KARINE PINTO E VAIRO

SARCOPHAGIDAE (DIPTERA) NECRÓFAGOS DO SUL DO BRASIL: Uma abordagem morfológica e comportamental

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Tese apresentada a Coordenação do Curso de Pós-Graduação em Ciências Biológicas, área de concentração em Entomologia da Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ciências Biológicas.

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Co- Orientadores:
Prof. Dr^{a}. Cátia Antunes de Mello-Patiu
Prof. Dr^{o}. Paulo Zarbin

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"SARCOPHAGIDAE (DIPTERA) NECRÓFAGOS DO SUL DO BRASIL: uma abordagem morfológica e comportamental"

Tese aprovada como requisito parcial para obtenção do grau de "Doutor em Ciências", no Programa de Pós-graduação em Ciências Biológicas, Área de Concentração em Entomologia, da Universidade Federal do Paraná, pela Comissão formada pelos professores:

Prof. Dr. Maurício Osvaldo Moura (Orientador)
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(Pós-doc UFPR)

Dra. Camila Borges da Cruz Martins
(UFPR)

Curitiba, 25 de fevereiro de 2015.

“Tudo ao seu tempo!
Nascimento,
Crescimento,
Evolução,
Tudo tem tempo!
Florescimento,
Amadurecimento,
Transformação,
Tudo tem tempo!
E você observou todas as etapas,
Ovos, Larvas, Pupas, Moscas.
E aí...
Conclusão,
Amor,
Persistência,
Esperança,
Competência,
Você as teve,
Tudo tem tempo!
Passado, Presente e Futuro,
Você vive!
Tudo ao seu tempo!”

Francisco Vairo

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APRESENTAÇÃO

Conforme formato requerido pelo Programa de Pós-Graduação em Entomologia da Universidade Federal do Paraná, esta tese está dividida em: *Introdução*, *Objetivos* e *Capítulos* (sob a forma de artigos científicos que serão submetidos logo após a análise, correções e sugestões da banca avaliadora). Este trabalho foi desenvolvido no Laboratório de Dinâmicas Ecológicas e Laboratório de Semioquímicos da Universidade Federal do Paraná; Laboratório – Diptera Sarcophagidae/DIPSARC do Museu Nacional do Rio de Janeiro; Laboratório do Dr. Thomas Pape no Natural History Museum of Denmark e Laboratório do Dr. Krzysztof Szpila na Copernicus University. A estudante recebeu bolsa de estudos concedida pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - 141487/2011-9 e bolsa período sanduíche concedida pela Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior (CAPES). Todos os experimentos apresentados nesse trabalho estão incluídos em projeto de pesquisa aprovado em seus aspectos éticos e metodológicos pelo Comitê de Ética da Universidade Federal do Paraná sob o número 581 processo 23075.109305/2011-79.

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RESUMO GERAL

Os vestígios entomológicos coletados em um local de morte podem ser de extrema importância para determinar o tempo de exposição do cadáver ao ambiente e consequentemente estimar o intervalo pós-morte (IPM). Massas de ovos, larvas e adultos de insetos encontrados relacionados a um cadáver podem guardar informações a respeito do que ocorreu no local e ainda, se houve ingestão de alguma substância previamente a morte. O início da análise dos insetos necrófagos por entomólogos forense se dá através da identificação dos espécimes. A identificação é um processo complexo realizado através de chaves de identificação e descrições. Qualquer erro no processo de identificação comprometerá todas as análises subsequentes que são baseadas em informações relativas ao desenvolvimento e ocorrência da espécie identificada. Sarcophagidae em comparação a outras famílias de dípteros muscóides é a que possui menos trabalhos de ecologia e biologia provavelmente devido à dificuldade na identificação. Além disso, apesar das moscas dessa família serem frequentemente coletadas em locais de morte, são subutilizadas para cálculo de IPM considerando tanto a falta de identificação quanto a dificuldade na obtenção de dados sobre o desenvolvimento. O IPM pode ser estimado através do tempo de desenvolvimento dos imaturos ou por sucessão entomológica. A sucessão entomológica pode ser compreendida pela relação da tanatoquímica com a atração dos insetos pelos compostos orgânicos voláteis eliminados ao longo da decomposição de um cadáver. Sendo assim, o objetivo desse trabalho foi abordar dois temas em Sarcophagidae: a falta de chaves de identificação e a falta de informações sobre a relação entre a atração dessas moscas e o processo de decomposição em cadáveres. Para isso, foram elaboradas chaves de identificação para fêmeas e imaturos de terceiro instar da região Sul do Brasil, e foram identificados os compostos responsáveis pela atração da espécie *Peckia* (*Sarcodexia*) *lambens*. As fêmeas de Sarcophagidae puderam ser diferenciadas principalmente pela morfologia da terminalia, através da análise do tergito 6 (dividido ou não), presença do tergito 8, epiprocto (inteiro ou dividido) e morfologia das espermatecas. Para as larvas, primeiramente foi realizada uma revisão terminológica necessária para a compreensão dos caracteres. Para a diferenciação das espécies foram analisados principalmente os escleritos do esqueleto cefálico, porém, a distribuição dos espinhos, morfologia das papilas anais e número de abertura do espiráculo anterior também foram considerados. Em relação à tanatoquímica, indol e acetofenona foram os compostos responsáveis pela atração de *P*. (*S*.) *lambens*.

