – Minor workers. Intracellular canaliculus (ic), microvilli (mic). Note lipid droplets near microvilli (arrow) as well as secretion granules containing proteins (sec). Scale bar = 4 $\mu$m. **i** – Major workers. Cross section of the secretory cell with several mitochondria (mi) in the cytoplasm, secretion (sec) near microvilli (mic) of the intracellular canaliculus (ic), vesicles (ve) and lipid droplets (li). Note the thin cuticular intima with spaces (arrow), due to the presence of microvilli. Scale bar = 4 $\mu$m. **j** – Major workers. Intracellular canaliculus (ic) with dilated microvillus (mic) due to the accumulation of secretion (sec). Scale bar = 2 $\mu$m. **k** – Major workers. Extracytoplasmic canaliculi (ec) in clusters and thick cuticle lining their wall (arrow), lumen (l) filled with secretion (sec). Scale bar = 2 $\mu$m. **l** – Minor workers. Reservoir (r) of the metapleural gland with lumen (l), reservoir wall with simple squamous epithelium, and nucleus (n), the arrow indicates a thin cuticle lining the reservoir wall. Note some secretory cells (sc) near the reservoir. Scale bar = 4 $\mu$m.


a
n
bl
sc
m
sec
b
vrer
gly
mic
vrer
c
li
v
mf
d
mi
v
e
G
f
sec
g
n
rerl
ser
h
sec
mic
ic
i
sec
ve
ic
mi
mic
li
j
ic
mic
k
ec
sec
l
sc
r

that varies in thickness towards the reservoir. In this transition area, microvilli decrease in size and a cuticular intima arises and thickens (fig. 1h-j). Fig. 1 shows a winding intracytoplasmic canaliculus. A large amount of secretion accumulates in the pericanalicular space. In some workers, this causes microvilli to dilate (fig. 1j). Lipid droplets and protein granules are observed near and between microvilli in minor and major workers (fig. 1c-i). In secretory cells of the three castes, mitochondria of various shapes are observed near the canaliculi (fig. 1d-i).

*Extracytoplasmic canaliculus*

In the metapleural gland of workers of *A. laevigata*, secretory cells are connected to the reservoir through the extracellular portion of canaliculi. The diameter of canaliculi and thickness of the cuticular intima (lumen) increase as they approach the reservoir (fig. 1k). In some regions, secretion is observed in the lumen (fig. 1k). Secretory cells of canaliculi (one for each canaliculus) are also observed (fig. 1), the nucleus exhibit heterochromatin and cytoplasm with many organelles (fig. 1).

*Storage portion (collecting chamber)*

The collecting chamber receives canaliculi (cluster of 20) and is characterized as a narrowing of the reservoir where infoldings are lined with a cuticle with perforations in the many sieve plates (fig. 1).

*Storage portion (reservoir)*

The reservoir is a "sac" consisted of a simple squamous epithelium. The heterochormatin of cells is sparse (fig. 1l) and the epithelium is lined by a cuticular intima, thinner than that of the collecting chamber (figs. 1 and 1l).

## Discussion

The metapleural gland of *Atta laevigata* workers consists of several secretory units composed of a secretory cell and a canaliculus, thus divided into a secretory and a storage portion connected by canaliculi. Recent studies have also shown the same arrangement in workers of *A. bisphaerica* and *A. sexdens rubropilosa* (Gusmão, 2001), *Acromyrmex subterraneus* (Souza et al., 2006), and *Ac. coronatus* (Vieira et al., 2010).

The secretory cells of the metapleural gland of *A. laevigata*, in all castes, exhibited a wider apical region in relation to the basal one. These portions are connected by connective tissue without fusing the cytoplasmic membranes. The same organization was observed in the ant *Dorylus* by Billen and Van Boven (1987) and in the attines *A. bisphaerica*, *A. sexdens sexdens*, and *A. sexdens rubropilosa* by Schoeters and Billen (1993). However, the few invaginations of the plasma membrane observed in the secretory cells of *A. laevigata* do not support the results reported for *Dorylus nigricans* by Billen and Van Boven (1987), which observed a large number

of invaginations. These authors suggested that the presence of these invaginations might facilitate the absorption of products from the hemolymph. Invaginations have also been commonly observed in pheromone glands of other ant species (Billen, 1985, 1986).

The ultrastructural organization of the secretory cells of metapleural glands of *A. laevigata* are classified as class III (Noirot and Quennedey, 1991), as they exhibit collecting canaliculi divided into intra and extracellular portions internally lined by a cuticular intima.

In workers of *A. laevigata* and *Ac. subterraneus* (Souza et al., 2006), *Ac. octospinosus* (Bot et al. 2001), as well as in other attines, such as *A. bisphaerica*, *A. sexdens rubropilosa* (Gusmão, 2000), the secretory portion is composed of round and/or oval-shaped cells. In *Diacamma rugosum* and *D. vagans* (Schoeters and Billen, 1992) of the subfamily Ponerinae, secretory cells are round-shaped.

In the cytoplasm of secretory cells of *A. laevigata*, large numbers of mitochondria of various shapes are observed near microvilli of intracellular canaliculi, supporting the results obtained in queens of *A. bisphaerica* and *A. sexdens rubropilosa* by Gusmão (2001), and Tulloch et al. (1962) in *Myrmecia nigrocincta*, and suggesting a high demand of energy in this area. Two hypothesis could explain the presence of these mitochondria near microvilli: the first hypothesis suggests that mitochondria might be involved in the synthesis of secretion, while the second hypothesis suggests that these organelles might play a role in processes of active transport, providing energy to the cells for the transport of products of secretion of the secretory cell into the intracellular canaliculis (Tulloch et al., 1962).

The lamellar rough endoplasmic reticulum in the three worker castes of *A. laevigata* was well developed, possibly due to its role in the transport of material from the nucleus to the cytoplasm during the synthesis of proteins (see Alberts et al., 2004). This organelle was also found in secretory cells of the metapleural gland of queens of *A. bisphaerica* and *A. sexdens rubropilosa* by Gusmão (2000), in workers of *A. sexdens rubropilosa* (Shoeters and Billen, 1993; Do Nascimento et al., 1996) and in *D. rugosum* and *D. vagans* (Shoeters and Billen, 1992). Using infrared spectrometry and the ninhydrin test, studies demonstrated that the secretion of the metapleural gland of workers and soldiers of *A. sexdens rubropilosa* consists of mainly proteins. According to Shoeters and Billen (1993), ants with well-developed RER probably produce volatile compounds, which may also have compounds containing proteins in the secretion. The present study clearly demonstrated that RER is more developed in cells of the metapleural gland of minor workers of *A. laevigata*, suggesting that it might be involved in the production of protein compounds, possibly due to the role these ants play in fungus growing in the colony.

The results presented here are similar to those by Billen and Van Boven (1987), who observed the presence of vesicular rough endoplasmic reticulum in army ants *Dorylus*. Yet, they are different from the findings reported by Shoeters and Billen (1992) in previous studies with *Diacamma*, in which the presence of vesicular rough endoplasmic reticulum was not observed.

Smooth endoplasmic reticulum in the secretory cells of the metapleural gland of *A. laevigata* was not commonly observed, contrary to the report for *D. nigricans* (Billen and Van Boven, 1987). In queens *A. bisphaerica* and *A. sexdens rubropilosa* (Gusmão, 2000) SER was more abundant than lamellar RER. SER participates in the synthesis of lipids and the amount in the cells indicates their role (Billen, 1984). According to Billen (1984) and Billen and Van Boven (1987), cells with well-developed SER may produce pheromones in addition to other substances without proteins and according to Han and Bordereau (1982), the presence of the two forms (lamellar and vesicular) in the same cell might indicate differences in function: the vesicular type is the most active and the lamellar is the least active. However, the ribosome is known to attach to membranes of the endoplasmic reticulum when the latter is actively synthesizing proteins. In *A. laevigata* workers, these metapleural gland cells probably produce a secretion consisting of proteins, as RER was found more frequently than SER.

Golgi regions were observed in secretory cells of the metapleural gland of *A. laevigata*, supporting the observed in *A. bisphaerica*, *A. sexdens sexdens*, and *A. sexdens rubropilosa* by Schoeters and Billen (1993) and in *A. bisphaerica* and *A. sexdens rubropilosa* (Gusmão, 2000). In *M. nigrocincta* (Tulloch et al., 1962), these organelles were not observed, suggesting that the materials produced in the reticulum were not stored or modified into other products.

The presence of many vacuoles with different contents in secretory cells of the metapleural gland of *A. laevigata* suggests cell "turnover", as also observed by Falco (1992) in the postpharyngeal gland of *Camponotus rufipes*.

In *A. laevigata*, the predominance of secretion granules of different sizes and electron densities was observed in the cytoplasm of secretory cells of minor workers, reflecting the diversity of secretions produced and supporting the reporte by Gusmão (2000) in other species of the genus *Atta*, which represent of proteins, lipids, among others. Secretion granules were also frequently observed near canaliculi in *M. nigrocincta* (Tulloch et al., 1962). However, previous studies on *A. laevigata* conducted by Vieira et al. (2010) demonstrated that the secretion of the metapleural gland consists of glycolipoproteins. Thus, the presence of glycogen in the secretory cells of major workers observed in this study confirms the histochemical results. Since glycogen is a polysaccharide used as the main energy source in animal cells (Junqueira and Carneiro, 2008), its abundance in the metapleural gland suggests that major workers use secretory cells to store it. This could be due to the tasks performed by them, such as collecting and transporting substrates and colony defense.

Morphological studies on exocrine glands of insects have demonstrated that cells contain many myelin figures arranged as lamellas that may indicate cell "turnover". These structures observed in *A. laevigata* were also found in *A. bisphaerica* and *A. sexdens rubropilosa* (Gusmão, 2000), and in *Dorylus* (Billen and Van Boven, 1987).

Regarding the activity of secretory cells of the metapleural gland, the uncondensed chromatin in the nucleus and the presence of nucleoli are important findings. According to Alberts et al. (2004), active cells with a wide diversity of protein synthesis exhibit large quantities of euchromatin, as well as nucleoli in the cells that produce large quantities of proteins, in addition of many pores in the nuclear envelope, due to the migration of precursor units to ribosomes from the nucleus to the cytoplasm (Amabis and Martho, 2003).

In the present study, the intracellular canaliculus projects invaginations from the cytoplasm of secretory cells, increasing the surface area to collect and modify the secretion. In addition, in secretory cells of the metapleural gland of *A. laevigata*, the canaliculus follows a winding path within the cell, but not around the nucleus, contrary to the reported by Tulloch et al. (1962) for the ant *M. nigrocincta*. Also regarding microvilli in the pericanalicular space in *A. laevigata*, as the canaliculus leaves the cytoplasmic portion of the cell, microvilli decrease in size and quantity, and the lumen becomes wider and the wall thicker due to the presence of the final secretion in the intracellular environment.

The extracellular portion of conducting canaliculi in *A. laevigata* did not exhibit microvilli, but rather a thicker cuticle, confirming their role only in the transport of compounds, similar to the observations in *D. rugosum* and *D. vagans* (Shoeters and Billen, 1992).

In the collecting chamber of the metapleural gland of *A. laevigata*, groups of approximately 20 canaliculi open into perforations in a sieve plate, as observed by Vieira et al. (2010) in *A. laevigata*, supporting the observations by Tulloch et al. (1962) in *M. nigrocincta*, but contrary to the observion in *M. nigrocincta*, in which only one canaliculus was observed per perforation. In *D. vagans*, only one single and large plate was observed (Schoeters and Billen, 1992).

Similar to the observations in *Diacamma* (Shoeters and Billen, 1992), the collecting chamber of *A. laevigata* is very thick and sclerotized, separating secretions, which can be acidic or consisting of similar compounds, to be conducted to the reservoir. This physical barrier prevents the contamination of the secretion by extracellular compounds and protects epithelial cells in the collecting chamber and of the reservoir from the secretion's action.

Based on these findings, the metapleural glands of minor, median, and major workers of *A. laevigata* are morphologically similar to the same type of gland of other attines of the genera *Atta* and *Acromyrmex*. However, in the ponerine *D. rugosum* and *D. vagans* (Schoeters and Billen, 1992), secretory cells are longer and/or cylindrical. The large number of mitochondria near microvilli is also a characteristic of secretory cells. The presence of well-developed RER in minor workers suggests that they are involved in the production of substances containing proteins, as RER was observed more frequently than SER. This was also supported by the presence of uncondensed chromatin in the nucleus. Vesicular rough endoplasmic reticulum has not been found in other attines, only in the Dorylinae *Dorylu*s (Billen and Van Boven, 1987). The amount of glycogen in secretory cells of the metapleural gland

of major workers indicates that secretory cells store glycogen to be used later. In addition, the predominance of secretion granules of different sizes and electron densities observed in secretory cells of minor workers of *A. laevigata* shows that they are more involved in the production of secretion.

## Acknowledgements

We thank the Center for the Study of Social Insects – CEIS-UNESP (São Paulo State University) for the support in the collection and identification of specimens used in this study and to FAPESP (State of São Paulo Research Foundation) for financial support (grant n° 2008/54301-3).

## References

Alberts, B., Johnson, A. & Walter, P. (2004) *Biologia Molecular da Célula*, 4th ed. Artmed, Porto Alegre, 1549 pp.

Amabis, J.M. & Martho, G.R. (2003) *Fundamentos da Biologia Moderna*. Moderna, São Paulo.

Beattie, A.J., Turnbull, C., Hough, T., Jobson, S. & Knox, R.B. (1985) The vulnerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. *Am. J. Bot.*, 72, 606-614.

Beattie, A.J., Turnbull, C., Hough, T. & Knox, R.B. (1986) Antibiotic production: a possible function for the metapleural glands of ants (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.*, 79, 448-450.

Billen, J.P.L. (1985) Comparative ultrastructural of the poison and Dufour glands in Old and New World army ants (Hymenoptera, Formicidae). *Actes Coll. Insectes Soc.*, 2, 79-86.

Billen, J.P.L. (1986) Comparative morphology and ultrastructure of the Dufour gland in ants (Hymenoptera, Formicidae). *Entomol. Gener.*, 11, 165-181.

Billen, J.P.L. & Peusens, G. (1984) Ultrastructure de la Glande Propharyngienne chez les Fourmis Formicines (Hymenoptera, Formicidae). *Actes Coll. Insectes Soc.*, 1, 121-129.

Billen, J.P.L. & Van Boven, J.K.A. (1987) The metapleural gland in Old Word army ants: a morphological and ultrastructural description (Hymenoptera, Formicidae). *Revue de Zoologie Africaine*, 101, 31-41.

Bolton, B. (2003) Synopsis and classification of Formicidae. *Am. Entomol. Inst.*, 71, 1-370.

Bot, A.N.M., Obermayer, M.L., Hölldobler, B. & Boomsma, J.J. (2001) Functional morphology of the metapleural gland in the leaf-cutting ant *Acromyrmex octospinosus*. *Insectes soc.*, 48, 63-66.

Brown Jr., W.L. (1968) An hypothesis concerning the function of the metapleural glands in ants. *The American Naturalist*, 102, 188-191.

Do Nascimento, R.R.D., Schoeters, E., Morgan, E.D., Billen, J. & Stradling, D.J. (1996) Chemistry of metapleural gland secretions of three attine ants, *Atta sexdens rubropilosa*, *Atta cephalotes*, and *Acromyrmex octospinosus* (Hymenoptera: Formicidae). *J. Chem. Ecol.*, 22, 987-1000.

Falco, J.R. (1992) *Comparação ultra-estrutural entre glândulas pós-faringeas de* Camponotus rufipes *(Hymenoptera: Formicidae) parasitadas e não parasitadas por nematódeis.* Monografia apresentada ao Instituto de Biociências, Universidade Estadual Paulista, Rio Claro.

Gusmão, L.G. (2000) *Estudo comparado da glândula metapleural e sua secreção em diferentes castas de três espécies de* Atta *(Hymenoptera: Formicidae)*. Tese (Doutorado). ESALQ-USP, Piracicaba-SP, 60 pp.

Gusmão, L.G., Caetano, F.H. & Nakano, O. (2001) Ultramorphology of the metapleural gland in three species of *Atta* (Hymenoptera, Formicidae). *Iheringia, Zool.*, 91, 33-36.

Han, S.H. & Bordereau, C. (1982) Origin and formation of the royal fat of body of higher termite queens. *J. Morphol.*, 173, 17-28.

Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. The Belknap Press of Harvard University Press, Cambridge, MA, 732 pp.

Junqueira, L.C.U. & Junqueira, L.M.M.S. (2008) *Histologia básica*. Guanabara Koogan, Rio de Janeiro, 540 pp.

Maschwitz, U. (1974) Vergleichende Untersuchungen zur Funktion der Ameisenmetathorakaldrüse. *Oecologia*, 16, 303-310.

Maschwitz, U., Koob, K. & Schildknecht, H. (1970) Ein Beitrag zur Funktion der Metathoracaldrüse der Ameisen. *J. Insect. Physiol.*, 16, 387-404.

Noirot, C. & Quennedey, A. (1991) Glands, gland cells, glandular units: some comments on terminology and classification. *Ann. Soc. Entomol. Fr.*, 27, 123-128.

Paiva Castro, U., Zamith, A.P.L. & Mariconi, F.A.M. (1961) Contribuição para o conhecimento da "saúva de vidro" *Atta laevigata* Fred. Smith, 1858. *Anais da ESALQ*, 18, 313-325.

Schoeters, E. & Billen, J. (1992) Morphological and ultrastructural study of metapleural gland in *Diacamma* (Hymenoptera, Formicidae). In: *Proceedings of the European Congress of Social Insects*, pp. 239-247. Leuven University.

Schoeters, E. & Billen, J. (1993) Anatomy and fine struture of the metapleural gland in *Atta*. *Belg. J. Zool.*, 123, 67-75.

Souza, A.L.B., Soares, I.M.F., Cyrino, L.T. & Serrão, J.E. (2006) The metapleural gland in two subspecies of *Acromyrmex subterraneus* (Hymenoptera: Formicidae). *Sociobiology*, 47, 19-25.

Tulloch, G.S., Shapiro, J.E. & Hershenov, B. (1962) The ultrastructure of the metasternal glands of ants. *Bull. Brooklyn Entomol. Soc.*, 57, 91-101.

Vieira, A.S., Bueno, O.C. & Camargo-Mathias, M.I. (2010) The functional morphology of the metapleural gland of the leaf-cutting ant *Atta laevigata* (Formicidae: Attini). *Micron*, 41, 149-157.

Vieira, A.S., Bueno, O.C. & Camargo-Mathias, M.I. (2011) Secretory profile of metapleural gland cells of the leaf-cutting ant *Acromyrmex coronatus* (Formicidae: Attini). *Microsc. Res. Tech.*, 74, 76-83.

Wilson, E.O. (1971) *The Insect Societies*. Belknap Press, Cambridge, MA, 548 pp.

# *Capítulo 3*

**Perfil secretor das células da glândula metapleural da formiga cortadeira *Acromyrmex coronatus* (Formicidae: Attini)**

**Autores**: Alexsandro Santana Vieira, Odair Corrêa Bueno e Maria Izabel Camargo-Mathias
**Periódico**: Microscopy Research and Technique, v. 74, p. 76–83, 2011.

## RESUMO

As formigas apresentam um par de glândulas metapleurais localizadas nas extremidades posterolaterais do alitronco. Devido à importância na organização social das mesmas, o presente estudo teve por objetivo descrever nas três castas de operárias de *Acromyrmex coronatus* a morfofisiologia desta glândula, com ênfase na sua atividade secretora, e fazendo uso de técnicas histológicas e histoquímicas. Os resultados obtidos revelaram que as porções secretora e armazenadora da glândula estão conectadas pelas porções extracitoplasmáticas dos canalículos que conduzem a secreção oriunda de cada célula secretora até a câmara coletora. Essa secreção possui perfil glicolipoproteíco, porém nas operárias mínimas é encontrada secreção com caráter polissacarídico maior quando comparada com aquelas das operárias maiores, fato que confirmaria as funções da glândula metapleural na manutenção do jardim de fungo. Os resultados revelaram também forte positividade para RNA tanto nos núcleos quanto no citoplasma das células secretoras mostrando que as mesmas estão ativas na síntese de proteínas, elementos estes que são encontrados na secreção final. A técnica variante de CEC mostrou a sincronia da atividade secretora da glândula como um todo.

**Palavras-chave**: *Acromyrmex coronatus*, Attini, glândula metapleural, células secretoras.

# Secretory Profile of Metapleural Gland Cells of the Leaf-Cutting Ant *Acromyrmex coronatus* (Formicidae: Attini)

ALEXSANDRO SANTANA VIEIRA,[1] ODAIR CORREA BUENO,[1,2] AND MARIA IZABEL CAMARGO-MATHIAS[1]*
[1]*Departamento de Biologia, Instituto de Biociências, UNESP. Av. 24A, n°1515. Cx. Postal 199, CEP 13506-900, Rio Claro, SP, Brazil*
[2]*Centro de Estudos de Insetos Sociais (CEIS), Instituto de Biociências, UNESP. Av. 24A, n°1515. Cx. Postal 199, CEP 13506-900, Rio Claro, SP, Brazil*

*KEY WORDS* *Acromyrmex coronatus*; Attini; metapleural gland; secretory cells

*ABSTRACT* Ants present a pair of metapleural glands located at the posterolateral end of the thorax. Because of its importance in the social organization of ants, the present study was aimed at describing the morphophysiology of this gland in three worker castes of *Acromyrmex coronatus*, focused on secretory activity using histological and histochemical techniques. Our findings revealed that the secretory and the storage portions of this gland are connected by extracytoplasmic portion of canaliculi that drain the secretion from each secretory cell to the collecting chamber. This secretion contains glycoproteins. In minor workers, the secretion contains higher levels of polysaccharides when compared to that of major workers, supporting the role of the metapleural gland in the maintenance of the fungus garden. The nucleus as well as cytoplasm of secretory cells were strongly positive for RNA indicating that these cells are active in the synthesis of proteins and lipids, compounds found in the final secretion. The variant of the CEC revealed that the secretory activity of the entire gland is synchronous, as all cells exhibit the result. *Microsc. Res. Tech. 74:76–83, 2011.* 

## INTRODUCTION

In social insects, 63 types of exocrine glands have been described (39 only in Formicidae, 21 in Apidae, 14 in Vespidae, and 11 in Isoptera) (Billen, 1994). Several of these glands function individually and most are associated with the social organization of colonies, producing digestive enzymes, defense and lubricating compounds, while some glands secrete antibiotic compounds, such as the metapleural gland examined in this study. These are paired structures located in the postolateral ends of the thorax, under an elevated area of the exoskeleton that can be seen from the outside of the insect and that houses the reservoir to the metapleural gland (Hölldobler and Wilson, 1990).

Some ant species build their nests and feed on the ground on decomposing wood, where a diverse and abundant community of pathogenic microbes thrives in moist environments and constant temperatures, as well as the absence of light (Hölldobler and Wilson, 1990; Thorne and Traniello, 2003; Wilson, 1971). The adaptation of ants to several pathogens is possible due to compounds with antibiotic properties secreted by the metapleural gland, inhibiting the development of pathogens (Beattie et al., 1985, 1986; Do Nascimento et al., 1996; Maschwitz et al., 1970; Maschwitz, 1974). This adaptation to several types of pathogens have been considered one of the main events in the evolution of the sociability and the diversification of ants (Wilson, 1971).

The role of the secretion produced by metapleural glands of leaf-cutting ants is more significant than in other ants, as the compounds present in the secretion control pathogens and microorganisms that eventually become competitors in the fungus garden and that can affect its development and maintenance (Bot et al., 2001, 2002; Hölldobler and Wilson, 1990).

Thus, this study was aimed at investigating the morphophysiology of the components of the metapleural gland (secretory cells, canaliculi, collecting chamber, and reservoir) to understand the dynamics of secretory activity in three worker castes of *Acromyrmex coronatus* (Fabricius, 1804), since once some studies have reported that the secretions produced by attines have antibiotic properties (Beattie et al., 1986; Do Nascimento et al., 1996; Maschwitz, 1974; Maschwitz et al., 1970), which could have the potential of suppressing the growth of fungi, bacteria, and other organisms that might interfere in the development and maintenance of the fungus garden of the colony, in general, however, its morphophysiology is little known in ants. Still, according to Bot and Boomsma (1996) and Wilson (1971) minor workers or gardeners perform tasks associated with fungus growing and tending the brood, media or generalists perform tasks within of nests, including refuse disposal and tending the queen, and major or foragers ones that cut and transport plant material to the fungus garden. The species *A. coronatus* is locally known as "quenquén de árvore" occurring basically in Brazil and Bolivia, besides being economically considered as an important pest insect (Gonçalves, 1961).

## MATERIALS AND METHODS

For this study, we used 24 workers of the three castes of *A. coronatus*. Ants were separated by caste based on

*Correspondence to: M. I. Camargo-Mathias, Departamento de Biologia, Instituto de Biociências, UNESP. Av. 24A, n° 1515. Cx. Postal 199, CEP 13506-900, Rio Claro, SP, Brazil. E-mail: micm@rc.unesp.br

Received 18 February 2010; accepted in revised form 6 April 2010

Contract grant sponsor: FAPESP (São Paulo Research Foundation); Contract grant number: 2008/54301-3

DOI 10.1002/jemt.20876

Published online 13 May 2010 in Wiley Online Library (wileyonlinelibrary.com).

head size. The average head size of minor workers was 0.934 ± 0.004 mm; for media: 1.360 ± 0.127 mm; and majors: 1.716 ± 0.092 mm. Workers were obtained from colonies ~2 years of age from the campus of the São Paulo State University/UNESP, Rio Claro-SP, Brazil, and maintained at CEIS/UNESP/SP/Brazil (Center for the Study of Social Insects).

To obtain the metapleural glands, the legs and heads were removed from ants in Petri dishes under Zeiss stereomicroscope with the aid of dissecting forceps and microscissors, remaining only the mesossoma. Glands were fixed with specific fixatives for each technique used. The material was dehydrated in a series of ethanol solutions 70, 80, 90, and 95%, during 15 min each, embedded in resin for 24 h, transferred to plastic molds previously filled with resin and catalyzer. After polymerization, the material was sectioned at 4 μm with a Leica RM 2145 microtome, hydrated, and placed on glass slides.

## HISTOLOGY

### Harris Hematoxylin—Aqueous Eosin Staining (According to Junqueira and Junqueira, 1983)

Six metapleural glands (two of each caste) were fixed with 4% paraformaldehyde for 24 h (Junqueira and Junqueira, 1983). Histological sections were hydrated for 1 min in distilled water and stained with hematoxylin for 10 min. The material was maintained immersed in a cuvette with water for 4 min and rinsed with tap water to allow the reaction to occur. Sections were stained with eosin for 5 min and washed with tap water. After drying, slides with sections were mounted with Canada balsam and slipcovered for later observation and photo documentation under photomicroscope Motic BA 300 connected to an Intel Pentium 4 computer.

### Histochemistry

PAS reaction (Periodic Acid Schiff) (McManus, 1946), for polysaccharide detection, with counterstaining with methyl green for RNA detection.

Six metapleural glands (two of each caste) were fixed with Bouin for 24 h. Histological sections were hydrated for 1 min with distilled water and immediately transferred to a solution of periodic acid for 10 min. The material was then washed with distilled water for 1 minute and immersed in Schiff's reagent for 1 h, washed for 30 min with tap water, counterstained with methyl green for 20 s and rinsed again with water. After drying, slides with sections were mounted in Canada balsam and within 48 h, the material was examined and photographed with a photomicroscope Motic BA 300 connected to an Intel Pentium 4 computer.

### Variant of the CEC (Critical Electrolyte Concentration) for DNA and RNA Detection, According to Mello et al. (1993) and Mello (1997)

Six metapleural glands (two of each caste) were fixed with Bouin for 24 h. A histological sections were stained with 0.025% Toluidine blue in McIlvane buffer (pH 4.0) for 40 min at room temperature. The material was transferred to aqueous solution of 0.05 M $MgCl_2$ for 4 min, and washed with distilled water. After drying, slides with sections were mounted with Canada balsam and slipcovered for observation and photographic documentation with a photomicroscope Motic BA 300 connected to an Intel Pentium 4 computer.

### Baker Staining to Detect Total Lipids According to Martoja and Martoja-Pierson (1967), Modified by Giovannetti (Personal Communication, 2009)

Six metapleural glands (two of each caste) were fixed with formol calcium for 24 h. Histological sections were stained with calcium dichromate (5 g of calcium dichromate, 1 g of anhydrous calcium chloride, 100 mL of distilled water) for 18 h at room temperature. The material was then washed three times with distilled water and treated with acid hematein (0.05 g of crystallized hematoxylin in 48 mL of distilled water, 1 mL of 1% aqueous solution of sodium iodate, 1 mL of acetic acid) for 5 h at room temperature. Sections were washed with distilled water (three times) and after drying, slides with sections were mounted in glycerinated gelatin and slipcovered for observation and photographic documentation with a photomicroscope Motic BA 300 connected to an Intel Pentium 4 computer.

### Bromophenol Blue Staining to Detect Total Proteins, According to Pearse (1985)

Six metapleural glands (two of each caste) were fixed with 4% paraformaldehyde and 0.9% NaCl in phosphate buffer 10% (0.1 M, pH 7.4) for 24 h. Sections were stained with bromophenol blue for 1 h at room temperature, and immersed in aqueous solution of 0.5% acetic acid for 5 min and then in tertiary butyl alcohol for 5 min. Slides were cleared in xylol and mounted in Canada balsam for observation and photographic documentation with a photomicroscope Motic BA 300 connected to an Intel Pentium 4 computer.

## RESULTS

### Histology

The present study revealed that the metapleural glands of *A. coronatus* ants of the three castes are paired structures composed of two portions that were termed in this study as (a) secretory portion consisted of secretory cells, and (b) storage portion consisted of the collecting chamber and the reservoir. The two portions are connected by extracytoplasmic canaliculi (Figs. 1A–1F). The secretory portion is innermost to the animal's body while the wall of the storage or reservoir is in contact with the integument, thus outermost in relation to the secretory portion (Figs. 2A–2F).

The secretory cells are oval or elongated with only one large nucleus and form clusters. From each cell, a canaliculus conducts the secretion and is divided into two distinct portions: (a) intracytoplasmic and, (b) extracytoplasmic. The extracytoplasmic portions of canaliculi form clusters (group of 16 canaliculi) that open in a perforation of the sieve plate of the collecting chamber (Fig. 1D). From this chamber, the secretion drains into the reservoir (Figs. 1A, 1C, and 1E). The diameter of the intracytoplasmic portion of canaliculi is smaller than that of the extracytoplasmic portion (Figs. 1C–1E). Both portions are lined internally by a cuticle, which is thicker in the extracytoplasmic portion (Figs. 1C–1E). Nuclei of cells that produce canaliculi are observed in Figures 1C and 1E.

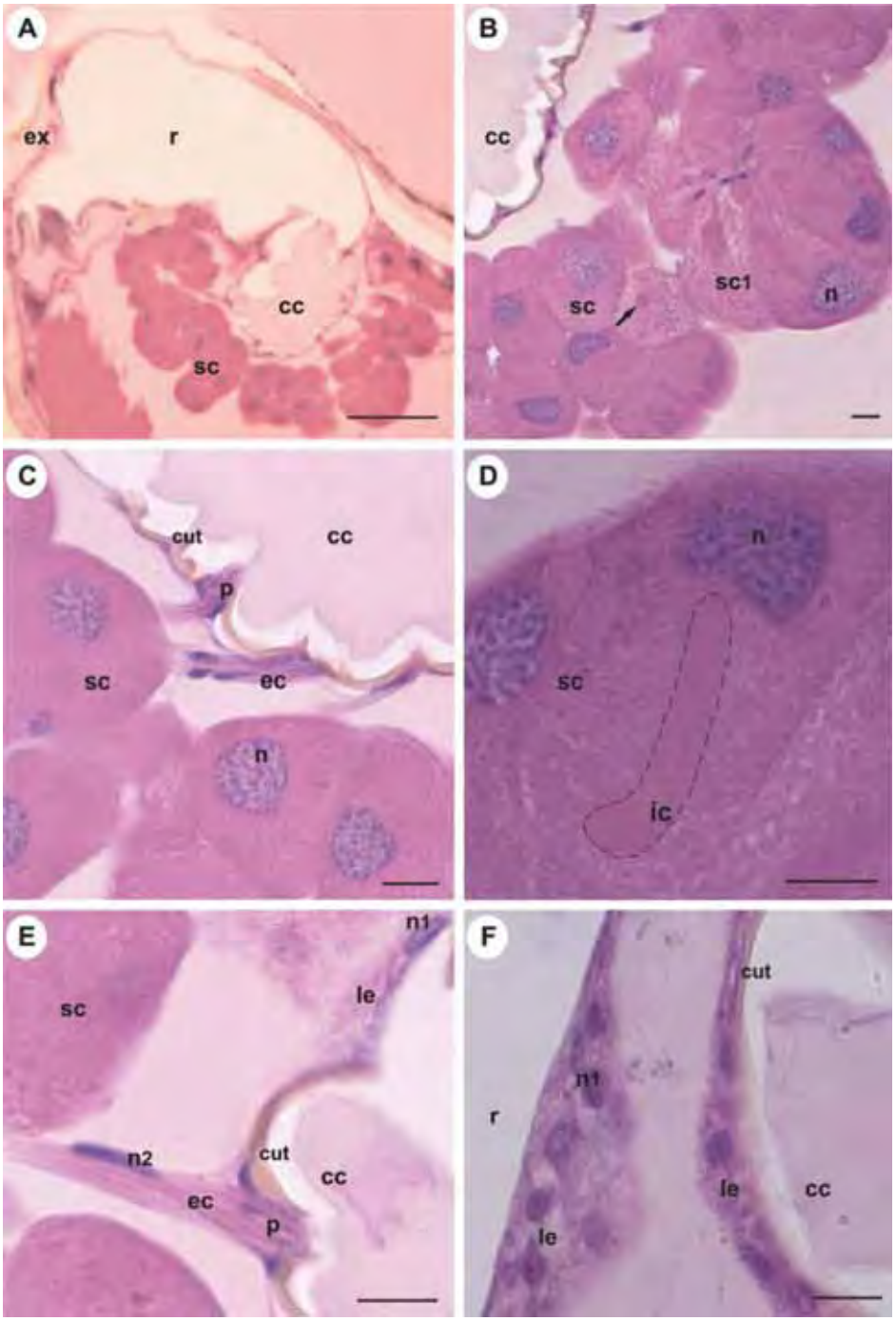



Fig. 1. Histological sections of the metapleural gland of minor, media, and major workers of *A. coronatus* stained with hematoxylin and eosin. (**A**) General view of the metapleural gland of media worker. Note secretory cells (sc), collecting chamber (cc), reservoir (r), and exoskeleton (ex). Scale bar = 100 μm. (**B**) Details of secretory cells (sc), nucleus (n), intracytoplasmatic canaliculus (arrow), and collecting chamber (cc) of major workers. Scale bar = 10 μm. Note cytoplasm of secretory cells (sc1) with heterogeneous contents. (**C**) Details of the extracytoplasmic portions of canaliculi (ec), sieve plates (p), cuticle (cut), secretory cells (sc), and nucleus (n) of media worker. Note canaliculi individually leaving secretory cells and opening in the sieve plate of the collecting chamber (cc). Scale bar = 10 μm. (**D**) Details of a longitudinal section of a secretory cell with intracytoplasmic canaliculus (ic) (dotted lines), and nuclei (n) of secretory cells (sc) of major worker. Scale bar = 10 μm. (**E**) View of extracytoplasmic canaliculi (ec) and nuclei (n2) of cells of canaliculi, lining epithelium (le) and corresponding nuclei (n1), collecting chamber (cc), cuticle (cut), sieve plate (p) in longitudinal section, and secretory cell (sc) of major worker. Scale bar = 10 μm. (**F**) Details of cells of the lining epithelium (le) with corresponding nuclei (n1), collecting chamber (cc), cuticle (cut), and reservoir (r) of major worker. Scale bar = 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

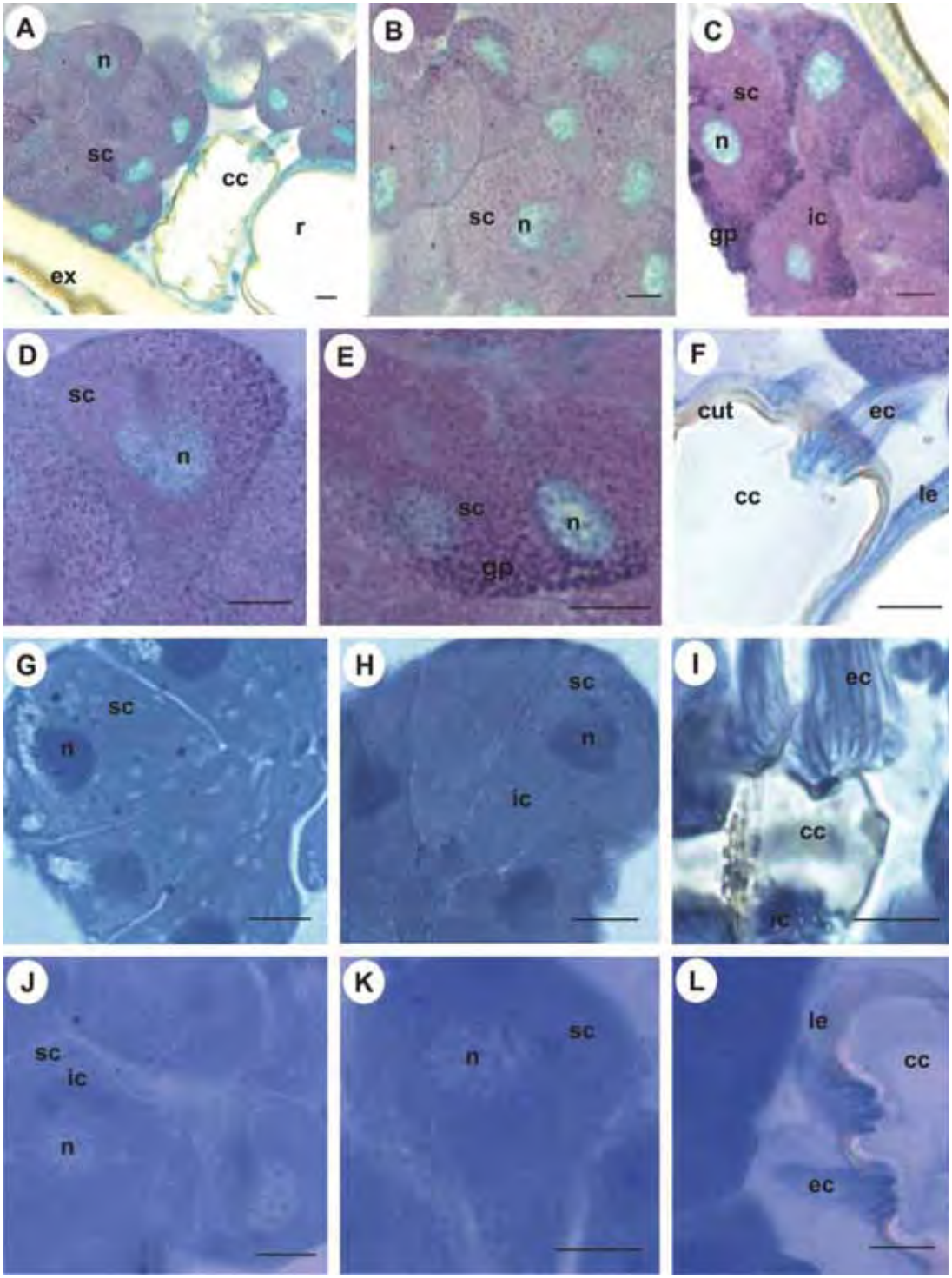



Fig. 2. Histological sections of the metapleural gland of minor, media, and major workers of *A. coronatus* stained with PAS/counterstained with methyl green (**A–F**), the Baker method (**G–I**), and bromophenol blue (**J–L**). (A–F) Details of secretory cells (sc) showing the cytoplasm, nucleus (n), intracytoplasmic canaliculi (ic) in cross section, extracytoplasmic canaliculi (ec) in longitudinal section, collecting chamber (cc) lining epithelium (le) of the collecting chamber (cc), cuticle (cut), reservoir (r), and exoskeleton (ex) of major worker. Note the presence of granules of polysaccharides (gp) in the cytoplasm, mainly in the region near the cell boundaries of minor workers (C and E): A, B, D, and F major workers. Scale bar = 10 μm. (G–I) Details of secretory cells (sc) showing cytoplasm, nucleus (n), intra (ic) and extracytoplasmic canaliculi (ec) in longitudinal and cross sections, collecting chamber (cc): G major workers, H and I minor workers. Scale bar = 10 μm. (J–L) Details of secretory cells (sc)showing cytoplasm, nucleus (n), intra (ic) and extracytoplasmic canaliculi (ec) in longitudinal section, collecting chamber (cc), lining epithelium (le) of major workers. Scale bar = 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Some secretory cells of the metapleural gland of major workers exhibit heterogeneous cytoplasm, as observed in Figure 1B.