Palavras-chave: Entomologia Forense, tanatoquímica, morfologia, larvas, fêmeas, *Oxysarcodexia*, *Peckia*, *Microcerella*, *Sarcophaga*.

ABSTRACT

The entomological evidence collected in a death place may be very important to determine the exposure time of the corpse to the environment and consequently estimate the *postmortem* interval (PMI). Insects related to a corpse can store information about what occurred at the site and, if the person ingested some chemicals prior to death. The analysis of insects by forensic entomologists is through the identification of specimens and its development. The identification is a complex process performed using identification keys and descriptions. Any error in the identification process will compromise all subsequent analyzes since each species has different information concerning development and behaviour. Sarcophagidae compared to other families of muscoid flies is the one that has least biology and ecology studies probably due to the difficulty in identification. In addition, despite the flies of this family are often collected in death sites it is underutilized for PMI estimative considering both the lack of identification as the difficulty in obtaining data on development and behavior. The PMI can be estimated by the development time of immature stages or entomological succession. Entomological succession can be understood by the tanatochemistry that influences the attraction of insects by volatile organic compounds along the decomposition process of a corpse. Thus, the aim of this study was to address two aspects in Sarcophagidae: the lack of identification keys and the lack of information about the relation between the attraction of these flies and the decomposition process. For this, identification keys were elaborated for females and third instar larvae of nine species collected in southern Brazil, and the compounds responsible for the attraction of *Peckia* (*Sarcodexia*) *lambens* were identified. Sarcophagidae females could be differentiated mainly by morphology of terminalia, through the analysis of tergite 6 (divided or not), presence of tergite 8, epiproct (divided or undivided) and morphology of spermathecae. For the larvae analysis, it was first performed a terminology review to understand the characters. For the differentiation of species the main characters analyzed were the cephaloskeleton sclerites, distribution of spines, morphology of anal papilla and the anterior and posterior spiracles. In relation to tanatochemistry, indole and acetophenone compounds were responsible for attractiveness of *P*. (S.) *lambens* to carcasses.

Keywords: Forensic Entomology, tanatochemistry, morphology, larvae, females, *Oxysarcodexia*, *Peckia, Microcerella*, *Sarcophaga*.

INTRODUÇÃO GERAL

A entomologia forense é o estudo dos insetos aplicado a investigações cíveis ou criminais (Oliveira-Costa 2010; Amendt *et al*. 2004). Dentro da área criminal, em casos envolvendo homicídios, os insetos podem auxiliar a responder algumas questões, principalmente relacionadas ao tempo decorrido da morte (Tomberlin *et al*. 2011). Quando ocorre um homicídio, durante o inquérito policial são levantadas provas essenciais para a ação penal e início do processo criminal. Nesse contexto, os vestígios entomológicos são tão importantes quanto qualquer outro vestígio coletado no local de morte. Os vestígios entomológicos, usualmente são insetos adultos e imaturos diretamente relacionados à decomposição humana e geralmente encontrados em grande quantidade no cadáver e no local (Gunn 2006).

A decomposição humana inicia aproximadamente quatro minutos após a morte e é primariamente dependente da temperatura e em menor grau, da umidade (Vass 2001). Além de fatores abióticos, a fauna associada também é responsável por acelerar o processo e, usualmente, é composta de micro organismos, carnívoros e insetos. Diversas ordens de insetos podem estar relacionadas a carcaças animais e cadáveres humanos, porém as mais abundantes e que são amplamente utilizadas na ciência forense são Diptera e Coleoptera (Byrd & Castner 2001).

As famílias de Diptera necrófagas de maior importância são Calliphoridae, Muscidae e Sarcophagidae, sendo esta última a que possui menor quantidade de informações taxonômicas e biológicas disponíveis, provavelmente devido à dificuldade na identificação das espécies. Nos adultos, os caracteres externos, em sua maioria, são muito uniformes, sendo necessário então, um estudo aprofundado das terminálias masculina e feminina (de Carvalho & Mello-Patiu 2008; Lopes 1941). Nos estágios imaturos, essa dificuldade também ocorre, já que somente um estudo acurado dos caracteres externos e do esqueleto cefálico mostra diferenças interespecíficas. Estudos morfológicos de larvas desta família são escassos, e para o Brasil, não existem chaves de identificação de imaturos. Em relação às fêmeas essa situação se

repete, não existindo chaves de identificação para espécies de fêmeas necrófagas.

A impossibilidade e/ou incorreta determinação da espécie pode gerar problemas em relação à análise do material coletado em local de morte. Para a entomologia forense, o primeiro passo na análise da evidência entomológica é a correta identificação da espécie. Se houver erro nessa etapa do trabalho, as informações biológicas levantadas e geradas sobre a espécie serão incorretas ocasionando falsas conclusões.

É notável que a falta de estudos morfológicos e de ferramentas que possam auxiliar na identificação de grupos como Sarcophagidae acarretam um entrave nas ciências aplicadas já que a ausência de conhecimento da diversidade e problemas na identificação a nível específico compromete a elaboração de trabalhos aplicados. Além disso, outra dificuldade em relação a estudos ecológicos envolvendo Sarcophagidae é sua estratégia reprodutiva, a viviparidade (Lopes 1941), que dificulta a realização de estudos de biologia e comportamento com um número amostral adequado em um período de tempo limitado.

Para a entomologia forense, além da correta identificação da espécie é necessário compreender os parâmetros biológicos e comportamentais das moscas que ocorrem em cadáveres. Isso porque, usualmente a pergunta “Quando a pessoa morreu?” não é respondida pelos peritos médicos legistas com assertividade. Quando um corpo é encontrado com mais de 72 horas do óbito, análises morfológicas e de temperatura corpórea podem não ser suficientes para determinar quando a morte ocorreu (Anderson 2005). Assim, nestes casos, os insetos são a principal ferramenta na datação do intervalo pós-morte, provendo informações mais robustas. Existem diversas evidências de congruência entre o tempo de desenvolvimento de espécies e o tempo decorrido desde a morte. Nesse contexto, a descrição detalhada de como o desenvolvimento varia com a temperatura é uma etapa fundamental do processo que permite relacionar o padrão de desenvolvimento das espécies de interesse forense com a estimativa do intervalo pós-morte (IPM) (Grassberger & Reiter 2002, Bourel et al. 2003, Ames & Turner 2003, Huntington et al. 2007). A segunda etapa desse processo é a validação dessas estimativas. Isso vem ocorrendo nos casos em que a entomologia forense foi determinante para

conclusão de investigações criminais (Anderson 2004, Benecke 1998, Turchetto et al. 2001, Pujol-Luz et al. 2006, Oliveira-Costa & Mello Patiu 2004; Vairo et al. 2015).

A idade dos estágios imaturos encontrados em um cadáver pode estimar a data da morte desde um dia até meses, dependendo das espécies envolvidas e das condições climáticas do local (Amendt et al.2004). Através do estudo de sucessão e ciclo de vida da espécie em questão, pode-se estimar quando ocorreu o óbito ou quanto tempo o cadáver ficou exposto ao ambiente (Turchetto & Vanin 2004). Há duas abordagens utilizadas para determinar quando a morte ocorreu utilizando evidências entomológicas. A primeira é baseada no desenvolvimento dos dípteros imaturos (IPM mínimo) e a segunda na análise da colonização/sucessão dos insetos decompositores na carcaça (Anderson 2005). No entanto, a utilização dos padrões de colonização, aplicado a corpos em avançado estado de decomposição, depende de um estudo prévio acerca da sucessão entomológica e comportamento das espécies de maneira mais local possível.

Para a compreensão da sucessão entomológica de maneira completa é importante levar em consideração a tanatoquímica, ou química da morte (Arroyo et al. 2004). Os insetos que são atraídos por cadáveres ou carcaças são estimulados pela presença de compostos orgânicos voláteis (COVs) que são eliminados quando se inicia o processo de decomposição e, também, pelos insetos que já se encontram no cadáver (Paczkowski et al. 2011). Esses COVs além de guiar os insetos para encontrar sítios de alimentação e reprodução, podem atrair predadores e parasitas (Reznik et al., 1992). Entender o perfil químico das etapas da decomposição e as causas da atratividade do inseto a determinados compostos podem auxiliar a responder questões comportamentais e ser de extrema importância para a estimativa do tempo de colonização do cadáver (Statheropoulos et al. 2007; Dekeirsschieter et al. 2009).

Sendo assim, para aprofundar o conhecimento acerca dos Sarcophagidae de importância forense é necessário investir em pesquisa básica, como a confecção de chaves de identificação para o grupo de maneira regional e ainda, compreender os mecanismos de atração dessa família a cadáveres traçando a melhor estratégia e analisando as metodologias mais

adequadas para tornar esse entendimento possível em um contexto mais amplo. Para isso, esse trabalho aborda os sarcofagídeos do sul do Brasil que ainda não foram estudados, ou seja, as fêmeas e larvas além de utilizar uma abordagem de ecologia química para detectar compostos voláteis atrativos à família utilizando uma espécie de Sarcophagidae como modelo biológico.

OBJETIVOS

Objetivo Geral

Possibilitar a identificação das fêmeas e larvas de Sarcophagidae (Diptera) envolvidas no processo de decomposição no sul do Brasil e determinar a atuação da tanatoquímica na atração de *Peckia* (*Sarcodexia*) *lambens*.

Objetivos específicos

1. Caracterizar as larvas de terceiro ínstar das espécies: *Oxysarcodexia paulistanensis* (Mattos, 1919), *Oxysarcodexia riograndensis* (Lopes, 1946), *Peckia* (*Pattonella*) *intermutans* (Walker, 1861), *Peckia* (*Pattonella*) *resona* (Lopes, 1935), *Peckia* (*Euboettcheria*) *australis* (Fabricius, 1805), *Peckia* (*Euboettcheria*) *florencioi* (Mattos, 1919), *Microcerella halli* (Prado & Fonseca 1932), e *Sarcophaga* (*Bercaea*) *africa* (Wiedemann, 1824).