The collecting chamber is a portion of the reservoir with irregular surface (sieve plates) in the region where clusters of extracytoplasmic canaliculi open (Figs. 1C and 1E). The sieve plate have approximately eight perforations/plate. The plate is internally lined by a simple squamous epithelium, with flattened cells and nuclei, and is lined by a cuticular intima that forms small folds (Figs. 1C and 1E). Attached to the collecting chamber is the reservoir, a membranous sac internally lined by a simple squamous epithelium. Similarly to the collecting chamber, the reservoir is also lined by cuticular intima (Figs. 1E–1F), but devoid of folds.

### Histochemistry

***Polysaccharide and RNA Detection.*** The PAS technique with counterstaining with methyl green revealed that in the metapleural glands of all workers of the three castes of *A. coronatus*, the cytoplasm of secretory cells are strongly positive for polysaccharides (Figs. 2A–2E). Granules containing polysaccharides are present in the entire cytoplasm. In the periphery of cells, granules are concentrated near the cell boundaries, especially in minor workers (Figs. 2C and 2E). The cytoplasm of secretory cells is not stained or is weakly stained for RNA. Unlike the cytoplasm, the nuclei of these cells are strongly stained for RNA, indicating the presence of ribonucleoproteins, as expected (Figs. 2A–2E).

The peripheral region of the intracytoplasmic canaliculi is moderately stained with PAS (Fig. 2C), while the extracytoplasmic portions are weakly stained in the three castes examined (Fig. 2F). The lumen of canaliculi in general is not stained for RNA.

The epithelial cells lining the collecting chamber and the reservoir are also negative to polysaccharides. However, weak to moderate staining for RNA is observed, indicating that its contents also contains ribonucleoproteins (Fig. 2A).

***DNA and RNA Detection.*** The CEC technique used in the metapleural glands of minor, media, and major workers of *A. coronatus* revealed that the nucleus and the cytoplasm of secretory cells contain ribonucleoproteins (Figs. 3A–3F). The nucleus of secretory cells exhibits ribosomal RNA. The cytoplasm of secretory cells also contain ribosomal RNA, but less intensively stained than the nucleus (Figs. 3C–3E).

The nuclei of cells of canaliculi are moderately stained for RNA in the three castes (Fig. 3F). The staining pattern of epithelial cells lining the collecting chamber and the reservoir also vary from weak to moderate for DNA and ribosomal RNA, respectively (Fig. 3F).

***Total Lipid Detection.*** The metapleural glands of *A. coronatus* workers contained total lipids (Figs. 2G–2I). The cytoplasm of secretory cells and extracytoplasmic canalliculi are strongly stained in the three worker castes (Figs. 2G–2I).

The periphery of the intracytoplasmic portion of canaliculi is strongly stained, while the lumen of intra and extracytoplasmic portions is weakly stained (Figs. 2H–2I). The epithelial cells lining the collecting chamber and the reservoir are moderately positive in the three castes (Fig. 2I).

***Protein Detection.*** Bromophenol blue staining revealed the presence of proteins in the metapleural glands of minor, media, and major workers (Figs. 2J–2L). The cytoplasm of secretory cells, the intracytoplasmic periphery of canaliculi, and the extracytoplasmic portions of canaliculi are strongly stained in all castes. The cytoplasm of secretory cells is more intensively stained than the nucleus. The epithelial cells lining the collecting chamber and the reservoir are moderately stained, while the nucleus of secretory cells and the lumen of canaliculi are weakly stained (Figs. 2J and 2L) in the three castes.

The results of histochemical tests were summarized in Table 1.

## DISCUSSION

The morphologic results obtained in the present study on the metapleural gland of minor, media, and major workers of *A. coronatus* support those by Hölldobler and Wilson (1990) for *Atta cephalotes*, which the gland is located in the ventral-postero portion of the thorax, under an elevated area of the exoskeleton that can be seen from the outside of the insect and that houses the reservoir to the metapleural gland in both sides of the metathorax.

More recent studies conducted by Vieira et al. (2010) also reported this morphology in *Atta laevigata* workers. As already described in the literature for other ant species, the metapleural gland is divided into two portions: a secretory and a store portion (Bot et al., 2001; Gusmão, 2000; Souza et al., 2006).

In *A. subterraneus* (Souza et al., 2006), *A. octospinosus* (Bot et al., 2001), as well as in other attines, such as *Atta bisphaerica*, *A. sexdens rubropilosa* (Gusmão, 2000), and *A. laevigata* (Vieira et al., 2010), the secretory portion exhibit round and or oval-shaped cells. In the subfamily Ponerinae, such as *Diacamma rugosum* and *D. vagans* (Schoeters and Billen, 1992), the secretory cells in the center of the metapleural gland are round, while those located in the periphery are elongated and/or cylindrical.

Secretory cells of the metapleural gland of *A. coronatus*, in all castes examined, are close to one another, but not fused and are bound together by only a loose conjunctive tissue. The same organization was observed in *A. bisphaerica*, *A. sexdens sexdens,* and *A. sexdens rubropilosa* by Schoeters and Billen (1993) and *A. laevigata* by Vieira et al. (2010). The presence of conjunctive tissue binding the secretory cells is justified, as each cell works as an independent unit with its own collecting canaliculus with the extracytoplasmic portion arriving at the reservoir along with others, forming clusters and opening in perforations in the sieve plate of the collecting chamber, region that precedes the lumen of the reservoir. This arrangement was also observed in the metapleural glands of *A. laevigata* (Vieira et al., 2010) and the mandibular glands of the ant *Atta sexdens* (Pavon and Camargo-Mathias, 2001).

The presence of heterogeneous content in the cytoplasm of some secretory cells indicates that the secretion is composed of various substances that form complexes.

In this study, the cytoplasm of secretory cells of the metapleural gland of *A. coronatus* in the three castes is strongly positive to polysaccharides, total lipids, and

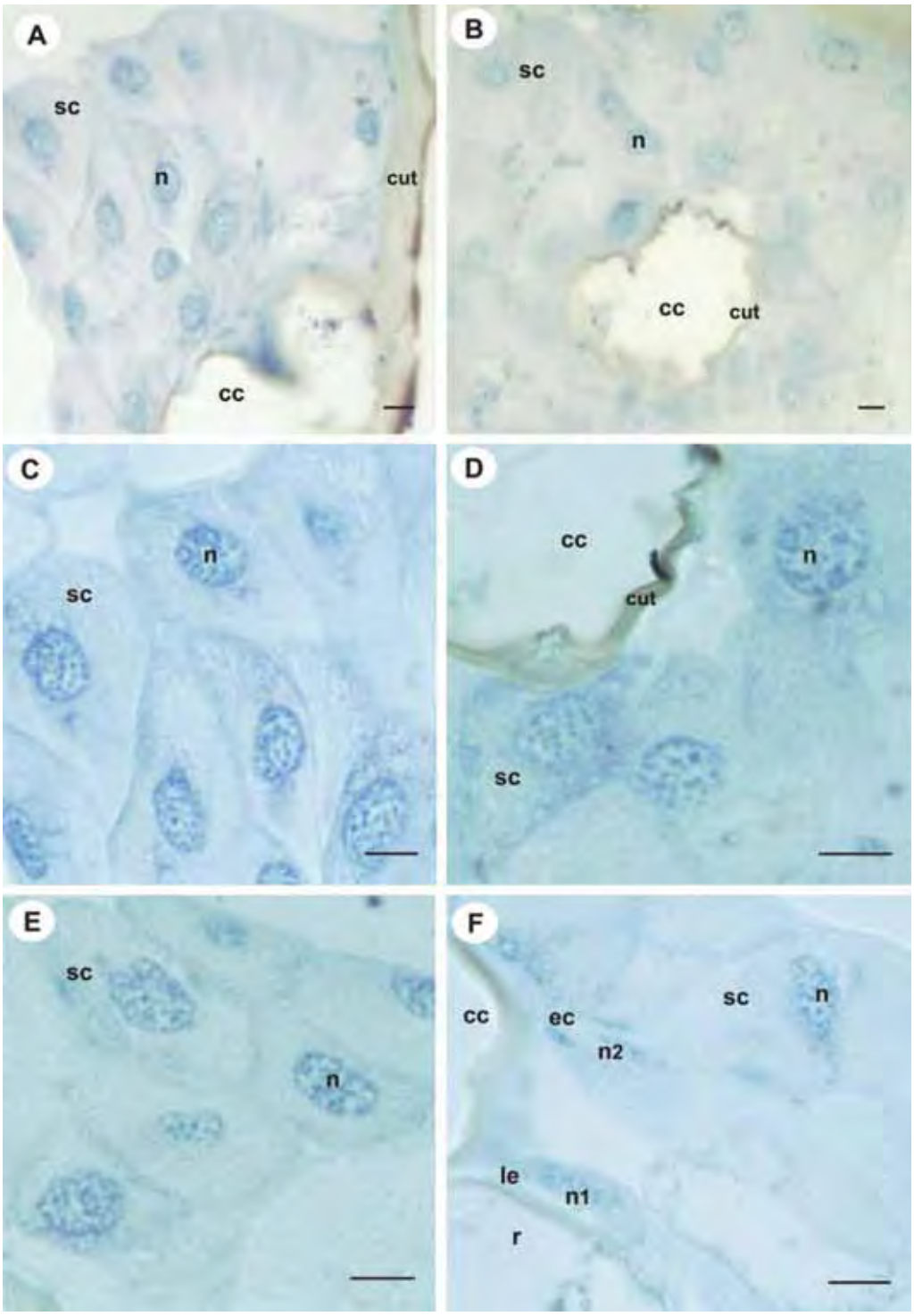



Fig. 3. Histological sections of the metapleural gland of minor, media, and major workers of *A. coronatus* stained with the variant of the CEC (Critical Electrolyte Concentration). (**A**, **B**) View of secretory cells (sc) and their corresponding nuclei (n), collecting chamber (cc), and cuticle (cut): (A) minor workers and, (B) media workers. Scale bar = 10 μm. (**C–E**) Details of secretory cells (sc) and their corresponding nuclei (n), collecting chamber (cc), and cuticle (cut): (C) minor workers, (D) media workers, and (E) major workers. Scale bar = 10 μm. (**F**) Details of secretory cells (sc) showing cytoplasm, nucleus (n), extracytoplasmic canaliculus (ec) and its corresponding nucleus (n2) in longitudinal section, and lining epithelium (le) and corresponding nuclei (n1) of the collecting chamber (cc) and reservoir (r) of minor workers. Scale bar = 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

*TABLE 1. Results of histochemical tests of the metapleural glands of* A. coronatus *workers*

| | Histochemical tests | | | | | |
|---|---|---|---|---|---|---|
| | | | CEC | | | |
| Regions of the metapleural gland | PAS | Methyl green | DNA | RNA | Baker | Bromophenol blue |
| Cytoplasm of secretory cells | Ma + + + | Ma − | Ma − | Ma + + | Me + + + | Mi + + + |
| | Me + + + | Me − | Me − | Me + + | Me + + + | Me + + + |
| | Mi + + + | Mi − | Mi − | Mi + + | Mi + + + | Mi + + + |
| Nucleus of secretory cells | Ma − | Ma + + + | Ma + | Ma + + + | Ma + + + | Ma + |
| | Me − | Me + + + | Me + | Me + + + | Me + + + | Me + |
| | Mi − | Mi + + + | Mi + | Mi + + + | Mi + + + | Mi + |
| Periphery of the intracytoplasmic canaliculus | Ma + + | Ma − | Ma − | Ma − | Ma + + + | Ma + + + |
| | Me + + | Me − | Me − | Me − | Me + + + | Me + + + |
| | Mi + + | Mi − | Mi − | Mi − | Mi + + + | Mi + + + |
| Extracytoplasmic canaliculus | Ma + | Ma − | Ma − | Ma + +[a] | Ma + + + | Ma + + + |
| | Me + | Me − | Me − | Me + +[a] | Ma + + + | Ma + + + |
| | Mi + | Mi − | Mi − | Mi + +[a] | Mi + + + | Mi + + + |
| Lumen of intra and extracytoplasmic canaliculi | Ma − | Ma − | Ma − | Ma − | Ma + | Ma + |
| | Me − | Me − | Me − | Me − | Me + | Me + |
| | Mi − | Mi − | Mi − | Mi − | Mi + | Mi + |
| Epithelial cells lining the collecting chamber | Ma − | Ma + | Ma +[a] | Ma + +[a] | Ma + + | Ma + + |
| | Me − | Me + | Me +[a] | Me + +[a] | Me + + | Me + + |
| | Mi − | Mi + | Mi +[a] | Mi + +[a] | Mi + + | Mi + + |
| Epithelial cells lining the reservoir | Ma − | Ma + + | Ma +[a] | Ma + +[a] | Ma + + | Ma + + |
| | Me − | Me + + | Me +[a] | Me + +[a] | Me + + | Me + + |
| | Mi − | Mi + + | Mi +[a] | Mi + +[a] | Mi + + | Mi + + |

Ma, major worker; Me, media worker; Mi, minor worker; +, weak staining; ++, moderate staining; +++, strong staining; −, negative.[a]Positive reaction only in the nucleus.

proteins, indicating that: (a) these cells produce these compounds in larger quantities or (b) in addition to producing these compounds, they can also to absorb part of these elements from the hemolymph. After being produced or absorbed, the compounds will make up the final secretion of the metapleural gland, used in the production of pheromones. Gusmão (2000) observed, using transmission electron microscopy (TEM), significant amounts of rough and smooth endoplasmic reticulum in the metapleural gland of *A. bisphaerica* and of *A. sexdens rubropilosa*, confirming its role in the production of lipids and proteins. Another role described in other studies (Bot et al., 2002) for the secretory cells of metapleural glands is the production of antibiotic compounds, supporting the need of synthesizing large quantities of lipids and proteins.

The secretory cells of the metapleural gland of minor workers of *A. coronatus* exhibit more polysaccharide granules compared to that of major workers, suggesting that the secretory activity of polysaccharides in gland cells of minor workers is higher, confirming their role in the maintenance of the fungus garden, while major workers are involved with tasks related to cutting and collecting substrates, as well as colony defense (Hölldobler and Wilson, 1990).

Another important finding of our study was the strong staining for ribosomal RNA in the nuclei (ribonucleuproteins) as well as in the cytoplasm of secretory cells of metapleural gland of *A. coronatus*. This and the presence of large nuclei suggest the continuous synthesis of compounds found in the final secretion. The nucleus varies in size according to the need of the cell to synthesize ribosomal RNA (rRNA), which is used to produce small and large ribosomal subunits. These subunits are synthesized in the nucleus and assembled in the cytoplasm, avoiding the formation of complete ribosomes in the nucleus and the risk of synthesis of proteins in the nucleus (De Robertis, 2008).

Thus, the secretory activity is probably continuous and controlled by the task performed by each individual in the colony. *A. coronatus* minor workers or gardeners have synthesis of secretion in highest level, due to their participation in the maintenance of the fungus garden, growing and caring the brood, and media or generalists performing tasks inside the nests, including refuse disposal and caring the queen, contrary to major ones, which collecting and transporting substrate, and make the defense of the colony, tasks these do not require a lot of secretion activity by the metapleural gland. In the present study, our results clearly demonstrated that secretory cells work in synchrony with the production of secretion, as all cells are at the same stage of activity, as revealed by CEC.

According to the classification of Noirot and Quennedey (1991), secretory cells of metapleural glands of *A. coronatus* are class III cells, as they have cuticular canaliculi divided into intra and extracellular portions. The intracellular portion of canaliculi drains the secretion produced by the cell and is probably responsible for modifying it, as class III secretory cells have microvilli surrounding the intracytoplasmic portion of canaliculi. The extracytoplasmic portion, which conducts the secretion from the cytoplasm of the cell to the collecting chamber, lacks these microvilli and only conducts the secretion. This was confirmed in *A. coronatus* by histochemical tests, as the periphery of intracytoplasmic canaliculi was strongly stained for proteins while the lumen of extracytoplasmic canaliculi was weak stained, showing that in addition to collecting and transporting the secretions, they also modify the composition of the secretion by reabsorbing elements that might be recycled by the individual. These results indicated that the secretion that leaves the cytoplasm of secretory cells does not have the same composition than that found in the extracytoplasmic portion of canaliculi, as also observed in *A. laevigata* (Vieira et al., 2010).

After being produced by metapleural gland cells, the secretion of *A. coronatus* is transported to the reservoir via canaliculi. Similarly to the observed in the present study, the presence of canaliculi conducting the secretion was also reported in other attines, such as *A. bisphaerica*, *A. s. sexdens A. s. rubropilosa* (Bot et al., 2001; Hölldobler and Engel-Siegel, 1984; Schoeters and Billen, 1993) and in *A. laevigata* (Vieira et al., 2010).

The collecting chamber of the metapleural gland of *A. coronatus* is lined by chitinous plates with various perforations, through which the clusters of canaliculi release the secretion produced by each secretory cell. Similar data were also observed in *A. laevigata* (Vieira et al., 2010), *A. bisphaerica*, *A. sexdens rubropilosa* (Gusmão, 2000), and *Myrmecia nigrocincta* (Tulloch et al., 1962). In the ponerine ant *D. vagans*, however, a single large plate is observed in the wall of the collecting chamber (Schoeters and Billen, 1992).

In *A. coronatus*, the reservoir and the collecting chamber, considered as the portions of the metapleural gland responsible storing the secretion, are lined by a single squamous epithelium. However, this epithelium also secretes the cuticular intima, which protects the epithelial wall from the acids of the secretion, and/or protects the secretion from contaminants that could minimize or inactivate its action, as also observed in *A. laevigata* (Vieira et al., 2010).

The epithelial cells lining the collecting chamber and the reservoir were weakly to moderate stained for RNA, and moderately stained for lipids and proteins, indicating that these cells are active and constantly being renovated, as the secretion is also in contact with the lining epithelium.

Thus, based on the results obtained in our study, the metapleural gland of minor, media, and major workers of *A. coronatus* have a morphology similar to that of other attines of the genera *Atta* and *Acromyrmex*, contrary from the observed in ponerine ants of the genera *Diacamma*, which have different secretory cells and perforated plates of the collecting chamber regarding their shape as well as structure (Schoeters and Billen, 1992). The secretion of the metapleural gland of *A. coronatus* is composed of glycolipoproteins, still indicating that minor workers are more actives in the synthesis than media and major ones. The presence of ribosomal RNA in nuclei as well as a large nucleus in each secretory cell indicates that they are synchronically synthesizing the secretion.

## ACKNOWLEDGMENTS

The authors thank the Center for the Study of Social Insects-CEIS-UNESP/SP/Brazil (São Paulo State University) for the aid in the collection and identification of specimens used in this study.

## REFERENCES

Beattie AJ, Turnbull C, Hough T, Jobson S, Knox RB. 1985. The vulnerability of pollen and fungal spores to ant secretions: Evidence and some evolutionary implications. Am J Bot 72:606–614.

Beattie AJ, Turnbull C, Hough T, Knox RB. 1986. Antibiotic production: A possible function for the metapleural glands of ants (Hymenoptera: Formicidae). Ann Entomol Soc Am 79:448–450.

Billen J. 1994. Morphology of exocrine glands in social insects: an update 100 years after Ch. Janet. In: Lenoir A, Arnold G, Lepage M, editors. "Les Insectes Sociaux." Publications Université Paris Nord, p. 214.

Bot ANM, Boomsma JJ. 1996. Variable metapleural gland size-allometries in *Acromyrmex* leafcutter ants (Hymenoptera: Formicidae). J Kans Entomol Soc 69:375–383.

Bot ANM, Obermayer ML, Hölldobler B, Boomsma JJ. 2001. Functional morphology of the metapleural gland in the leaf-cutting ant *Acromyrmex octospinosus*. Insectes Soc 48:63–66.

Bot ANM, Ortius-Lechner D, Finster K, Maile R, Boomsma JJ. 2002. Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. Insectes Soc 49:363–370.

De Robertis, Jr, Hib J, Ponzio R. 2008. In: Trad Paulo AFD, Alves JGG, Neto JVG, Ierusalimschy R, editors. De Robertis Biologia celular e Molecular. Transcrição do DNA. Rio de Janeiro: Guanabara Koogan. pp. 275–291.

Do Nascimento RRD, Schoeters E, Morgan ED, Billen J, Stradling DJ. 1996. Chemistry of metapleural gland secretions of three attine ants, *Atta sexdens rubropilosa*, *Atta cephalotes*, and *Acromyrmex octospinosus* (Hymenoptera: Formicidae). J Chem Ecol 22:987–1000.

Gonçalves C. 1961. O gênero *Acromyrmex* no Brasil (Hym. Formicidae). Studia Ent 4:113–180.

Gusmão LG. 2000. Estudo comparado da glêndula metapleural e sua secreção em diferentes castas de três espécies de *Atta* (Hymenoptera: Formicidae). Tese (Doutorado): ESALQ-USP, Piracicaba-SP. 60 p.

Hölldobler B, Engel-Siegel H. 1984. On the metapleural gland in ants. Psyche 91:201–224.

Hölldobler B, Wilson EO. 1990. The Ants. Cambridge: Harvard Univ. Press.

Junqueira LCU, Junqueira LMMS. 1983. Técnicas básicas de citologia e histologia. Livraria Editora Santos. pp. 48–81.

Martoja R, Martoja-Pierson M. 1967. Initiation aux techneques de l'histologie animale. Masson et cie, éditeurs, bd St-Germain, Paris IV.

Maschwitz U. 1974. Vergleichende Untersuchungen zur Funktion der Ameisenmetathorakaldrüse. Oecologia 16:303–310.

Maschwitz U, Koob K, Schildknecht H. 1970. Ein Beitrag zur Funktion der Metathoracaldrüse der Ameisen. J Insect Physiol 16:387–404.

McManus JFA. 1946. Histological demonstration of mucin after periodic acid. Nature 158:202.

Mello MLS. 1997. Cytochemistry of DNA. RNA and nuclear proteins. Braz J Genet 20:257–264.

Mello MLS, Vidal BC, Dantas MM, Monteiro ALP. 1993. Discrimination of the nucleolus by a critical electrolyte concentration method. Acta Histochem Cytochem 26:1–3.

Noirot C, Quennedey A. 1991. Glands, gland cells, glandular units: Some comments on terminology and classification. Ann Soc Entomol Fr 27:123–128.

Pavon LF, Camargo-Mathias MI. 2001. A morpho-histological and ultrastructural studies of the mandibular glands of *Atta sexdens* worker ants (Hymenoptera: Formicidae). Sociobiology 38:449–464.

Pearse AGE. 1985. Histochemistry. Theoretical and applied, 4th ed. London: J & A Churchill.

Souza ALB, Soares IMF, Cyrino LT, Serrão JE. 2006. The metapleural gland in two subspecies of *Acromyrmex subterraneus* (Hymenoptera: Formicidae). Sociobiology 47:19–25.

Schoeters E, Billen J. 1992. Morphological and ultrastructural study of metapleural gland in *Diacamma* (Hymenoptera, Formicidae). In: European Congress of Social Insects, 1o, Leuven, 1991. Leuven-Proceedings Leuven, Leuven University. pp. 239–247.

Schoeters E, Billen J. 1993. Anatomy and fine struture of the metapleural gland in *Atta*. Belg J Zool 123:67–75.

Thorne BL, Traniello JFA. 2003. Comparative social biology of basal taxa of ants and termites. Annu Rev Entomol 48:283–306.

Tulloch GS, Shapiro JE, Hershenov B. 1962. The ultrastructure of the metasternal glands of ants. Bull Brooklyn Entomol Soc 57:91–101.

Vieira AS, Bueno OC, Camargo-Mathias MI. 2010. The functional morphology of the metapleural gland of the leaf-cutting ant *Atta laevigata* (Formicidae: Attini). Micron 41:149–157.

Wilson EO. 1971. The Insect Societies. Cambridge, MA: Harvard University Press.

# *Capítulo 4*

**Diferenças morfofisiológicas da glândula metapleural entre formigas cultivadoras e não cultivadoras de fungos (Hymenoptera, Formicidae)**

**Autores**: Alexsandro Santana Vieira, Odair Corrêa Bueno e Maria Izabel Camargo-Mathias
**Periódico**: Submetido ao periódico Plos one (2012)
**Situação**: corrigido e resubmetido

## RESUMO

A glândula metapleural órgão exclusivo das formigas tem como principal papel o de produzir secreções que inibem a proliferação de diferentes tipos de patógenos. O presente estudo investigou as diferenças morfofisiológicas entre a glândula metapleural de três formigas não cultivadoras de fungos das tribos Ectatommini, Mymicini e Blepharidattini, e de formigas cultivadoras de fungos, duas atines basais e três derivadas. A glândula metapleural das formigas não cultivadoras de fungos (Ectatommini, Mymicini e Blepharidattini) e das atine basais possuem menor número de células secretoras do que aquelas das formigas atines derivadas (formigas cortadeiras). Também, a glândula metapleural tem mais agrupamentos de células secretoras e placas perfuradas, confirmando maior capacidade de produção e de armazenamento de secreção em formigas derivadas cultivadoras de fungos. Suas glândulas também produzem maiores teores de polissacarídeos e de lipídios ácidos do que Mymicini, Blepharidattini e Attini basal. Os resultados confirmaram diferenças morfofisiológicas sugerindo que as glândulas metapleurais das atines derivadas (formigas cortadeiras) são mais desenvolvidas para produzir grande quantidade de secreção ativa contra a proliferação de fungos e bactérias não desejados no jardim de fungo, quando comparado com atines basal e formigas não cultivadoras de fungos, provavelmente as formigas cortadeiras pode ter evoluído glândulas metapleurais desenvolvidas em resposta a forte pressão de parasitas.

**Palavras-chave**: glândulas exócrinas, histologia, histoquímica, morfologia interna.

# Morphophysiological Differences between the Metapleural Glands of Fungus-Growing and Non–fungus-Growing Ants (Hymenoptera, Formicidae)

Alexsandro Santana Vieira[1], Odair Correa Bueno[2] & Maria Izabel Camargo-Mathias[1]

[1]UNESP – Universidade Estadual Paulista, Campus Rio Claro. Departamento de Biologia. Av. 24A, nº1515. Cx. Postal 199 – CEP: 13506-900 – Rio Claro, SP- Brazil

[2] Centro de Estudos de Insetos Sociais. Instituto de Biociências, UNESP - Univ Estadual Paulista, Campus Rio Claro. CEP: 13506-900 – Rio Claro, SP- Brazil

Corresponding author: M. I. Camargo-Mathias. E-mail: micm@rc.unesp.br

Keywords: exocrine glands, histology, histochemistry, internal anatomy

**Abstract**

The metapleural gland is an organ unique to ants. Its main role is to produce secretions that inhibit the proliferation of different types of pathogens. The aim of the present study was to examine the morphophysiological differences between the metapleural gland of 3 non–fungus-growing ants of the tribes Ectatommini, Myrmicini, and Blepharidattini and that of 5 fungus-growing ants from 2 basal and 3 derived attines. The metapleural gland of the non–fungus-growing ants and the basal attine ants has fewer secretory cells than that of the derived attine ants (leaf-cutting ants). In addition, the metapleural gland of the latter had more clusters of secretory cells and sieve plates, confirming a greater storage capacity and demand for secretion in these more advanced farming ants. The glands of the derived attine ants also produced higher levels of polysaccharides and acidic lipids than those of Myrmicini, Blepharidattini, and basal attines. Our results confirm morphophysiological differences between the metapleural glands of the derived attines and those of the basal attines and non–fungus-growing ants, suggesting that the metapleural glands of the derived attines (leaf-cutting ants) are more developed to produce greater secretions against the proliferation of unwanted fungi and bacteria in the fungal garden. It is possible that leaf-cutting ants may have evolved more developed metapleural glands in response to stronger pressure from parasites.

## Introduction

Fungus-growing ants (Attini) are especially interesting biological models for comparative studies since they comprise a monophyletic tribe [1] and show distinct evolutionary transitions. One of these transitions involves fungus-growing behavior and the other involves the gradual change in colony-size. Eight of the 12 genera (basal attines, such as *Apterostigma* and *Mycetarotes*) form small colonies (<100 individuals) that use several types of detritus, including dead insects and droppings, as substrate to cultivate their fungus [2,3]. The other 4 genera (derived attines), including *Trachymyrmex* [2,3], *Sericomyrmex* [4], and leaf-cutting ants, cultivate their fungi on plants and exhibit advanced levels of mutualism, since the fungi produce food bodies rich in proteins. Another derived trait of leaf-cutting ants is multiple-queen mating. The leaf-cutting ants *Acromyrmex* and *Atta* are considered apomorphic among the derived attines and are distinctly different since they have polymorphic workers that exclusively use fresh vegetation as a substrate for their fungi. In addition, their colonies are formed of tens of thousands (*Acromyrmex*) to millions (*Atta*) of individuals [2,3]. On the basis of morphology, Attini are phylogenetically close to *Blepharidatta* and *Wasmannia* [5,6], which do not cultivate fungus [7]. Further, the basal ants of the subfamily Ectatomminae are not fungus growers but use their sting to hunt arthropods (insects, among other invertebrates) [6]. In addition, the species *Ectatomma brunneum* has monomorphic workers with a small number of individuals in the colony, reaching approximately 100 [8]. A possible structural synapomorphy of the metapleural gland may exist between Ectatomminae and Myrmicinae, in which the external and internal morphologies might be very similar among the species of these subfamilies [8].

The fungus-growing behavior exhibited by attines shows major evolutionary changes that are unique to ants: (1) The transition to fungus farming by attine ants ~50

million years ago (MYA) and possibly the need for extra defenses for their fungal crops, a novel challenge that no other ant has ever faced; (2) The transition within attines to rearing a single clade of coevolving gongylidia-producing fungi at the base of higher attines (approximately ~20 MYA) that may have required more elaborate hygienic defenses when the fungi were more vulnerable and had to be maintained in larger gardens; (3) The transition to active herbivory ~10 MYA that brought even less diversity into clonal crops, large-scale fungus farming, and possibly novel pathogens that came with fresh leaves [9].

Adaptation against several types of pathogens has been considered one of the main events in the evolution and elaboration of ant societies [10]. The metapleural gland is an organ unique to ants and is arranged as a paired structure located at the posterolateral ends of the metathorax [6]. Four hypotheses have been proposed regarding metapleural gland function [11]: (a) species or colony recognition, (b) territory marking, (c) antisepsis, and (d) chemical defense. Poulsen et al. [12] have presented the best evidence for the importance of the metapleural gland in disease suppression. Sumner et al. [13] demonstrated that socially parasitic ants have smaller metapleural glands and are more susceptible to disease. However, Hughes et al. [14] used only the externally visible bulla (indirect measurements) to infer a major transition in metapleural gland-size at the base of leaf-cutting ants. Therefore, further studies on the internal morphology of the metapleural gland need to be performed in order to compare attine ants with non–fungus-growing outgroups.

Most recently, studies on the metapleural gland have focused on the chemical composition of its secretions [12,15-18], while few studies have examined its internal morphology [19-21]. Fernandez-Marin et al. [18] observed that ants exposed to

infection with fungal spores cleaned the opening of the metapleural gland more frequently than control ants; this confirmed the importance of this gland in the hygiene of the colonies. In addition, fungi and bacteria varied regarding their sensitivity to the compounds produced by the metapleural gland of the leaf-cutting ant *Acromyrmex octospinosus* [17]. However, in *A. octospinosus*, the organic secretions produced by the metapleural gland inhibit infections and the development of different types of pathogens [17].

The aim of the present study was to compare morphological differences in the metapleural gland of 3 non–fungus-growing ants of the tribes Ectatommini (*Ectatomma brunneum*), Myrmicini (*Pogonomyrmex naegeli*), and Blepharidattini (*Wasmannia auropunctata*) and 5 fungus-growing ants from 2 basal (*Apterostigma pilosum* and *Mycetarotes parallelus*) and 3 derived lineages (*Trachymyrmex fuscus*, *Acromyrmex coronatus*, and *Atta laevigata*). This information is relevant because, so far, studies have largely concentrated on evidence from a single species rather than on comparative evidence. In addition, the secretions of the metapleural gland of the different species were examined with specific staining techniques, giving new comparative insights into the chemical composition of the secretions.

**Methods**

In the current study, 36 workers of each species were used. The ants were separated according to head-size: *E. brunneum* (2.075 ± 0.132 mm), *P. naegeli* (1.035 ± 0.13 mm), *W. auropunctata* (0.378 ± 0.038 mm), *A. pilosum* (0.716 ± 0.054 mm), *M. parallelus* (0.710 ± 0.017 mm), *T. fuscus* (1.149 ± 0.031 mm), and polymorphic workers of *A. coronatus* (majors: 1.716 ± 0.092 mm, medias 1.360 ± 0.127 mm; minors: 0.934 ±

0.004 mm) and of *A. laevigata* (majors: 3.282 ± 0.335 mm, medias: 2.048 ± 0.172 mm, minors: 0.773 ± 0.016 mm) [22,23].

To obtain the metapleural glands, the legs and heads were removed from the ants in Petri dishes containing saline solution (NaCl, 7.5 $g \cdot L^{-1}$; $Na_2HPO4$, 2.38 $g \cdot L^{-1}$; and $KH_2PO_2$, 2.72 $g \cdot L^{-1}$) under a Zeiss stereomicroscope with the aid of dissecting forceps and microscissors, leaving only the mesosoma. Glands were fixed with specific fixatives, depending on the technique used (see below). The material was dehydrated in a series of ethanol solutions (70, 80, 90, and 95%) for 15 min each, embedded in resin for 24 h, and transferred to plastic molds previously filled with resin and catalyzer [24]. After polymerization, the material was sectioned at 4 µm with a Leica RM 2145 microtome, hydrated, and placed on glass slides.

After the procedures for each technique, dry slides with the sections were mounted using Canada balsam and covered with a cover slip for later observation and photo documentation under a photomicroscope Leica connected to an Intel Pentium 4 computer.

***Histology***

*Harris hematoxylin—aqueous eosin staining [24]*

Six metapleural glands of each species were fixed with 4% paraformaldehyde for 24 h. Histological sections were hydrated for 1 min in distilled water and stained with hematoxylin for 10 min. The material was maintained immersed in a cuvette with water for 4 min and rinsed with tap water to allow the reaction to occur. The sections were stained with eosin for 5 min and washed with tap water.

***Histochemistry***

*Periodic acid Schiff (PAS) reaction [25] for polysaccharide detection and counterstaining with methyl green for RNA detection*

Six metapleural glands of each species were fixed with Bouin's aqueous mixture for 24 h. Histological sections were hydrated for 1 min with distilled water and immediately transferred to a solution of 0.4% periodic acid for 10 min. The material was then washed with distilled water for 1 min and immersed in Schiff's reagent for 1 h, washed for 30 min with tap water, counterstained with methyl green for 20 s, and rinsed again with water.

*Bromophenol blue staining to detect total proteins [26]*

Six metapleural glands of each species were fixed with 4% paraformaldehyde and 0.9% NaCl in 10% phosphate buffer (0.1 M, pH 7.4) for 24 h. The sections were stained with bromophenol blue for 1 h at room temperature and immersed in an aqueous solution of 0.5% acetic acid for 5 min and then in tertiary butyl alcohol for 5 min.

*Nile blue staining to detect acidic lipids [27]*

Six metapleural glands of each species were removed and fixed with calcium formol for 24 h. Sections were stained with Nile blue for 5 min at 37°C, rinsed in tap water, and immersed in 1% acetic acid for 1 min. After drying, slides with the gland sections were mounted in glycerinated gelatin and covered using a cover slip for observation and photographic documentation.

## Results

### *Morphology*

The metapleural glands of *E. brunneum*, *P. naegeli*, *W. auropunctata*, *A. pilosum*, *M. parallelus*, and *T. fuscus* are divided into secretory and storage portions connected by canaliculi (Figures 1–6). The morphology of these glands has previously been described for *A. laevigata* [22] and *A. coronatus* [23]; thus, in the present study, we only described the details that differed from those reported in the literature.

The secretory portion of the metapleural glands of the examined ants exhibited clusters of secretory cells that varied in number across species. In *E. brunneum*, 47 secretory cells formed a single cluster; in *P. naegeli*, 30 secretory cells were divided into 2 groups of 15; in *W. auropunctata*, 15 secretory cells were grouped in a single cluster; in *A. pilosum*, 42 cells were distributed in 3 groups of 14; in *M. parallelus*, 30 secretory cells were divided into 2 groups of 15; and in *T. fuscus*, 60 secretory cells were divided into 3 clusters of 20 (Figures 1–6).

Two cell shapes were observed in the secretory portion of the metapleural glands. Round-shaped cells were observed in *E. brunneum* and *W. auropunctata*, while oval-shaped cells were observed in *P. naegeli*, *A. pilosum*, *M. parallelus*, and *T. fuscus* (Figures 1–6).

In all the examined species, the canaliculus that connected the secretory portion to the storage portion was subdivided into 2 distinct portions: (a) intracytoplasmic and (b) extracytoplasmic portions (Figures 1–6). In *E. brunneum* and *P. naegeli*, the intracytoplasmic portion surrounded the nucleus of the secretory cell (Figures 1A, F–G; 2A–C, G), while in *W. auropunctata*, *A. pilosum*, *M. parallelus* and *T. fuscus*, the

canaliculus meandered in the cytoplasm of the secretory cell (Figures 3A-B; 4C-D;5C-D; 6A–C).

The intracytoplasmic canaliculi arose individually from each secretory cell in all the examined species. However, the extracytoplasmic portions of the canaliculi remained either separated or clustered, opening in the sieve plate. Canaliculi were separated in *E. brunneum*, *W. auropunctata*, and *P. naegeli*, and grouped in *A. pilosum*, *M. parallelus*, and *T. fuscus* (Figures 1–6).

The number of canaliculi in each metapleural gland corresponds to the number of secretory cells, since each cell gives rise to 1 canaliculus. In all the examined species, the diameter of the intracytoplasmic portion of the canaliculi was narrower than that of the extracytoplasmic one and was lined by a thin cuticular intima. The extracytoplasmic portion was wider in diameter and had a thicker cuticular intima (Figures 1–6).

The sieve plate (single or multiple) is located on the wall of the collecting chamber, where the extracytoplasmic canaliculi open. In *E. brunneum* and *W. auropunctata* the sieve plate was a single structure, while 2 sieve plates were observed in *P. naegeli* and *M. parallelus* and 3 plates in *A. pilosum* and *T. fuscus* (Figures 1–6). Moreover, in *E. brunneum*, *W. auropunctata*, *A. pilosum*, *M. parallelus*, and *T. fuscus* (Figures 1, 3–6) the cuticular intima of the plate exhibited folds, which were absent in *P. naegeli* (Figure 2).

In all the examined species, the collecting chamber and the reservoir were lined by a simple squamous epithelium with cells with flat nuclei and cuticular intima (Figures 1–6). A summary of the morphohistological results is present in table 1.

***Histochemistry***

The histochemistry techniques (PAS, bromophenol blue, and Nile blue) applied to the metapleural glands of the workers of *E. brunneum*, *P. naegeli*, *W. auropunctata*, *A. pilosum*, *M. parallelus*, and *T. fuscus* revealed that the secretory cells of all the species contained polysaccharides, proteins, and acidic lipids in the cytoplasm. However, differences in staining intensity were observed.

*Cells of the secretory portion*

Moderate staining of polysaccharides was observed in the cytoplasm of secretory cells of the metapleural gland of *P. naegeli* (Figures 2C-D), *W. auropunctata* (Figures 3C-D), *A. pilosum* (Figures 4B-C), and *M. parallelus* (Figures 5C-D). In *E. brunneum* (Figure 1D) and *T. fuscus* (Figure 6B), the cells were strongly stained for polysaccharides. In *P. naegeli*, stained granules were concentrated near the cell borders (Figures 2C-D).

The cytoplasm of the secretory cells of the metapleural gland of *E. brunneum*, *P. naegeli*, *A. pilosum*, and *T. fuscus* was weakly stained with methyl green (Figures 1D; 2C-D; 4B, and C; 6B), while the nuclei were strongly stained (Figures 1D; 2C-D; 4B and C; 6B).

The secretory cells were moderately stained for proteins in *W. auropunctata* (Figures 3E-F) and *M. parallelus* (Figures 5E-F), unlike in *E. brunneum* (Figures 1F), *P. naegeli* (Figures 2E-F), *A. pilosum* (Figures 4E and F), and *T. fuscus* (Figures 6C), where the secretory cells were strongly stained for proteins. For all the species, secretory cells were strongly stained for acidic lipids (Figures 1G-H; 2G-H; 3G-H; 4G-H; 5G-H; 6D-E). A summary of the histochemical results is present in table 2.

*Intracytoplasmic and extracytoplasmic canaliculi*

The pericanalicular region of the intracytoplasmic canaliculi of the secretory cells was strongly stained for polysaccharides in *E. brunneum*, *P. naegeli*, *W. auropunctata*, *A. pilosum*, and *T. fuscus* (Figures 1D, 2C-D, 3C, 4B-C, 6B) and moderately stained in *M. parallelus* (Figures 5C-D). The lumen of the intracytoplasmic canaliculi was strongly stained for polysaccharides in *P. naegeli*, *W. auropunctata*, and *M. parallelus* (Figures 2C-D, 3C, 5C-D), moderately stained in *E. brunneum* and *T. fuscus* (Figures 1D, 6B), and weakly stained in *A. pilosum* (Figures 4B-C). The lumen of the extracytoplasmic canaliculi was moderately stained in *E. brunneum*, *P. naegeli*, *W. auropunctata*, *M. parallelus*, and *T. fuscus* (Figure 1D), while it was unstained in *A. pilosum*.