2. Elaborar uma chave de identificação para as larvas de terceiro instar citadas anteriormente incluindo *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830).

3. Caracterizar as fêmeas das espécies: *Oxysarcodexia paulistanensis* (Mattos, 1919), *Oxysarcodexia riograndensis* (Lopes, 1946), *Peckia* (*Pattonella*) *intermutans* (Walker 1861), *Peckia* (*Pattonella*) *resona* (Lopes, 1935), *Peckia* (*Euboettcheria*) *australis* (Fabricius, 1805), *Peckia* (*Euboettcheria*) *florencioi* (Mattos, 1919), *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830), *Microcerella*

halli (Prado & Fonseca 1932), e *Sarcophaga* (*Bercaea*) *africa* (Wiedemann, 1824).

4. Elaborar uma chave de identificação para as fêmeas citadas anteriormente.

5. Analisar os compostos voláteis da decomposição de carcaças de ratos (*Rattus norvegicus*) e testar a atratividade química da espécie *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830) (Diptera: Sarcophagidae).

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CAPÍTULO I

Comparative morphology and identification key for females of nine Sarcophagidae species (Diptera) with forensic importance in Southern Brazil

Comparative morphology and identification key for females of nine Sarcophagidae species (Diptera) with forensic importance in Southern Brazil

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Abstract

Comparative morphology and identification key for Sarcophagidae (Diptera) females with forensic importance in Southern Brazil. The identification of female flesh flies was always considered a difficult task since morphological descriptions and keys for females are rare. Even in a forensic entomology framework, where females play a major role, flesh flies females are usually not identified. In order to fill this gap in Southern Brazil fauna we provide detailed descriptions and key for female of nine species included in four genera: *Microcerella halli* (Engel), *Oxysarcodexia paulistanensis* (Mattos), *Oxysarcodexia riograndensis* (Lopes), *Peckia* (*Euboettcheria*) *australis* (Townsend), *Peckia* (*Euboettcheria*) *florencioi* (Prado & Fonseca), *Peckia* (*Pattonella*) *intermutans* (Walker), *Peckia* (*Pattonella*) *resona* (Lopes), *Peckia* (*Sarcodexia*) *lambens* (Wiedemann), and *Sarcophaga* (*Bercaea*) *africa* (Wiedemann). These species are distinguished mainly by genital characters as

tergite 6 divided or undivided, presence of tergite 8, spermatechae morphology and vaginal plate shape.

Key-Words: forensic entomology, *Microcerella*, *Oxysarcodexia*, *Peckia*, *Sarcophaga*.

Resumo

Morfologia comparada e chave de identificação para nove espécies de Sarcophagidae (Diptera) de importância forense do Sul do Brasil. A identificação de fêmeas de Sarcophagidae (Diptera) foi sempre considerada difícil principalmente pela falta de descrições morfológicas e chaves de identificação. Mesmo com grande importância para a entomologia forense, as fêmeas usualmente não são identificadas. Sendo assim, buscando mudar esse panorama para o Sul do Brasil, foram elaboradas descrições detalhadas e chave de identificação para fêmeas de nove espécies incluídas em quatro gêneros: *Microcerella halli* (Engel), *Oxysarcodexia paulistanensis* (Mattos), *Oxysarcodexia riograndensis* (Lopes), *Peckia* (*Euboettcheria*) *australis* (Townsend), *Peckia* (*Euboettcheria*) *florencioi* (Prado & Fonseca), *Peckia* (*Pattonella*) *intermutans* (Walker), *Peckia* (*Pattonella*) *resona* (Lopes), *Peckia* (*Sarcodexia*) *lambens* (Wiedemann) e *Sarcophaga* (*Bercaea*) *africa* (Wiedemann). Os principais caracteres utilizados dizem respeito à terminália, como tergito 6 dividido ou inteiro, presença do tergito 8, morfologia das espermatecas e formato da placa vaginal.

Palavras-chave: entomologia forense, *Microcerella*, *Oxysarcodexia*, *Peckia*, *Sarcophaga*.

Introduction

Sarcophagidae Hagen, 1881 is widely distributed with about 3,100 described species in 400 genera. Although it has worldwide geographic distribution, Sarcophagidae richness is remarkably concentrated in regions of tropical and warm temperate climate (Shewell 1987; Pape 1996) and in

Neotropical region more than 800 species are found. There are three subfamilies, Miltogramminae, Paramacronychiinae and Sarcophaginae, but only Sarcophaginae has species of forensic and medical importance in the neotropics (Pape 1996).

The external morphology of most Sarcophaginae adults is extremely similar. Species share three gray black stripes pattern in the mesonotum, meron with bristles, undeveloped subscutellum, abdomen checkered or spotted and medium to large size, ranging from eight to 14 mm (Carvalho & Mello-Patiu 2008). Probably because of this morphological similarity and the lack of keys this group is considered of difficult identification (Barros *et al*. 2008; Mulieri *et al.* 2010; Vairo *et al*. 2011).

Fleshfly females are much more abundant than males on carcasses. They use the corpse not only as source of food and mating site but also as larviposition site. In forensic entomology, the species that rear on corpses are considered the most important data source. The biological data from these species is essential to estimate the minimum *post mortem* interval (PMI), which corresponds to the period of insect activity on corpse (Tomberlin *et al.* 2011). In addition their use in applied sciences, such as forensic entomology, females have their own place in Sarcophagidae systematics and can provide important characters for map the group evolution (Lopes 1941,Lopes 1957; Tibana & Mello-Patiu 1985; Mello-Patiu & Santos 2001) although females are still unknown in many species. However, despite their importance, Sarcophagidae females are usually neglected in taxonomic and applied research.

In southern Brazil, forensic entomology is well disseminated (Vairo *et al*. 2015; Correa *et al*. 2014) but there are no available keys for all necrophagous

fleshflies females, making this group underutilized in forensic cases. Mulieri et al. (2010) provided a key to male and female adults of Sarcophaginae from Buenos Aires Province including 39 species, that can be used partially to fauna from southern Brazil, but only four species herein analyzed were included among them. Nevertheless, a more detailed comparison of females of most species of forensic importance is essential to provide a greater number of characters and minimize the difficulties in the problematic task of female identification, especially by non-taxonomists, in medical, veterinary and forensic applications (Mulieri et al. 2010, Carvalho & Mello-Patiu 2008). Therefore, as a first step to filling this gap, we present a pictorial key for females of nine necrophagous species of Sarcophaginae from southern Brazil.

Material and Methods

All species chosen met two criteria: can be reared in organic matter, thus being necrophagous, and have their geographic range reaching Southern Brazil. Those species are: *Oxysarcodexia paulistanensis* (Mattos, 1919), *Microcerella halli* (Engel, 1931), *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830), *Peckia* (*Pattonella*) *resona* (Lopes, 1935), *Peckia* (*Pattonella*) *intermutans* (Walker, 1861), *Oxysarcodexia riograndensis* Lopes, 1946, *Peckia* (*Euboettcheria*) *australis* (Townsend, 1927), *Peckia* (*Euboettcheria*) *florencioi* (Prado & Fonseca, 1932) and *Sarcophaga* (*Bercaea*) *africa* (Wiedemann, 1824). The first five species have larvae already sampled on carcasses and/or human corpses (Salviano 1996; Moura *et al*. 1997; Moura *et al*. 1998; Carvalho & Linhares 2001; Moura *et al*. 2005; Oliveira & Vasconcelos 2010; Vairo *et al.* 2011) and the last four have adults sampled in Paraná, Santa Catarina and Rio Grande do Sul and reared in laboratory with putrefied bovine meat.

Although 22 species of fleshflies with potential forensic importance were already registered in Southern Brazil (Vairo *et al*. 2010), in this work we were interested in species that could be used to estimate the minimum post mortem interval i.e., not only species attracted by carrion, but those species in which the larvae are reared on carcasses or corpses

To start the colonies we collected specimens from Curitiba (Paraná), Campinas (São Paulo) and Bombas (Santa Catarina). Females were captured using a butterfly bait trap which allows the researcher to choose flies in the field. All females were reared individually in small cages until larviposition, thus producing an isolineage. The larvae were reared in putrefied bovine meat until the emergence of adults. After the emergence, males were identified based on Vairo *et al*. (2011), thus ensuring the correct identification of females. Colonies were established and maintained at the Universidade Federal do Paraná, Centro Politécnico, Curitiba, Paraná, Brazil, except the colony of *P. intermutans* established at the Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. The females were mounted and the abdomens removed and cleared in 10% potassium hydroxide, washed a few times in distilled water and immersed in 10% acetic acid. Photographs were taken with a Leica DFC 500 digital camera and Auto-Montage Pro Digital Imaging System (Syncropy), using a Leica MZ16 stereomicroscope. The illustrations were produced using drawing tube and edited with GIMP 2.8. We adopted the terminology of Shewell (1987) for general morphology and Lopes (1939) for “vaginal plate”. Synonymic information for each species is available in Pape (1996). Updated distribution data after Pape (1996) are also provided (Barros *et al*. 2008. Barbosa *et al*. 2009, Rosa *et al*. 2009, Souza *et al*. 2011, Vairo *et al*. 2011, Buenaventura &

Pape 2013, Vairo *et al*. 2014). Vouchers are deposited in Coleção Padre Jesus Santiago Moure, Universidade Federal do Paraná (DZUP) and Coleção Entomológica do Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ).

Results

The results are divided in descriptions of each species with illustrations and the identification key. Figure 1 is a general sketch of the female terminalia showing the main structures used in species identification.