In the pericanalicular region of the intracytoplasmic canaliculi, strong staining for total proteins was observed in *P. naegeli*, *A. pilosum*, and *T. fuscus* (Figures 2F, 4E, 6C), moderate staining was observed in *M. parallelus* (Figure 5F), and weak staining was observed in *W. auropunctata* (Figure 3E). Staining for proteins was not observed in the lumen of *E. brunneum*. The lumen of the intracytoplasmic canaliculi was strongly stained for proteins in *M. parallelus* (Figure 5F), moderately stained in *W. auropunctata* (Figure 3E), and weakly stained in *E. brunneum*, *P. naegeli*, *A. pilosum* and *T. fuscus* (Figures 2F, 4E, 6C). In the extracytoplasmic portion, strong staining for proteins was observed in *M. parallelus* (Figures 5E-F) and weak staining was observed in *E. brunneum*, *P. naegeli*, *W. auropunctata*, *A. pilosum*, and *T. fuscus* (Figures 1F, 2E-F, 4F, 6C).

Furthermore, the pericanalicular region of the intracytoplasmic portion of the canaliculus was strongly stained for acidic lipids in *W. auropunctata*, *A. pilosum*, *M. parallelus*, and *T. fuscus* (Figures 3G, 4H, 5G-H, 6E), weakly stained in *P. naegeli*

(Figure 2G), and unstained in *E. brunneum*. The lumen of the intracytoplasmic canaliculi was strongly positive for lipids in *W. auropunctata* (Figure 3G), moderately positive in *M. parallelus* and *T. fuscus* (Figures 5G-H, 6E), and weakly positive in *E. brunneum, P. naegeli,* and *A. pilosum* (Figures 2G, 4H). The lumen of the extracytoplasmic canaliculi was strongly positive in *W. auropunctata* (Figure 3G), moderately positive in *M. parallelus* and *T. fuscus* (Figures 5G-H, 6D), and weakly positive in *E. brunneum, P. naegeli,* and *A. pilosum* (Figures 1H, 2G, 4G).

*Storage portion (collecting chamber and reservoir)*

The histochemical techniques revealed that the epithelial cells lining the collecting chamber and reservoir were weakly positive for polysaccharides in all the examined species (Figures 1C, 2D, 3C, 4C, 5C, and 6B). However, a secretion consisting of polysaccharides (Figure 1E) was observed in the reservoir of *E. brunneum*. Moderate staining for RNA was observed in the epithelial cells of *A. pilosum*, while weak staining for RNA was observed in *E. brunneum* and *P. naegeli* (Figures 1C, 2D). Strong staining for proteins was detected in the epithelial cells of *E. brunneum* (Figure 1F), moderate staining in *A. pilosum* (Figure 4F), and weak staining in *P. naegeli, W. auropunctata, M. parallelus,* and *T. fuscus* (Figures 2F, 3E). The epithelial cells were also strongly stained for lipids in *E. brunneum* (Figure 1H), moderately stained in *W. auropunctata, A. pilosum,* and *T. fuscus* (Figures 4H, 6E), and weakly stained in *P. naegeli* and *M. parallelus* (Figures 2G, 5H). A summary of the histochemical results is present in table 2.

## Discussion

The results of the present study on the metapleural gland of ants from the groups Ectatommini, Myrmicini, Blepharidattini, and Attini (basal and derived attines) confirmed the presence of secretory and storage portions connected by canaliculi, supporting the results obtained for the leaf-cutting ants *Atta bisphaerica* and *Atta sexdens rubropilosa* [28], *A. octospinosus* [19], *Atta subterraneus* [21], as well as *A. laevigata* and *A. coronatus* [22,23].

The non–fungus-growing ants (Ectatommini, Myrmicini, Blepharidattini) had fewer secretory cells than the leaf-cutting ants. Blepharidattini ants (*W. auropunctata*) may be an evolutionary lineage that experienced a reduction in the number of secretory cells as a result of the use of antimicrobial venom, such as in *Polyrhachis dives* [29], or a reduction in parasite pressure. Some ants, such as *Camponotus*, are devoid of metapleural glands, possibly because of a lower susceptibility to pathogens as they build arboreal nests [30,31]. However, Walker and Hughes [32] demonstrated that arboreal species were not less resistant to *Metarhizium anisopliae* than ground-dwelling species, and the species that inhabited both arboreal and ground habitats had the greatest resistance. The Myrmicini (*P. naegeli*) and Ectatommini (*E. brunneum*) are non–fungus-growers that build nests in the ground, and their metapleural gland may be physiologically modified to undertake other roles. For example, in many myrmicine ants, the metapleural gland secretes territory markers and nest-entrance compounds or regulates aggressive interactions between neighboring colonies [33-36].

On the basis of the literature, attines are phylogenetically divided into basal and derived ant lineages [37]. However, results of the current study indicates that the number of secretory cells in the metapleural gland supports the phylogenetic description

by Brady et al. [37], who demonstrated that basal attines (*A. pilosum* and *M. parallelus*) have fewer secretory cells, an intermediary number between those of the derived attine *T. fuscus*. In addition, the leaf cutting ants *A. laevigata* and *A. coronatus* have more secretory cells, indicating higher secretory capacity. The primary role of the metapleural gland [38,11] is to produce antiseptic and cleaning compounds to avoid contamination of ants and nests by microorganisms. However, Poulsen et al. [39] reported that in some ants (leaf-cutting ants), the metapleural gland may also have a primary role in producing antimicrobial compounds to protect the ants and their mutualistic fungal crop against parasites and support the growth of Actinomycetes on the ant cuticle.

The current study demonstrated that in Ectatommini, Blepharidattini, Myrmicini, and the basal attine *M. parallelus*, the secretory cells are arranged in a single group or 2 groups, while in the basal attine *A. pilosum* and the derived attine *T. fuscus*, the secretory cells are arranged in 3 groups. In the derived attines *A. laevigata* and *A. coronatus* [22,23], several groups of secretory cells (7 or 8) were found, suggesting that the number of secretory cells is associated with the evolutionary lineage of the tribe. Therefore, derived attines have more groups of secretory cells, and as a consequence, they have higher demands for secretion.

The shape of the secretory cells may also indicate the secretory capacity of the metapleural gland in ants. For example, cells are round-shaped in Ectatommini and Blepharidattini and oval-shaped in Myrmicini and Attini. This oval morphology has also been observed in the attines *A. bisphaerica*, *A. sexdens rubropilosa* [28], *A. octospinosus* [19], *A. subterraneus* [21], *A. laevigata*, and *A. coronatus* [22,23], and the round-shaped cells have been observed in *Diacamma rugosum* and *Diacamma vagans* [40]. According to Junqueira and Carneiro [41], cells taller rather than shorter (oval)

have the physiology typical of secretory cells, suggesting that oval-shaped secretory cells of the metapleural gland of attines produce secretions more actively than the metapleural glands of Ectatommini and Blepharidattini.

In the present study, Ectatommini, Myrmicini, and Attini (basal and derived ants) exhibited metapleural glands with secretory cells that stained strongly for the presence of proteins, while in Blepharidattini, the secretory cells were moderately stained for proteins. Ultrastructural analysis has also revealed protein compounds in the secretion of cells of the metapleural gland on the basis of the presence of abundant rough endoplasmic reticulum [38,42]. This suggests that these glands are also capable of synthesizing protein compounds that stain intensely, but in Blepharidattini, the proteins stain to a lesser extent. These protein compounds probably contribute to most of the antibacterial and antifungal properties, but further investigation of their full composition and function is required.

Staining for the presence of acidic lipids and polysaccharides was observed in the secretory cells of all the examined groups, similar to that previously observed in *A. laevigata* and *A. coronatus* [22,23]. However, in *E. brunneum*, *T. fuscus*, and leaf-cutting ants [22-23], the secretory cells were strongly stained for polysaccharides. Gusmão [28], who studied *A. bisphaerica* and *A. sexdens rubropilosa*, and Vieira et al. [42], who studied *A. laevigata*, observed a significant amount of smooth endoplasmic reticulum. These results demonstrate that derived attines (leaf-cutting ants) and fungus-growers synthesize more polysaccharides and acidic lipids than the non–fungus-growing ants (Ectatommini, Myrmicini, and Blepharidattini), which may provide antibacterial and antifungal properties or energy reserves for future use.

The canaliculi connect the secretory portion to the storage portion of the metapleural gland in ants and are divided into intracytoplasmic and extracytoplasmic portions [43]. The intracytoplasmic portion in Ectatommini and Myrmicini surrounds the nuclei, whereas in Blepharidattini and Attini (basal and derived attines, *T. fuscus*), this portion meanders around the nucleus, possibly to increase the surface area for the collection of elements synthesized by the secretory cells. Our results concur with those of previous studies in which the intracytoplasmic portion in the leaf-cutting ants *A. laevigata* and *A. coronatus* was observed to meander around the nucleus [22,23]. Likewise, according to Vieira et al. [42], the intracytoplasmic portion of the canaliculi of the metapleural gland of *A. laevigata* has microvilli to increase the surface area, consequently collecting more secretions produced by the secretory cells. These findings are consistent with a higher demand for secretions in these more advanced farming ants.

The secretions produced by the secretory cells of the metapleural gland in all the examined groups, as well as in *A. laevigata* and *A. coronatus* [22,23], differed from those found in the lumen (inside canaliculi) of the extracytoplasmic portion of the canaliculi. Polysaccharides, proteins, and acidic lipids were strongly stained in the cytoplasm and weakly or moderately stained in the lumen of the extracytoplasmic portion, indicating that in addition to collecting and transporting the secretions, these structures also modify their composition.

The number of canaliculi in each metapleural gland corresponds to the number of secretory cells, since each cell gives rise to 1 canaliculus. In Ectatommini, Blepharidattini, Myrmicini, and Attini (basal), fewer canaliculi were observed than those in the derived attines. Furthermore, the sieve plate, located in the collecting chamber of the storage portion of the metapleural gland, varied in number, depending

on the group. In Ectatommini, Blepharidattini, Myrmicini, and Attini (basal), fewer plates were observed than those in the derived attines. A greater number of plates have also been observed in the derived attines *A. octospinosus* [19], *A. sexdens* [30], *A. bisphaerica*, and *A. sexdens rubropilosa* [21]. In the ponerine ant *D. vagans* and the doryline (*Dorylus* spp. [44]) and dolichoderine ants (*Dolichoderus quadripunctatus*, *Linepithema humile*, *Tapinoma erraticum* [45]), a single large sieve plate was observed, suggesting that derived attines have more plates in order to receive more extracytoplasmic canaliculi because of a larger number of secretory cells. However, our results showed that the closest outgroup, *W. auropunctata*, had the lowest number of plates and canaliculi, suggesting that the disease pressure in this species is not severe. From this species onwards, the number of plates and canaliculi increases throughout the attines. In addition, our results highlight that smaller increases in plate number in the more distant outgroups are appropriate: *Ectatomma* as a predator/scavenger [46] has probably rather dirty (infectious) food and *Pogonomyrmex* has independently evolved multiple-queen mating [47], quite possibly also in response to higher disease pressure.

The cuticular intima of the collecting chamber of the metapleural glands had infoldings in the area of the sieve plates in Ectatommini, Blepharidattini, and Attini (basal and derived), suggesting that these structures may direct the flow of secretions towards the reservoir (storage portion). A narrow ridge has been observed in the reservoir wall of *A. sexdens*, and it is thought to guide secretions from the collecting chamber toward the opening of the metapleural gland [48]. Nevertheless, the reservoir and the collecting chamber were lined by a simple squamous epithelium in all the tribes examined in this study. According to Vieira et al. [22], lining may not be the only role of these cells, which possibly secrete compounds of the cuticular intima, a protective

structure of the epithelial wall, to protect against its own secretion and/or from possible contaminants that could minimize or inactivate its action.

Three hypotheses have been proposed to explain the presence of more secretory cells, clusters of secretory cells, and sieve plates in leaf-cutting ants than in non–fungus-growing ants (Ectatommini, Myrmicini, and Blepharidattini) and the basal attines. First, the queens of leaf-cutting ants mate with multiple males, whereas those of other attines mate only once [49]. Hamilton [50], Schmid-Hempel [51], and Hughes and Boomsma [52] suggested that polyandrous queens of social insects may have evolved because, the more genetically diverse colonies they produce, the more resistant to parasites they may become. According to Hughes et al. [14], both polyandry and large metapleural gland reservoirs may have evolved in response to stronger pressure from parasites Second, Hughes et al. [14] suggested that leaf-cutting ants may be able to invest more in resistance since they use fresh vegetation as a substrate for their fungus, which in turn provides more resources for them to use. Third, leaf-cutting ant workers are polymorphic and include individuals specialized in cutting leaves, unlike other lower attines [14]. However, our results did not show an abrupt evolutionary transition in the secretory defense in fungus-growing ants, in which the diameter of the bulla is significantly enhanced only at the final transition to leaf-cutting ants, as reported by Hughes et al. [14]. Our findings indicated that glandular capacity was already partially enhanced in *Trachymyrmex,* suggesting that secretion capacity increased before reservoir capacity.

In conclusion, morphophysiological differences showed that the derived attines (leaf-cutting ants) had more secretory cells, cluster cells, and sieve plates, as well as higher levels of polysaccharides and acidic lipids, than Ectatommini, Myrmicini,

Blepharidattini, and basal Attini. This suggests that the metapleural glands of derived attines are more developed and have a greater capacity to produce secretions that are effective against unwanted fungi and bacteria in the fungal garden. When compared with basal attines and non–fungus-growing ants, it is probable that leaf-cutting ants may have evolved more developed metapleural glands in response to stronger pressure from parasites.

**Acknowledgments**

We are grateful to the Centro de Estudos de Insetos Sociais – CEIS (UNESP-Campus Rio Claro-SP, Brazil) for assistance in the collection and identification of specimens examined in this study, and to FAPESP (grant # 2008/54301-3) and CNPq for financial support.

**Literature cited**

1. North RD, Jackson CW, Howse PE (1997) Evolutionary aspects of anti-fungus interactions in leaf-cutting ants. Tree 12: 386–389.
2. Weber NA (1972) Gardening ants: The Attines. Philadelphia (PA): American Philosophical Society 92: 146p.
3. Mueller UG (2002) Ant versus fungus versus mutualism: Ant-cultivar conflict and the deconstruction of the attine ant-fungus symbiosis. The American Naturalist 160:S67-S98.
4. Villesen P, Murakami T, Schultz TR, Boomsma JJ (2002) Identifying the transition between single and multiple mating of queens in fungus-growing ants. Proc R Soc Lond B 269:1541–1548.
5. Emery C (1899) Végétarianisme chez les fourmis. Archives Sciences Physiques et Naturalles 8: 488-490. In: Mueller, U. G.; T. R. Schultz; C. R. Currie; R. M. Adams &

D. Malloch. 2001. The origin of the attine ant-fungus mutualism. The Quarterly Review of Biology 76(2): 169-197.

6. Hölldobler B, Wilson EO (1990) The Ants. Cambridge: Harvard University Press. pp. 732.

7. Longino JT, Fernández F (2007) Taxonomic review of the genus *Wasmannia*, pp. 271-289. In: Snelling, R. R., B. L. Fisher, and P. S. Ward (eds). *Advances in ant systematics (Hymenoptera: Formicidae)*: homage to E. O. Wilson – 50 years of contributions. Mem Am Entomol Inst, 80.

8. Bolton B (2003) Synopsis and Classification of Formicidae. Mem Am Entomol Inst 71: 1-370.

9. Schultz TR, Brady SG (2008) Major evolutionary transitions in ant agriculture. Proc Natl Acad Sci 105: 5435–5440.

10. Wilson EO (1971) The Insect Societies. Cambridge, MA: Harvard University Press. pp. 514.

11. Yek SH, Mueller UG (2011) The metapleural gland of ants. Biological Reviews 86: 774-791.

12. Poulsen M, Bot ANM, Nielsen MG, Boomsma JJ (2002) Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. Behav ecol sociobiol 52:151-157.

13. Sumner S, Hughes WOH, Boomsma JJ (2003) Evidence for differential selection and potential adaptive evolution in the worker caste of an inquiline social parasite. Behav Ecol Sociobiol 54:256–263.

14. Hughes WOH, Pagliarini R, Madsen HB, Dijkstra MJ, Boomsma JJ (2008) Antimicrobial defence shows an abrupt evolutionary transition in the fungus-growing ants. Evolution 62:1252–1257.

15. Do Nascimento RRD, Schoeters E, Morgan ED, Billen J, Stradling DJ (1996) Chemistry of metapleural gland secretions of three attine ants, *Atta sexdens rubropilosa*, *Atta cephalotes*, and *Acromyrmex octospinosus* (Hymenoptera: Formicidae). J chem ecol 22: 987-1000.

16. Ortius-Lechner D, Maile R, Morgan ED, Boomsma JJ (2000) Metapleural gland secretion of the leaf-cutter ant *Acromyrmex octospinosus*: New compounds and their functional significance. J chem ecol 26:1667-1683.

17. Bot ANM, Ortius-Lechner D, Finster K, Maile R, Boomsma JJ (2002) Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. Ins Soc 49: 363-370.

18. Fernandez-Marin H, Zimmerman J, Rehner S, Wcislo W (2006). Active use of the metapleural glands by ants in controlling fungal infection. Proc R Soc Lond B Biol sci 273:1689-1695.

19. Bot ANM, Obermayer ML, Hölldobler B, Boomsma JJ (2001) Functional morphology of the metapleural gland in the leaf-cutting ant *Acromyrmex octospinosus*. Ins Soc 48: 63-66.

20. Gusmão LG, Caetano FH, Nakano O (2001). Ultramorphology of the metapleural gland in three species of *Atta* (Hymenoptera, Formicidae). Iheringia, Zool 91: 33-36.

21. De Souza ALB, Soares IMF, Cyrino LT, Serrão JE (2006) The Metapleural Gland in Two Subspecies of *Acromyrmex subterraneus* (Hymenoptera: Formicidae). Sociobiology 47: 19-25.

22. Vieira AS, Bueno OC, Camargo-Mathias MI (2010) The functional morphology of the metapleural gland of the leaf-cutting ant *Atta laevigata* (Formicidae: Attini). Micron 41:149–157.

23. Vieira AS, Bueno OC, Camargo-Mathias MI (2011) Secretory profile of metapleural gland cells of the leaf-cutting ant *Acromyrmex coronatus* (Formicidae: Attini). Microsc res tech 74:76–83.

24. Junqueira LCU, Junqueira LMMS (1983) Técnicas básicas de citologia and histologia. Livraria Editora Santos, pp. 48-81.

25. McManus JFA (1946) Histological demonstration of mucin after periodic acid. Nature 158: 202.

26. Pearse AGE (1985) Histochemistry. Theoretical and Applied, 4th ed. J & A Churchill, London.

27. Lison L. 1960. Histochimie et Cytochimie Animales, Gauthier-Villars, Éditeur, Paris.

28. Gusmão LG (2000) Estudo comparado da glândula metapleural e sua secreção em diferentes castas de três espécies de *Atta* (Hymenoptera: Formicidae). 60p. *Tese* (Doutorado) - ESALQ-USP, Piracicaba-SP.

29. Graystock P, Hughes WOH (2011) Disease resistance in a weaver ant, Polyrhachis dives, and the role of antibiotic-producing glands. Behav Ecol Sociobiol 65(12): 2319-2327.

30. Hölldobler B, Engel-Siegel H (1985) On the metapleural gland in ants. Psyche 91: 201–224.

31. Johnson RN, Agapow P-M, Crozier RH (2003) A tree island approach to inferring phylogeny in the ant subfamily Formicinae, with especial reference to the evolution of weaving. Mol phylogenet evol 29, 317–330.

32. Walker TN, Hughes WOH (2011) Arboreality and the evolution of disease resistance in ants. Ecol Entomol 36: 588–595.

33. Tulloch GS, Shapiro JE, Hershenov B (1962) The ultrastructure of the metasternal glands of ants. Bull Brooklyn Entomol Soc 57: 91-101.

34. Jaffe K, Puche H (1984) Colony-specific territorial marking with the metapleural gland secretion in the ant *Solenopsis geminata* (Fabr). J insect physiol 30: 265–270.

35. Jaffe K, Lopez ME, Aragort W (1986) On the communication systems of the ants *Pseudomyrmex termitarius* and *P. triplarinus*. Ins Soc 33:105–117.

36. Cammaerts M-C, Cammaerts R (2001) Marking of nest entrances and vicinity in two related *Tetramorium* ant species (Hymenoptera: Formicidae). J Ins Behav 14: 247–269.

37. Brady SG, Schultz TR, Fisher BL, Ward PS (2006) Evaluating alternative hypotheses for the early evolution and diversification of ants", PNAS 103(48): 18172–18177.

38. Maschwitz U, Koob K, Schildknecht H (1970) Ein Beitrag zur Funktion der Metathoracaldrüse der Ameisen. J Ins Physiol 16: 387–404.

39. Poulsen M, Bot ANM, Boomsma JJ (2003) The effect of metapleural gland secretion on the growth of a mutualistic bacterium on the cuticle of leaf-cutting ants. Naturwissenschaften 90, 406–409.

40. Schoeters E, Billen J (1992) Morphological and ultrastructural study of metapleural gland in *Diacamma* (Hymenoptera, Formicidae). In: European Congress of Social Insects, 1o, Leuven (1991) Proceedings Leuven, Leuven University. pp. 239-247.

41. Junqueira LC, Carneiro J (2008). Histologia básica, 11 ed. Guanabara Koogan, Rio de Janeiro. pp. 524.

42. Vieira AS, Bueno OC, Camargo-Mathias MI (2012) Ultrastructural profile of metapleural gland cells of the ant *Atta laevigata* (F. Smith, 1858) (Formicidae: Attini). Animal Biology 62: 1–11.

43. Noirot C, Quennedey A (1991) Glands, gland cells, glandular units: some comments on terminology and classification. Ann Soc Entomol Fr 27: 123-128.

44. Billen JPJ, Van Boven JKA (1987) The metapleural gland in Old World army ants: a morphological and ultrastructural description (Hymenoptera, Formicidae). Revue de Zoologie Africaine 101: 31–41.

45. Fanfani A, Valcurone Dazzini M (1991). Metapleural glands of some Dolichoderinae ants. Ethol ecol evol S1: 95–98.

46. Almeida AJ (1987) Descrição de seis fêmeas do gênero Ectatomma Smith, 1858 (Hymenoptera, Formicidae, Ponerinae). An Soc. Nord. Zool 1:175-183.

47. Gadau J, Strehl C-P, Oettler J, Hölldobler B (2003) Determinants of intracolonial relatedness in *Pogonomyrmex rugosus* (Hymenoptera; Formicidae): mating frequency and brood raids. Mol Ecol 12:1931–1938.

48. Schoeters E, Billen J. 1993. Anatomy and fine structure of the metapleural gland in *Atta*. Belg J Zool 123: 67–75.

49. Sumner S, Hughes WHO, Pedersen JS, Boomsma JJ (2004) Ant parasite queens revert to mating singly. Nature 428:35–36.

50. Hamilton WD (1987) Kinship, recognition, disease, and intelligence: constraints of social evolution. Pp. 81–100. In: Y. Ito, J. L. Brown, and J. Kirkkawa, ed. Animal societies: theories and facts. Japan Scientific Societies Press, Tokyo.

51. Schmid-Hempel P (2000) Mating, parasites and other trials of life in social insects. Microb. Infect. 2:515–520.

52. Hughes WHO, Boomsma JJ (2004) Genetic diversity and disease resistance in leaf-cutting ant societies. Evolution 58:1251–1260.

**Figure 1**. **Schematic representation, histology, and histochemistry of the metapleural gland of the basal ant *Ectatomma brunneum*.** Secretory cells (**sc**) and their nuclei (**n**), extracytoplasmic portions of canaliculi (**ec**) and their nuclei (**n**), collecting chamber (**cc**), sieve plate (**p**) and reservoir (**r**) with opening (**og**), lined by epithelium (**ep**) and cuticular intima (**cut**) are shown. **A–C:** Histological sections of the metapleural gland stained with hematoxylin and eosin (staining in nucleus and cytoplasm). Details of secretory cells (**sc**) and their nuclei (**n**), intracytoplasmic (**arrow**) and extracytoplasmic portions of canaliculi (**ec**) with nuclei (**n**). Note canaliculi surrounding the nucleus of the secretory cell and arising individually from each cell and opening in the sieve plate (**p**) of the collecting chamber (**cc**) lined by the cuticular intima (**cut**), and the reservoir storing secretion (**sec**). Scale bar = 25 µm. **D-E:** Histological sections of the metapleural gland stained with PAS/methyl green (for detecting polysaccharides and RNA). Details of the secretory cells (**sc**) showing the cytoplasm, nucleus (**n**), intracytoplasmic (**arrow**) and extracytoplasmic (**ec**) canaliculi, collecting chamber (**cc**), and the lining epithelium (**arrow**) and nuclei of cells (**arrow**). Note the presence of secretion (**sec**) containing polysaccharides in the collecting chamber and reservoir. Scale bar = 25 µm. **F:** Histological sections of the metapleural gland stained with bromophenol blue (for detecting total proteins). Details of the secretory cells (**sc**), intracytoplasmic (**arrow**) and extracytoplasmic (**ec**) portions of canaliculi, and collecting chamber (**cc**) lined by epithelium (**ep**). Note the presence of secretion (**sec**) in the collecting chamber. Scale bar = 25 µm. **G-H:** Histological sections of the metapleural gland stained with Nile blue (for detecting acidic lipids). Details of the secretory cells (**sc**), intracytoplasmic (**arrow**) and extracytoplasmic (**ec**) portions of canaliculi, lining epithelium (**arrow**) of the collecting chamber (**cc**), and the reservoir (**r**). Note the presence of secretion (**sec**) containing acidic lipids in the reservoir. Scale bar = 25 µm.

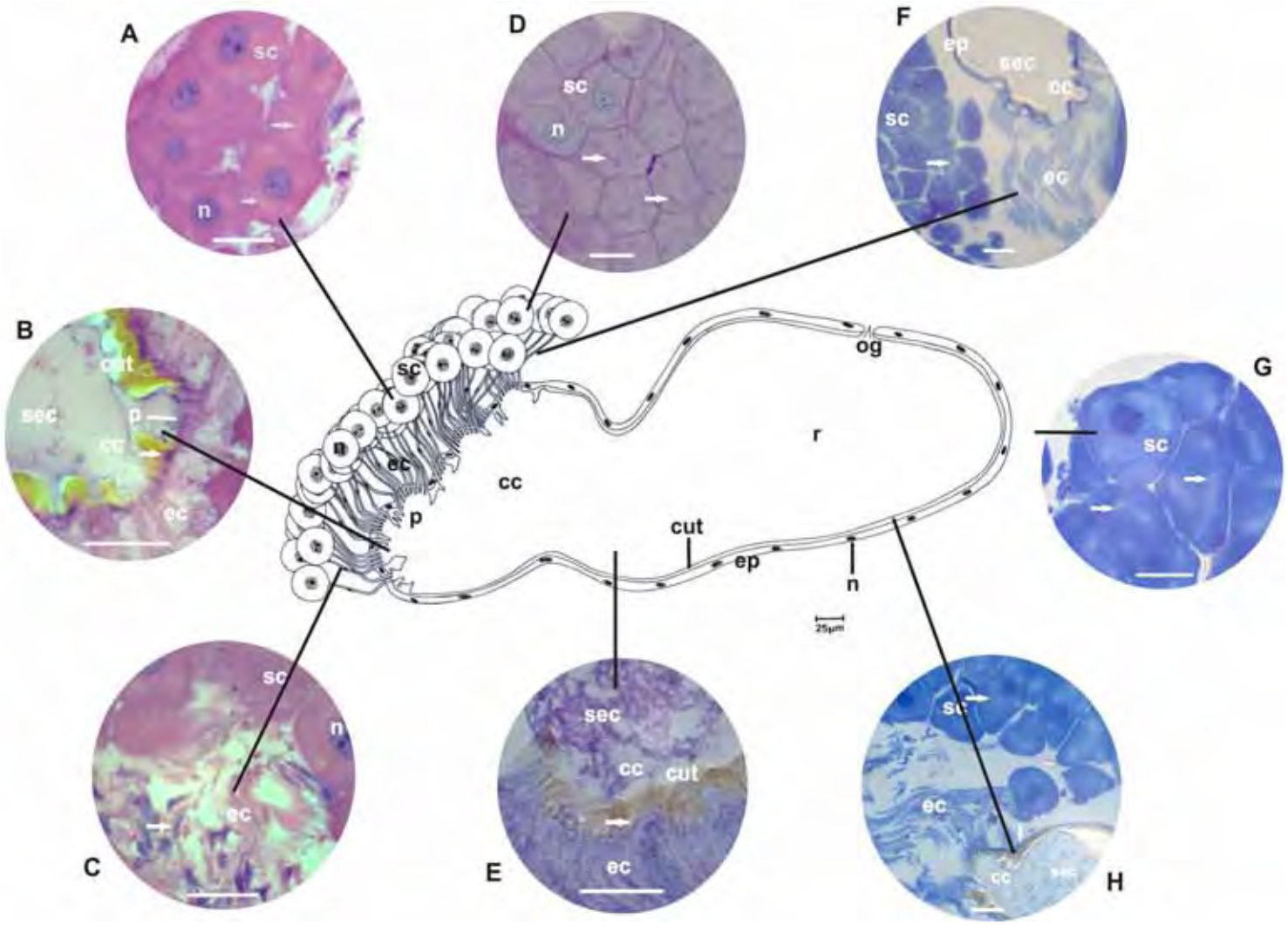

A
sc
n
D
sc
n
F
ep
sec
cc
sc
og
B
cut
sec
p
cc
ec
sc
n
ec
cc
p
og
r
cut
ep
n
25µm
G
sc
C
sc
n
ec
E
sec
cc
cut
ec
H
sc
ec
cc

**Figure 2**. **Schematic representation, histology, and histochemistry of the metapleural gland of *Pogonomyrmex naegeli* (Myrmicini).** Secretory cells (**sc**) and their nuclei (**n**), extracytoplasmic portions of canaliculi (**ec**) and their nuclei (**n**), collecting chamber (**cc**) and the sieve plate (**p**) and reservoir (**r**) with opening (**og**) lined by epithelium (**ep**), and cuticular intima (**cut**) are shown. **A**–**B:** Histological sections of the metapleural gland stained with hematoxylin and eosin (staining in nucleus and cytoplasm). Details of secretory cells (**sc**) and nuclei (**n**), intracytoplasmic (**arrow**) and extracytoplasmic portions of canaliculi (**ec**), and their nuclei (**n**). Note the intracytoplasmic portion of canaliculi (**arrow**) surrounding the nucleus of the secretory cells and arising individually from cells to open in the sieve plate (**p**) of the collecting chamber (**cc**) lined by a cuticular intima (**cut**). Scale bar = 25 µm. **C**–**D:** Histological sections of the metapleural gland stained with PAS/methyl green (for detecting polysaccharides and RNA). Details of the secretory cells (**sc**) showing the cytoplasm, nuclei (**n**), intracytoplasmic (**arrow**) and extracytoplasmic (**ec**) canaliculi, collecting chamber (**cc**), lining epithelium (**ep**), and nuclei (**n**). Note the presence of granules containing polysaccharides (**dark arrow**) in the cytoplasm of the secretory cell. Scale bar = 25 µm. **E-F:** Sections of the metapleural gland stained with bromophenol blue (for detecting total proteins). Details of secretory cells (**sc**), extracytoplasmic portion (**ec**) of canaliculi, and collecting chamber (**cc**) lined by epithelium (**ep**). Scale bar = 25 µm. **G–H:** Histological sections of the metapleural gland stained with Nile blue (for detecting acidic lipids). Details of the secretory cells (**sc**), intracytoplasmic (**arrow**) and extracytoplasmic portions (**ec**) of canaliculi, and collecting chamber (**cc**). Scale bar = 25 µm.

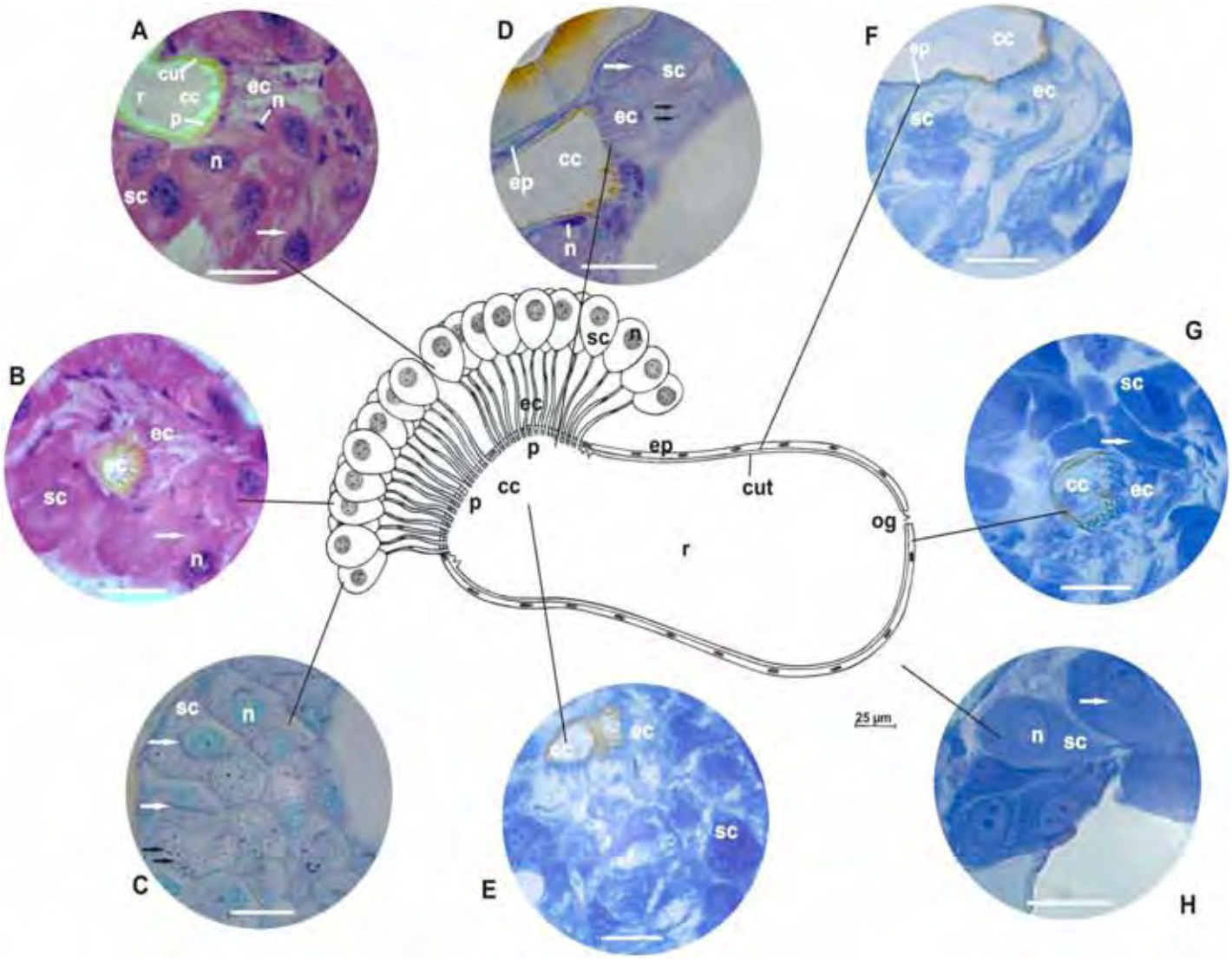

A
cut
r
cc
ec
n
p
n
sc
D
sc
ec
cc
ep
n
F
ep
cc
ec
sc
B
ec
cc
sc
n
sc
n
ec
p
ep
cut
p
cc
og
r
G
sc
cc
ec
C
sc
n
E
ec
cc
sc
25 µm
n
sc
H

**Figure 3. Schematic representation, histology, and histochemistry of the metapleural gland of *Wasmannia auropunctata* (Blepharidattini)**. Secretory cells (**sc**) and their nuclei (**n**), extracytoplasmic portion of canaliculi (**ec**) and their nuclei (**n**), collecting chamber (**cc**) and the sieve plate (**p**) and reservoir (**r**) with opening (**og**), lined by epithelium (**ep**) and cuticular intima (**cut**) are shown. **A**–**B:** Histological sections of the metapleural gland stained with hematoxylin and eosin (staining in nucleus and cytoplasm). Details of the secretory cells (**sc**) and nuclei (**n**), intracytoplasmic portion (**arrow**) of canaliculi (**ec**), reservoir (**r**) lined by epithelium (**ep**) and cuticular intima (**cut**). Scale bar = 20 µm. **C**–**D:** Histological sections of the metapleural gland stained with PAS/methyl green (for detecting polysaccharides and RNA). Details of secretory cells (**sc**) showing the cytoplasm, nucleus (**n**), intracytoplasmic canaliculi (**arrow**), and reservoir (**r**) lined by epithelium (**ep**). Note the presence of few granules containing polysaccharides (**dark arrow**) located in the periphery of the secretory cells. Scale bar = 20 µm. **E-F:** Histological sections of the metapleural gland stained with bromophenol blue (for detecting total proteins). Details of secretory cells (**sc**), nuclei (**n**), intracytoplasmic portion of the canaliculi (**arrow**), and reservoir (**r**) lined by a cuticular intima (**cut**). Scale bar = 20 µm. **G–H:** Histological sections of the metapleural gland stained with Nile blue (for detecting acidic lipids). Details of secretory cells (**sc**) and collecting chamber (**cc**). Scale bar = 20 µm.

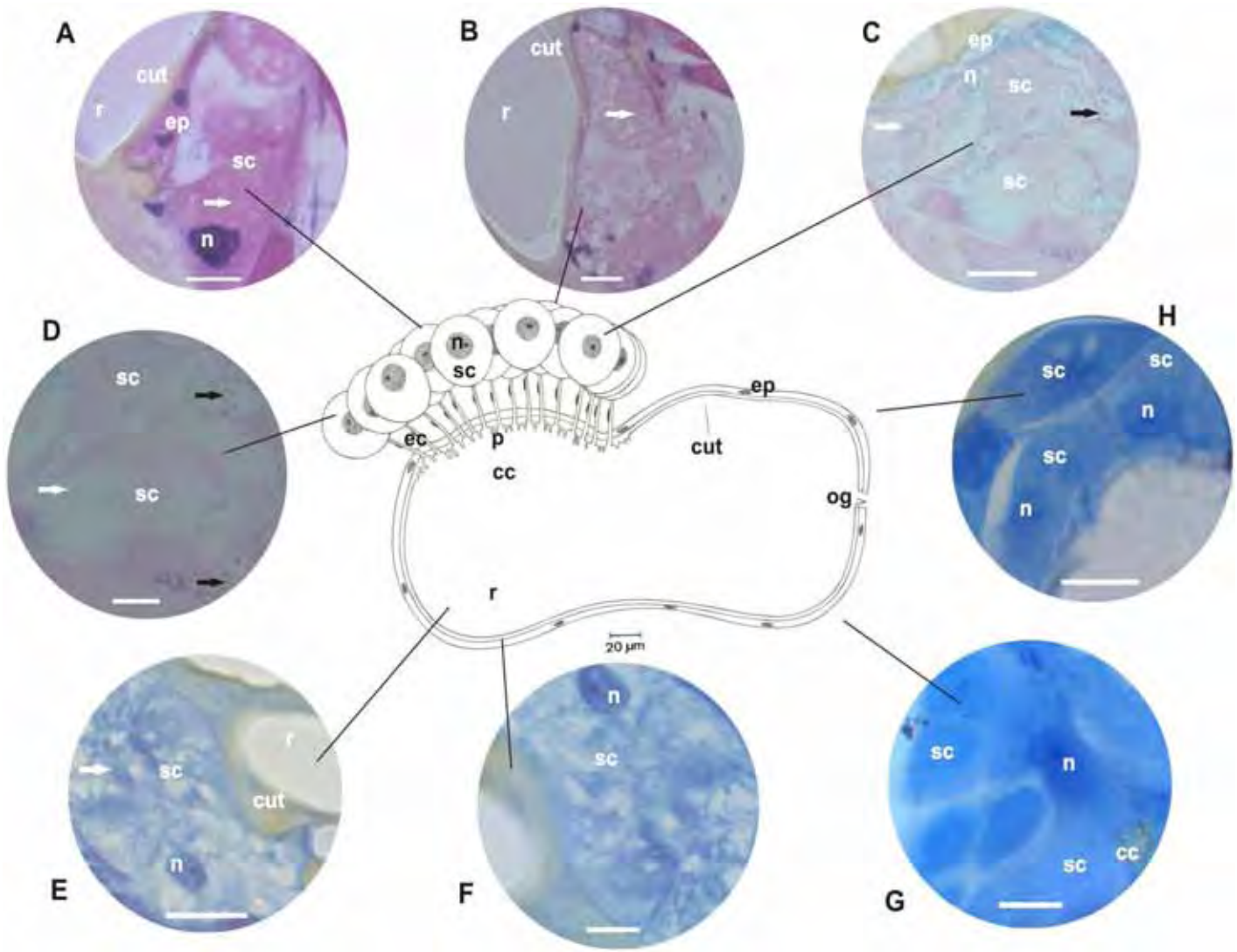

A
cut
r
ep
sc
n
B
cut
r
C
ep
n
sc
sc
D
sc
sc
n
sc
ec
p
cc
ep
cut
og
r
20 µm
H
sc
sc
n
sc
n
E
r
sc
cut
n
F
n
sc
G
sc
n
sc
cc

**Figure 4**. **Schematic representation, histology, and histochemistry of the metapleural gland *Apterostigma pilosum* (Attini)**. Secretory cells (**sc**) and their nuclei (**n**), extracytoplasmic portion of canaliculi (**ec**) and their nuclei (**n**), collecting chamber (**cc**) and the sieve plate (**p**), and reservoir (**r**) with opening (**og**), lined by epithelium (**ep**) and cuticular intima (**cut**) are shown. **A:** Histological sections of the metapleural gland stained with hematoxylin and eosin (staining in nucleus and cytoplasm). Details of secretory cells (**sc**) and nuclei (**n**), intra (**arrow**) and extracytoplasmic portions of canaliculi (**ec**) and their nuclei (**n**). Scale bar = 25 µm. **B–D:** Histological sections of the metapleural gland stained with PAS/methyl green (for detecting polysaccharides and RNA). Details of the secretory cells (**sc**) showing the cytoplasm, nuclei (**n**), cross section of intracytoplasmic canaliculi (**arrow**), longitudinal section of extracytoplasmic canaliculi (**ec**), collecting chamber (**cc**), and reservoir (**r**) lined by epithelium (**ep**) and their nuclei (**arrow**). Scale bar = 25 µm. **E-F:** Histological sections of the metapleural gland stained with bromophenol blue (for detecting total proteins). Details of secretory cells (**sc**) and nuclei (**n**), and intracytoplasmic (**arrow**), lumen (**dark arrow**), and extracytoplasmic portions (**ec**) of canaliculi, and collecting chamber (**cc**) lined by epithelium (**arrow**). Scale bar = 25 µm. **G–H:** Histological sections of the metapleural gland stained with Nile blue (for detecting acidic lipids). Details of secretory cells (**sc**) and nuclei (**n**), intracytoplasmic (**dark arrow**) and extracytoplasmic portions of canaliculi (**ec**), and reservoir (**r**) lined by epithelium (**arrow**). Note the presence of secretion (**sec**) containing acidic lipids in the reservoir. Scale bar = 25 µm.

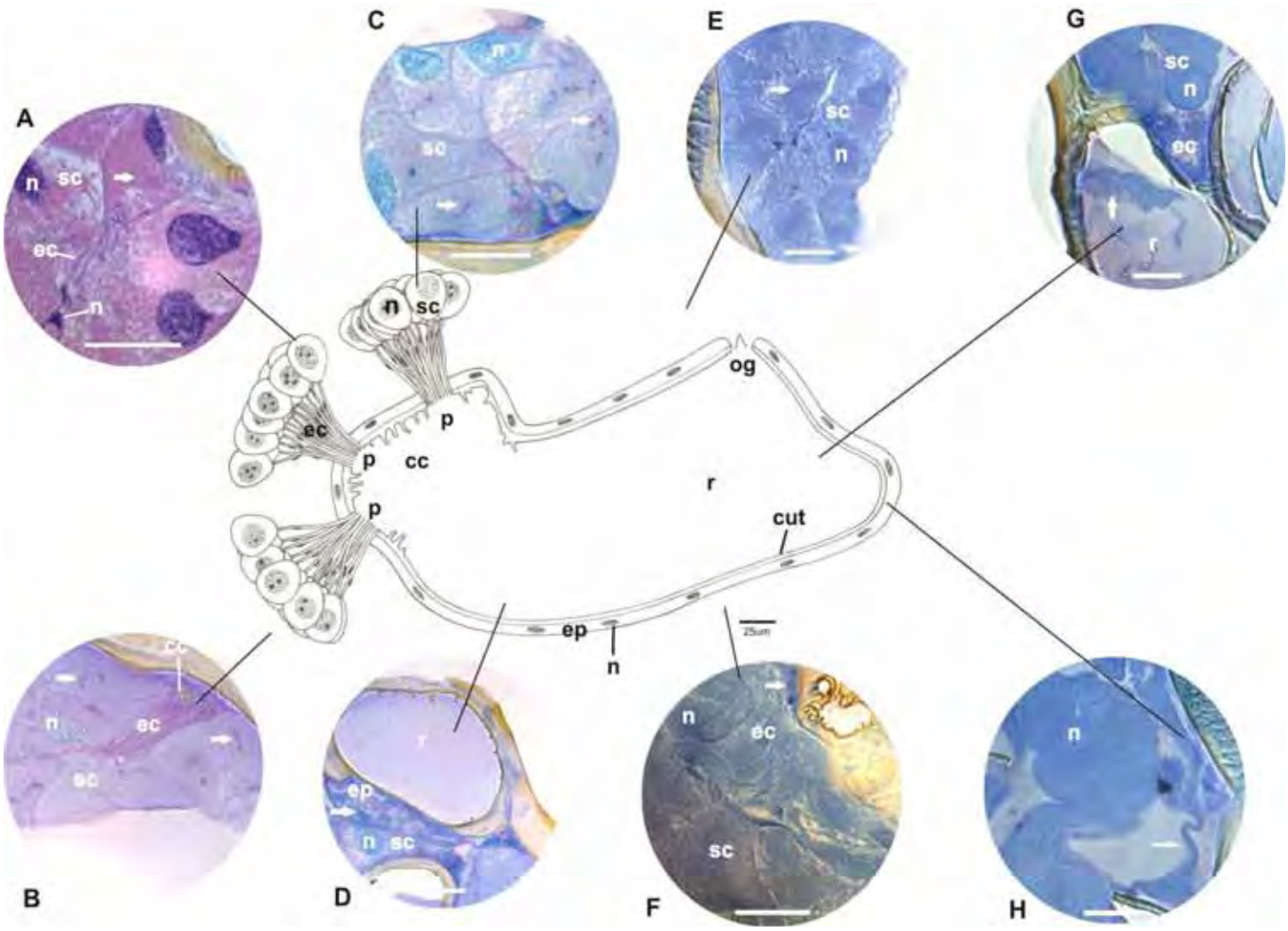

A
n
sc
ec
n
C
n
sc
E
sc
n
G
sc
n
ec
r
n
sc
ec
p
p
cc
p
og
r
cut
ep
n
25um
B
n
ec
sc
D
ep
n
sc
F
n
ec
sc
H
n

**Figure 5**. **Schematic representation, histology, and histochemistry of the metapleural gland *Mycetarotes parallelus* (Attini)**. Secretory cells (**sc**), nuclei (**n**), extracytoplasmic portion of canaliculi (**ec**), collecting chamber (**cc**) and the sieve plate (**p**), and reservoir (**r**) with opening (**og**), lined by epithelium (**ep**) and cuticular intima (**cut**) are shown. **A**–**B:** Histological sections of the metapleural gland stained with hematoxylin and eosin (staining in nucleus and cytoplasm). Details of the secretory cells (**sc**) and nuclei (**n**), reservoir (**r**) lined by epithelium (**ep**) and cuticular intima (**cut**). Scale bar = 20 µm. **C**–**D:** Histological sections of the metapleural gland stained with PAS/methyl green (for detecting polysaccharides and RNA). Details of the secretory cells (**sc**) and canaliculi intracytoplasmic (**arrow**). Scale bar = 20 µm. **E**–**F:** Histological sections of the metapleural gland stained with bromophenol blue (for detecting total proteins). Details of the secretory cells (**sc**) and nuclei (**n**), extracytoplasmic canaliculi (**ec**), collecting chamber (**cc**), and reservoir (**r**), lined by a cuticular intima (**cut**). Note the presence of secretion (**sec**) containing proteins in the reservoir. Scale bar = 20 µm. **G–H:** Histological sections of the metapleural gland stained with Nile blue (for detecting acidic lipids). Details of the secretory cells (**sc**) and nuclei (**n**), and extracytoplasmic canaliculi (**arrow**). Scale bar = 20 µm.

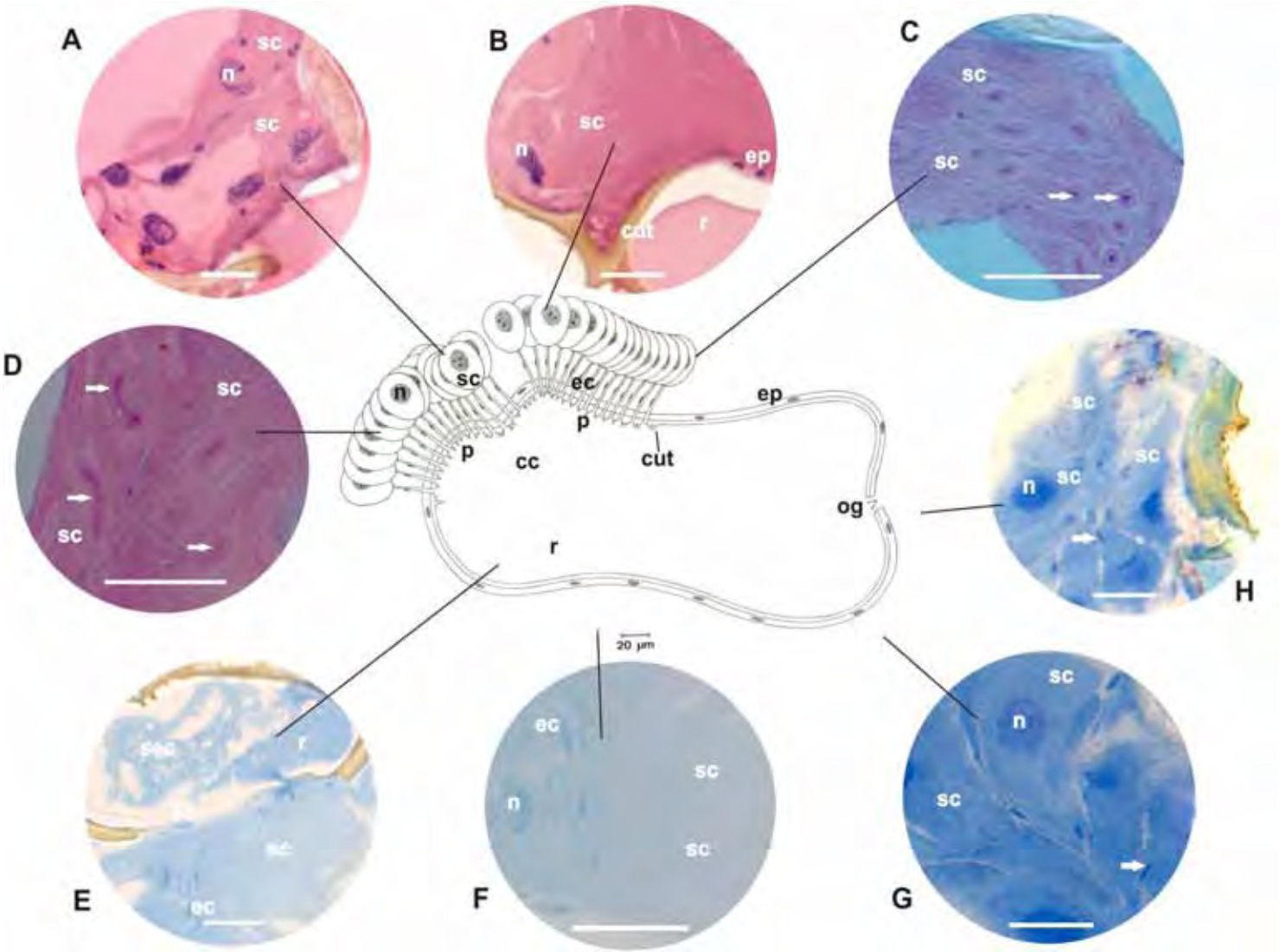

A
sc
n
sc
B
sc
n
ep
cut
r
C
sc
sc
D
sc
sc
n
sc
ec
p
p
cc
cut
ep
og
r
H
sc
sc
n
sc
E
r
ec
F
20 µm
ec
sc
n
sc
G
sc
n
sc

**Figure 6**. **Schematic representation, histology, and histochemistry of the metapleural gland of *Trachymyrmex fuscus* (Attini).** Secretory cells (**sc**) and nuclei (**n**), extracytoplasmic portion of canaliculi (**ec**), collecting chamber (**cc**) and the sieve plate (**p**), and reservoir (**r**) with opening (**og**), both lined by epithelium (**ep**) and cuticular intima (**cut**) are shown. **A:** Histological sections of the metapleural gland stained with hematoxylin and eosin (staining in nucleus and cytoplasm) Details of secretory cells (**sc**) and nuclei (**n**), intracytoplasmic portion of canaliculi (**arrow**). Scale bar = 25 µm. **B:** Histological sections of the metapleural gland stained with PAS/methyl green (for detecting polysaccharides and RNA). Details of secretory cells (**sc**) and nuclei (**n**), intracytoplasmic portion of canaliculi (**arrow**). Note the presence of granules containing polysaccharides in the periphery of the secretory cells (**dark arrow**). Scale bar = 25 µm. **C:** Histological sections of the metapleural gland stained with bromophenol blue (for detecting total proteins). Details of secretory cells (**sc**) and nuclei (**n**) and the intracytoplasmic portion of canaliculi (**arrow**). Scale bar = 25 µm. **D:** Histological section of the metapleural gland stained with Nile blue (for detecting acidic lipids). Details of the secretory cells (**sc**) and nuclei (**n**), extracytoplasmic portion of canaliculi (**ec**), collecting chamber (**cc**) and the sieve plate (**p**), and reservoir (**r**) lined by epithelium (**ep**) and cuticular intima (**cut**). Note the presence of secretion (**sec**) containing acidic lipids in the reservoir. Scale bar = 25 µm.

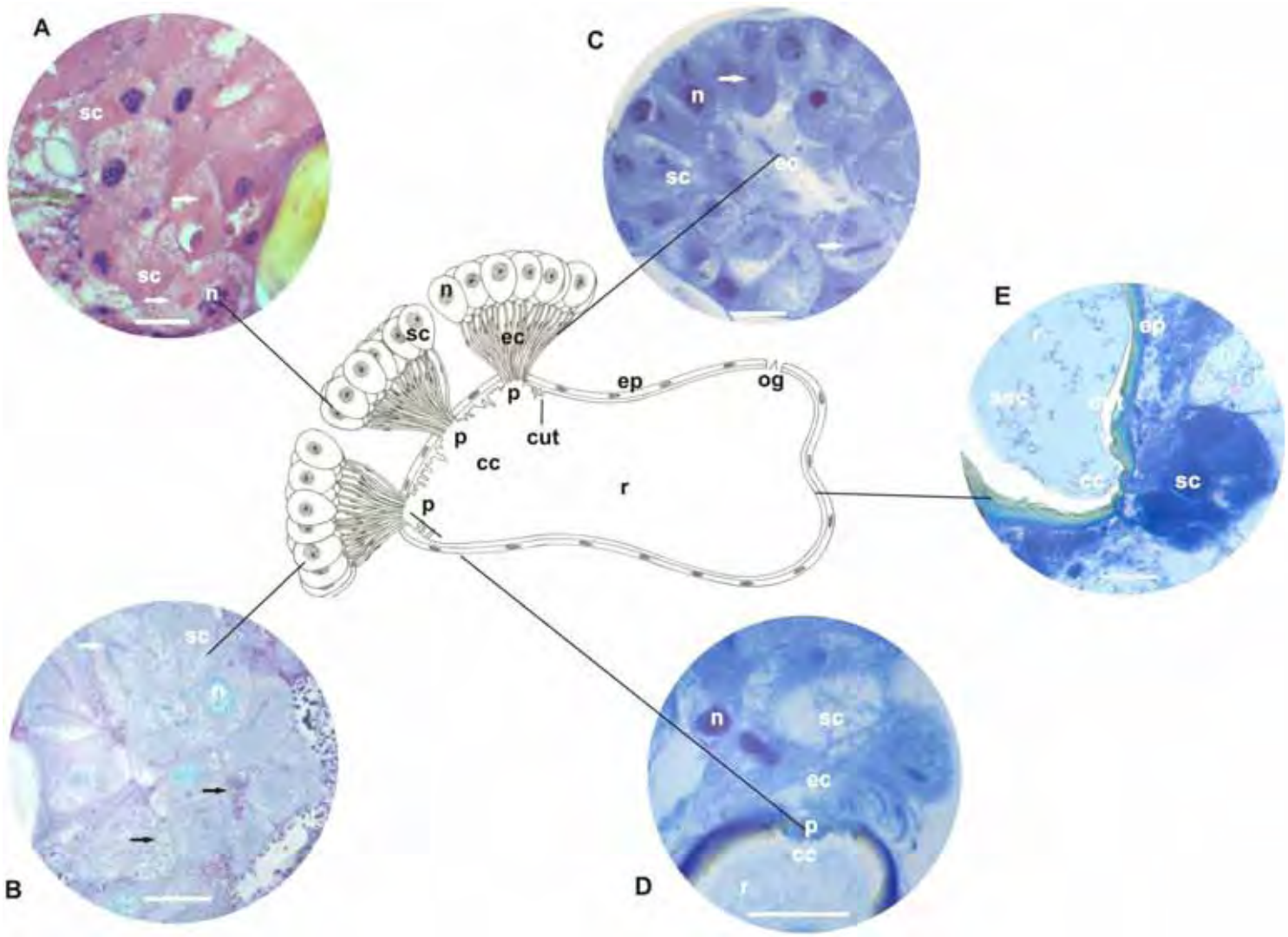

A
sc
sc
n
C
n
sc
ec
n
sc
ec
p
ep
og
cut
p
cc
r
p
E
ep
sc
cc
sc
B
sc
n
D
n
sc
ec
p
cc
r

**Table 1**. Morphological comparison of the metapleural glands of monomorphic workers of *Ectatomma brunneum*, *Pogonomyrmex naegeli*, *Wasmannia auropunctata*, *Apterostigma pilosum*, *Mycetarotes parallelus*, and *Trachymyrmex fuscus*, and polymorphic workers of the leaf-cutting ants *Acromyrmex coronatus* and *Atta laevigata.*

| | Secretory cells | | | Intracellular canaliculi | Extracellular canaliculi | | Reservoir | |
|---|---|---|---|---|---|---|---|---|
| **Species** | **Total number of cells of each gland** | **Number of groups of secretory cells** | **Shape of secretory cells** | **Sinuous or surrounding the nucleus** | **Clustered or separate** | **Canaliculi in each gland** | **Number of sieve plates** | **Absence/presence of infoldings** |
| ***Ectatomma brunneum*** | 47 | single group of cells | round | surrounding the nucleus | separate | 47 | 01 | present |
| ***Pogonomyrmex naegeli*** | 30 | 2 groups of 15 cells | oval | surrounding the nucleus | separate | 30 | 02 | absent |
| ***Wasmannia auropunctata*** | 15 | single group of cells | round | sinuous | separate | 15 | 01 | present |
| ***Apterostigma pilosum*** | 42 | 3 groups of 14 cells | oval | sinuous | clustered | 42 | 03 | present |
| ***Mycetarotes parallelus*** | 30 | 2 groups of 15 cells | oval | sinuous | clustered | 30 | 02 | present |
| ***Trachymyrmex fuscus*** | 60 | 3 groups of 20 cells | oval | sinuous | clustered | 60 | 03 | present |
| ****Acromyrmex coronatus*** (Vieira et al. 2011) | ≥136 | 8 groups of 17 cells | oval | sinuous | clustered | ≥136 | ≥08 | present |
| ****Atta laevigata*** (Vieira et al. 2010) | ≥140 | 7 groups of 20 cells | oval | sinuous | clustered | ≥140 | ≥07 | present |

*already published data, see details in Vieira et al. (2010, 2011) for *Atta laevigata* and *Acromyrmex coronatus* (minor workers)

**Table 2**. Results of the histochemical tests on the metapleural gland of monomorphic workers of *Ectatomma brunneum*, *Pogonomyrmex naegeli*, *Wasmannia auropuncata*, *Apterostigma pilosum*, *Mycetarotes parallelus*, and *Trachymyrmex fuscus*, and polymorphic workers of the leaf-cutting ants *Acromyrmex coronatus* and *Atta levigata*.

| Species | Histochemical tests | Portions of the metapleural gland | | | | | |
|---|---|---|---|---|---|---|---|
| | | Target compounds | Cytoplasm of secretory cells | Periphery of intracytoplasmic canaliculi | Lumen of intracytoplasmic canaliculi | Lumen of extracytoplasmic canaliculi | Lining epithelial cells |
| | **PAS** | polysaccharides | +++ | +++ | ++ | ++ | + |
| | **Methyl Green** | RNA | + | - | - | - | + |
| ***Ectatomma brunneum*** | **Bromophenol Blue** | total proteins | +++ | - | + | + | +++ |
| | **Nile Blue** | acidic lipids | +++ | - | + | + | +++ |
| | **PAS** | polysaccharides | ++ | +++ | +++ | ++ | + |
| | **Methyl Green** | RNA | + | - | - | - | + |
| ***Pogonomyrmex naegeli*** | **Bromophenol Blue** | total proteins | +++ | +++ | + | + | + |
| | **Nile Blue** | acidic lipids | +++ | + | + | + | + |
| | **PAS** | polysaccharides | ++ | +++ | +++ | ++ | + |
| | **Methyl Green** | RNA | - | - | - | - | - |
| ***Wasmannia auropunctata*** | **Bromophenol Blue** | total proteins | ++ | + | ++ | + | + |
| | **Nile Blue** | acidic lipids | +++ | +++ | +++ | +++ | ++ |
| | **PAS** | polysaccharides | ++ | +++ | + | - | + |
| | **Methyl Green** | RNA | + | - | - | - | ++ |
| ***Apterostigma pilosum*** | **Bromophenol Blue** | total proteins | +++ | +++ | + | + | ++ |
| | **Nile Blue** | acidic lipids | +++ | +++ | + | + | ++ |
| | **PAS** | polysaccharides | ++ | ++ | +++ | ++ | + |
| | **Methyl Green** | RNA | - | - | - | - | - |
| ***Mycetarotes parallelus*** | **Bromophenol Blue** | total proteins | ++ | ++ | +++ | +++ | + |
| | **Nile Blue** | acidic lipids | +++ | +++ | ++ | ++ | + |
| | **PAS** | polysaccharides | +++ | +++ | ++ | ++ | + |
| | **Methyl Green** | RNA | + | - | - | - | - |
| ***Trachymyrmex fuscus*** | **Bromophenol Blue** | total proteins | +++ | +++ | + | + | + |
| | **Nile Blue** | acidic lipids | +++ | +++ | ++ | ++ | ++ |
| | **PAS** | polysaccharides | +++ | ++ | - | - | - |
| | **Methyl Green** | RNA | - | - | - | - | + |
| ****Acromyrmex coronatus*** **(Vieira et al. 2011)** | **Bromophenol Blue** | total proteins | +++ | +++ | + | + | ++ |
| | **Baker** | total lipids | +++ | +++ | + | + | ++ |
| | **PAS** | polysaccharides | + | - | - | - | + |
| | **Alcian Blue** | glycosaminoglycans | +++ | - | +++ | +++ | ++ |
| ****Atta laevigata*** **(Vieira et al. 2010)** | **Bromophenol Blue** | total proteins | +++ | +++ | + | + | +++ |
| | **Nile Blue** | acidic lipids | +++ | - | +++ | +++ | ++ |

*already published data, see details in Vieira et al. (2010, 2011) for *Atta laevigata* and *Acromyrmex coronatus* (minor workers) ; (+) weakly positive; (++) moderately positive; (+++) strongly positive; (-) negative.

# *Capítulo 5*

**Ultraestrutura comparada da glândula metapleural em formigas cultivadoras (Attini) e não cultivadoras de fungos (Blepharidattini e Ectatommini) (Hymenoptera: Formicidae)**

**Autores**: Alexsandro Santana Vieira, Odair Corrêa Bueno e Maria Izabel Camargo-Mathias
**Periódico**: Submetido ao periódico Insectes Sociaux (2012)

## RESUMO

As glândulas metapleurais são consideradas sinapomórficas no grupo das formigas. O presente trabalho teve como objetivo investigar as diferenças ultraestruturais das glândulas metapleurais de formigas cultivadoras de fungos Attini derivada e basal, e formigas não cultivadoras de fungos Blepharidattini e Ectatommini, por meio da microscopia eletrônica de transmissão. Observou-se nas células das glândulas de todas as espécies, aqui estudadas, a presença de invaginações na membrana plasmática que facilitaria a absorção de material extracelular, oriundo da hemolinfa. A presença de vesículas nas células secretoras de *Acromyrmex coronatus* e *Apterostigma pilosum* e, das não cultivadoras de fungos *Wasmannia auropunctata* e *Ectatomma brunneum* poderia indicar a ocorrência de processos de endo e/ou exocitose, confirmados pela presença de secreção no interior das vesículas. Verificou-se a presença de vacúolos digestivos (autofagocitose), em todas as espécies estudadas, organelas responsáveis por realizar a ciclagem celular. As Attini derivadas e basais diferiram das não cultivadoras de fungos, por possuírem nas glândulas metapleurais as células secretoras com forma oval, o que poderia indicar maior atividade na produção de secreção. Ainda, as células secretoras da glândula metapleural das formigas cortadeiras apresentaram muitas mitocôndrias próximas as microvilosidades da porção intracitoplasmática do canalículo, sugerindo maior envolvimento da glândula metapleural na atividade de produção e transporte de secreção. Desta forma, as glândulas metapleurais das formigas cortadeiras apresentaram-se mais ativas na produção de secreção do que as das atines basais e as das formigas não cultivadoras de fungos.

**Palavras-chave**: célula secretora, glândula exócrina, organelas.

# Comparative ultrastructure of the metapleural glands of fungus-growing (Attini) and non-fungus-growing ants (Blepharidattini and Ectatommini) (Hymenoptera: Formicidae)

Alexsandro Santana Vieira[1]; Odair Corrêa Bueno[1,2] and Maria Izabel Camargo-Mathias[1]

[1]UNESP – Universidade Estadual Paulista –Departamento de Biologia, Rio Claro, SP, Brazil. Av. 24A, 1515, Bela Vista, CEP 13506-900.

[2] Centro de Estudos de Insetos Sociais. Instituto de Biociências, UNESP - Univ Estadual Paulista, Campus Rio Claro. CEP: 13506-900 – Rio Claro, SP- Brazil.

Corresponding author; M.I. Camargo-Mathias: e-mail: micm@rc.unesp.br

ABSTRACT

The metapleural glands are considered synapomorphic structures in ants. The present study was aimed at investigating the ultrastructural differences of the metapleural glands of fungus-growing ants from derived and basal lineages, and non-fungus-growing ants from the tribes Blepharidattini and Ectatommini with transmission electron microscopy. The gland cells of all species examined exhibited invaginations in the plasma membrane that could facilitate the absorption of extracellular material from the hemolymph. The presence of vesicles in the secretory cells of *Acromyrmex coronatus* and *Apterostigma pilosum*, and the non-fungus-growing ants *Wasmannia auropunctata* and *Ectatomma brunneum* indicated the occurrence of endo and/or exocytosis, which was confirmed with the observation of secretion in the vesicles. Digestive vacuoles (autophagocytosis), organelles responsible for cell recycling, were present in all species examined. Derived and basal attines differed from non-fungus-growing ants, by the presence of oval secretory cells in the metapleural glands, which suggests a higher production of secretion. Also, the secretory cells of the metapleural gland of leaf-cutting ants exhibited many mitochondria near microvilli of the intracytoplasmic portion of the canaliculus, suggesting an important role of the metapleural gland in the production and transport of secretion. Therefore, metapleural glands of leaf-cutting ants were more active in the production of secretion than those of basal attines and non-fungus-growing ants.

Keywords: secretory cell, exocrine gland, organelles.

INTRODUCTION

Some ant species build their nests in the ground or decomposing wood, and feed on the soil, where a diverse and abundant community of pathogenic microbes prospers due to constant humidity and temperature, and absence of light (Wilson 1971; Hölldobler and Wilson 1990; Thorne and Traniello 2003). The adaptation against several types of pathogens, considered one of the main events in the evolution of sociability and diversification of ants (Wilson 1971), was possible due to the action of compounds with antibiotic properties secreted mainly by the metapleural gland of leaf-cutting ants. The role of these compounds is to inhibit the development of pathogens (Maschwitz et al. 1970, 1974; Beattie et al. 1985, 1986; Do Nascimento et al. 1996; Hölldobler and Wilson 1990; Bot et al. 2002; Poulsen et al. 2002; Fernandez-Marin et al. 2006; Vieira et al. 2010).

The metapleural glands are considered synapomorphic in ants (Bolton 2003). They are paired structures located at the postolateral ends of the metathorax of ants (Hölldobler and Wilson 1990). According to Yek and Muller (2011), four hypothesis could explain the role of its secretions: a) species or colony recognition; b) nest entrance and territory marking; c) chemical defense, and d) antisepsis. The metapleural gland is the organ responsible for the production of pheromones of recognition and identification of nest mates, as well as foreign individuals (Brown, 1968). The secretion of the metapleural gland is used to mark the nest entrance or territory in some ant species including *Tetramorium caespitum*, *T. impurum* (Cammaerts and Cammaerts 2001), *Pheidole pallidula* (Cammaerts and Cammaerts 1998), *Solenopsis geminata* (Jaffe and Puche 1984) and *Pseudomyrmex triplarinus* (Jaffe et al. 1986). The use of metapleural gland secretion in chemical defense has been reported for *Crematogaster* subgenus *Physocrema*, which exhibits a hypertrophied metapleural gland (Janet 1898;

Donisthorpe 1941; Hosoishi and Ogata 2008), with a secretion consisted of a mixture of phenolic compounds that are harmful to predators (Attygale et al. 1989; Jones et al. 2005). Maschwitz et al. (1970) and Maschwitz (1974) reported antibiotic properties of secretion produced by the metapleural gland of ants. Some antibiotics produced by this gland have been described for *Atta sexdens*, such as phenylacetic acids (Maschwitz et al. 1970), β-hydroxydecanoic acids (myrmicacin), and indoleacetic acid (Schildknecht and Koob 1971).

Recent studies by Vieira et al. (2010, 2011) have examined the morphology of the metapleural glands in *Atta laevigata* and *Acromyrmex coronatus*. These authors described a secretory and a storage portion connected by canaliculi. However, only one recent study, also by Vieira et al. (2012), described the ultrastructure of the metapleural gland of *A. laevigata*. Earlier studies revealed that in *Diacamma rugosum, D. vagans*, *A. bisphaerica*, and *A. sexdens rubropilosa*, the cytoplasm of secretory cells of the metapleural gland exhibit large quantities of mitochondria of various shapes and sizes, ranging from elongated to thin, with more or less electron density (Schoeters and Billen 1992; Gusmão 2000). Regions of the Golgi were also commonly observed. However, in minor workers of *A. sexdens sexdens* and *A. sexdens rubropilosa*, workers of *A. bisphaerica*, and queens of *A. laevigata*, these organelles were not frequently found (Shoeters and Billen 1993). Smooth endoplasmic reticulum has been observed in secretory cells of *D. rugosum* and *D. vagans* (Shoeters and Billen 1992). In *A. sexdens rubropilosa* workers (Shoeters and Billen 1993), the endoplasmic reticulum was well developed with a typical lamellar organization, and in *A. laevigata* (Vieira et al. 2012) vesicular rough endoplasmic reticulum was observed. In queens of *A. bisphaerica* and *A. sexdens rubropilosa* (Gusmão 2000), however, both types have been found. Smooth

endoplasmic reticulum was more abundant than rough endoplasmic reticulum, suggesting ultrastructural intra and interespecific differences.

Fungus-growing ants (Attini) are considered biological models especially interesting in comparative studies, as they are part of a monophyletic tribe (North et al., 1997). The tribe Attini is subdivided in basal attines (*Apterostigma pilosum* and *Mycetarotes parallelus*) (Hölldobler and Wilson 1990; Mueller et al. 2001; Solomon et al. 2004), which use insect droppings and remnants as substrate for their symbiotic fungi; derived attines (*Trachymyrmex fuscus*), which use vegetation as substrate for their fungus (Weber 1972; Mueller 2002); and derived leaf-cutting Attini (*Atta laevigata* and *Acromyrmex coronatus*) that use only fresh vegetation as substrate for their fungus (Weber 1972; Muller 2002). Blepharidattini, represented by *Wasmannia auropunctata* does not cultivate fungus, although it grows on nest walls, and Ectatommini (*Ectatomma brunneum*) are not fungus growers, but rather predators (Bolton 2003).

Given the importance of the secretions produced by the metapleural gland in the biology of ants, the present study was aimed at investigating the ultrastructural differences of the metapleural glands of fungus-growing ants, the derived (*T. fuscus, A. laevigata* and *A. coronatus*) and basal attines (*A. pilosum, M. parallelus*), and the non-fungus growers Blepharidattini (*W. auropunctata*) and Ectatommini (*E. brunneum*), since there are ultrastructural studies on the metapleural gland of leaf-cutting ants, but not on other attines (basal) or non-fungus-growing ants.

## MATERIALS AND METHODS

For this study, head measurements were taken for 36 specimens of the Attini group, consisting of monomorphic workers of *A. pilosum* (0.716 ± 0.054mm), *M.*

*parallelus* (0.710 ± 0.017mm), *T. fuscus* (1.149 ± 0.031mm), and polymorphic workers of *A. coronatus* (minors: 0.934 ± 0.004 mm; media: 1.360 ± 0.127 mm; and majors: 1.716 ± 0.092 mm). Results on the metapleural gland of *A. laevigata* published by Vieira et al. (2012) were used. Also, nine monomorphic workers of each non-fungus-growing ant species of *W. auropunctata* (0.378 ± 0.038mm) and *E. brunneum* (2.075 ± 0.132 mm) were measured. These colonies were collected in the Rio Claro campus and maintained in the laboratory of the Center for Studies on Social Insects - UNESP, Rio Claro campus, SP/Brazil.

**Ultrastructural analysis**

*Transmission Electron Microscopy (TEM)*

To obtain metapleural glands, the legs and heads of the individuals were removed and placed in Petri dishes with colored wax and physiological solution (NaCl 7.5 g/L, Na2HPO4 2.38 g/L and KH2PO2 2.72 g/L) under a ZEISS stereomicroscope with the aid of dissecting forceps and microscissors. Metapleural glands (mesossoma) were carefully removed and fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 24 h. After fixation, the material was rinsed twice with 0.1 M sodium cacodylate buffer for 15 minutes, and post-fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.2) for two hours. The material was rinsed twice with 0.1 M sodium cacodylate buffer for 15 min and then with 10% ethanol solution for 15 minutes. Contrast enhancement was carried out with 2% uranyl acetate in 10% ethanol for 12 hours. The material was dehydrated in a series of acetone solutions (50%, 60%, 70%, 80%, 90%, and 95%), five minutes each and finally 100% acetone, twice for five minutes each. After dehydration, the material was included in Epon Araldite and oven-dried at 60°C for 24 hours. After polymerization, blocs were sectioned with a

Sorvall-Porter Blum MT2-B ultramicrotome. Ultrathin sections were collected with a copper screen, contrasted with 2% uranyl acetate and 0.4% lead citrate for 45 and 10 minutes, respectively. The material was examined and photographed under a transmission electron microscope PHILIPS CM 100.

## RESULTS

The ultrastructure of the metapleural glands of the Attini ants (*A. pilosum*, *M. parallelus*, *T. fuscus*, and *A. coronatus*-minor, medium, and major workers) (Figure 1-4), as well as those of the groups Blepharidattini (*W. auropunctata*) and Ectatommini (*E. brunneum*) (Fig. 5-6) revealed that they are divided in two portions: **secretory** and **storage** portions connected by canaliculi. The ultrastructure of the metapleural gland of *A. laevigata* has been previously described by Vieira et al. (2012).

### *Secretory Portion*

All species examined in the present study (*A. coronatus*, *T. fuscus*, *M. parallelus*, *A. pilosum*, *W. auropunctata*, and *E. brunneum*) exhibit secretory cells very close to each other (Fig. 3A, 4A-B, 5A-B, 6B), but plasma membranes are not fused, only the basal lamina. The lateral region of the plasma membrane of secretory cells can exhibit small invaginations toward the cytoplasm. In *E. brunneum* these invaginations are more evident (Fig. 6A).

Vesicles near the plasma membrane of secretory cells are observed in *A. coronatus* (Fig. 1A-B), *A. pilosum* (Fig. 4A) *W. auropunctata* (Fig. 5A), and *E. brunneum* (Fig. 6A).

The cells of the secretory portion are round-shaped in *W. auropunctata* (Fig. 5A) and *E. brunneum* (Fig. 6B), and oval (apical region wider than the basal area) in *A. coronatus, T. fuscus, M. parallelus,* and *A. pilosum*, (Fig. 1C, 3A, 4A).

Mitochondria are also observed in the secretory cells of the metapleural gland of all species examined in the present study. In *A. coronatus* (Fig. 1C, D and E), they occur mainly near the intracellular portion of the canaliculus, while in *T. fuscus* (Fig. 2B-C, E, F and G), *M. parallelus* (Fig. 3B-C), *A. pilosum* (Fig. 4B-C and E), *W. auropunctata* (Fig. 5D) and *E. brunneum* (Fig. 6B), they are distributed throughout the cytoplasm. These mitochondria exhibit various shapes, sizes and arrangements. In *A. coronatus* (Fig. 1C, D and E), *T. fuscus* (Fig. 2B-C), *A. pilosum* (Fig. 4B), *W. auropunctata* (Fig. 5D) and *E. brunneum* (Fig. 6C and E), mitochondria are large, round and elongated. In *M. parallelus* (Fig. 3B-C) fewer round and elongated mitochondria are observed.

Around the nucleus of secretory cells, the rough endoplasmic reticulum (RER) is lamellar and well developed in *A. coronatus* (Fig. 1E), *A. pilosum* (Fig. 4B), *W. auropunctata* (Fig. 5E-F), and *E. brunneum* (Fig. 6C). In *T. fuscus* (Fig. 2C) and *M. parallelus* (Fig. 3), however, RER is little developed.

The smooth endoplasmic reticulum (SER) is found in the metapleural glands of all species. In *A. coronatus* (Fig. 1D), *A. pilosum* (Fig. 4A) *M. parallelus* (Fig. 3) and *W. auropunctata* (Fig. 5F) SER is not well developed, unlike in *T. fuscus* (Fig. 2D). In *E. brunneum*, it was not observed.

In the regions where the Golgi complex is located, flattened cisternae are layered and exhibit dilated ends. In *A. coronatus* (Fig. 1F), *T. fuscus* (Fig. 2G), *A.*

*pilosum* (Fig. 4C) and *E. brunneum* (Fig. 6D) the regions of the Golgi are well developed. In *M. parallelus* (Fig. 3D) they are little developed and absent in *W. auropunctata.*

Vacuoles with different contents are observed in the periphery or near intracytoplasmic canaliculus of the secretory cells of the metapleural gland of *A. coronatus* (Fig. 1A-B and H), *T. fuscus* (Fig. 2E-F), *A. pilosum* (Fig. 4E), *M. parallelus* (Fig. 3C-D), *W. auropunctata* (Fig. 5B, F-H), and *E. brunneum* (Fig. 6D-E). In *W. auropunctata* many vacuoles are distributed throughout the cytoplasm (Fig. 5C-F).

Also, digestive vacuoles are observed as phagosomes and autophagosomes in all species examined, *A. coronatus* (Fig. 1G), *T. fuscus* (Fig. 2F), *M. parallelus* (Fig. 3C), *A. pilosum* (Fig. 4E), *W. auropunctata* (Fig. 5G), and *E. brunneum* (Fig. 6E).

Myelin figures with multilamellar arrangement are also found in secretory cells of metapleural glands of *A. coronatus* (Fig. 1G), *T. fuscus*, *A. pilosum* (Fig. 4D), and *W. auropunctata* (Fig. 5G), but absent in *M. parallelus* and *E. brunneum*.

In the cytoplasm of secretory cells, secretion granules of different electron densities are located in the periphery of the cell or near the intracellular portion of the canaliculus. In *A. coronatus* (Fig. 1C, G and H), *A. pilosum* (Fig. 4D-E), and *W. auropunctata* (Fig. 5B, G-H), secretions granules are large, but small in *T. fuscus* (Fig. 2E and G), *M. parallelus* (Fig. 3E) and *E. brunneum* (Fig. 6D-E) .