Oxysarcodexia paulistanensis (Mattos, 1919)

(Figures 2, 11A and 12A)

Description – Differs from male in the following: Two proclinate orbital setae, the superior one with half length of the inferior; inner vertical setae differentiated from postocellar setae. Tergite 5 with a dorsolateral light golden spot. Tergite 6 divided, the median region connecting the two plates are sclerotized; spiracle 6 in membrane and 7 within the sclerites. 6-8 strong marginal setae accompanied by thin setae. Tergite 7 absent. Tergite 8 as two lateral bare plates, relatively pigmented, centrally extended and tapered at the top and bottom, joined by a membrane. Epiproct absent. Sternites 2-6 rectangular with rounded corners with strong setae in the posterior margin and weak setae in the median part; sternite 6 shorter and wider comparing to sternite 5; sternite 7 wider than 6 with 3 strong setae in each lateral and some setulae; sternites 6, 7 and 8 fused; sternite 8 broadly membranous with an small marginal sclerotized area with setulae. Vaginal plate present, well sclerotized, almost the same size as hipoproct, rectangular, with concave posterior margin and central area with a

depression. Spermatheca elongated and slightly oval with transversal striations in all extension.

Distribution: Argentina (Buenos Aires, Córdoba, Entre Ríos), Brazil (Distrito Federal, Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, São Paulo), Chile (Santiago).

Material examined: Eight females from colonies initiated by specimens collected in Brazil, Paraná, Curitiba, ii.2011. K. Vairo col.

Oxysarcodexia riograndensis (Lopes, 1946)

(Figures 3, 11B and 12B)

Description – Differs from male in the following: Two proclinate orbital setae, the superior one with similar size as frontals and the inferior one two times the size as the superior; inner vertical setae differentiated from the postocellar setae. Tergite 5 with a dorsolateral golden light spot. Tergite 6 undivided; spiracle 6 in membrane and spiracle 7 within the sclerite, with 6-9 strong marginal setae. Tergite 7 absent. Tergite 8 as two lateral sclerotized bare plates, two times the cercus size. Epiproct absent. Sternites 1-5 dark-brown, darker compared to the others; sternites 2 and 5 with square shape, posterior corners rounded, strong setae in the posterior margin and some setulae in the median part; sternite 5 shorter than 6; sternite 6, 7 and 8 fused; sternite 6 wider than 5 with one row of setae, 3 strong setae in each side and with many setulae in central part; sternite 7 almost 1.5 times the size of sternite 5, posterior margin concave, marginal setae being 3 strong lateral ones and other small weak setae; sternite 8 membranous with median area rounded and pigmented, margin with some

setulae. Vaginal plate sub-rectangular, posterior margin slightly concave. Spermathecae slightly elongated with transversal striations in all extension.

Distribution: Argentina (Jujuy), Brazil (Paraná, Rio de Janeiro, Rio Grande do Sul).

Material examined: Six females from colonies initiated by specimens collected in Brazil, Paraná, Curitiba, ii.2011. K. Vairo col.

Peckia (*Pattonella*) *intermutans* (Walker, 1861)

(Figures 4, 11G and 12C)

Description – Differs from male in the following: Two well-developed proclinate orbital setae; inner vertical setae differentiated from postocellar setae. Tergite 5 with one lateral golden spot and a light golden coloration at posterior margin in dorsal view. Tergite 6 divided in two big plates separated by a narrow membrane; spiracle 6 and 7 within the sclerite; 10-12 strong setae on posterior margin. Tergite 7 absent. Tergite 8 as two small bare plates, slightly larger than cercus. Epiproct absent. Sternites 2-5 square shaped with strong setae on posterior margin; sternites 6, 7 and 8 separated; sternite 6 square shaped, a bit smaller than sternite 5, with numerous strong marginal and premarginal setae; sternite 7 square with setae more concentrated on posterior margin, with a strong pair on each side; sternite 8 membranous, not well pigmented, about half of length of sternite 7, with 5 long setae. Vaginal plate membranous, slightly pigmented; anterior margin rounded and posterior margin with a median depression. Spermatheca elongated with a segmental constriction separating a narrower proximal part and a not striated distal part.

Distribution: Brazil (Amazonas, Ceará, Distrito Federal, Goiás, Mato Grosso, Minas Gerais, Pará, Rio de Janeiro, Paraná, Santa Catarina, São Paulo), Costa Rica, Ecuador, Guatemala, Guiana, Honduras, Mexico (Jalisco), Panama, Paraguay, Peru, St. Lúcia, Trinidad & Tobago (Tobago, Trinidad).

Material examined**:** Nine females from colonies initiated by specimens collected in Brazil, São Paulo, Mogi Guaçu, iv.2011. M. Grella col.

Peckia (*Pattonella*) *resona* (Lopes, 1935)

(Figures 5, 11C and 12D)

Description – Differs from male in the following: Two proclinate well developed orbital setae, both two times the size of frontal setae; inner vertical setae distinguish from the postocellar setae. Tergite 5 with an anterior silver spot in dorsal view. Tergite 6 divided in two big plates separated by a narrow membrane; spiracle 6 and 7 within the sclerite; 12 strong marginal setae concentrated in the median region. Tergite 7 absent. Tergite 8 as two small and narrow bare plates, a bit bigger than cercus. Epiproct absent. Sternites 2-6 squared shaped with strong and long setae on the posterior margin; sternites 6, 7 and 8 individualized; sternite 6 square, a bit smaller than sternite 5, with strong and long setae concentrated on the posterior third; sternite 7 with the half length of sternite 6, with long setae on the posterior half and strong posterior marginal setae; sternite 8 membranous; sparsely pigmented, with a similar length of sternite 7, with long and thin setae on posterior margin. Vaginal plate absent or probably completely membranous and not apparent.