Lipid droplets are observed in the secretory cells of the metapleural glands. In *A. coronatus*, secretory cells of the metapleural gland of major workers differ from those of minors (Fig. 1C and H), where large lipid droplets are observed around the intracellular canaliculus, unlike the observed in major and media workers. In *W.*

*auropunctata* (Fig. 5H) large lipid droplets are also present, while in *T. fuscus* (Fig. 2G), *M. parallelus* (Fi. 3E) and *A. pilosum* (Fig. 4E), small lipid droplets are distributed throughout the cytoplasm. In *E. brunneum*, lipid droplets are absent.

In all species examined in this study, the nuclei of secretory cells are large; the heterochromatin is not evident, with predominance of euchromatin (Fig.1-6). The nuclear envelope is irregular in some secretory cells of *A. coronatus* (Fig. 1D), *T. fuscus* (Fig. 2B), *A. pilosum* (Fig. 4B and E), *W. auropunctata* (Fig. 5E), and regular in *M. parallelus* (Fig. 3A) and *E. brunneum* (Fig. 6B).

Table 1 presents an ultrastructural comparison of the morphological characteristics of the secretory portion of the metapleural glands of all species examined.

*Intracytoplasmic or intracellular canaliculus*

From each secretory cell of the metapleural gland, a canaliculus originates and divides into intra and extracytoplasmic portions. The latter is connected to the gland reservoir (Fig. 1H, 2G, 3E, 4E and F, 5H, 6E).

The intracellular portion of canaliculi exhibit microvilli (pericanalicular space) and is internally lined by the cuticular intima that becomes thicker toward the reservoir (Fig. 1H, 2G). In *A. coronatus* (Fig. 1H), *T. fuscus* (Fig. 2G), *M. parallelus* (Fig. 3E), *A. pilosum* (Fig. 4E), *W. auropunctata* (Fig. 5H), the intracytoplasmic canaliculus meanders the cytoplasm, while in *E. brunneum* (Fig. 6E), it surrounds the nucleus.

*Extracytoplasmic or extracellular canaliculus*

In all species examined in this study, the extracellular portion of canaliculi connects the secretory cells to the reservoir of the metapleural gland and is internally

lined by the cuticular intima (lumen). Along with the extracytoplasmic portion of the canaliculus, the squamous cell that originates this structure exhibits a round nucleus (Fig. 1I, 3F, 4F, 6F). In *A. coronatus* and *A. pilosum* secretion is present in the lumen (Fig. 1I).

*Storage Portion (Collecting Chamber)*

The collecting chamber is the area that receives canaliculi (narrowing of the reservoir). In all species examined in this study, this portion is internally lined by cuticle and exhibits sieve plates, where canaliculi open into the reservoir (Fig. 3F).

*Storage Portion* (*Reservoir*)

In all species, the reservoir is a sac lined by a wall of a single squamous epithelium, nuclei exhibit sparse heterochromatin (Fig. 2H, 3F). The epithelium is lined by the cuticular intima, thinner than that observed in the collecting chamber.

DISCUSSION

The metapleural glands of ants of basal and derived attines, and Blepharidattini and Ectatommini exhibited two portions: secretory and storage portions connected by canaliculi. The morphological organization was similar to that described in previous studies on workers of *A. bisphaerica* and *A. sexdens rubropilosa* (Gusmão et al. 2001), *A. subterraneus* (De Souza et al. 2006), *A. laevigata* (Vieira et al. 2010, 2012) and *A. coronatus* (Vieira et al. 2011).

The plasma membrane exhibited specializations, characterized as invaginations of secretory cells in *A. coronatus, T. fuscus, W. auropunctata, M. parallelus,* and *A. pilosum,* and *A. laevigata* (Vieira et al. 2012). In *E. brunneum,* these invaginations were very prominent. According to our observations, these invaginations might facilitate the

absorption of the extracellular material from the hemolymph into the cytoplasm, forming vesicles. This has also been reported by Billen and van Boven (1987) and Caetano (1998) for the metapleural glands of nomad ants *Dorylus* spp. and the post-pharyngeal gland of *Dinoponera australis*, respectively. The invaginations have also been frequently observed in pheromone producing glands of other ant species (Billen 1985, 1986). The role of these invaginations may be to increase the surface area between cells and the extracellular environment.

In the present study, we observed the presence of vesicles in the secretory cells of the metapleural gland of *A. coronatus*, *A. pilosum*, *W. auropunctata* and *E. brunneum*. According to Junqueira and Salles (1975) these vesicles contain fluids and could be the result of endocytosis or exocytosis.

The secretory cells of the metapleural glands of Ectatommini and Blepharidattini examined in the present study were round, as well as those of *Diacamma rugosum* and *D. vagans* (Schoeters and Billen 1992). In attines, these cells were oval, as also observed in *A. substerraneus* (De Souza et al. 2006), *A. octospinosus* (Bot et al. 2001), *A. bisphaerica* and *A. sexdens rubropilosa* (Gusmão, 2000), and *A. laevigata* (Vieira et al. 2012). According to Junqueira and Carneiro (2008), the role of cells taller than wider (columnar cells) is mainly synthesize and secrete compounds, which suggests that the level of synthesis in the metapleural gland cells of attines is higher than in those of Ectatommini and Blepharidattini.

Mitochondria, important organelles in the physiology of secretory cells, exhibited various shapes and were located in different areas of the cell. In the derived attines *A. coronatus* and *A. laevigata* (Vieira et al. 2012), mitochondria were observed mainly near microvilli of the intracytoplasmic portion of canaliculi (pericanalicular). In

the derived attine *T. fuscus*, and basal attine, and Ectatommini and Blepharidattini, this organelle was distributed throughout the cytoplasm, suggesting that the metapleural glands of leaf-cutting ants (derived attines) are more involved in the synthesis of secretion than those of other species. According to Tulloch et al. (1962), and Vieira et al. (2012), two hypothesis could explain the presence of mitochondria in the pericanalicular space: a) they could be involved in the synthesis of secretion (mainly lipids), and thus provide energy for the synthesis of cellular material and b) they could be involved in the production of energy required by the active transport of secretion produced in the cytoplasm of secretory cells to the intracellular canaliculi.

The presence of well-developed lamellar RER in the leaf-cutting ants *A. coronatus* and *A. laevigata* (Vieira et al. 2012), as well as the basal attine *A. pilosum* and Blepharidattini and Ectatommini, has been associated with the transport of the RNA from the nucleus to the cytoplasm during the synthesis of proteins (Alberts et al. 2004). This type of reticulum was not frequently observed in *T. fuscus* and *M. parallelus*, indicating that the secretory cells of these species may be less active in the synthesis of proteins. In *A. bisphaerica* and *A. sexdens rubropilosa* (Gusmão et al. 2001), and other groups such as *D. rugosum* and *D. vagans* (Schoeters and Billen 1992) some regions exhibited well-developed RER for the production of proteins compounds that will make up the final secretion. This is in agreement with our findings regarding the presence of large nuclei and predominance of euchromatin. In some secretory cells of the metapleural gland of leaf-cutting ants, and the attines *T. fuscus* and *A. pilosum*, and the Blepharidattini *W. auropunctata*, the nucleus is irregular. According to Alberts et al. (2004) cells with irregular nuclei have an increased nuclear surface, possibly

intensifying the transport of RNAm from the nucleus to the cytoplasm and consequently the synthesis of proteins by ribosomes.

The smooth endoplasmic reticulum was little developed and not frequently observed in the secretory cells of the metapleural glands of leaf-cutting ants and basal attines, unlike in *T. fuscus*. According to Billen (1984), the smooth endoplasmic reticulum (SER) participates in the synthesis of lipids and its level of development indicates the intensity of synthesis. However, although SER is not well developed in minor workers of *A. coronatus* and *A. laevigata* (Vieira et al. 2012) and *W. auropunctata*, lipid droplets were observed in the cytoplasm of secretory cells of their metapleural glands, suggesting the existence of another source of synthesis of lipids. Therefore, the metapleural gland of leaf-cutting ants and Blepharidattini may be more active in the production of lipids than in other attines and ectatommines.

In the leaf-cutting ants *A. coronatus* and *A. laevigata* (Vieira et al. 2012) and in Ectatommini, regions of the Golgi were well developed compared to those of *M. parallelus* and *W. auropunctata*, in which this organelle were not always observed. In the leaf-cutting ants *A. bisphaerica*, *A. sexdens sexdens*, and *A. sexdens rubropilosa* (Schoeters and Billen 1993) the Golgi complex was also observed, unlike in *M. nigrocincta* (Tulloch et al. 1962). According to Carvalho and Recco-Pimentel (2001), the role of the Golgi complex is to store and modify the secretions produced in the RER, suggesting that in all species examined in this study, except in *W. auropunctata*, the Golgi complex probably modifies the secretions of RER into compounds with antibiotic properties. Chromatographic tests conducted by Ortius-Lechner (2000) demonstrated the presence of various volatile substances, including the antibiotic compound in the secretion of the metapleural gland of *A. octospinosus*.

The presence of vacuoles with different contents in the cytoplasm of secretory cells of the metapleural glands of all species examined in our study, as well as *A. laevigata* (Vieira et al. 2012), suggest cell turnover. Therefore recycling processes of components of secretory cells may occur, as suggested by the presence of many digestive vacuoles as phagosomes and autophagosomes. These vacuoles were more abundant in *A. coronatus* and *A. laevigata* (Vieira et al. 2012), and less evident in *T. fuscus*, *A. pilosum* and in the Blepharidattini *W. auropunctata* and the Ectatommini *E. brunneum.* According to Junqueira and Salles (1975), phagosomes containing hydrolytic enzymes fuse with materials of the cell and form autophagosomes, where intracellular digestion takes place (autophagocytosis). Myelin figures were then formed as a result of the intracellular digestion, as observed in the secretory cells of the metapleural glands of leaf-cutting ants and *T. fuscus*, *A. pilosum*, and *W. auropunctata*.

Large secretion granules were observed in the cytoplasm of secretory cells of the metapleural glands of the leaf-cutting ant *A. coronatus*, *A. laevigata* (Vieira et al. 2012), the basal attine *A. pilosum*, and the Blepharidattini *W. auropunctata* compared to those of attines *T. fuscus* and *M. paralelus.* In *E. brunneum*, on the other hand, small granules were observed, suggesting the capacity of these ants to produce secretion consisted of different chemistry compounds.

Leaf-cutting ants and basal attines (fungus-growers), as well as non-fungus growers examined in the present study exhibit intra and extracanaliculi with similar morphology. The intracytoplasmic portion exhibited microvilli (pericanalicular space), whose main role is to increase the surface area for collection and later modification of the secretion (Vieira et al. 2012). Also, in all attines, the intracytoplasmic portion meanders in the secretory cells, while in *E. brunneum* and *M. nigrocincta* (Tulloch et al.

1962), canaliculi surround the nucleus. This morphological arrangement suggest an additional increase in the area of collection of the secretion throughout the cytoplasm of secretory cells of the metapleural glands.

The extracytoplasmic portion was devoid of microvilli and the cuticle lining the lumen was thick, confirming the role of this region in the transport and preservation of the secretion produced in secretory cells. According to Vieira et al. (2012), the thick cuticle might act as a physical barrier preventing the contamination or modification of the secretion by agents external to the canaliculus.

The collecting chamber and the reservoir of all species examined in the present study exhibited simple squamous epithelium lined by a cuticle. Schoeters and Billen (1992) observed a similar morphology in *Diacamma*. Also, according to Schoeters and Billen (1992) and Vieira et al. (2012), the sclerotized cuticle acts as a barrier separating the secretion, which can be acidic or made up of similar compounds, protecting the epithelial cells of the collecting chamber and the reservoir from the secretion and preventing the contamination of the secretion by extracellular compounds.

Thus, the present ultrastructural study demonstrates that the metapleural glands of derived and basal attines differ from non-fungus-growing ants by exhibiting oval secretory cells, which suggests a higher secretory capacity in attines. In addition, secretory cells of the metapleural glands of leaf-cutting ants exhibit many mitochondria located near microvilli of the intracytoplasmic portion of the canaliculus, indicating more involvement in the production of lipids and transport of the secretion. Therefore, the metapleural glands of leaf-cutting ants were more active in the production of secretion than those of basal attines and non-fungus-growing ants.

ACKNOWLEDGEMENTS

We thank the Center of Social Studies – São Paulo State University (UNESP), Rio Claro, SP-Brazil for assistance in the collection and identification of specimens used in this study, FAPESP (São Paulo Research Foundation) for financial support (grant # 2008/54301-3), and CNPq.

REFERENCES

Alberts B, Johnson A, Walter P (2004) Biologia Molecular da Célula. 4 ed. Porto Alegre: Artmed. 1549p.

Attygalle AB, Siegel B, Vostowsky O, Bestmann HJ, Maschwitz U (1989) Chemical composition and function of metapleural gland secretion of the ant, *Crematogaster deformis* Smith (Hymenoptera: Myrmicinae). J Chem Ecol 15:317–328

Beattie AJ, Turnbull C, Hough T, Jobson S, Knox RB (1985) The vulnerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. Am J Bot 72:606–614

Beattie AJ, Turnbull C, Hough T, Knox RB (1986) Antibiotic production: A possible function for the metapleural glands of ants (Hymenoptera: Formicidae). Ann Entomol Soc Am 79:448–450

Billen JPL, Peusens G (1984) Ultrastructure de la Glande Propharyngienne chez les Fourmis Formicines (Hymenoptera, Formicidae). Act Coll Insectes Soc 1:121-129

Billen JPL (1985) Comparative Ultrastructural of the Poison and Dufour Glands in Old and New World Army Ants (Hymenoptera, Formicidae). Act Coll Insectes Soc 2:79-86

Billen JPL (1986) Comparative Morphology and Ultrastructure of the Dufour Gland in Ants (Hymenoptera, Formicidae). Entomol Gen 11:165-181

Billen JPL, Van Boven (1987) The metapleural gland in Old Word army ants: a morphological and ultrastructural description (Hymenoptera, Formicidae). Rev Zool Afr 101:31-41

Bolton B (2003) Synopsis and Classification of Formicidae. Mem Am Entomol Inst 71:1-370

Bot ANM, Obermayer ML, Hölldobler B, Boomsma JJ (2001) Functional morphology of the metapleural gland in the leaf-cutting ant *Acromyrmex octospinosus*. Insectes Soc 48:63-66

Bot ANM, Ortius-Lechner D, Finster K, Maile R, Boomsma JJ (2002) Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. Insectes Soc 49:363-370

Brown WLJr (1968) An Hypothesis Concerning the Function of the Metapleural Glands in Ants. Am Nat 102:188-191

Caetano FH (1998) Aspectos ultramorfológicos, ultra-estruturais e enzimológicos da glândula pós-feringea de *Dinoponera australis* (Formicidae: Ponerinae). Tese de Livre-Docência em Histologia. Rio Claro – UNESP – Instituto de Biociências, Brasil. 134p.

Cammaerts M-C, Cammaerts R (1998) Marking of nest entrances and vicinity in the ant *Pheidole pallidula* (Formicidae, Myrmecinae). Behav Processes 42:19-41

Cammaerts M-C, Cammaerts R (2001) Marking of nest entrances and vicinity in two related *Tetramorium* ant species (Hymenoptera: Formicidae). J Insect Behav 14:247–269

Carvalho HF, Recco-Pimentel SM (2007) A Célula. 2ª ed. Editora Manole Ltda: Barueri, São Paulo, Brasil. 287p.

De Souza ALB, Soares IMF, Cyrino LT, Serrão JE (2006) The Metapleural Gland in Two Subspecies of *Acromyrmex subterraneus* (Hymenoptera: Formicidae). Sociobiology 47:19-25.

Do Nascimento RRD, Schoeters E, Morgan ED, Billen J, Stradling DJ (1996) Chemistry of metapleural gland secretions of three attine ants, *Atta sexdens rubropilosa*, *Atta cephalotes*, and *Acromyrmex octospinosus* (Hymenoptera: Formicidae). J Chem Ecol 22:987-1000

Donisthorpe H (1941) Description of a new species of *Crematogaster* Lund, subgenus *Physocrema* Forel, with a list of, and a key to, the known species of the subgenus. The Entomologist 74:225-227

Fernandez-Marin H, Zimmerman J, Rehner S, Wcislo W (2006) Active use of the metapleural glands by ants in controlling fungal infection. Proc Roy Soc Lond B Biol Sci 273:1689-1695

Gusmão LG (2000) Estudo comparado da glândula metapleural e sua secreção em diferentes castas de três espécies de *Atta* (Hymenoptera: Formicidae). 60p. Tese (Doutorado) - ESALQ-USP, Piracicaba-SP

Gusmão LG, Caetano FH, Nakano O (2001) Ultramorphology of the metapleural gland in three species of *Atta* (Hymenoptera, Formicidae). Iheringia, Zool 91:33-36

Hölldobler B, Wilson EO (1990) The Ants. The Belknap Press of Harvard University Press, Cambridge, Mass, 732p

Hosoishi S, Ogata K (2008) The ant genus *Crematogaster* Lund, subgenus *Physocrema* Forel, in the Indochinese Peninsula (Hymenoptera: Formicidae). Asian Myrmecol 2:1-10

Jaffé K, Puche H (1984) Colony-specific territorial marking with the metapleural gland secretion in the ant *Solenopsis geminata* (Fabr.). J Insect Physiol 30:265–270

Jaffe K, Lopez ME, Aragort W (1986) On the communication systems of the ants *Pseudomyrmex termitarius* and *P. triplarinus*. Insectes Soc 33:105–117

Janet CH (1898) Système glandulaire tègumentaire de la *Myrmica rubra*: observations diverses sur les fourmis, in: Janet C (eds), Études sur les Fourmis, les Guèpes et les Abeilles, Note 17, p. 1-28. Carré et Naud, Paris.

Jones TH, Brunner SR, Edwards AA, Davidson DW, Sneling RR 2(005) 6-Alkylsalicylic acids and 6-alkylresorcylic acids from ants in the genus *Crematogaster* from Brunei. J Chem Ecol 31:407-417

Junqueira LCU, Junqueira LMMS (2008) Histologia básica. Rio de Janeiro, Guanabara Koogan

Junqueira LCU, Salles LMM (1975) Ultra-estrutura e função celular. ed. Guanabara Koogan, São Paulo, Brasil

Maschwitz U, Koob K, Schildknecht H (1970) Ein Beitrag zur Funktion der Metathoracaldrüse der Ameisen. J Insect Physiol 16:387–404

Maschwitz U (1974) Vergleichende Untersuchungen zur Funktion der Ameisenmetathorakaldrüse. Oecologia 16:303–310

Mueller UG (2002) Ant versus fungus versus mutualism: Ant-cultivar conflict and the deconstruction of the attine ant-fungus symbiosis. Am Nat 160:S67-S98

Mueller UG, Schultz TR, Currie CR, Adams RM, Malloch D (2001) The origin of the attine ant-fungus mutualism. Q Rev Biol 76:169-197

North RD, Jackson CW, Howse PE (1997) Evolutionary aspects of anti-fungus interactions in leaf-cutting ants. Tree 12:386–389

Ortius-Lechner D, Maile R, Morgan ED, Boomsma JJ (2000) Metapleural gland secretion of the leaf-cutter ant *Acromyrmex octospinosus*: New compounds and their functional significance. J Chem Ecol 26:1667-1683

Poulsen M, Bot ANM, Nielsen MG, Boomsma JJ (2002) Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. Behav Ecol Sociobiol 52:151-157

Schildknecht H, Koob K (1971) Myrmicacin, the first insect herbicide. Angew Chem Int Ed 10:124–125

Schoeters E, Billen J (1992) Morphological and ultrastructural study of metapleural gland in *Diacamma* (Hymenoptera, Formicidae), in: European Congress of Social Insects, 1o, Leuven, 1991. Proceedings Leuven, Leuven University. pp. 239-247

Schoeters E, Billen J (1993) Anatomy and fine struture of the metapleural gland in *Atta*. Belg J Zool 123:67–75

Solomon SE, Mueller UG, Schultz TR, Currie CR, Price SL (2004) Nesting biology of the fungus growing ants *Mycetarotes* Emery (Attini, Formicidae). Insectes Soc 51:333-338

Thorne BL, Traniello JFA (2003) Comparative social biology of basal taxa of ants and termites. Ann Rev Entomol 48283–306

Tulloch GS, Shapiro JE, Hershenov B (1962) The ultrastructure of the metasternal glands of ants. Bull Brooklyn Entomol Soc 57:91-101

Vieira AS, Bueno OC, Camargo-Mathias MI (2010) The functional morphology of the metapleural gland of the leaf-cutting ant *Atta laevigata* (Formicidae: Attini). Micron 41:149–157

Vieira AS, Bueno OC, Camargo-Mathias MI (2011) Secretory Profile of Metapleural Gland Cells of the Leaf-Cutting Ant *Acromyrmex coronatus* (Formicidae: Attini). Microsc Res Tech 74:76–83

Vieira AS, Bueno OC, Camargo-Mathias MI (2012) Ultrastructural profile of metapleural gland cells of the ant *Atta laevigata* (F. Smith, 1858) (Formicidae: Attini). Anim Biol 62:1–11

Weber NA (1972) Gardening ants: The Attines. Philadelphia (PA): American Philosophical Society, 92, 146p

Wilson EO (1971) The Insect Societies. Belknap Press, Harvard University, Cambridge Massachusetts.

Yek SH, Mueller UG (2011) The metapleural gland of ants. Biological Reviews, 86, 774-791.

**Figure 1. Schematic representation and transmission electron microscopy (TEM) of the metapleural gland of *Acromyrmex coronatus* (leaf-cutting ant). A – B)** Detail of two secretory cells (**sc**), in **A** shows the plasma membrane (**m**), with invaginations (**i**), and vesicles (**ve**) and in **B** another vesicle (**ve**). The plasma membrane is not fused, but the areas of contact between two secretory cells (**sc**), the basal laminae are (**bl**). Minor worker. **A** (scale bar= 1 µm) and **B** (scale bar= 2 µm). (**C)** General view of the oval-shaped secretory cells **(sc**), in addition to the intracytoplasmic portion of the canaliculus (**ic**) and its microvilli(**mic**), secretion (**sec**), lipid droplets (**li**) and mitochondria (**mi**) near this canaliculus. Minor worker. Scale bar= 6 µm. **D)** Detail of secretory cell (**sc**) with irregular shaped nucleus (**n**) (**white arrow**), in addition to mitochondria (**mi**), various shapes: elongated and round; and smooth endoplasmic reticulum (**dark arrow**). Major worker. Scale bar= 2 µm. **E)** Detail of a secretory cell (**sc**) with lamellar rough endoplasmic reticulum (**rer**) near the nucleus (**n**) and mitochondria (**mi**). Major worker. Scale bar= 2 µm. **F)** Regions of the Golgi (**G**) with typical organization in flattened and layered cisternae with flattened ends and vacuoles (**v**). Minor worker. Scale bar= 1 µm. **G)** Myelin figure (**mf**) with multilamellar content, in addition to vacuoles (**v**) and autophagosome (**ap**) with contents of different electron densities. Minor worker. Scale bar= 2 µm. **H)** Intracytoplasmic canaliculus (**ic**), microvilli (**mic**). Note near microvilli lipid droplets (**li**), vacuoles (**v**), and secretion (**sec**) of different electron densities and electron lucent. Minor worker. Scale bar= 2 µm. **I)** Cluster of intracytoplasmic canaliculi (**ec**) with round nucleus (**n**) and thick cuticle lining its wall (**white arrow)** showing the lumen (**l**) filled with secretion. Minor worker. Scale bar= 3 µm.

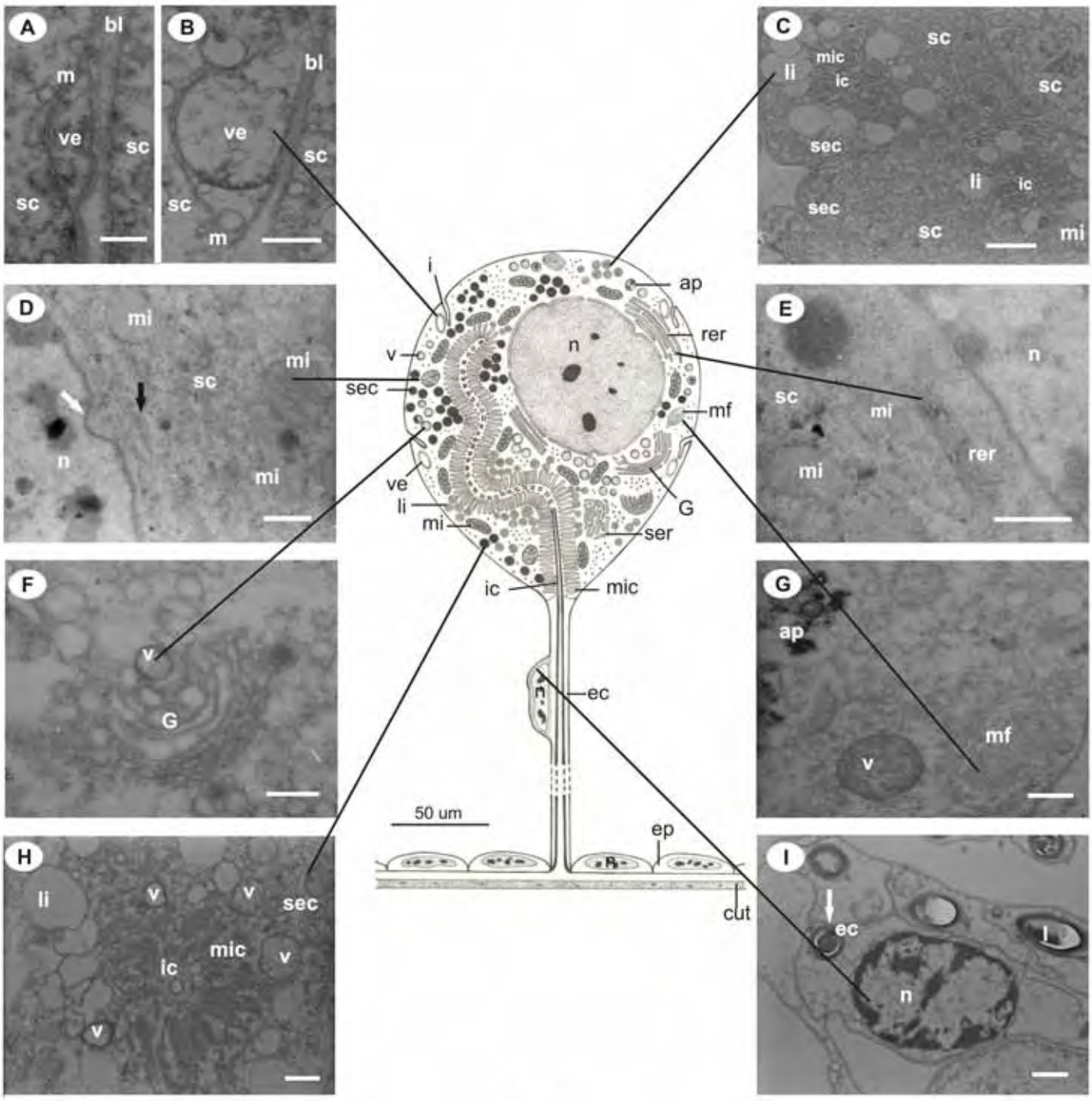

A
bl
m
ve
sc
sc
B
bl
ve
sc
sc
m
C
sc
mic
li
ic
sc
sec
li
ic
sec
sc
mi
D
mi
mi
sc
n
mi
E
n
sc
mi
mi
rer
F
v
G
G
ap
mf
v
H
li
v
v
sec
mic
ic
v
v
i
ap
rer
n
v
sec
mf
ve
li
G
mi
ser
ic
mic
ec
50 um
ep
cut
I
ec
l
n

Fig. 1

**Figure 2. Schematic representation and transmission electron microscopy (TEM) of the metapleural gland of *Trachymyrmex fuscus* (derived Attini)**. **A)** Detail of secretory cell (**sc**), showing the invaginations (**i**) of the plasma membrane (**m**) and mitochondria (**mi**). Scale bar= 4 µm. **B)** A secretory cell **(sc**) with irregular-shaped nucleus (**n**) (**white arrow**). Round and elongated mitochondria (**mi**) near the nucleus. Scale bar= 4 µm. **C)** Detail of secretory cell showing the lamellar rough endoplasmic reticulum(**white arrow**) near the nucleus (**n**), mitochondria (**mi)** and lipid droplets (**li**). Scale bar= 2 µm. **D)** Detail of a secretory cell (**sc**) with nucleus (**n**) with lamellar smooth endoplasmic reticulum (**ser**). Scale bar= 2 µm. **E)** Golgi regions (**G**) with typical organization in flattened and layered cisternae and with flattened ends and vacuoles (**v**), and mitochondria **(mi**). Scale bar= 2 µm. **F)** Detail of secretory cell (**sc**) with nucleus (**n**), autophagosome (**ap**) and mitochondrium (**mi**). Scale bar= 1 µm. **G)** Intracytoplasmic canaliculus (**ic**), microvilli (**mic**) and cuticular intima (**white arrow**). Note near microvilli lipid droplets (**dark arrow**) and mitochondria (**mi**). Scale bar= 4 µm. **H)** Detail of the simple squamous epithelium (**ep**) of the reservoir with cuticular intima (**cut**) and nucleus (**n**). Scale bar= 4 µm.

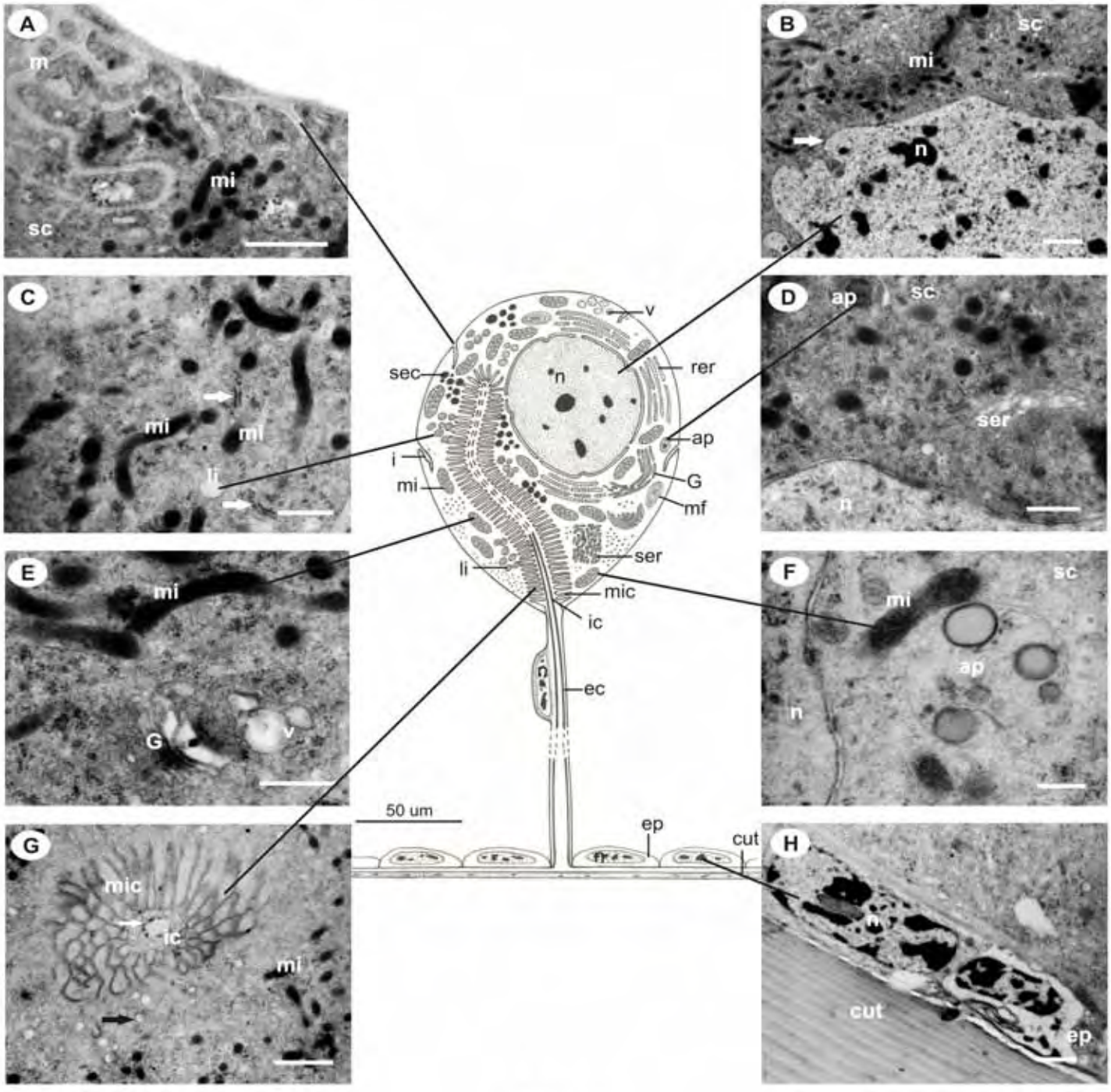

A
m
mi
sc
B
sc
mi
n
C
mi
mi
li
D
ap
sc
ser
n
v
sec
n
rer
ap
i
mi
G
mf
ser
li
mic
ic
ec
E
mi
G
v
F
sc
mi
ap
n
50 um
ep
cut
G
mic
ic
mi
H
cut
ep

Fig. 2

**Figure 3. Schematic representation and transmission electron microscopy (TEM) of the metapleural gland of *Mycetarotes parallelus* (basal Attini)**. **A)** General view of a secretory cell (**sc**), regular-shaped nucleus (**n**) (**white arrow**), secretion (**sec**), extracytoplasmic canaliculi (**ec**) and simple squamous epithelium (**ep**) of the reservoir (**r**) and nucleus (**n**). Scale bar= 10 µm. **B)** Detail of a secretory cell (**sc**) showing the plasma membrane (**m**) with invaginations (**i**), basal lamina (**bl**) and mitochondria(**mi**). Scale bar= 2 µm. **C)** Detail of vacuoles (**v**) and mitochondria (**mi**) in secretory cells and digestive vacuole as phagosome (**ph**). Scale bar= 2 µm. **D)** Regions of Golgi (**G**) with typical organization in flattened and layered cisternae and with flattened ends, and vacuoles (**v**). Scale bar= 2 µm. **E)** Intracytoplasmic canaliculus (**ic**), microvilli (**mic**) and cuticular intima (**white arrow**). Note lipid droplets (**li**) near microvilli lipid droplets, and secretion (**sec**). Scale bar= 2 µm. **F)** Detail of the storage portion of the metapleural gland, the collecting chamber (**cc**), in which extracytoplasmic canaliculi (**ec**) open, internally lined by a cuticular intima (**white arrow**), lumen (**l**), and sieved plates (**dashed**) where canaliculi attach. The collecting chamber is a narrowing of the reservoir (**r**), which exhibit a thick cuticular intima (**cut**). Scale bar= 6 µm.

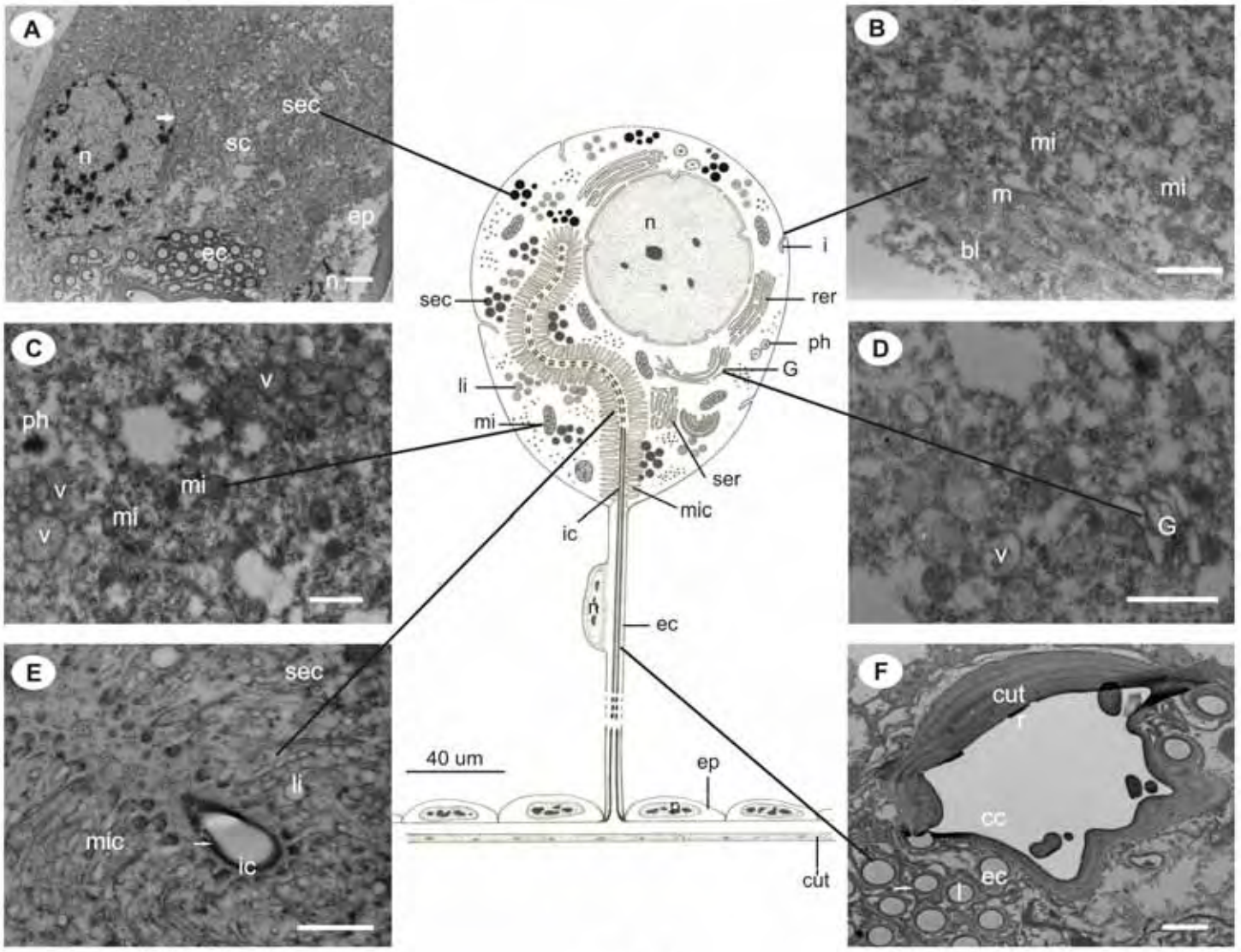

A
sec
sc
n
ep
ec
n
B
mi
m
mi
bl
C
v
ph
v
mi
mi
v
D
G
v
E
sec
li
mic
ic
F
cut
cc
ec
n
sec
i
rer
ph
G
li
mi
ser
mic
ic
ec
40 um
ep
cut

Fig. 3

**Figure 4. Schematic representation and transmission electron microscopy (TEM) of the metapleural of *Apterostigma pilosum* (basal Attini)**. **A)** Detail of secretory cell (**sc**), showing the plasma membrane (**arrow**) with invaginations (**i**), vesicles (**arrow**) and smooth endoplasmic reticulum(**ser**). Scale bar= 2 µm. **B)** Detail of a secretory cell (**sc**) with irregular nucleus (**n**) (**arrow**). Lamellar rough endoplasmic reticulum (**rer**), and mitochondria (**mi**) of various shapes: round and elongated. Scale bar= 1 µm. **C)** Regions of Golgi (**G**) with typical organization as flattened and layered cysternae and flattened ends. Mitochondria (**mi**) are also observed. Scale bar= 1 µm. **D)** Detail of secretory cell (**sc**) showing autophagosome (**ap**) and secretion (**sec**). Scale bar= 2 µm. **E)** Intracytoplasmic canaliculus (**ic**), microvilli (**mic**). Note lipid droplets (**li**), secretion (**sec**), vacuole (**v**), mitochondria (**mi**) and digestive vacuole as phagosome (**ph**) and irregular nucleus (**n**)(**arrow**). Scale bar= 4 µm. **F)** Detail of a cluster (**dashed line**) of the extracytoplasmic portion of the canaliculus(**ec**) showing the cuticular intima (**arrow**), lumen (**l**), and secretion (**sec**) inside the extracytoplasmic canaliculus. Scale bar= 2 µm.

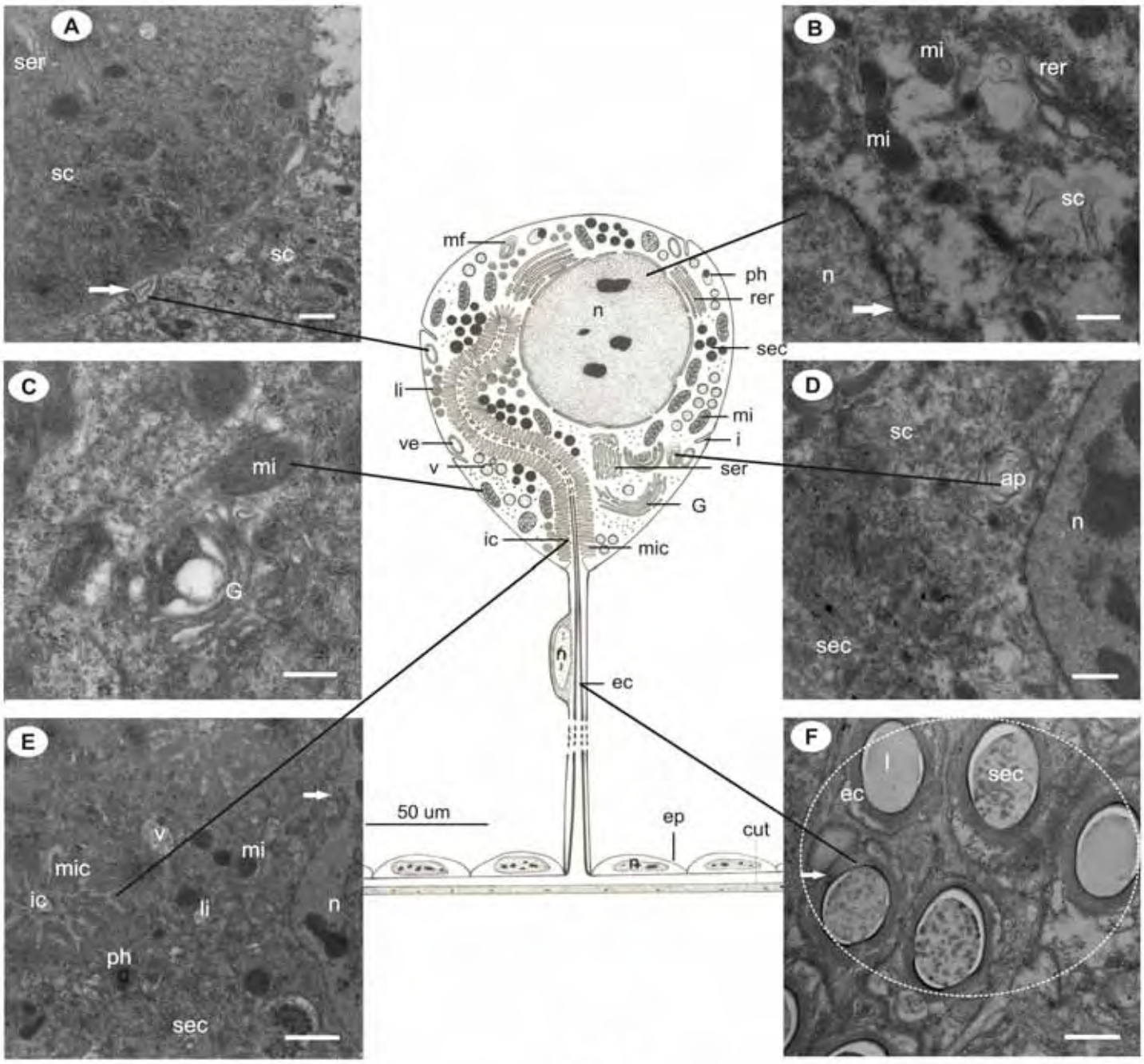

A
ser
sc
sc
B
mi
rer
mi
sc
n
C
mi
G
D
sc
ap
n
sec
E
v
mi
mic
ic
li
n
ph
sec
F
l
sec
ec
mf
ph
rer
n
sec
li
mi
ve
i
v
ser
G
ic
mic
ec
50 um
ep
cut

Fig. 4

**Figure 5. Schematic representation and transmission electron microscopy (TEM) of the metapleural gland of *Wasmannia auropunctata* (Blepharidattini)**. **A)** General view of secretory cells (**sc**), nucleus (**n**), intracytoplasmic canaliculus (**ic**) and pericanalicular space with microvilli(**mic**), extracytoplasmic canaliculus (**ec**), and vesicles (**ve**). Scale bar= 20 µm. **B)** Detail of a secretory cell (**sc**) showing invaginations (**i**) in the plasma membrane (**m**), basal lamina (**bl**), secretion (**sec**), and vacuole (**v**). Scale bar= 4 µm. **C)** Detail of plasma membrane (**m**) of the secretory cell. The plasma membrane is not fused, but the contact areas between the two secretory cells (**sc**), the basal laminae (**bl**) are. Vacuoles (**v**) and vesicles (**ve**) are also present. Scale bar= 1 µm. **D)** Detail of mitochondria (**mi**) and vacuoles (**v**) in the secretory cell. Scale bar= 1 µm. **E)** Detail of a secretory cell (**sc**) com irregular-shaped nucleus (**n**) (**arrow**), lamellar rough endoplasmic reticulum (**rer**), and vacuoles (**v**). Scale bar= 2 µm. **F)** Apical region of a secretory cell (**sc**) with large nucleus (**n**), lamellar rough endoplasmic reticulum(**rer**), smooth endoplasmic reticulum(**ser**), and vacuole(**v**). Scale bar= 3 µm. **G)** Detail of a secretory cell (**sc**) showing myelin figure (**mf**), digestive vacuole as autophagosome (**ap**), secretion (**sec**), and vacuole (**v**). Scale bar= 1 µm. **H)** Intracytoplasmic canaliculus (**ic**) and microvilli (**mic**). Note lipid droplets (**arrow**), secretion (**sec**), and vacuole (**v**). Scale bar= 4 µm.

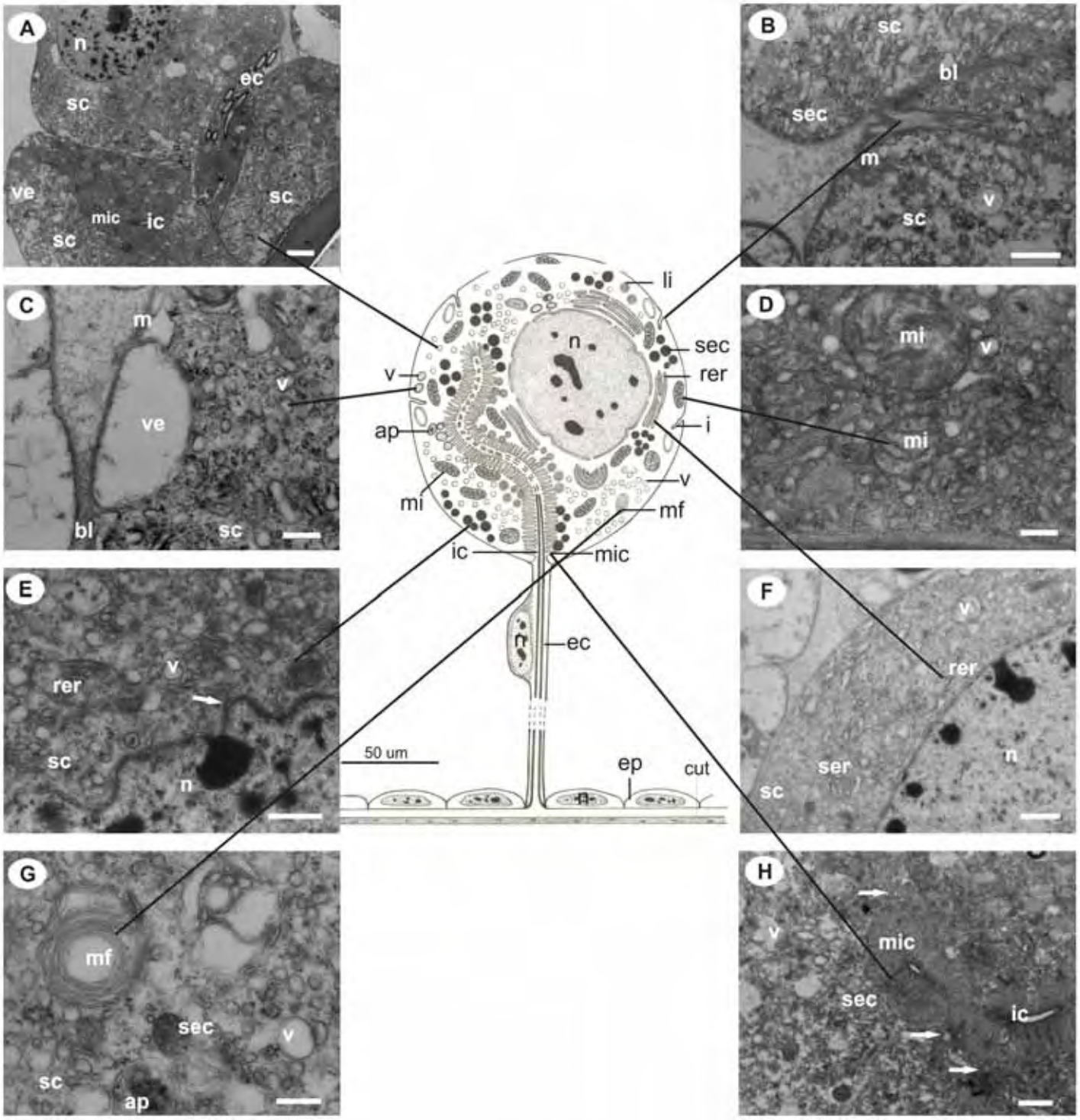

A
n
ec
sc
ve
mic
ic
sc
sc
B
sc
bl
sec
m
v
sc
C
m
v
ve
bl
sc
li
n
sec
rer
v
i
ap
v
mi
mf
ic
mic
ec
50 um
ep
cut
D
mi
v
mi
E
rer
v
sc
n
F
v
rer
ser
n
sc
G
mf
sec
v
sc
ap
H
v
mic
sec
ic

Fig. 5

**Figure 6. Schematic representation and transmission electron microscopy (TEM) of the metapleural gland of *Ectatomma brunneum* (Ectatommini)**. **A)** Detail of the plasma membrane (**m**) and invaginations (**i**) of the secretory cell (**sc**). The plasma membranes of adjacent cells are not fused, but the contact areas between the two secretory cells (**sc**), the basal laminae (**bl**) are. Mitochondria (**mi**) and vesicles (**ve**) are present. Scale bar= 4 µm. **B)** Detail of a secretory cell (**sc**) with regular nucleus (**n**) (**dark arrow**), mitochondria (**white arrow**), and digestive vacuoles as phagosomes (**ph**) and autophagosomes (**ap**). Scale bar= 6 µm. **C)** Detail of a secretory cell (**sc**) showing lamellar endoplasmic reticulum (**rer**) and mitochondria (**mi**). Scale bar= 2 µm. **D)** Regions of Golgi (**G**) with typical organization of flattened and layered cysternae with flattened ends. Vacuoles (**v**) and secretion (**sec**) are also observed. Scale bar= 1 µm. **E)** Intracytoplasmic canaliculus (**ic**), microvilli (**mic**). Note the presence of secretion (**sec**), digestive vacuole as autophagosome (**ap**), and mitochondria (**mi**). Scale bar= 2 µm. **F)** Detail of the extracytoplasmic portion of the canaliculus(**ec**), nucleus (**n**), cuticular intima (**arrow**), lumen (**l**), and simple squamous epithelium(**ep**) of the reservoir (**r**). Scale bar= 4 µm.

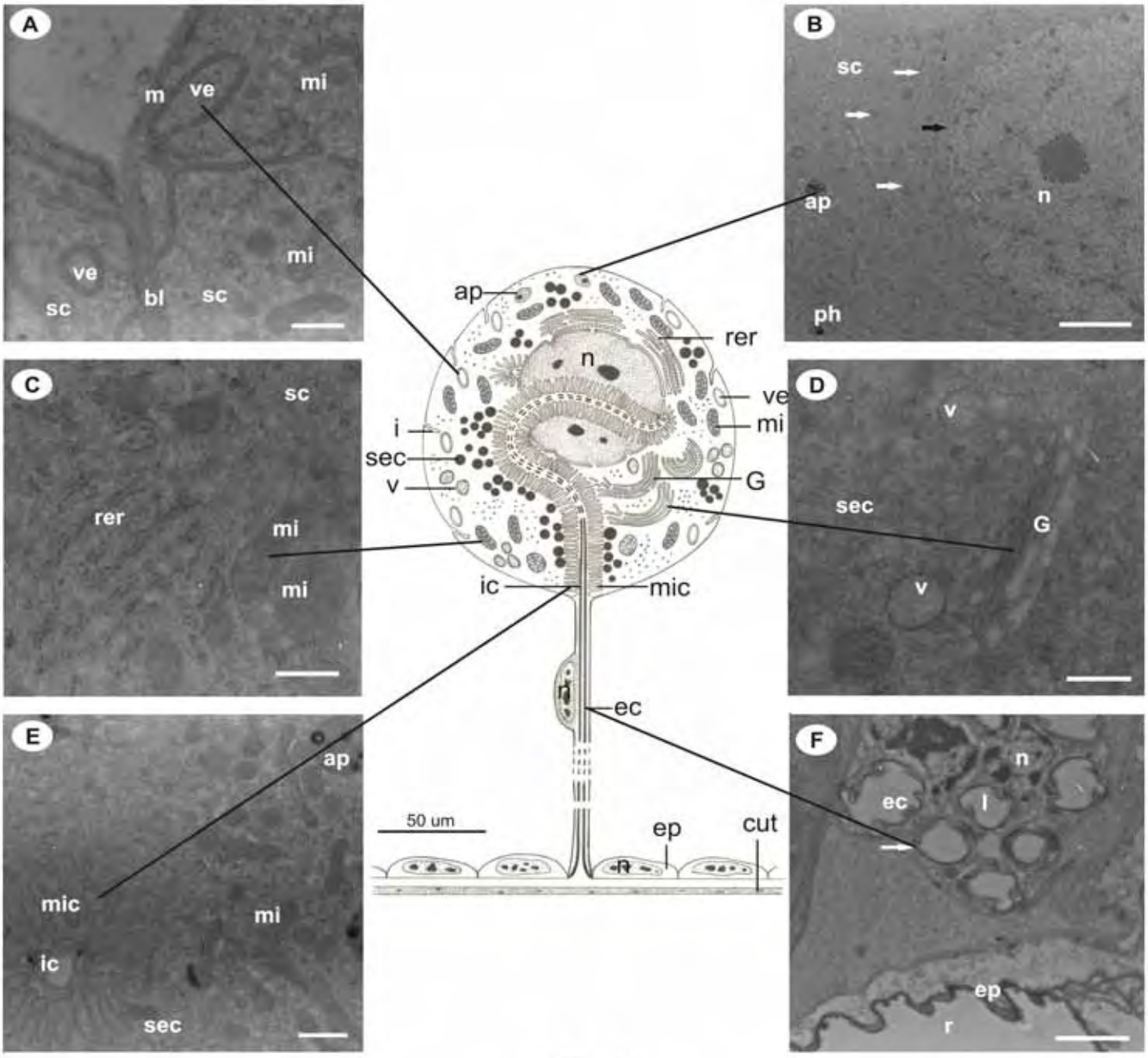

A
m
ve
mi
mi
ve
bl
sc
sc
B
sc
ap
n
ph
C
sc
rer
mi
mi
D
v
sec
G
v
E
ap
mic
mi
ic
sec
F
n
ec
l
ep
r
ap
rer
n
ve
i
mi
sec
v
G
ic
mic
ec
50 um
ep
cut

Fig. 6

**Table 1**. Ultrastructural results of the secretory portion of the metapleural glands of monomorphic workers of *Ectatomma brunneum*, *Wasmannia auropuncata*, *Apterostigma pilosum*, *Mycetarotes parallelus*, *Trachymyrmex fuscus*, and polymorphic workers of *Acromyrmex coronatus* and *Atta laevigata*.

| | Secretory Cells | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **Shape of secretory cells** | **Invaginations of the plasma membrane** | **Mitochondria** | **Lamellar RER** | **SER** | **Regions of Golgi** | **Myelin figure** | **Digestive vacuole** | **vacuoles** | **Secretion granules** | **lipid droplets** | **nucleus** |
| ***Ectatomma brunneum*** | R | P | P | D | NO | D | NO | PH/AP | P | S | NO | RE |
| ***Wasmannia auropunctata*** | R | P | P | D | LD | NO | P | AP | P | L | L | IR |
| ***Apterostigma pilosum*** | O | P | P | D | LD | D | P | PH | P | L | S | IR |
| ***Mycetarotes parallelus*** | O | P | - | LD | LD | LD | NO | PH | P | S | S | RE |
| ***Trachymyrmex fuscus*** | O | P | P | LD | D | D | P | AP | P | S | S | IR |
| ***Acromyrmex coronatus*** | O | P | P | D | LD | D | P | AP | P | L | L | IR |
| ****Atta laevigata*** | O | P | P | D | LD | D | P | AP | P | L | L | IR |

*published data, see details in Vieira et al. (2012,) for *Atta laevigata* (minor workers); P= present; NO= not observed; D= developed; LD= little developed; RE= regular; IR= irregular; R= round; O= oval; PH= phagosome; AP= autophagosome; L= large; S= Small;

# *Capítulo 6*

**Composição química das secreções das glândulas metapleurais em formigas cultivadoras e não cultivadoras de fungos (Hymenoptera, Formicidae)**

**Autores**: Alexsandro Santana Vieira, E. David Morgan, Falko P. Drijfhout e Maria Izabel Camargo-Mathias
**Periódico**: Submetido ao periódico Journal of Chemical Ecology (2012)

## RESUMO

A glândula metapleural é única no grupo das formigas, e incomum entre as glândulas exócrinas, não possuindo um mecanismo para fechamento e retenção de sua secreção. Até agora, nenhuma conclusão clara tem sido estabelecida sobre a sua função. O presente estudo teve por objetivo investigar a composição química da secreção da glândula metapleural de diferentes grupos de espécies, de formigas cultivadoras de fungos, Attini derivadas *Trachymyrmex fuscus*, *Atta laevigata*, e *Acromyrmex coronatus*, as Attini basais *Apterostigma pilosum* e *Mycetarotes parallelus*, e as formigas não cultivadoras de fungos *Ectatomma brunneum* e *Pogonomyrmex naegeli*. Os resultados mostraram que a secreção das formigas cortadeiras (*A. laevigata* e *A. coronatus*) contém maior variedade de compostos voláteis do que as das formigas myrmicine e ectatommine. Os compostos mais abundantes encontrados na glândula metapleural de *A. laevigata* e *A. coronatus* foram hidroxiácidos, e ácido fenilacético (somente em *A. laevigata*). O composto indole estava presente em todos os grupos, enquanto skatole foi encontrado em grandes quantidades somente nas atines. Cetonas e aldeídos estiveram presentes na secreção de algumas atines. Ésteres foram encontrados na secreção da glândula metapleural de todas as espécies examinadas, principalmente de *A. laevigata*, *A. coronatus* e *T. fuscus*. As formigas cortadeiras, bem como *T. fuscus* tiveram maiores quantidades de compostos voláteis quando comparadas com outras atines e formigas não cultivadoras de fungos, indicando a maior capacidade das glândulas metapleurais dessas formigas (principalmente das formigas cortadeiras) em produzirem compostos ácidos, provavelmente com papel antibiótico e antifúngico.

**Palavras-chave**: compostos antibióticos, compostos antifúngicos, glândulas exócrinas, cromatografia em fase gasosa.

# Chemical composition of metapleural glands secretions of fungus-growing and non–fungus-growing ants (Hymenoptera, Formicidae)

Alexsandro S. Vieira[1], E. David Morgan[2], Falko P. Drijfhout[2] and Maria I. Camargo-Mathias[1]

[1] UNESP – Universidade Estadual Paulista, Campus Rio Claro. Av. 24A, nº1515. Cx. Postal 199 – CEP: 13506-900 – Rio Claro, SP- Brazil.

[2] Chemical Ecology Group, School of Physical and Geographical Sciences, Keele University, Staffordshire ST5 5BG, England-UK.

*Corresponding author: micm@rc.unesp.br (M.I. Camargo-Mathias).

**Abstract**. The metapleural gland is unique to ants, and unusual among exocrine glands in having no mechanism for closure and retention of its secretion. As yet, no clear conclusion has bee reached concerning its function. The present study has investigated the volatile part of the metapleural gland secretions of different groups of species, of fungus-growing ants, represented by the derived attines *Trachymyrmex fuscus, Atta laevigata,* and *Acromyrmex coronatus,* the basal attines *Apterostigma pilosum* and *Mycetarotes parallelus*, and non-fungus-growing ants of the tribes Ectatommini (*Ectatomma brunneum*) and Myrmicini (*Pogonomyrmex naegeli*). Our results showed that the secretions of leaf-cutting ants (*A. laevigata* and *A. coronatus*) and derived attine- *T. fuscus* contain a greater varity and largest quantities of volatile compounds than those of myrmicine and ectatommine ants. The most abundant compounds found in the metapleural glands of *A. laevigata* and *A. coronatus* were hydroxy acids, and phenylacetic acid (only in *A. laevigata*). The compound indole was present in all groups examined in this study, while skatole was found in large quantities only in attines. Ketones and aldehydes, present in the secretion of some attines. Esters are present in the metapleural gland secretion of all species examined, though mainly in *A. laevigata*, *A. coronatus* and *T. fuscus*. The metapleural glands of these ants (mainly leaf-cutting ants) produce chiefly secretion containing acidic compounds, probably with an antibiotic or antifungal function, when compared with basal attines and non-fungus-growing ants.

Keywords: antibiotic compounds, antifungal compounds, exocrine gland, gas chromatography.

## INTRODUCTION

The metapleural gland is a paired structure unique to ants, located in the posterolateroal end of the metathorax (Hölldobler and Wilson, 1990) and consists of a secretory and a storage portion connected by canaliculi (Vieira et al., 2010, 2011). The gland does not have a sphincter or means of closure. Its secretion must therefore be in continuous contact with the external surface of the ant or be spread by movement of the legs or thorax (Hölldobler and Wilson, 1990). Its study challenges the curiosity of the investigator. No general function of the gland has yet been recognized. Yek and Muller (2011) proposed four hypothesis regarding the function of its secretions: a) species or colony recognition; b) nest entrance and territory marking; c) antisepsis, and d) chemical defense. The best evidence available indicates that it is responsible for the production of secretions inhibiting the development of antagonistic bacteria and fungi, especially in leaf-cutting ants (Hölldobler and Wilson, 1990; Bot et al., 2002; Poulsen et al., 2002; Fernandez-Marin et al., 2006; Vieira et al., 2010).

Several studies have been made of metapleural glands of various species, but till now, no comparative studies have been made. Studies of a small number of leaf-cutting ants, such as *Acromyrmex subterraneus* and *Atta sexdens* (Schildknecht and Koob, 1970, 1971), *A. sexdens rubropilosa*, *A. cephalotes* and *A. octospinosus* (do Nascimento et al., 1996; Ortius-Lechner et al., 2000) have been conducted, and on a few species of Formicinae, such as *Messor babarus* and *Labidus coecus* (Beattie et al., 1984), *Camponotus femoratus* (Seidel et al., 1990), *Crematogaster* (Maschwitz, 1974; Attygalle et al., 1989; Jones et al., 2005), on Myrmicinae, such as *Pogonomyrmex rugosus* (Fales et al., 1992), *Solenopsis invicta* and *S. geminata* (Cabrera et al., 2004; Jaffé and Puche, 1984), and *Crematogaster difformis* (Attygalle et al., 1989).

Though unique to ants, it is not found in every species. It is particularly absent from some formicine and myrmicine species, and is sometimes absent from males while present in workers (Hölldobler and Wilson, 1990), and different castes sometimes have different compounds, for example, workers and soldiers of *Atta cephalotes* (do Nascimento et al., 1996).

Do Nascimento et al. (1996) found that the metapleural gland secretion of the leaf-cutting ants they studied contained a large proportion of proteins, which are much more difficult to identify and study. The volatile part, which lends itself readily to study by gas chromatography linked to mass spectrometry (GC-MS), have revealed a total of 43 compounds in leaf-cutting ants, 19 in fire ants, and 16 in *Crematogaster* species. The chemical composition varied significantly among these three groups (Yek and Muller, 2011). Fatty and long chain carboxylic acids are the dominant compounds in fire ants and leaf-cutting ants, while phenolic compounds are the most abundant in *Crematogaster* (Attygalle et al., 1989; Yek and Muller, 2011). Five compounds were common to metapleural gland secretions of leaf-cutting ants: 1) 3-hydroxydecanoic acid (myrmicacin), 2) indoleacetic acid (IAA), 3) phenylacetic acid (PAA), 4) 3-hydroxydodecanoic acid, and 5) heptadecanoie acid. For example, in *A. sexdens* (Maschwitz et al., 1970; Schildknecht and Koob, 1971) PAA, myrmicacin, IAA and other β-hydroxyacids were found, while in *A. octospinosus* (Do Nascimento et al., 1996), myrmicacin and IAA. In another group of ants examined, *Myrmica laevinodis* (Beattie et al., 1984, 1985), the main compounds were PAA, IAA and myrmicacin.

Fungus-growing ants (Attini) have been considered biological models particularly interesting for comparative studies, as they are members of a monophyletic tribe (North et al., 1997). The Attini group is subdivided into basal attines (exemplified

by *Apterostigma pilosum* and *Mycetarotes parallelus*) (Hölldobler and Wilson, 1990; Mueller et al., 2001; Solomon et al., 2004), which use droppings and dead insects as substrate for their symbiotic fungi; derived attines (*Trachymyrmex fuscus*) that use vegetation as a substrate to cultivate their fungi (Weber, 1972; Mueller, 2002); and derived attines (leaf-cutting ants) (such as *Atta laevigata* and *Acromyrmex coronatus*) that use exclusively fresh vegetation as substrate in their fungal garden (Weber, 1972; Muller, 2002). Non-fungus-growing ants, represented by the myrmicine ant *Pogonomyrmex naegeli*, collect and store seeds (Muller et al. 2001), while the ectatommine ant *Ectatomma brunneum* exhibits a predatory behavior (Bolton, 2003).

The fungus-growing behavior exhibited by attines shows major evolutionary changes that are unique to ants: 1) The transition to fungus farming by attine ants about 50 million years ago (MYA), and possibly the need for extra defenses for their fungal crops, were novel challenges that no other ant ever faced. 2) The transition within attines to rearing a single clade of coevolving gongylidia-producing fungi as the base of higher attines, approximately 20 MYA, might have required more elaborate hygienic defenses when fungi were more vulnerable and had to be maintained in larger gardens. 3) The transition to active herbivory ~10 MYA, brought even less diversity into clonal crops, large scale fungus farming, and possibly novel pathogens that came with fresh leaves (Schultz and Brady, 2008).

The present study was aimed at investigating and comparing the chemical composition of these secretions in fungus-growing attines, represented by derived (*T. fuscus*, *A. laevigata* and *A. coronatus*) and basal species (*A. pilosum*, *M. parallelus*), and non-fungus-growing related ants of the tribe Ectatommini (*E. brunneum*) and Myrmicini (*P. naegeli*). It gives new comparative insights on their composition, as the few studies

available have focused on the secretions of the metapleural gland of leaf-cutting ants species and have neglected others attines as well as non-fungus-growing ants.

## METHODS AND MATERIALS

Seven species of ant were used in this study. The colonies of *A. laevigata* were collected at Corumbataí farm (22º17'S, 47º40'W), Rio Claro, São Paulo State, Brazil. The remaining ant species were collected on the UNESP (São Paulo State University) campus in Rio Claro, São Paulo State, Brazil. After being transferred to the Center for the Study of Social Insects (CEIS/Centro de Estudos de Insetos Sociais) at UNESP, each colony was housed in a nest box in a rearing room with a constant temperature of 25ºC and air humidity of 70%. The ants were fed twice a week with rose leaves and flowers, depending on the ant species. Ten individual workers of each caste of *A. laevigata* (head size for majors: 3.282 ± 0.335 mm, medium: 2.048 ± 0.172 mm, minors: 0.773 ± 0.016 mm) and *A. coronatus* (majors: 1.716 ± 0.092 mm, medium: 1.360 ± 0.127 mm; minors: 0.934 ± 0.004 mm) were used. For *E. brunneum* (2.075 ± 0.132 mm), *P. naegeli* (1.035 ±0.13mm), *A. pilosum* (0.716 ±0.054mm), *M. parallelus* (0.710 ±0.017mm), and *T. fuscus* (1.149 ±0.031mm), 10 individual monomorphic workers were used for each species.

The metapleural gland was extracted from each worker by removing the posterolateral region of the thorax, termed the metathorax, with a sterile scalpel (Ortius-Lechner et al., 2000). The tissues of the metapleural gland were individually placed in soft glass capillary tubes, and immediately sealed for gas chromatographic analysis (Morgan, 1990).

### *Chemical Analysis: Gas Chromatography–Mass Spectrometry*

The chemical analysis was carried out at Keele University (England-UK), with an Agilent Technologies 6890N Network GC with a SGE HT5 capillary column (30m x 0.25 mm ID, 0.25 µm film thickness) and coupled to an Agilent 5973 Network Mass Selective Detector. The GC was coupled to a computer and data processed with Agilent Chemstation software. Elution was carried out with helium at 1 mL/min.

The samples were injected in splitless mode (injection temperature 250ºC) by crushing the capillary tubes after 1 minute inside the injector port after insertion (Maile et al., 1998). The oven temperature was programmed to be held initially at 40ºC for 5 minutes then raised to 300ºC at a rate of 10ºC/min. The mass spectrometer was operated in Electron Ionization at 70 eV, scanning from m/z 40 to m/z 500 at 1.5 scans $s^{-1}$.

The peaks of the chromatogram were identified by comparing their mass spectra with those in the NIST-Wiley (NIST08) library. The identification of compounds was confirmed whenever possible by comparison of the retention times and mass spectra of synthetic standards (Sigma-Aldrich, UK). Compounds were quantified by acquiring calibration curves from six different concentrations for each compound under the same chromatographic conditions. Because aromatic compounds like skatole have very different mass spectral responses from aliphatic compounds like 2-nonanone, which again have responses different from those of long-chain compounds, where all three or two types of compound were found together in the glands, the amount of each compound as recorded by the total ion current was first converted to nanogram (ng) to give a more accurate measure of weight proportion for percentage composition. 2-Nonanone was used as a quantitative standard for simple aliphatic compounds, skatole for the aromatic compounds, and methyl oleate for the long chain esters and acids.

RESULTS

The analysis of the metapleural gland secretions of all the species examined by GC-MS revealed the presence of several compounds in each species. The secretion of *A. laevigata* was characterized by the presence of 12 compounds (2-nonanone, methyl phenylacetate, indole, phenylacetic acid, skatole, 3-hydroxyhexanoic acid, methyl 3-indoleacetate, ethyl palmitate, methyl linoleate, methyl oleate, methyl stearate, and oleic acid - Table 1). The secretion of *A. coronatus* consisted of 9 compounds (2-nonanone, 4-hydroxyoctanoic acid lactone, skatole, 3-hydroxydecanoic acid, methyl 3-indoleacetate, methyl palmitate, methyl linoleate, methyl oleate, methyl stearate - Tab. 2). Ten compounds were found in the secretion of *T. fuscus* (nonanal, indole, skatole, methyl 3-indoleacetate, 2-heptadecanone, ethyl palmitate, methyl linoleate, methyl oleate, methyl stearate, and ethyl oleate - Table 3); eight compounds in the secretion of *M. parallelus* (indole, skatole, 2-heptadecanone, methyl palmitate, methyl oleate, methyl stearate, ethyl oleate, and ethyl stearate - Table 4); four compounds in the secretion of *A. pilosum* (indole, skatole, octadecanal, and methyl 3-indoleacetate - Table 4), four compounds in the secretion of *P. naegeli* (indole, ethyl palmitate, methyl oleate, ethyl oleate - Table 5); and six compounds in the secretion of *E. brunneum* (m-cresol, octanoic acid, 1-tridecene, indole, an unidentified compound, and oleic acid - Table 5).

In the metapleural glands of *A. laevigata*, large quantities of compounds were found in major workers (average of 1203 ng), with less in medium (456 ng), and minor workers (126 ng). Skatole, phenylacetic acid, indole and 3-hydroxyhexanoic acid were the most abundant compounds in the metapleural glands of the three castes (minor, medium, and major workers) (Table1), with less of methyl linoleate, methyl oleate and methyl stearate (Table1). The compound 3-hydroxyhexanoic acid was detected in large quantities in major and medium ants, but it was not present in minor workers (Table 1).

In major workers of *A. coronatus*, large quantities of compounds (278 ng) were found per metapleural gland, followed by medium (147 ng), and minor workers (66 ng). Skatole, 4-hydroxyoctanoic acid lactone, 3-hydroxydecanoic acid and 2-nonanone were the most abundant compounds in the three castes examined (Table 2). Methyl stearate, methyl linoate, methyl oleate and methyl palmitate were less abundant in the secretion of *A. coronatus* (Table 2).

The metapleural gland of monomorphic workers of *T. fuscus* contained on average 90 ng, of which skatole (68.5 %), methyl 3-indoleacetate (10.9 %) and indole (7.9 %) were the most abundant, with less methyl palmitate and methyl linoate (Table 3). The metapleural gland of monomorphic workers of *M. parallelus* contained skatole (55.9%), indole (13.1) and ethyl oleate (15.5%), while methyl stearate and methyl palmitate (Table 4) were less abundant, with a mean of 31 ng of secretion (Table 4). In *A. pilosum* the metapleural gland contained on average 184 ng of secretion. Skatole (95.5%) was the most abundant compound, with less octadecanal and indole (Table 4). The metapleural gland of *P. naegeli* contained 52 ng of secretion (Table 5), with indole (85.7%) and ethyl oleate (11.6%) as the most abundant components, and less ethyl palmitate (Table 5). In *E. brunneum* a mean of 69 ng of compounds were found (Table 5). Oleic acid (41.8%), m-cresol (29.6) and indole (20.1%) were the most abundant with some octanoic acid (Table 5). Some compounds frequently found in the metapleural gland secretion examined here varied considerably in quantity, so standard deviations of percentage are included (Table 6). Other compounds are found only in the metapleural gland secretions of one species (Table 1-5), such as methyl phenylacetate, 3-hydroxyhexanoic acid, phenylacetic acid in *A. laevigata*; 4-hydroxyoctanoic acid and 3-

hydroxydecanoic acid in *A. coronatus*; nonanal in *T. fuscus*; octadecanal in *A. pilosum*; m-cresol and octanoic acid in *E. brunneum*.

The chemical structure of the most important compounds identified in the metapleural glands of the species of the tribes Attini, Myrmicini and Ectatommini are represented in figure 1.

## DISCUSSION

In a study of the morphology and function of the metapleural gland secretion of some ant species (basal and derived, fungus and non-fungus-growing ants), the present work has focused on the volatile portion of the secretion. Vieira et al. (2010, 2011) have demonstrated in *A. laevigata* and *A. coronatus* the presence of secretory cells strongly staining for proteins.

A greater variety of compounds were found in the Attini group (12 compounds in *A. laevigata;* nine compounds in *A. coronatus;* ten compounds in *T, fuscus;* eight compounds in *M. parallelus;* four compounds in *A. pilosum*) compared to Myrmicini (four compounds in *P. naegeli*) and Ectatommini (six compounds in *E. brunneum*). This shows that the derived ants of the Attini (leaf-cutting ants) had a greater variety of compounds when compared with basal ants (Attini basal) and those not cultivating fungi, indicating its greater capacity for synthesis of compounds.

In addition, the leaf-cutting ants (derived attines) *A. laevigata* and *A. coronatus* had the largest quantities of volatile compounds per worker (1203 ng in major workers of *A. laevigata*; 278 ng in major workers of *A. coronatus*) when compared to other Attini (90 ng in *T. fuscus*; 31 ng in *M. parallelus*; 184 ng in *A. pilosum*) and others groups e.g. Myrmicini (52 ng in *P. naegeli*) or Ectatommini (69 ng in *E. brunneum*),

indicating a greater capacity of the metapleural glands of these ants (mainly leaf-cutting ants) to produce compounds and in greater variety. This corroborated the study of Vieira et al. (2012, unpublished data) showing that the shape of the secretory cells of the metapleural glands might also indicate their secretory capacity. In Ectatommini, cells are round, while in Myrmicini and Attini, they are oval. This oval morphology has also been observed in the *A. bisphaerica, A. sexdens rubropilosa* (Gusmão, 2000), *A. octospinosus* (Bot et al., 2001), *A. subterraneus* (de Souza et al., 1996), *A. laevigata* and *A. coronatus* (Vieira et al., 2010, 2011), and round-shaped cells in *Diacamma rugosum* and *D. vagans* (Schoeters and Billen, 1992). According to Junqueira and Carneiro (2008) cells taller rather than shorter, have a physiology typical of secretory cells, suggesting that oval-shaped secretory cells of the metapleural gland of attines produce secretion more actively than Ectatommini tribes.

We have found from morphophysiology that non-fungus growing ants (Ectatommini, Myrmicini) have fewer secretory cells than derived (leaf-cutting) attines, indicating higher secretory capacity of the metapleural glands in leaf-cutting ants (Vieira et al., 2012, unpublished). This could produce a reduction in parasite pressure in the derived species. Some ants, such as *Camponotus*, are devoid of metapleural glands possibly due to a lower susceptibility to pathogens, as they build arboreal nests (Hölldobler and Engel-Siegel, 1985; Johnson et al., 2003). The Myrmicini (*P. naegeli*) and Ectatommini (*E. brunneum*) are non-fungus growers that built nests in the ground and their metapleural gland might be physiologically modified, with other roles, such as, in many myrmicines, territorial markers, nest entrance compounds, or regulation of aggressive interactions between neighboring colonies (Tulloch et al., 1962; Jaffe and Puche, 1984; Jaffe et al., 1986; Cammaerts and Cammaerts, 2001). Poulsen et al. (2003)

reported that in some leaf-cutting ants, the metapleural gland also has a primary role to produce antimicrobial compounds to protect the ants and their mutualistic fungal crop against parasites.

The metapleural gland secretion of major and medium workers of *A. laevigata* contained 3-hydroxyhexanoic acid ($C_6$), while those of *A. coronatus* contain 4-hydroxyoctanoic acid lactone ($C_8$) and 3-hydroxydecanoic acid ($C_{10}$). Schildknecht (1976) and do Nascimento et al. (1996) demonstrated that the largest quantities of acidic and volatile compounds present in the secretion of *A. sexdens rubropilosa* were phenylacetic acid, followed by 3-hydroxydecanoic acid ($C_{10}$). Both groups found $C_6$, $C_8$, $C_{10,}$ and $C_{12}$ acids, and $C_{10}$, $C_{12}$, $C_{14}$, and $C_{16,}$ β-hydroxyacids in the metapleural gland secretion of *A. sexdens rubropilosa* and *A. cephalotes*. Ortius-Lechner et al. (2000) detected the presence of many acids in the metapleural gland secretion of *A. octospinosus*, which were believed to reduce the pH in the fungus garden. Unpublished morphophysiological results of Vieira et al. demonstrated that derived attines (leaf-cutting ants), and fungus-growers, synthesise more polysaccharides and acidic lipids than non-fungus-grower ants (Ectatommini, Myrmicini, and Blepharidattini). Morever, Vieira et al. (2010, 2011) have demonstrated in *A. laevigata* and *A. coronatus* the presence of secretory cells strongly stained for proteins, as well as in Ectatommini, Myrmicini, basal and derived Atini (Vieira et al., 2012, unpublished data). Do Nascimento et al. (1996) studying the species *A. sexdens rubropilosa*, *A. cephalotes*, and *A. octospinosus* demonstrated by infrared spectrometry and confirmed by the ninhydrin test the secretions contain chiefly proteins, and they may well provide much of the antibacterial and antifungal properties.

The amount of secretion in all multi-cast species varies with the size of the workers. Bot et al. (2001) found minor workers of *A. octospinosus* have less secretory cells (200-300 cells), than medium (350-450) and majors (500-600) workers, and major workers of *A. laevigata* have larger metapleural gland reservoirs than minor and medium workers (Vieira et al. 2010).

Iwanawi (1978) showed that myrmicacin (3-hydroxydecanoic acid) can act on mitosis at various stages even after metaphase. Beattie et al. (1984) found myrmicacin in the non-leaf cutting ants *M. barbarus* and *L. coecus*. In the present study, myrmicacin was found in the secretion of *A. laevigata.*

Indole was detected in the metapleural gland secretion of derived and basal attines. It was also found in the secretion of the ants of the tribe Myrmicini and Ectatommini, although in small quantities. It has been identified as an attractant or pheromone in widely scattered insects, particularly among Diptera and Coleoptera (pherobase.com). Phenylacetic acid (PAA) was found in large quantities in *A. laevigata*, *A. sexdens*, and *A. cephalotes* (do Nascimento et al., 1996), but was absent from the secretion of *Acromyrmex*, as demonstrated in *A. octospinosus* (do Nascimento et al., 1996; Ortius-Lechner et al., 2000) and *A. subterraneus* (Schildknecht and Koob, 1970), *A. coronatus*. It is also absent from the basal attines and myrmicine and ectatommine species examined in the present study. According to Yek and Muller (2011), PAA might contribute to the acidity of the metapleural gland secretion and thus act as an antimicrobial. Skatole was another compound detected in the metapleural glands of derived and basal attine ants. In *A. laevigata*, *A. coronatus*, and *A. pilosum*, this compound is present in large quantities. Do Nascimento et al. (1996) detected

skatole in the metapleural gland secretion of *A. cephalotes* and suggested as probably responsible for the distinct odor of the secretion.