Spermatheca elongated with a segmental constriction separating a narrower proximal part, and a rounded not striated distal part.

Distribution: Argentina (Corrientes), Brazil (Rio de Janeiro, Rio Grande do Sul, Santa Catarina, Paraná, Minas Gerais, São Paulo).

Material examined**:** Two females from colonies initiated by specimens collected in Brazil, Paraná, Curitiba, v.2012. K. Vairo col.

Peckia (*Euboettcheria*) *australis* (Townsend, 1927)

(Figures 6, 11H and 12E)

Description – Differs from male in the following: Two proclinate orbital setae well developed, superior with half of the length of inferior; inner vertical setae differentiated of postocellar setae. Tergite 5 with a light golden microtomentum. Tergite 6 divided in two plates connected by a broad membrane; spiracle 6 in membrane and spiracle 7 within the sclerite, near the margin; 15-17 strong and long marginal setae. Tergites 7 and 8 not absent. Epiproct entire, narrow, with numerous setae on median region. Sternites 2-5 squared shaped with strong marginal setae; sternites 6 separated, 7 and 8 fused; sternite 6 larger than 5, but shorter in length, with strong marginal setae; sternite 7 with a depressed central area, sternite 8 represented by a narrow posterior membranous area with setulae, separated of the sternite 7 by a semicircular, swollen, and setose area. Vaginal plate absent. Spermatheca spherical not striated.

Distribution: Argentina (Misiones), Brazil (Mato Grosso, Rio Grande do Sul, Santa Catarina, Paraná, São Paulo), Paraguay.

Material examined**:** Eight females from colonies initiated by specimens collected in Brazil, Paraná, Curitiba, vii. 2011. K. Vairo col.

Peckia (*Euboettcheria*) *florencioi* (Prado & Fonseca, 1932)

(Figures 7, 11D and 12F)

Description – Differs from male in the following: Two proclinate orbital setae well developed; inner vertical setae differentiated of postocellar setae. Tergite 5 with light golden microtomentum in dorsal view. Tergite 6 divided in two plates with a broad connecting membrane; spiracle 6 in membrane and spiracle 7 within the sclerite near the margin; 12-15 strong and long marginal setae. Tergites 7 and 8 not absent. Epiproct entire, short, median region depigmented, with strong and long setae. Sternites 6, 7 and 8 fused; sternite 7 with the same width as sternite 6, anteriorly rounded, without setae; sternite 8 narrower than sternite 7, posterior margin slightly swollen with sparse setulae. Vaginal plate present, well-sclerotized, with a digitiform discal apophysis projecting inwards. Spermatheca spherical not striated, with a postero-ventral unsclerotized area.

Distribution: Argentina (Misiones, San Luis), Brazil (Mato Grosso, Rio Grande do Sul, Santa Catarina, Paraná, São Paulo).

Material examined**:** Eight females from colonies initiated by specimens collected in Brazil, Paraná, Curitiba, vi.2012. K. Vairo col.

Peckia (*Sarcodexia*) *lambens* (Wiedemann, 1830)

(Figures 8, 11E and 12G)

Description – Differs from male in the following: Posterior femur without a patch of black short setae in the apical third of the anterior surface (male femoral organ). Tergite 5 with golden microtomentum in lateral and dorsal view. Tergite 6 undivided; spiracle 6 in membrane and 7 within the sclerite; 14-16 marginal strong setae accompanied by some setulae. Tergites 7 and 8 absent. Epiproct entire, with some fine setulae along the margin and one conspicuous strong setae in each side. Hipoproct broad with a conspicuous hollow at the medium part. Sternite 2 with 1.5 times the size of sternites 3 and 4; sternite 5 subrectangular with rounded corners and several developed setae; sternite 6 two times the sternite 5 width, with strong marginal setae and sparse discal setulae; sternites 7 and 8 narrower than sternite 6, both linked to the sternite 6 by a lateral conspicuous membranes; sternite 7 with no setae and sternite 8 broadly membranous, represented by a swollen and setulose marginal area. Vaginal plate absent. Spermatheca circular not striated with a postero-ventral unsclerotized area.

Distribution: Argentina (Misiones, Tucumán), Bahamas (Grand Bahamas, New Providence), Bolivia, Brazil (Amazonas, Ceará, Mato Grosso, Rio de Janeiro, Santa Catarina, São Paulo, Paraná), Chile (Tarapacá), Colombia, Costa Rica, Cuba, El Salvador, Guyana, Haiti, Jamaica, Mexico (Jalisco, Nuevo Leon, Tamaulipas), Panamá, Paraguay, Peru, Puerto Rico, St. Vincent, Trinidad & Tobago (Tobago).