In the present study, two ketones and two aldehydes were also found in the metapleural gland secretion: 2-nonanone in *A. laevigata* and *A. coronatus,* and 2-heptadecanone in *T. fuscus,* with nonanal in *T. fuscus* and octadecanal in *A. pilosum*. Nonanal has been found widely in insects, but there is little evidence of its use in chemical communication in ants. There is no clear evidence of pheromonal use of the other compounds.

Among the compounds found in the species examined here, the long chain esters methyl palmitate, ethyl palmitate, methyl linoleate, methyl oleate, and methyl stearate are chiefly the products of derived attines. Ortius-Lechner et al. (2000) also found esters in metapleural glands of *A. octospinosus,* but suggest they may be artifacts produced from the acids during heating. The metapleural gland of *Solenopsis geminata* contains fatty acids (Jaffé and Puche, 1984), which might have a role in territory marking, and an anti-bacterial effect. The antibiotic activity increases with chain length for these acids (Cowles, 1941; Spoehr et al., 1949). *E. brunneum* (Ectatommini) also has fatty acids (octanoic acid and oleic acid), and the one phenol, m-cresol, found here, which was also found in the mandibular gland of the Formicinae *Camponotus quadrisectus* (Voegtle et al., 2008) and there might act as an alarm or defense pheromone.

Too little is yet known of the semiochemistry of the compounds found here, to reach firm conclusions. Three hypotheses can be used to explain the greater capacity of the metapleural glands of leaf-cutting ants to produce volatile compounds compared to other attines and non-fungus-growing ectatommine and myrmicinae ants. First, leaf-cutting ant queens mate with multiple males whereas those of other attines mate only

once (Sumner et al., 2004). Hamilton et al. (1987), Schmid-Hempel (2000) and Hughes and Boomsma (2004) suggested that polyandrous queens of social insects may have evolved because the more genetically diverse colonies they produce, the more resistant to parasites they may be. According to Hughes et al. (2008) both polyandry and large metapleural gland reservoirs may have evolved in response to stronger pressure from parasites. Secondly, Hughes et al. (2008) suggested that leaf-cutting ants may be able to invest more in parasite resistance, as they use fresh vegetation as substrate for their fungus, which in turn provides more resources for them to use. Thirdly, leaf-cutting ant workers are polymorphic and include individuals specialized in a greater variety of tasks; unlike others basal attines (Hughes et al., 2008).

In conclusion, it is clear that these leaf-cutting ants possess a considerable variety of compounds in their metapleural glands. However, gas chromatography, powerful as it is, is not the solution to all analytical problems. These glands require further investigation of their full composition and function.

ACKNOWLEDGEMENTS

The authors thank the Center for Study of Social Insects (Centro de Estudos de Insetos Sociais – CEIS – UNESP/SP/Brazil – São Paulo State University) for assistance in the collection and identification of specimens used in this study. IAV thanks Keele University for study support, and FAPESP (São Paulo Research Foundation) for financial support (grant 2008/54301-3) and CNPq (researcher grant).

REFERENCES

ATTYGALLE, A. B., and MORGAN, E. D. 1984. Chemicals from the glands of ants. *Chem. Soc. Rev.* 13: 245Y278.

ATTYGALLE, A. B., SIEGEL, B., VOSTOWSKY, O., BESTMANN, H. J., and MASCHWITZ, U. 1989. Chemical composition and function of metapleural gland secretion of the ant, *Crematogaster deformis* Smith (Hymenoptera: Myrmicinae). *J. Chem. Ecol*. 15:317Y328.

BEATTIE, A. J., TURNBULL, C., KNOX, R. B., and WILLIAMS, E. G. 1984. Ant inhibition of pollen function: a possible reason why ant pollination is rare. *Am. J. Bot.* 71:421Y426.

BEATTIE, A. J., TURNBULL, C., HOUGH, T., JOBSON, S., and KNOX, R. B. 1985. The vulnerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. *Am. J. Bot.* 72:606Y614.

BEATTIE, A. J., TURNBULL, C., HOUGH, T., and KNOX, R. B. 1986. Antibiotic production: A possible function for the metapleural glands of ants (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* 79, 448Y450.

BOLTON, B. 2003. Synopsis and Classification of Formicidae. *Mem. Am. Entomol. Inst.* 71: 1Y370.

BOT, A. N. M., OBERMAYER, M. L., HÖLLDOBLER, B., and BOOMSMA, J. J. 2001. Functional morphology of the metapleural gland in the leaf-cutting ant *Acromyrmex octospinosus*. *Ins. Soc*. 48: 63Y66.

BOT, A. N. M., ORTIUS-LECHNER, D., FINSTER, K., MAILE, R., and BOOMSMA, J. J. 2002. Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. *Ins. Soc*. 49:363Y370.

CABRERA, A., WILLIAMS, D., HERNÁNDEZ, J. V., CAETANO, F. H., and JAFFÉ, K. 2004. Metapleural-and postpharyngeal-gland secretions from workers of the ants *Solenopsis invicta* and S. geminate. *Chem. Biodiv.* 1:303Y311.

CAMMAERTS, M.-C., and CAMMAERTS, R. 2001. Marking of nest entrances and vicinity in two related *Tetramorium* ant species (Hymenoptera: Formicidae). *J. Ins. Behav.* 14: 247Y269.

COWLES, P. B. 1941. The germicidal action of the hydrogen ion and of the lower fatty acids. *Yale J. Biol. Med*. 13:571Y578.

DE SOUZA, A. L. B., SOARES, I. M. F., CYRINO, L. T., and SERRÃO, J. E. 2006. The Metapleural Gland in Two Subspecies of *Acromyrmex subterraneus* (Hymenoptera: Formicidae). *Sociobiology*. 47: 19Y25.

DO NASCIMENTO, R. R. D., SCHOETERS, E., MORGAN, E. D., BILLEN, J., and STRADLING, D. J. 1996. Chemistry of metapleural gland secretions of three attine ants, *Atta sexdens rubropilosa*, *Atta cephalotes*, and *Acromyrmex octospinosus* (Hymenoptera: Formicidae). *J. Chem. Ecol.* 22:987Y1000.

FALES, H. M., JONES, T. H., JAOUNI, T., BLUM, M. S., and SCHMIDT, J. O. 1992. Phenylalkenals in ponerine (*Leptogenys* sp.) and myrmicine (*Pogonomyrmex* sp.). *J. Chem. Ecol*. 18:847Y854.

FERNANDEZ-MARIN, H., ZIMMERMAN, J., REHNER, S., and WCISLO, W. 2006. Active use of the metapleural glands by ants in controlling fungal infection. *Proc. R. Soc. Lond. B. Biol. Sci.* 273:1689Y1695.

GUSMÃO, L. G. 2000. Estudo comparado da glândula metapleural e sua secreção em diferentes castas de três espécies de *Atta* (Hymenoptera: Formicidae). 60p. *Tese* (Doutorado) - ESALQ-USP, Piracicaba-SP.

HAMILTON, W. D. 1987. Kinship, recognition, disease, and intelligence: constraints of social evolution. Pp. 81Y100. In: Y. Ito, J. L. Brown, and J. Kirkkawa, ed. Animal societies: theories and facts. Japan Scientific Societies Press, Tokyo.

HÖLLDOBLER, B., and WILSON, E. O. 1990. The Ants. Cambridge: Harvard University Press. pp. 732.

HÖLLDOBLER, B., and ENGEL-SIEGEL, H. 1985. On the metapleural gland in ants. *Psyche* 91:201Y224.

HUGHES, W. H. O., and BOOMSMA, J. J. 2004. Genetic diversity and disease resistance in leaf-cutting ant societies. *Evolution* 58:1251Y1260.

HUGHES, W. O. H., PAGLIARINI, R., MADSEN, H. B., DIJKSTRA, M. J., and BOOMSMA, J. J. 2008. Antimicrobial defence shows an abrupt evolutionary transition in the fungus-growing ants. *Evolution* 62:1252Y1257.

IWANAWI, Y. 1978. Myrmicacin, a new inhibitor for mitotic progression after metaphase. *Protoplasma* 95:267Y271.

JAFFÉ, K., and PUCHE, H. 1984. Colony-specific territorial marking with the metapleural gland secretion in the ant *Solenopsis geminata* (Fabr.). *J. Insect. Physiol.* 30:265Y270.

JAFFÉ, K., LOPEZ, M. E., and ARAGORT, W. 1986. On the communication systems of the ants *Pseudomyrmex termitarius* and *P. triplarinus*. *Ins. Soc.* 33:105Y117.

JONES, T. H., BRUNNER, S. R., EDWARDS, A. A., DAVIDSON, D. W., and SNELING, R. R. 2005. 6-Alkylsalicylic acids and 6-alkylresorcylic acids from ants in the genus *Crematogaster* from Brunei. *J. Chem. Ecol.* 31:407Y417.

JOHNSON, R. N., AGAPOW, P.-M., and CROZIER, R. H. 2003. A tree island approach to inferring phylogeny in the ant subfamily Formicinae, with especial reference to the evolution of weaving. *Mol. Phylogenet. Evol.* 29:317Y330.

JUNQUEIRA, L. C., and CARNEIRO, J. 2008. Histologia básica, 11 ed. Guanabara Koogan, Rio de Janeiro. pp. 524.

MAILE, R., DANI, F. R., JONES, G. R., MORGAN, E. D., and ORTIUS, D. 1998. Sampling techniques for gas chromatographic–mass spectrometric analysis of long-chain free fatty acids from insect exocrine glands. *J. Chromatogr. A* 816:169Y175.

MASCHWITZ, U. 1974. Vergleichende Untersuchungen zur Funktion der Ameisenmetathorakaldrüse. *Oecologia* 16:303Y310.

MASCHWITZ, U., KOOB, K., and SCHILDKNECHT, H. 1970. Ein Beitrag zur Funktion der Metathorakaldrüse der Ameisen. *J. Insect. Physiol.* 16:387Y404.

MORGAN, E. D. 1990. Preparation of small-scale samples from insects for chromatography. *Anal.Chim. Acta* 236:227Y235.

MUELLER, U. G., SCHULTZ, T. R., CURRIE, C. R., ADAMS, R. M., and MALLOCH, D. 2001. The origin of the attine ant-fungus mutualism. *Q. Rev. Biol.* 76(2): 169Y197.

MUELLER, U. G. 2002. Ant versus fungus versus mutualism: Ant-cultivar conflict and the deconstruction of the attine ant-fungus symbiosis. *Amer. Nat.* 160:S67YS98.

NORTH, R. D., JACKSON, C. W., and HOWSE, P. E. 1997. Evolutionary aspects of anti-fungus interactions in leaf-cutting ants. *Tree* 12:386Y389.

ORTIUS-LECHNER, D., MAILE, R., MORGAN, E. D., and BOOMSMA, J. J. 2000. Metapleural gland secretion of the leaf-cutter ant *Acromyrmex octospinosus*: New compounds and their functional significance. *J. Chem. Ecol.* 26:1667Y1683.

POULSEN, M., BOT, A. N. M., NIELSEN, M. G., and BOOMSMA, J. J. 2002. Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. *Behav. Ecol. Sociobiol.* 52:151Y157.

POULSEN, M., BOT, A. N. M., and BOOMSMA, J. J. 2003. The effect of metapleural gland secretion on the growth of a mutualistic bacterium on the cuticle of leaf-cutting ants. *Naturwissenschaften* 90:406Y409.

QUINLAN, R. J., and CHERRETT, J. M. 1979. The role of fungus in the diet of the leaf-cutting ant *Atta cephalotes* (L.). *Ecol. Entomol.* 4:151Y160.

SCHILDKNECHT, H., and KOOB, U. 1970. Plant bioregutators in the metathoracic glands of myrmicine ants, Angew. *Chem. Int. Ed. Engl.* 9:173.

SCHILDKNECHT, H., and KOOB, K. 1971. Myrmicacin, the first insect herbicide. *Angew. Chem.* 10:124Y125.

SCHILDKNECHT, H., REED, P. B., REED, F. D., and KOOB, K. 1973. Auxin activity in the symbiosis of leaf-cutting ants their fungus. *Insect. Biochem.* 3:439Y442.

SCHMID-HEMPEL, P. 2000. Mating, parasites and other trials of life in social insects. *Microb. Infect.* 2:515Y520.

SCHOETERS, E., and BILLEN, J. 1992. Morphological and ultrastructural study of metapleural gland in *Diacamma* (Hymenoptera, Formicidae), *in* European Congress of Social Insects, 1o, Leuven (1991) Proceedings Leuven, Leuven University. pp. 239Y247.

SCHULTZ, T. R., and BRADY, S. G. 2008. Major evolutionary transitions in ant agriculture. *Proc. Natl. Acad. Sci.* 105: 5435Y5440.

Seidel, J. L., Epstein, W. W., and Davidson, D. W. 1990. Neotropical ant gardens: I. Chemical constituents. *J. Chem. Ecol.* 16:1791-1816.

SOLOMON, S. E., MUELLER, U. G., SCHULTZ, T. R., CURRIE, C.R., and PRICE, S. L. 2004 Nesting biology of the fungus growing ants *Mycetarotes* Emery (Attini, Formicidae). *Ins. Soc.* 51:333Y338.

SPOEHR, H. A., SMITH, J. H. C., STRAIN, H. H., MILNER, H. W., and HARDIN, G. J. 1949. Fatty Acid Antibacterials from Plants. Carnegie Institution of Washington Publications, Washington, D.C.,pp. 36Y68.

SUMNER, S., HUGHES, W. H. O., PEDERSEN, J. S., and BOOMSMA, J. J. 2004. Ant parasite queens revert to mating singly. *Nature* 428:35Y36.

TULLOCH, G. S., SHAPIRO, J. E., and HERSHENOV, B. 1962. The ultrastructure of the metasternal glands of ants. *Bull. Brooklyn. Entomol. Soc.* 57: 91Y101.

VIEIRA, A. S., BUENO, O. C., and CAMARGO-MATHIAS, M. I. 2010. The functional morphology of the metapleural gland of the leaf-cutting ant *Atta laevigata* (Formicidae: Attini). *Micron* 41:149Y157.

VIEIRA, A. S., BUENO, O. C., and CAMARGO-MATHIAS, M. I. 2011. Secretory Profile of Metapleural Gland Cells of the Leaf-Cutting Ant *Acromyrmex coronatus* (Formicidae: Attini). *Microsc. res. tech.* 74:76Y83.

VIEIRA, A. S., BUENO, O. C., and CAMARGO-MATHIAS, M. I. 2012. Ultrastructural profile of metapleural gland cells of the ant *Atta laevigata* (F. Smith, 1858) (Formicidae: Attini). *Anim. Biol.* 62:1Y11.

VIEIRA, A. S., BUENO, O. C., and CAMARGO-MATHIAS, M. I. 2012. Morphophysiological differences between the metapleural glands of fungus-growing and non-fungus growing ants (Hymenoptera, Formicidae). *Submitted to Plos one*.

YEK, S. H., and MUELLER, U. G. 2011. The metapleural gland of ants. *Biol. rev.* 86: 774Y791.

WEBER, N. A. 1972. Gardening ants: The Attines. Philadelphia (PA): American Philosophical Society 92: 146p.

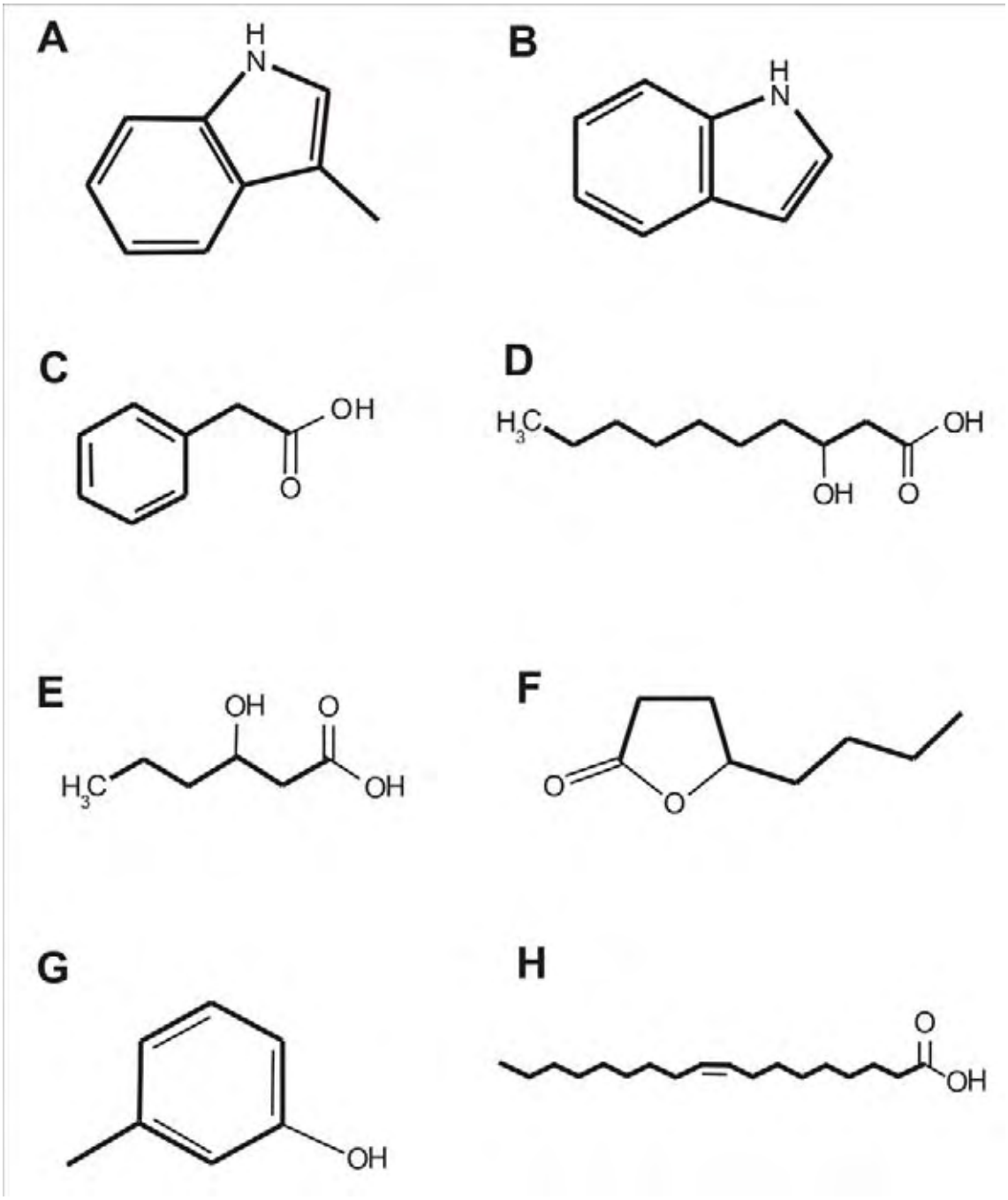



Figure 1. Chemical structure of some of the compounds identified in the metapleural gland secretion of *Atta laevigata* (**A**, **B**, **C**, **E** and **H**), *Acromyrmex coronatus* (**A**, **D** and **F**), *Trachymyrmex fuscus* (**A** and **B**), *Mycetarotes parallelus* (**A** and **B**), *Apterostigma pilosum* (**A** and **B**), *Pogonomyrmex naegeli* (**B**), *Ectatomma brunneum* (**G** and **H**). **A**=Skatole; **B**= Indole; **C**= Phenylacetic acid; **D**= 3-Hydroxydecanoic acid; **E**= 3-Hydroxyhexanoic acid; **F**= 4-Hydroxyoctanoic acid lactone; **G**= m-Cresol; **H**= Oleic acid.

**Table 1.** Compounds detected in metapleural gland secretion of minor, medium, and major workers of the leaf-cutting ant *Atta laevigata*

| | Mean secretion amount | | | | | |
|---|---|---|---|---|---|---|
| | Major worker (*N*= 10) | | Medium worker (*N*= 10) | | Minor worker (*N*= 10) | |
| | Nanogram (±SD) | % | Nanogram (±SD) | % | Nanogram (±SD) | % |
| **2-Nonanone** | 25.5 ±16.4 | 2.1 | 12.7 ±5.3 | 2.8 | 16.8 ±10.7 | 13.3 |
| **Methyl phenylacetate**[#] | 28.1 ±12,6 | 2.3 | 13.5 ±3.6 | 3.0 | 6.4 ±2.0 | 5.0 |
| **Indole** | 95.4 ±36.3 | 7.9 | 30.9 ±13.4 | 6.8 | 14.5 ±4.1 | 11.5 |
| **Phenylacetic acid**[#] | 353.5 ±238.4 | 29.4 | 128.2 ±93.4 | 28.1 | 38.4 ±32.5 | 30.4 |
| **Skatole (3-methylindole)** | 472.3 ±269.6 | 39.3 | 183.1 ±126.4 | 40.1 | 37.0 ±21.9 | 29.3 |
| **3-Hydroxyhexanoic acid**[#] | 90.9 ±35.8 | 7.6 | 55.8 ±13.6 | 12.2 | _ | _ |
| **Methyl 3-indoleacetate*** | 46.8 ±13.5 | 3.9 | 24.1 ±10.1 | 5.3 | 7.6 ±1.7 | 6.0 |
| **Ethyl palmitate** | 33.4 ±15.3 | 2.8 | 1.3 ±0.2 | 0.3 | 0.8 ±0.5 | 0.6 |
| **Methyl linoleate** | 3.1 ±1.5 | 0.3 | 1.5 ±0.5 | 0.3 | 0.6 ±0.3 | 0.4 |
| **Methyl oleate** | 6.4 ±3.0 | 0.5 | 1.9 ±0.8 | 0.4 | 0.9 ±0.4 | 0.7 |
| **Methyl stearate** | 5.3 ±2.1 | 0.4 | 1.7 ±0.7 | 0.4 | 0.6 ±0.3 | 0.5 |
| **Oleic acid** | 42.7 ±28.5 | 3.6 | 2.1 ±1.1 | 0.5 | 2.8 ±2.2 | 2.2 |
| **Total** | **1203** | **100** | **456** | **100** | **126** | **100** |

* Identification not confirmed by injection of standard samples; SD= standard deviation; [#] exclusive substance.

**Table 2.** Compounds detected in metapleural gland secretion of minor, medium, and major workers of the leaf-cutting ant *Acromyrmex coronatus*

| | Mean secretion amount | | | | | |
|---|---|---|---|---|---|---|
| | Major worker (*N*= 10) | | Medium worker (*N*= 10) | | Minor worker (*N*= 10) | |
| | Nanogram (±SD) | % | Nanogram (±SD) | % | Nanogram (±SD) | % |
| **2-Nonanone** | 30.3 ±13.7 | 10.9 | 19.3 ±8.9 | 13.2 | 7.2 ±3.0 | 10.9 |
| **4-Hydroxyoctanoic acid lactone**[#] | 76.1 ±32.3 | 27.4 | 43.6 ±10.2 | 29.8 | 23.8 ±12.8 | 36.0 |
| **Skatole (3-methylindole)** | 118.8 ±83.4 | 42.8 | 58.4 ±38.0 | 39.8 | 26.6 ±14.6 | 40.3 |
| **3-Hydroxydecanoic acid** [#] | 41.8 ±20.0 | 15.1 | 16.0 ±5.3 | 10.9 | 6.5 ±4.6 | 9.9 |
| **Methyl 3-indoleacetate*** | 6.5 ±3.4 | 2.4 | 5.3 ±2.3 | 3.6 | - | - |
| **Methyl palmitate** | 0.6 ±0.2 | 0.2 | 0.8 ±0.6 | 0.6 | 0.3 ±0.1 | 0.5 |
| **Methyl linoleate** | 0.8 ±0.2 | 0.3 | 0.6 ±0.3 | 0.4 | 0.4 ±0.1 | 0.6 |
| **Methyl oleate** | 1.7 ±0.5 | 0.6 | 1.9 ±1.5 | 1.3 | 0.8 ±0.4 | 1.3 |
| **Methyl stearate** | 0.9 ±0.2 | 0.3 | 0.6 ±0.3 | 0.4 | 0.4 ±0.1 | 0.5 |
| **Total** | **278** | **100** | **147** | **100** | **66** | **100** |

* Identification not confirmed by injection of standard samples; SD= standard deviation; [#] exclusive compound.

**Table 3.** Compounds detected in metapleural gland secretion of monomorphic workers in the derived attine *Trachymyrmex fuscus*

| | **Mean secretion amount (*N*=10)** | |
|---|---|---|
| | **Nanogram (±SD)** | **%** |
| **Nonanal**[#] | 2.2 ±1.0 | 2.4 |
| **Indole** | 7.1 ±2.5 | 7.9 |
| **Skatole (3-methylindole)** | 61.3 ±30.9 | 68.5 |
| **Methyl 3-indoleacetate*** | 9.7 ±4.3 | 10.9 |
| **2-Heptadecanone*** | 3.5 ±1.4 | 4.0 |
| **Ethyl palmitate** | 0.4 ±0.3 | 0.5 |
| **Methyl linoleate** | 0.5 ±0.2 | 0.5 |
| **Methyl oleate** | 1.0 ±0.4 | 1.1 |
| **Methyl stearate** | 0.6 ±0.4 | 0.7 |
| **Ethyl oleate** | 3.2 ±1.8 | 3.6 |
| **Total** | **90** | **100** |

* Identification not confirmed by injection of standard samples; SD= standard deviation; [#]exclusive compound.

**Table 4**. Compounds detected in metapleural gland secretion of monomorphic workers in the basal attines *Mycetarotes parallelus* and *Apterostigma pilosum*

| | **Mean secretion amount** (*N*=10) | | **Mean secretion amount (***N*=10) | |
|---|---|---|---|---|
| | *Mycetarotes parallelus* | | *Apterostigma pilosum* | |
| | **Nanogram (±SD)** | **%** | **Nanogram (±SD)** | **%** |
| **Indole** | 4.0 ±2.0 | 13.1 | 2.5 ±1.1 | 1.4 |
| **Skatole (3-methylindole)** | 17.2 ±4.1 | 55.9 | 175.1 ±121.7 | 95.5 |
| **2-heptadecanone*** | 1.7 ±0.3 | 5.6 | - | - |
| **Methyl palmitate** | 0.4 ±0.2 | 1.4 | - | - |
| **Methyl oleate** | 1.3 ±0.7 | 4.3 | - | - |
| **Methyl 3-indoleacetate*** | - | - | 4.2 ±2.8 | 2.3 |
| **Methyl stearate** | 0.6 ±0.3 | 1.8 | - | - |
| **Ethyl Oleate** | 4.8 ±1.3 | 15.5 | - | - |
| **Ethyl stearate** | 0.7 ±0.6 | 2.4 | - | - |
| **Octadecanal*#** | - | - | 2.0 ±0.5 | 1.1 |
| **Total** | **31** | **100** | **184** | **100** |

* Identification not confirmed by injection of standard samples; SD= standard deviation. #exclusive compound.

**Table 5.** Compounds detected in metapleural gland secretion of monomorphic workers in the non-fungus-growing ants *Pogonomyrmex naegeli and Ectatomma brunneum*

| | **Mean secretion amount** | | | |
|---|---|---|---|---|
| | *Pogonomyrmex naegeli* (N=10) | **%** | *Ectatomma brunneum* (N=10) | **%** |
| | **Nanogram (±SD)** | | **Nanogram (±SD)** | |
| **Indole** | 44.7 ±14.6 | 85.7 | 13.9 ±4.8 | 20.1 |
| **m-cresol**[#] | - | - | 20.5 ±9.7 | 29.6 |
| **Octanoic acid**[#] | - | - | 1.8 ±0.8 | 2.6 |
| **Unidentified compound*** | - | - | 3.8 ±1.8 | 5.5 |
| **Unidentified compound*** | - | - | 0.3 ±0.0 | 0.4 |
| **Ethyl palmitate** | 0.7 ±0.4 | 1.4 | - | - |
| **Methyl oleate** | 0.7 ±0.2 | 1.3 | - | - |
| **Ethyl oleate** | 6.1 ±4.0 | 11.6 | - | - |
| **Oleic acid** | - | - | 29.0 ±28.0 | 41.8 |
| **Total** | **52** | **100** | **69** | **100** |

* Identification not confirmed by injection of standard samples; SD= standard deviation; [#] exclusive compound.

Table 6. Common compounds in the metapleural gland secretion of *Atta laevigata*, *Acromyrmex coronatus*, *Trachymyrmex fuscus*, *Mycetarotes paralleluls*, *Apterostigma pilosum*, *Pogonomyrmex naegeli* and *Ectatomma brunneum*.

| | Mean secretion amount Nanogram (±SD) | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***Atta laevigata**** | ***Acromyrmex coronatus**** | ***Trachymyrmex fuscus*** | ***Mycetarotes parallelus*** | ***Apterostigma pilosum*** | ***Pogonomyrmex naegeli*** | ***Ectatomma brunneum*** |
| **Indole** | 95.4 ±36.3 | - | 7.1 ±2.5 | 4.0 ±2.0 | 2.5 ±1.1 | 44.7 ±14.6 | 13.9 ±4.8 |
| **Skatole** | 472.3 ±269.6 | 118.8 ±83.4 | 61.3 ±30.9 | 17.2 ±4.1 | 175.1 ±121.7 | - | - |
| **Oleic acid** | 42.7 ±28.5 | - | - | - | - | - | 29.0 ±28.0 |
| **2-nonanone** | 25.5 ±16.4 | 30.3 ±13.7 | - | - | - | - | - |
| **2-heptadecanone** | - | - | 3.5 ±1.4 | 1.7 ±0.3 | - | - | - |
| **Methyl 3-indoleacetate** | 46.8 ±13.5 | 6.5 ±3.4 | 9.7 ±4.3 | - | 4.2 ±2.8 | - | - |
| **Methyl palmitate** | - | 0.6 ±0.2 | - | 0.4 ±0.2 | - | - | - |
| **Ethyl palmitate** | 33.4 ±15.3 | - | 0.4 ±0.3 | - | - | 0.7 ±0.4 | - |
| **Methyl linoleate** | 3.1 ±1.5 | 0.8 ±0.2 | 0.5 ±0.2 | - | - | - | - |
| **Methyl oleate** | 6.4 ±3.0 | 1.7 ±0.5 | 1.0 ±0.4 | 1.3 ±0.7 | - | 0.7 ±0.2 | - |
| **Methyl stearate** | 5.3 ±2.1 | 0.9 ±0.2 | 0.6 ±0.4 | 0.6 ±0.3 | - | - | - |

* major worker; SD= standard deviation.

# *Discussão geral*

## V. DISCUSSÃO GERAL

O presente trabalho trouxe os resultados morfológicos obtidos das glândulas metapleurais de operárias monomórficas das espécies *Ectatomma brunneum* (Ectatommini), *Pogonomyrmex naegeli* (Myrmicini), *Wasmannia auropunctata* (Blepharidatiini), *Apterostigma pilosum*, *Mycetarotes parallelus* (Attini basal), *Trachymyrmex fuscus* (Attini derivada), bem como das operárias mínimas, médias e maiores de *Atta laevigata* e de *Acromyrmex coronatus*, que corroboraram aqueles de Hölldobler e Wilson (1990) para *Atta cephalotes* mostrando que as glândulas metapleurais são órgãos pares e observados em ambos os lados posterior-ventral do metatórax das formigas. Sob uma elevação externa do exoesqueleto foi encontrado o reservatório (HÖLLDOBLER; WILSON, 1990).

A morfologia interna da glândula metapleural das formigas *E. brunneum*, *P. naegeli*, *W. auropunctata*, *A. pilosum*, *M. parallelus*, *T. fuscus*, *A. laevigata* e *A. coronatus*, mostrou a presença de duas porções: a secretora formada por várias células secretoras, e o reservatório, porção armazenadora constituída de um saco epitelial, ambas conectadas entre si por meio de canalículos. Morfologia semelhante também foi observada nas formigas cortadeiras *A. bisphaerica* e *A. sexdens rubropilosa* (GUSMÃO, 2000), *Acromyrmex octospinosus* (BOT et al., 2001), *A. subterraneus* por De Souza et al. (2006).

Foi aqui observado que a porção secretora da glândula metapleural, nas espécies *E. brunneum* e *W. auropunctata* possui as células secretoras com forma arredondada, assim como também observado em *Diacamma rugosum* e em *D. vagans* (SCHOETERS; BILLEN,

1992), enquanto que em *P. naegeli*, *A. pilosum*, *M. parallelus*, *T. fuscus*, *A. laevigata* e *A. coronatus* estas apresentaram-se ovaladas, assim como também observado em *A. substerraneus* (DE SOUZA et al., 2006), *A. octospinosus* (BOT et al., 2001), *A. bisphaerica* e *A. sexdens rubropilosa* (GUSMÃO, 2000). De acordo com Junqueira e Carneiro (2008) células colunares (mais altas do que largas) teriam principalmente a função de sintetizar e de secretar produtos o que poderia sugerir que nas glândulas metapleurais das Attini, de forma geral, ocorreria maior atividade de síntese de secreção do que nas das Ectatommini e Blepharidattini.

Os resultados também mostraram que as células secretoras da glândula metapleural dos representantes de todos os grupos aqui estudados, encontraram-se localizadas muito próximas umas das outras, porém não foi observada fusão da membrana plasmática, apenas a presença de um frouxo tecido conjuntivo com função de mantê-las unidas. Essa mesma organização foi observada em *A. bisphaerica*, *A. sexdens sexdens,* e em *A. sexdens rubropilosa* por Schoeters e Billen (1993). Por outro lado, observou-se a presença de especializações da membrana plasmática nas células secretoras de *A. laevigata*, *A. coronatus*, *T. fuscus*, *M. parallelus*, *A. pilosum*, *W. auropunctata* e *E. brunneum*, sob a forma de invaginações. Essas especializações agiriam como facilitadoras da absorção do material extracelular oriundo da hemolinfa para o interior do citoplasma, onde depois de internalizado (sob a forma de vesículas) este seria processado, resultados estes também relatados por Billen e Van Boven (1987) e por Caetano (1998) ao estudarem a glândula metapleural das formigas nômades *Dorylus* spp. e a glândula pós-faríngea de *Dinoponera australis*, respectivamente. Essas invaginações também foram frequentemente observadas em glândulas produtoras de feromônios de outras espécies de formigas (BILLEN, 1985, 1986), e nestas teriam o papel de aumentar a superfície de contato entre as células e o meio extracelular.

As formigas não cultivadoras de fungos *E. brunneum*, *P. naegeli*, *W. auropunctata* apresentaram respectivamente 47, 30 e 15 células secretoras, as *A. pilosum*, *M. parallelus* (Attini basal) 42 e 30, e *T. fuscus* (Attini derivada) 60 células, números estes menores do que os observados nas formigas cortadeiras *A. laevigata* e *A. coronatus* (140 e 136). Formigas *W. auropunctata*, provavelmente seriam oriundas de uma linhagem evolutiva que sofreu redução no número de células secretoras, talvez por consequência da menor pressão dos parasitas ou, ainda, teriam desenvolvido outras formas de combater os patógenos, como por exemplo, produzindo substâncias antimicrobianas via glândula de veneno, assim como relatado para *Polyrhachis dives* (GRAYSTOCK; HUGHES, 2011). Neste contexto, as formigas

*Camponotus* seriam exemplos de formigas que não apresentariam glândulas metapleurais, uma vez que estariam menos susceptíveis ao ataque de patógenos, por terem seus ninhos construídos em árvores (HÖLDOBLER; ENGEL-SIEGEL, 1985; JOHNSON; AGAPOW; CROZIER, 2003. Assim, o número de células secretoras encontrado em *P. naegeli* e *E. brunneum* se justificaria pelo fato delas precisarem de uma glândula metapleural funcional por estarem vulneráveis ao ataque de patógenos.

A literatura registrou para o grupo das formigas Attini uma divisão filogenética em espécies basais e derivadas (BRADY et al., 2006). No presente estudo os resultados mostrando o número de células secretoras das glândulas metapleurais dos diferentes grupos confirmaram essa divisão e ainda que *A. pilosum* e *M. parallelus* teriam menor número, *T. fuscus*, *A. laevigata* e *A. coronatus* teriam maior número de células secretoras, sugerindo a maior capacidade secretora das glândulas metapleurais das atines, principalmente nas formigas cortadeiras, o que se justificaria pelo fato das mesmas estarem mais vulneráveis ao ataque de patógenos, uma vez que a tarefa de cultivar fungos necessitaria de maiores investimentos na resistência destes microrganismos.

Outro resultado importante obtido por meio do desenvolvimento deste estudo foi com relação às formigas *E. brunneum*, *W. auropunctata*, *P. naegeli* e *M. parallelus* as quais apresentaram as células secretoras dispostas diferentemente de *A. pilosum, T. fuscus, A. laevigata* e de *A. coronatus*. Nas Ectatommini (*E. brunneum*) e Blepharidattini (*W. auropunctata*) estas células encontraram-se dispostas em um único grupo, na Myrmicini (*P. naegeli*) e Attini basal (*M. parallelus*) em dois grupos, nas Attini (*A. pilosum* e *T. fuscus*) em três grupos, e nas cortadeiras (*A. laevigata* e *A. coronatus*) estas foram encontradas em diversos agrupamentos contendo cada um deles cerca de 7 a 8 células secretoras. Esses dados permitiram inferir que o número de grupos de células secretoras também poderia estar diretamente relacionado com a linhagem evolutiva do grupo, sendo assim encontrado nas formigas cortadeiras maior número de grupos de células secretoras, devido à maior demanda de produção de secreção com funções antibiótica e antifúngica.

Em todas as espécies aqui estudadas os resultados revelaram positividade para RNA tanto nos núcleos quanto no citoplasma das células secretoras das glândulas metapleurais. Estes dados associados à presença de núcleos volumosos poderiam sugerir a ocorrência de processos contínuos de síntese de secreção. Ainda, a técnica variante de CEC aqui utilizada mostrou sincronia da atividade nas células da glândula metapleural de *A. coronatus*. Ou seja, os agrupamentos celulares produziriam secreção de forma sincrônica.

Resultados mais refinados utilizando técnicas de microscopia eletrônica de transmissão foram também aqui obtidos. Pode-se observar que nas células secretoras das glândulas metapleurais as mitocôndrias, organelas importantes na fisiologia celular apresentaram várias formas e localizações, principalmente próximas às microvilosidades (espaço pericanalicular) da porção intracitoplasmática dos canalículos tanto em *A. coronatus*, como em operárias mínimas de *A. laevigata*. Já nas espécies *T. fuscus, A. pilosum*, *M. parallelus*, *E. brunneum* e *W. auropunctata* essas organelas foram observadas distribuídas por todo o citoplasma, indicando que nas glândulas metapleurais das formigas cortadeiras, principalmente das operárias mínimas, elas estariam envolvidas no fornecimento de energia para a síntese de secreção, mais do que ocorreria nas outras espécies. De acordo com os dados obtidos neste trabalho e corroborando Tulloch, Shapiro e Hershenov (1962), duas seriam as hipóteses que poderiam explicar a significativa presença de mitocôndrias próxima do espaço pericanalicular: a) o envolvimento destas organelas tanto na síntese de secreção (principalmente aquela de origem lipídica), como no fornecimento de energia para os processos de síntese e, b) no fornecimento de energia (alta demanda) para realização dos processos de transporte ativo quando da passagem e modificação da secreção produzida no citoplasma das células secretoras para o interior dos canalículos.

Ainda, por meio deste estudo ultraestrutural verificou-se no citoplasma das células secretoras a presença de vacúolos com diferentes conteúdos em todas as espécies aqui estudadas, sugerindo a ocorrência de "turnover" de componentes celulares (processos de reciclagem), dados que foram confirmados pela presença significativa de vacúolos digestivos na forma de fagossomos e de autofagossomos mais evidentes em *A. laevigata* e *A. coronatus*, e menos em *T. fuscus, A. pilosum* e, *W. auropunctata* e *E. brunneum*. De acordo com Junqueira e Salles (1975) os fagossomos seriam organelas que conteriam enzimas hidrolíticas, se fundiriam com o material da própria célula e formariam os autofagossomos, local este onde ocorreria a digestão intracelular (autofagocitose). A partir dos resíduos desta digestão formar-se-iam as figuras mielínicas, estruturas observadas em *A. laevigata* e *A. coronatus* (formigas cortadeiras), *T. fuscus*, *A. pilosum* e em *W. auropunctata*.

A evidente presença de RER do tipo lamelar nas espécies *A. coronatus, A. laevigata*, *A. pilosum*, *W. auropunctata* e *E. brunneum* foi atribuída ao papel desta organela no transporte de RNA do núcleo para o citoplasma, durante a síntese de proteínas (ALBERTS et al., 2004). Essa forma de retículo foi pouco encontrada em *T. fuscus* e em *M. parallelus*, indicando que as células secretoras destas duas espécies estariam menos ativas na síntese de

elementos protéicos. Em *A. bisphaerica* e *A. sexdens rubropilosa* (GUSMÃO; CAETANO; NAKANO, 2001), bem como registrado por Schoeters e Billen (1992) em outras espécies de outros grupos (*D. rugosum* e *D. vagans*) foi demonstrada a presença de regiões com RER desenvolvido, as quais foi atribuída também participação nos processos de produção de compostos protéicos pelas células secretoras e que poderiam fazer parte dos componentes finais da secreção com provável função antibiótica ou antifúngica.

Associada a esses resultados a morfofisiologia da glândula metapleural mostrou que nas formigas *E. brunneum, P. naegeli, A. pilosum, M. parallelus, T. fuscus, A. laevigata* e *A. coronatus* houve forte marcação de proteínas nas células secretoras, enquanto que em *W. auropunctata* essa marcação foi moderada, dados estes que corroboraram Maschwtiz, Koob e Schildknecht (1970) que também detectaram a presença de componentes protéicos nas células secretoras da glândula metapleural de *A. sexdens* e confirmado pela observação de retículo endoplasmático rugoso bem desenvolvido.

O presente estudo morfofisiológico mostrou que as espécies *A. laevigata, A. coronatus* e *T. fuscus* seriam capazes de sintetizar mais compostos polissacarídeos do que as *A. pilosum, M. parallelus* (Attini basal), *P. naegeli* (Myrmicini) e *W. auropunctata* (Blepharidattini). Além disso, nas células secretoras da glândula metapleural das operárias mínimas de *A. coronatus* e de *A. laevigata* foi observada granulação polissacarídica fortemente positiva em contraste com as maiores, sugerindo que as células secretoras das glândulas metapleurais das Attini derivadas e as das operárias mínimas poderiam também servir como depósitos de material energético, os quais poderiam ser requisitados posteriormente para utilização.

Por outro lado, o retículo endoplasmático liso, além de pouco desenvolvido foi pouco observado nas células secretoras da glândula metapleural de *A. coronatus, A. pilosum* e *M. parallelus*. Ao contrário de *A. laevigata* e de *T. fuscus* que apresentaram esta organela bem desenvolvida. De acordo com Billen (1984) o retículo endoplasmático liso (REL) participaria na síntese de lipídios na célula e o seu grau de desenvolvimento indicaria o grau de intensidade desta síntese. Apesar do REL ter sido encontrado pouco desenvolvido nas operárias de *A. coronatus* e de *W. auropunctata* foram observadas grandes gotas lipídicas no citoplasma das células secretoras de suas glândulas metapleurais, fato que poderia sugerir existência de outra fonte de lipídios, provavelmente as mitocôndrias. A síntese de lipídios por mitocôndrias já foi descrita por outros autores, como Caetano, Zara e Gregório (2002) nas glândulas pós-faríngeas de *Dinoponera australis*, Caperucci e Camargo-Mathias (2006) nos

ovócitos de *Neoponera villosa*, e Denardi et al. (2011) em células secretoras de glândulas salivares do carrapato *Amblyomma cajennense*.

Nas formigas *A. coronatus* e *A. laevigata*, bem como em *E. brunneum* foram observadas regiões de Golgi bem desenvolvidas quando comparadas com *M. parallelus* e *W. auropunctata*, espécies estas em que esta organela (algumas vezes) não foi observada. De acordo com Carvalho e Recco-Pimentel (2001) o Golgi seria responsável por armazenar e modificar as secreções produzidas pelo RE, sugerindo então que nas espécies aqui estudadas, exceto em *W. auropunctata* o Golgi estaria participando da síntese/modificação da secreção original em compostos com potencial antibiótico.

O presente estudo ultraestrutural revelou também no citoplasma das células secretoras da glândula metapleural das operárias mínimas de *A. laevigata* apresentou predominância de grânulos de secreção quando comparou-se com as operárias maiores, onde apenas diferenças no tamanho e na eletrondensidade destes foram observados, o que sinalizou a diversidade de conteúdo e, confirmou que a glândula metapleural na casta das operárias mínimas estaria mais envolvida na produção de secreção, visto que apresentaram maior variedade na composição e no tamanho dos grânulos. Os grandes grânulos de secreção foram observados nas células das glândulas metapleurais de *A. coronatus, A. laevigata, A. pilosum* e, *W. auropunctata*, ao contrário do observado em *T. fuscus* e *M. parallelus*, e *E. brunneum* onde pequenos grânulos estiveram presentes, indicando que diferentes espécies de formigas produzem secreções de diferentes naturezas. Os resultados histoquímicos confirmaram essa diversidade por meio da detecção de complexos de natureza lipoprotéica, os quais poderiam também ter ação antibiótica e/ou antifúngica, principalmente nas formigas cultivadoras de fungos.

Diferentemente do observado na porção intracitoplasmática dos canalículos das células secretoras a porção extracitoplasmática em todas as espécies aqui estudadas, não apresentou microvilosidades, porém, apresentou uma espessa cutícula revestindo o seu lúmen, confirmando o papel exclusivo destas regiões no transporte da secreção coletada das células secretoras. A espessa cutícula funcionaria como uma barreira mecânica impedindo a contaminação ou modificação da secreção por agentes externos ao canalículo, o que garantiria sua eficácia. O estudo morfofisiológico também mostrou que a secreção produzida pelas células das glândulas metapleurais em todos os indivíduos aqui estudados, não apresentou a mesma composição encontrada na secreção presente no lúmen da porção extracitoplasmática do canalículo, pois observou-se que no citoplasma os polissacarídeos, proteínas e lipídios ácidos foram fortemente marcados, porém foram moderada ou fracamente no lúmen da

porção extracitoplasmática do canalículo, dados que confirmaram que além de coletora a porção intracitoplasmática do canalículo teria também capacidade de modificar a secreção.

A porção armazenadora da glândula metapleural nos indivíduos, aqui estudados, apresentou placas perfuradas, estruturas quitinosas presentes no início da câmara coletora, que em *E. brunneum* e *W. auropunctata* foi única, em *M. parallelus* e *P. naegeli* duas, em *A. pilosum* três e, em *A. laevigata* e *A. coronatus* sete e oito placas, respectivamente. Desta forma um maior número de placas sendo encontrado nas formigas cortadeiras, sugeriria que provavelmente as mesmas produziriam mais secreção do que as outras espécies. Estudos realizados com *A. octospinosus* (BOT et al., 2001), *A. sexdens* (HÖLLDOBLER; ENGEL-SIEGEL, 1985), *A. bisphaerica* e *A. sexdens rubropilosa* (GUSMÃO, 2000) demonstraram a presença de maior número de placas na câmara coletora destas espécies. Ao contrário, em *D. vagans* (SCHOETERS; BILLEN, 1992), *Dorylus* spp., (BILLEN; VAN BOVEN, 1987), e *Dolichoderus quadripunctatus*, *Linepthema humile*, *Tapinoma erraticum* (FANFINI; DAZZINI, 1991) a placa perfurada apresentar-se-ia como estrutura única. Assim os dados aqui obtidos permitiram sugerir que espécies apresentando maior número de células secretoras também possuiriam maior número de placas perfuradas, como foi o exemplo das formigas cortadeiras, aqui analisadas, uma vez que quanto mais secreção é produzida maior necessidade de estruturas capazes de escoá-la se faz necessário.

Na câmara coletora das glândulas metapleurais também foram observadas dobras na cutícula de *E. brunneum*, *W. auropunctata*, *A. pilosum*, *M. parallelus*, *T. fuscus*, *A. laevigata* e *A. coronatus*, porém em *P. naegeli* essas dobras não foram observadas, sugerindo que nas primeiras elas teriam a função de direcionar o fluxo da secreção produzida na porção secretora em direção ao reservatório. Semelhantemente outros trabalhos Schoeters e Billen (1993) descreveram a ocorrência de um estreito sulco na parede do reservatório de *A. sexdens* ao qual foi atribuído a função de guiar a secreção da câmara coletora para a abertura da glândula metapleural.

Além da câmara coletora também o reservatório da glândula metapleural de todas as espécies, aqui estudadas, apresentaram epitélio simples pavimentoso fazendo o revestimento interno, função esta que não seria a única destas células, ficando a elas também atribuída a de secretar os componentes que formariam a cutícula, protetora sobre o epitélio, evitando que a secreção danificasse as células epiteliais, bem como preservando a secreção da ação de agentes externos, os quais poderiam minimizar ou inativar a sua ação.

O estudo químico da secreção revelou que as Attini derivadas apresentaram maior variedade de compostos nas glândulas metapleurais, tendo sido encontrados 12 em *A. laevigata,* nove em *A. coronatus,* e 10 em *T, fuscus.* Poucos compostos foram detectados nas atines basais: oito em *M. parallelus,* quatro em *A. pilosum,* e nas ectatommine e myrmicine: quatro em *P. naegeli,* seis em *E. brunneum*. Isto sugeriu que as glândulas metapleurais nas atines derivadas seriam mais ativas e produziriam mais compostos voláteis do que nas espécies das atines basais e das não cultivadoras de fungos, o que possivelmente traria maior eficiência no combate de patógenos.

Na análise química da secreção da glândula metapleural foi encontrada a presença de compostos produzidos exclusivamente pelas formigas cortadeiras como: o ácido 3-hidroxihexanoico ($C_6$) em operárias maiores e médias de *A. laevigata*, o ácido 4-hidroxioctanoico lactona ($C_8$), ácido 3- hidroxidecanoico ($C_{10}$) em *A. coronatus*. Schildknecht (1976) e Do Nascimento et al. (1996) em estudos anteriores já haviam demonstrado a presença de grande quantidade de ácido fenilacético, ácido 3- hidroxidecanoico ($C_{10}$) na secreção de *A. sexdens rubropilosa*. Também Schildknecht (1976) e Do Nascimento et al. (1996) encontraram $C_6$, $C_8$, $C_{10,}$ $C_{12}$, e $C_{10}$, $C_{12}$, $C_{14}$, $C_{16,}$ β-hidroxiácidos em *A. sexdens rubropilosa* e em *A. cephalotes*. Ortius-Lechner et al. (2000) detectaram em *A. octospinosus* a presença de muitos ácidos, e sugeriram que estes atuariam como redutores do pH do jardim do fungo (pH ácido inibiria a proliferação de fungos e de bactérias).

Nas formigas, aqui estudadas, outros compostos foram encontrados, tais como: myrmicacina (ácido 3-hidroxihexanoico) em *A. laevigata;* indole em *A. pilosum, M. parallelus, T. fuscus, A. laevigata* e *A. coronatus* e em pequenas quantidades em *P. naegeli* e *E. brunneum*; ácido fenilacético em *A. laevigata;* skatole em *A. pilosum, M. parallelus, T. fuscus, A. laevigata* e *A. coronatus*; e m-cresol em *E. brunneum,* cada um deles provavelmente desempenhando funções diferentes. Ou seja, myrmicacina agiria sobre os processos de divisão celular do fungo (IWANAWI, 1978); o indole atuaria como feromônio (MORGAN et al. 1999, 2003); o ácido fenilacético agiria como antibiótico contribuindo no controle da acidez da secreção da glândula metapleural de *A. sexdens* e *A. cephalotes* (DO NASCIMENTO et al., 1996; YEK; MULLER, 2011); o skatole seria responsável pelo odor distinto da secreção das glândulas metapleurais de *A. cephalotes* (DO NASCIMENTO et al., 1996); o m-cresol (fenol) em *E. brunneum,* assim como *Camponotus quadrisectus* (VOEGTLE et al., 2008) poderia estar agindo como feromônio de alarme ou de defesa.

Assim, diante dos resultados aqui obtidos o presente estudo deixou clara a existência de diferenças morfofisiológica, ultraestrutural e química (Tabela 1) entre as glândulas metapleurais das formigas *A. pilosum*, *M. parallelus*, *T. fuscus*, *A. laevigata* e *A. coronatus* (Attini), *E. brunneum* (Ectatommini), *W. auropunctata* (Blepharidattini), e *P. naegeli* (Myrmicini), tendo *A. laevigata* e *A. coronatus* as glândulas metapleurais mais desenvolvidas do que as *T. fuscus*, *A. pilosum*, *M. parallelus*, *E. brunneum*, *P. naegeli* e *W. auropunctata*. O aumento do tamanho e na capacidade secretora das glândulas metapleurais poderia, então, ser consequência: 1) da ocorrência de poliandria, o que traria maior diversidade genética para as colônias, conferindo-lhes maior resistência aos parasitas (HAMILTON, 1987; HUGHES; BOOMSMA, 2004); 2) do uso de vegetação fresca (corte e transporte) como substrato para os fungos, o que deixaria os indivíduos vulneráveis ao maior ataque de patógenos. Consequentemente estas espécies (formigas cortadeiras) precisariam ter maior investimento nos mecanismos de resistência a parasitas (HUGHES et al., 2008); 3) da presença de operárias polimórficas, uma vez que a glândula metapleural teria seu tamanho proporcional ao tamanho do corpo da formiga, e assim indivíduos especializados no corte de folhas teriam glândulas metapleurais maiores (HUGHES et al., 2008).

De forma geral, os resultados aqui obtidos permitiram inferir que em resposta a forte pressão de parasitas deva ter ocorrido o maior desenvolvimento da glândula metapleural nas formigas cortadeiras, o que capacitou-as a ter maior atividade de síntese de secreções com propriedades antibióticas e/ou antifúngicas.

**Tabela 1**. Resumo dos resultados morfofisiológicos, ultraestruturais e químicos obtidos das glândulas metapleurais das formigas cultivadoras (Attini) e não cultivadoras de fungos (Ectatommini, Blepharidattini, Myrmicini).

| Grupos | Espécies | Número médio de células secretoras | Forma das células secretoras | Número de placas perfuradas | Dobras presente/ ausente | Elementos mais frequente encontrados | RER | REL | Grânulos de secreção | Compostos detectados | Principais compostos |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Ectatommini** | *Ectatomma brunneum* | 47 | redonda | 01 | presente | polissacarídeos<br>proteínas | D | NO | P | 06 | ésteres<br>ácidos |
| **Blepharidattini** | *Wasmannia auropunctata* | 15 | redonda | 01 | presente | lipídios | D | PD | G | - | - |
| **Myrmicini** | *Pogonomyrmex naegeli* | 30 | oval | 02 | ausente | proteínas<br>lipídios | - | - | - | 04 | ésteres |
| **Attini basal** | *Apterostigma pilosum* | 42 | oval | 03 | presente | proteínas<br>lipídios | D | PD | G | 04 | ésteres |
| | *Mycetarotes parallelus* | 30 | oval | 02 | presente | lipídios | PD | PD | P | 08 | ésteres |
| **Attini derivada** | *Trachymyrmex fuscus* | 60 | oval | 03 | presente | polissacarídeos<br>proteínas<br>lipídios | PD | D | P | 10 | cetonas/<br>aldeídos/<br>ésteres |
| | *Acromyrmex coronatus** | ≥136 | oval | ≥08 | presente | polissacarídeos<br>proteínas<br>lipídios | D | PD | G | 09 | ácidos/<br>cetonas/<br>ésteres |
| | *Atta laevigata** | ≥140 | oval | ≥07 | presente | polissacarídeos<br>proteínas<br>lipídios | D | D | G | 12 | ácidos/<br>cetonas/<br>ésteres |

* dados para operárias mínimas; RER= retículo endoplasmático rugoso; REL= retículo endoplasmático liso; NO= não observado; PD= pouco desenvolvido; D= desenvolvido; P= menor; G= maior; ⁻ = dados não observados

# *Conclusões*

## VI. CONCLUSÕES

- A glândula metapleural de algumas espécies de formigas cultivadoras ou não de fungos possui organização morfológica semelhante, ou seja, está dividida em uma porção secretora e uma armazenadora, conectadas entre si por canalículos condutores.
- As glândulas metapleurais das espécies *E. brunneum*, *P. naegeli* e *W. auropunctata* (não cultivadores de fungos), *A. pilosum*, *M. parallelus* e *T. fuscus* possuem menor número de células secretoras do que as das *A. laevigata* e *A. coronatus*. Ainda, as espécies *A. laevigata* e *A. coronatus* possuem maior número de agrupamentos de células secretoras, bem como maior número de placas perfuradas, indicando que as glândulas metapleurais de *A. laevigata* e de *A. coronatus* seriam maiores e mais ativas na produção de secreção do que as das outras atines e as das não cultivadoras de fungos.
- Ao longo da evolução as glândulas metapleurais foram se tornando mais complexas, sendo de forma simples nas formigas basais (não cultivadoras de fungos) e de forma complexas nas cultivadoras de fungos, principalmente nas formigas cortadeiras (Attini derivada), possuindo nestas últimas os maiores números de células secretoras, agrupamentos celulares, placas perfuradas, indicando que o cultivo de fungos conduziu a essa maior complexidade das glândulas metapleurais.
- As células secretoras da glândula metapleural das espécies *A. laevigata* e *A. coronatus* sintetizam maiores teores de polissacarídeos e de lipídios ácidos do que as de *E.*

*brunneum* e *W. auropunctata*, *A. pilosum*, *M. parallelus*, e *T. fuscus*. A espécie *E. brunneum* sintetiza menores teores de lipídio. Isto indica que esses lipídios produzidos pela glândula metapleural fazem parte da secreção com função antibiótica.

- As células secretoras da glândula metapleural de *E. brunneum*, *P. naegeli*, *A. pilosum*, *M. parallelus*, *T. fuscus*, *A. laevigata* e *A. coronatus* tem um ciclo de atividade de síntese da secreção sincrônico.
- Nas operárias mínimas de *A. laevigata* e de *A. coronatus* as células secretoras da glândula metapleural apresentaram maiores regiões com RER desenvolvido, bem como grandes grânulos de secreção de natureza lipoprotéica, indicando sua maior capacidade de síntese de secreção com funções antibióticas e antifúngicas.
- A presença de vacúolos digestivos nas células secretoras das glândulas metapleurais de todas as espécies aqui estudadas demonstrou a ocorrência de processos de ciclagem celular.
- *A. laevigata*, *A. coronatus*, e *T. fuscus* apresentaram as maiores quantidades de compostos voláteis quando comparadas com *A. pilosum*, *M. parallelus*, *P. naegeli* e *E. brunneum*, sugerindo que as glândulas metapleurais nas atines derivadas produzam mais compostos voláteis do que as atines basais e não cultivadoras de fungos, por serem maiores e mais ativas.
- As glândulas metapleurais de *A. laevigata* e de *A. coronatus* apresentaram-se maiores e mais ativas na produção de secreção do que as das *A. pilosum*, *M. parallelus*, *T. fuscus*, *P. naegeli*, *W. auropunctata* e *E. brunneum*, em resposta a forte pressão de parasitas.

# *Referências*

## VII. REFERÊNCIAS

ALBERTS, B.; BRAY, D.; LEWIS, J.; RALF, M.; ROBERTS, K.; WATSON, J. D. **Molecular Biology of the Cell**. New York: Garland Publish, 4th ed. 2002.1294p.

ANGUS, C. J.; JONES, M. K.; BEATTIE, A. J. A possible explanation for size differences in the metapleural glands of ants (Hymenoptera, Formicidae). **Journal of the Australian Entomological Society**, v. 32, p. 73-77, 1993.

ASTRUC, C.; JULIEN, J. F.; ERRARD, C.; LENOIR, A. Phylogeny of ants (Formicidae) based on morphology and DNA sequence data. **Molecular Phylogenetics and Evolution,** v. 31, p. 880-893, 2004.

ATTYGALLE, A. B.; SIEGEL, B.; VOSTOWSKY, O.; BESTMANN, H. J.; MASCHWITZ, U. Chemical composition and function of metapleural gland secretion of the ant, *Crematogaster deformis* Smith (Hymenoptera: Myrmicinae). **Journal of Chemical Ecology**, v. 15, p. 317–328, 1989.

BAILEY I. W. Some relations between ants and fungi. **Ecology**, v. 1, p. 174-189, 1920.
BEATTIE, A. J.; TURNBULL, C.; HOUGH, T.; JOBSON, S.; KNOX, R. B. The vulnerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. **American Journal of Botany**, v. 72, p. 606–614, 1985.

BEATTIE, A. J.; TURNBULL, C.; HOUGH, T.; KNOX, R. B. Antibiotic production: A possible function for the metapleural glands of ants (Hymenoptera: Formicidae). **Annals of the Entomological Society of America**, v. 79, p. 448–450, 1986.

BILLEN, J. P. L.; PEUSENS, G. Ultrastructure de la glande propharyngienne chez les fourmis formicines (Hymenoptera, Formicidae). **Actes des Colloques Insectes Sociaux**, v. 1, p. 121-129, 1984.

BILLEN, J. P. L. Comparative ultrastructural of the poison and Dufour glands in old and new world army ants (Hymenoptera, Formicidae). **Actes des Colloques Insectes Sociaux**, v. 2, p. 79-86, 1985.

BILLEN, J. P. L. Comparative morphology and ultrastructure of the Dufour gland in ants (Hymenoptera, Formicidae). **Entomologia Generalis,** v. 11, p. 165-181, 1986.

BILLEN, J. P. J.; VAN BOVEN. The metapleural gland in old word army ants: a morphological and ultrastructural description (Hymenoptera, Formicidae). **Revue de Zoologie Africaine**, v. 101, p. 31-41, 1987.

BOLTON, B. **Identification guide to the ant genera of the world**. Cambridge, Harvard University Press, 1994. 222 p.

BOLTON, B. Synopsis and Classification of Formicidae. **The American Entomological Institute**, v. 71, p. 1-370, 2003.

BOT, A. N. M.; BOOMSMA, J. J. Variable metapleural gland size-allometries in *Acromyrmex* leafcutter ants (Hymenoptera: Formicidae). **Journal of the Kansas Entomological Society**, v. 69, p. 375-383, 1996.

BOT, A. N. M.; OBERMAYER, M. L.; HÖLLDOBLER, B.; BOOMSMA, J. J. Functional morphology of the metapleural gland in the leaf-cutting ant *Acromyrmex octospinosus*. **Insectes Sociaux**, v. 48, p. 63-66, 2001.

BOT, A. N. M.; ORTIUS-LECHNER, D.; FINSTER, K.; MAILE, R.; BOOMSMA, J. J. Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. **Insectes Sociaux**, v. 49, p. 363-370, 2002.

BRADY S. G.; SCHULTZ, T. R.; FISHER, B. L.; WARD, P. S. Evaluating alternative hypotheses for the early evolution and diversification of ants", **PNAS**, v. 103, n. 48, p. 18172–18177, 2006.

BROWN, W. L. JR. An Hypothesis Concerning the Function of the Metapleural Glands in Ants. **The American Naturalist**, v. 102, n. 924, p. 188-191, 1968.

CAETANO, F. H. **Aspectos ultramorfológicos, ultra-estruturais e enzimológicos da glândula pós-feringea de *Dinoponera australis* (Formicidae: Ponerinae)**. Tese (Livre-Docência em Histologia). Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, Brasil. 134p. 1998.

CAETANO, F. H.; JAFFÉ, K.; ZARA, F. J. **Formigas: biologia e anatomia**. Rio Claro: Editora Topázio, 2002. 131 p.

CAETANO, F. H.; ZARA, F. J.; GREGÓRIO, E. A. The origin of lipid droplets in the post-pharyngeal gland of *Dinoponera australis* (Formicidae: Ponerinae). **Cytologia**, v. 67, p. 301-308, 2002.

CAPERUCCI, D.; CAMARGO-MATHIAS, M. I. Lipids in oocytes of ants *Neoponera villosa* (Hymenoptera: Ponerinae). **Sociobiology**, v. 47, p. 531-541, 2006.

CARVALHO, H. F.; RECCO-PIMENTEL, S. M. **A Célula.** 2ª ed. Editora Manole Ltda: Barueri, São Paulo, Brasil. 287p, 2007.

DENARDI, S. E.; FURQUIM. K. C. S.; BECHARA, G. H.; CAMARGO-MATHIAS, M. I. Salivary glands of *Amblyomma cajennense* (Acari: Ixodidae): a histological and an ultrastructural overview. **Experimental and Applied Acarology**, v. 54, p. 177–189, 2011.

DE SOUZA, A. L. B.; SOARES I. M. F.; CYRINO, L. T.; SERRÃO, J. E. The metapleural gland in two subspecies of *Acromyrmex subterraneus* (Hymenoptera: Formicidae). **Sociobiology**, v. 47, n. 1, p. 19-25, 2006.

DINIZ, J. L. M; BRANDÃO, C. R. F.; YAMAMOTO, C. I. Biology of *Blepharidatta* ants, the sister group of the Attini: a possible origin of fungus-ants symbiosis. **Naturwissenchaften,** v. 85, p. 270-274, 1998.

DO NASCIMENTO, R. R. D.; SCHOETERS, E.; MORGAN, E. D.; BILLEN, J.; STRADLING, D. J. Chemistry of metapleural gland secretions of three attine ants, *Atta sexdens rubropilosa*, *Atta cephalotes*, and *Acromyrmex octospinosus* (Hymenoptera: Formicidae). **Journal of Chemical Ecology**, v. 22, p. 987-1000, 1996.

DONISTHORPE, H. Description of a new species of *Crematogaster* Lund, subgenus *Physocrema* Forel, with a list of, and a key to, the known species of the subgenus. **The Entomologist**, v. 74, p. 225-227, 1941.

EMERY, C. Végétarianisme chez les fourmis. Archives Sciences Physiques et Naturalles 8: 488-490. In: MUELLER, U. G.; SCHULTZ, T. R.; CURRIE, C. R.; ADAMS, R. M.; MALLOCH, D. The origin of the attine ant-fungus mutualism. **The Quarterly Review of Biology**, v. 76, n. 2, p. 169-197, 2001.

FANFINI, A.; DAZZINI M. V. Le glandole metatoraciche di *Crematogaster striatula* (Hymenoptera, Formicidae, Myrmicinae). **Fragmenta Entomologica**, v. 23, p. 191-200, 1991.

FARQUHARSON, C. O. The growth of fungi on the shelters builds over coccide by *Crematogaster* ants. Transactions of the Entomological Society of London 62: 42-50. In: MUELLER, U. G.; SCHULTZ, T. R.; CURRIE, C. R.; ADAMS, R. M.; MALLOCH, D. The origin of the attine ant-fungus mutualism. **The Quarterly Review of Biology**, v. 76, n. 2, p. 169-197, 2001.

FERNANDEZ-MARIN, H.; ZIMMERMAN, J.; REHNER, S.; WCISLO, W. Active use of the metapleural glands by ants in controlling fungal infection. **Proceedings of the Royal Society of London Biological Sciences**, v. 273, p. 1689-1695, 2006.

FOWLER, H. G.; FORTI, L. C.; BRANDÃO, C. R. F.; DELABIE, J. H. C.; VASCONCELOS, H. L. Ecologia Nutricional de Formigas. In: PANIZZI, A. R.; PARRA J. R. P. **Ecologia Nutricional de Insetos e suas implicações no manejo de pragas**. Brasília: CNPq, p. 427-433, 1991.

GARLING, L. Origin of ant-fungus mutualism: a new hypothesis. **Biotropica**, v. 11, p. 284-291, 1979.

GRAYSTOCK, P.; HUGHES, W. O. H. Disease resistance in a weaver ant, *Polyrhachis dives*, and the role of antibiotic-producing glands. **Behavioural Ecology and Sociobiology**, v. 65, n. 12, p. 2319-2327, 2011.

GUSMÃO, L. G. **Estudo comparado da glândula metapleural e sua secreção em diferentes castas de três espécies de *Atta* (Hymenoptera: Formicidae)**. 60p. Tese (Doutorado) – ESALQ - Universidade de São Paulo, Piracicaba, Brasil, 2000.

GUSMÃO, L. G.; CAETANO, F. H.; NAKANO, O. Ultramorphology of the metapleural gland in three species of *Atta* (Hymenoptera, Formicidae). **Iheringia, Série Zoologia**, v. 91, p. 33-36, 2001.

HAMILTON, W. D. Kinship, recognition, disease, and intelligence: constraints of social evolution. Pp. 81–100. In: ITO, Y.; BROWN, J. L.; KIRKKAWA, **Journal of Animal Societies: theories and facts**. Japan Scientific Societies Press, Tokyo, 1987.

HÖLLDOBLER, B.; WILSON, E. O. **The Ants**. Cambridge: Harvard Univ. Press. 732 p, 1990.

HÖLLDOBLER, B.; ENGEL-SIEGEL, H. On the metapleural gland in ants. **Psyche**, v. 91, p. 201–224, 1985.

HOSOISHI, S.; OGATA, K. The ant genus *Crematogaster* Lund, subgenus *Physocrema* Forel, in the Indochinese Peninsula (Hymenoptera: Formicidae). **Asian Myrmecology**, v. 2, p. 1-10, 2008.

HUGHES, W. O. H.; EILENBERG, J.; BOOMSMA, J. J. Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. **Proceedings of the Royal Society of London Biological Sciences**, v. 269, p. 1811-1819, 2002.

HUGHES, W. H. O.; BOOMSMA, J, J. Genetic diversity and disease resistance in leaf-cutting ant societies. **Evolution**, v. 58, p. 1251–1260, 2004.

HUGHES, W. O. H.; PAGLIARINI, R.; MADSEN, H. B.; DIJKSTRA, M. J.; BOOMSMA, J. J. Antimicrobial defence shows an abrupt evolutionary transition in the fungus-growing ants. **Evolution**, v. 62, p. 1252–1257, 2008.

IIZUKA, T.; IWADARE, T.; ORITO, K. Antibacterial activity of myrmicacin a compound in the secretion of South American leaf-cutting ant *Atta sexdens* and related compounds on pathogenic bacteria in silkworm larvae, *Streptococcus faecalis* AD-4. **Journal of the faculty of agriculture**, Hokkaido, v. 59, p. 262-266, 1979.

ITO, F.; HASHIM, R.; HUEI, Y. S.; KAUFMANN, E.; AKINO, T.; BILLEN, J. Spectacular batesian mimicry in ants. **Naturwissenschaften**, v. 91, n. 10, p. 481-484, 2004.

IWANAWI, Y. Myrmicacin, a new inhibitor for mitotic progression after metaphase. **Protoplasma**, v. 95, p. 267–271, 1978.

JAFFÉ, K.; PUCHE; H. Colony-specific territorial marking with the metapleural gland secretion in the ant *Solenopsis geminata* (Fabr.). **Journal of Insect Physiology**, v. 30, p. 265–270, 1984.

JAFFE, K.; LOPEZ, M. E.; ARAGORT, W. On the communication systems of the ants *Pseudomyrmex termitarius* and *P. triplarinus*. **Insectes Sociaux**, v. 33, p. 105–117, 1986.

JANET, C. H. Système glandulaire tègumentaire de la *Myrmica rubra*: observations diverses sur les fourmis. In: **Études sur les Fourmis, les Guèpes et les Abeilles**, Note 17 (C. JANET), p. 1-28. Carré et Naud, Paris. 1898.

JOHNSON, R. N.; AGAPOW, P-M.; CROZIER, R. H. A tree island approach to inferring phylogeny in the ant subfamily Formicinae, with especial reference to the evolution of weaving. **Molecular Phylogenetics and Evolution**, v. 29, p. 317–330, 2003.

JONES, T. H.; BRUNNER, S. R.; EDWARDS, A. A.; DAVIDSON, D. W.; SNELING, R. R. 6-Alkylsalicylic acids and 6-alkylresorcylic acids from ants in the genus *Crematogaster* from Brunei. **Journal of Chemical Ecology**, v. 31, p. 407-417, 2005.

JUNQUEIRA, L. C. U.; JUNQUEIRA, L. M. M. S. **Técnicas básicas de citologia e histologia**. São Paulo: Editora Santos, 1983. p. 48-81.

JUNQUEIRA, L. C.; CARNEIRO, J. **Histologia básica**. Rio de Janeiro: 11 ed. Guanabara Koogan, 2008.

JUNQUEIRA, L. C. U.; SALLES, L. M. M. **Ultra-estrutura e função celular**. Editora Guanabara Koogan, São Paulo, Brasil. 1975, 120p.

LISON, L. **Histochimie et Cytochimie Animales**, Gauthier-Villars, Éditeur, Paris, 1960.

LONGINO J. T.; FERNÁNDEZ, F. Taxonomic review of the genus *Wasmannia*. In: Snelling, R. R.; Fisher, B. L; Ward P. S. **Advances in ant systematics (Hymenoptera: Formicidae)**: homage to E. O. Wilson – 50 years of contributions. **Memoirs of the American Entomological Institute**, v. 80. p. 271-289, 2007.

LOPES, B. C.; SANTOS, R. A. Aspects of the ecology of ants (Hymenoptera: Formicidae) on the mangrove vegetation of Rantones, Santa Catarina Island, SC, Brazil. **Boletim Entomológico Venezuelano N. S.**, v. 11, n. 2), p 123, 1996.

MAILE, R.; DANI, F. R.; JONES, G. R.; MORGAN, E. D.; ORTIUS-LECHNER, D. Sampling techniques for gas chromatographic–mass spectrometric analysis of long-chain free fatty acids from insect exocrine glands. **Journal of Chromatography**, v. A, p. 816:169–175, 1998.

McMANUS, J. F. A. **Histological demonstration of mucin after periodic acid**. Nature, v. 158, 202p, 1946.

MARKL, H. Peripheres Nervensystem und Muskulatur im Thorax der Arbeiterin von *Apis mellifera* L., *Formica polyctena* Foerster und *Vespa vulgaris* L. und der Grundplan der Innervierung des Insektenthorax. **Zoologische Jahrbücher. Abtheilung für Anatomie und Ontogenie der Thiere**, v. 83, p. 107–184, 1966.

MARTOJA, R.; MARTOJA-PIERSON, M. **Initiation aux techniques de l'histologie animale**. Masson et c[ie], éditeurs, bd St-Germain, Paris IV, 1967.

MASCHWITZ, U.; KOOB, K.; SCHILDKNECHT, H. Ein Beitrag zur Funktion der Metathoracaldrüse der Ameisen. **Journal of Insect Physiology**, v. 16, p. 387–404, 1970.

MASCHWITZ, U. Vergleichende Untersuchungen zur Funktion der Ameisenmetathorakaldrüse. **Oecologia**, v. 16, p. 303–310, 1974.

MELLO, M. L. S. Cytochemistry of DNA. RNA and nuclear proteins. **Brazilian Journal of Genetics**, v. 20, p. 257–264, 1997.

MELLO, M. L. S.; VIDAL, B. C.; DANTAS, M. M.; MONTEIRO, A. L. P. Discrimination of the nucleolus by a critical electrolyte concentration method. **Acta Histochem Cytochem**, v. 26, p. 1–3, 1993.

MORGAN, E. D. Preparation of small-scale samples from insects for chromatography. **Anal.Chim. Acta**, v. 236, p. 227–235, 1990.

MORGAN, E. D.; DO NASCIMENTO, R. R.; KEEGANS, S. J.; BILLEN, J. Comparative study of mandibular gland secretions of workers of ponerine ants. **Journal of Chemical Ecology**, v. 25, p. 1395-1409, 1999.

MORGAN, E. D.; JUNGNICKEL. H.; KEEGANS, S. J.; DO NASCIMENTO, R. R.; BILLEN, J.; GOBIN, B.; ITO, F. Comparative survey of abdominal gland secretions of the ant subfamily ponerinae. **Journal of Chemical Ecology**, v. 29, p. 95-114, 2003.

MUELLER, U. G.; SCHULTZ, T. R.; CURRIE, C. R.; ADAMS, R. M.; MALLOCH, D. The origin of the attine ant-fungus mutualism. **The Quarterly Review of Biology**, v. 76, n. 2, p. 169-197, 2001.

MUELLER, U. G. Ant versus fungus versus mutualism: Ant-cultivar conflict and the deconstruction of the attine ant-fungus symbiosis. **The American Naturalist**, v. 160, p. S67-S98, 2002.

NOIROT, C.; QUENNEDEY, A. Glands, gland cells, glandular units: some comments on terminology and classification. **Annual of Society Entomology France**, v. 27, p. 123-128, 1991.

NORTH, R. D.; JACKSON, C.W.; HOWSE, P. E. Evolutionary aspects of anti-fungus interactions in leaf-cutting ants. **TREE**, v. 12, p. 386–389, 1997.

ORTIUS-LECHNER, D.; MAILE, R.; MORGAN, E. D.; BOOMSMA, J. J. Metapleural gland secretion of the leaf-cutter ant *Acromyrmex octospinosus*: New compounds and their functional significance. **Journal of Chemical Ecology**, v. 26, p. 1667-1683, 2000.

PEARSE, A. G. E. **Histochemistry. Theoretical and Applied**. 4th ed. J & A Churchill, London, 1985.

POULSEN, M.; BOT, A. N. M.; NIELSEN, M. G.; BOOMSMA, J. J. Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. **Behavioral Ecology and Sociobiology**, v. 52, p. 151-157, 2002.

POULSEN, M.; HUGHES, W. O. H.; BOOMSMA, J. J. Differential resistance and the importance of antibiotic production in *Acromyrmex echinatior* leaf-cutting ant castes towards the entomopathogenic fungus *Aspergillus nomius*. **Insectes Sociaux**, v. 53, p. 349-355, 2006.

SANTSCHI, F. Formicides nouveaux ou peu conus du Congo français. Annales de la Société Entomologique de France 78: 349-400. In: Mueller, U. G.; Schultz, T. R.; Currie, C. R.; Adams, R. M.; Malloch, D. The origin of the attine ant-fungus mutualism. **The Quarterly Review of Biology**, v. 76, n. 2, p. 169-197, 2001.

SCHILDKNECHT, H. Chemical ecology – a chaper of modern natural products chemistry. **Angewandte Chemie**, v. 15, p. 214-222, 1976.

SCHILDKNECHT, H.; KOOB K. Myrmicacin, the first insect herbicide. **Angewandte Chemie International Edition,** v. 10, p. 124–125, 1971.

SCHOETERS, E.; BILLEN, J. Morphological and ultrastructural study of metapleural gland in *Diacamma* (Hymenoptera, Formicidae). In: **European Congress of Social Insects**, 1o, Leuven, 1991. Proceedings... Leuven, Leuven University. p.239-247, 1992.

SCHOETERS, E.; BILLEN, J. Anatomy and fine struture of the metapleural gland in *Atta*. **Belgian Journal of Zoology**, v. 123, p. 67–75, 1993.

SCHULTZ, T. R.; BRADY, S. G. Major evolutionary transitions in ant agriculture. **Proceedings of the National Academy of Sciences**, v. 105, p. 5435–5440, 2008.

SUMNER, S.; HUGHES, W. O. H.; BOOMSMA, J. J. Evidence for differential selection and potential adaptive evolution in the worker caste of an inquiline social parasite. **Behavioural Ecology and Sociobiology**, v. 54, p. 256–263, 2003.

SUMNER, S; HUGHES, W. H. O.; PEDERSEN, J.S.; BOOMSMA, J. J. Ant parasite queens revert to mating singly. **Nature**, v. 428, p. 35–36, 2004.

TERRA, W. R. A digestão dos insetos. **Ciência Hoje**., v. 12, n. 70, p. 28-38, 1991.

THORNE, B. L.; TRANIELLO, J. F. A. Comparative social biology of basal taxa of ants and termites. **Annual Review Entomology**, v. 48, p. 283–306, 2003.

TULLOCH, G. S; SHAPIRO, J. E.; HERSHENOV, B. The ultrastructure of the metasternal glands of ants. **Bulletin of Brooklyn Entomology Society**, v. 57, p. 91-101, 1962.

VEAL, D. A.; TRIMBLE, J. E.; BEATTIE, A. J. Antimicrobial properties of secretions from the metapleural glands of *Myrmecia gulosa* (the Australian bull ant). **Journal Applied Bacterial**, v. 72, p. 188–194, 1992.

VOEGTLE, H. L.; JONES T. H.; DAVIDSON, D. W.; SNELLING, R. R. E-2-Ethylhexenal, E-2-Ethyl-2-Hexenol, Mellein, and 4-Hydroxymellein in *Camponotus* Species from Brunei. **Journal of Chemical Ecology**, v. 34, n. 2, p. 215-219, 2008.

VON IHERING H. Die Ameisen von Rio Grande do Sul. Berliner Entomologische Zeitschrift 39: 321-446. In: MUELLER, U. G.; SCHULTZ, T. R.; CURRIE, C. R.; ADAMS, R. M.; MALLOCH, D. The origin of the attine ant-fungus mutualism. **The Quarterly Review of Biology**, v. 76, n. 2, p. 169-197, 2001.

VON IHERING H. Die Anlagen neuer Colonien Und Pilzgarten bei *Atta sexdens*. Zoologicher Anzeiger 21: 238-245. In: Mueller, U. G.; Schultz, T. R.; Currie, C. R.; Adams, R. M.; Malloch, D. The origin of the attine ant-fungus mutualism. **The Quarterly Review of Biology**, v. 76, n. 2, p. 169-197, 2001.

WEBER, N. A. **Gardening ants: The Attines**. Philadelphia (PA): American Philosophical Society, v. 92, 146p, 1972.

WHEELER, W. M. The fungus-growth ants of North America. **Bulletin of the American Museum of Natural History**, v. 23, p. 669-807, 1907.

WHEELER, W. M. 1910. Ants: Structure, Development and Behavior. New York: Columbia University Press. In: HÖLLDOBLER, B.; WILSON, E. O. **The Ants**. Cambridge: Harvard Univ. Press. 1990, 732 p.

WILSON, E. O. **The Insect Societies**. Cambridge, MA: Harvard Univ. Press. 1971. 548 p.

YEK, S. H.; MUELLER, U. G. The metapleural gland of ants. **Biological Reviews**, v. 86, p. 774-791, 2011.