Material examined**:** Seven females from colonies initiated by specimens collected in Brazil, Paraná, Curitiba, iv.2011. K. Vairo col.

Microcerella halli (Engel, 1931)

(Figures 9, 11F and 12H)

Description – Differs from male in the following: Two proclinate orbital setae well developed; no row of small and strong setae on anteroventral part of trochanter 3; tibia 2 with tree anterior setae and presence of a reddish sensorial area on posterior part of femur. Tergite 5 black with silver microtomentum. Tergite 6 undivided; reddish brown to orange, contrasting with the dark tergite 5; spiracle 6 in membrane and spiracle 7 within the sclerite; 20-24 strong marginal setae accompanied of small ones. Tergite 7, tergite 8 and epiproct absent. Sternites 1-5 reddish brown, darker than the others; sternites 2-6 squared shaped with a row of strong setae on posterior margin; sternites 6, 7 and 8 fused; sternite 6 wider and shorter than the sternite 5; sternite 7 quadrangular; central surface sligthly depressed relative to the posterior margin, without setae; sternite 8 swollen, widely membranous except for the sclerotized posterior margin, posterior angles expanded with 3 apical setae each. Vaginal plate absent or probably completely membranous and not apparent. Spermatheca divided in two parts by a constriction, a narrow and cylindrical proximal part and a rounded distal one, less striated than the proximal and 2.0 times its width.

Distribution: Argentina (no further data), Bolivia, Brazil (Ceará, Minas Gerais, São Paulo, Paraná, Rio Grande do Sul).

Material examined**:** Ten females from colonies initiated by specimens collected in Brazil, Paraná, Curitiba, vi.2011. K. Vairo col.

Sarcophaga (*Bercaea*) *africa* (Wiedemann, 1824)

(Figures 10, 11I and 12I)

Description – Differs from male in the following: Two proclinate orbital setae well developed; inner vertical setae differentiated of postocellar setae. Tergite 5 with golden microtomentum more conspicuous in lateral view. Tergite 6 divided in two plates well separated and dorsally folded; spiracle 6 in membrane and spiracle 7 within the sclerite; 15-16 strong and long marginal setae. Tergites 7 and 8 absent. Epiproct represented by two small dorsal plates without setae. Sternites 2-4 squared shaped with posterior margin rounded; two strong setae in each angle of posterior margin; sternite 5 quadrangular with strong marginal angular setae. Sternites 6, 7 and 8 fused; Sternite 6 almost two times wider than sternite 5, with a medially interrupted row of setae on posterior margin; sternite 7 with a noticeably elevated central area; sternite 8 like a narrow and swollen range fused with the posterior margin of sternite 7, with two lateral groups of setae, two strongest setae and many setulae. Vaginal plate well sclerotized, darker than the sternites, and very long, from the hipoproct to the middle of sternite 6 with a median suture. Spermatheca oval and slightly elongated with transversal striations in all surface.

Distribution: Argentina (Buenos Aires), Brazil (Rio de Janeiro, Paraná, Rio Grande do Sul), Costa Rica, Cuba, Mexico, Paraguay.

Material examined**:** Ten females from colonies initiated by specimens collected in Brazil, Paraná, Curitiba, viii.2012. K. Vairo col.

Identification key for flesh flies females with forensic importance in Southern Brazil

1. Tergite 6 undivided 2

1'. Tergite 6 divided in two plates 4

2. Mid tibia with long median anterior seta that extends beyond the apex of tibia; spermatheca rounded; epiproct present …….. ..... *Peckia* (*Sarcodexia*) *lambens*

2'. Mid tibia without a long median anterior seta that extends beyond the apex of tibia; spermatheca not rounded, with a different shape as above; epiproct absent ……..... 3

3. Tergite 8 well sclerotized, vaginal plate conspicuous ..... *Oxysarcodexia riograndensis*

3'. Tergite 8 absent, vaginal plate absent or completely membranous ..... *Microcerella halli*

4. Tergite 6 as two separated plates dorsally folded ….……..... *Sarcophaga* (*Bercaea*) *africa*

4'. Tergite 6 as two plates separated by a membrane or by a sclerotized area, not folded dorsally 5

5. Vaginal plate well sclerotized, almost the same size as hipoproct, rectangular, with concave posterior margin and central area with a depression; tergite 6 as two plates separated by a sclerotized area ..... *Oxysarcodexia paulistanensis*

5' Vaginal plate absent or, if present, not as described above; tergite 6 as two plates separated by a membrane 6

6. Spermatheca spherical, without striations and segmental constrictions; tergite 8 absent..... 7

6’. Spermatheca with segmental constrictions, divided in proximal and distal part; tergite 8 present 8

7. Vaginal plate absent ..... *Peckia* (*Euboettcheria*) *australis*

7’. Vaginal plate present, with a median finger-like projection ..... *Peckia* (*Euboettcheria*) *florencioi*

8. Tergite 8 wider than long; tergite 5 with two lateral golden spots ..... *Peckia* (*Pattonella*) *intermutans*

8’. Tergite 8 longer than wide; tergite 5 with no lateral golden spots ..... *Peckia* (*Pattonella*) *resona*

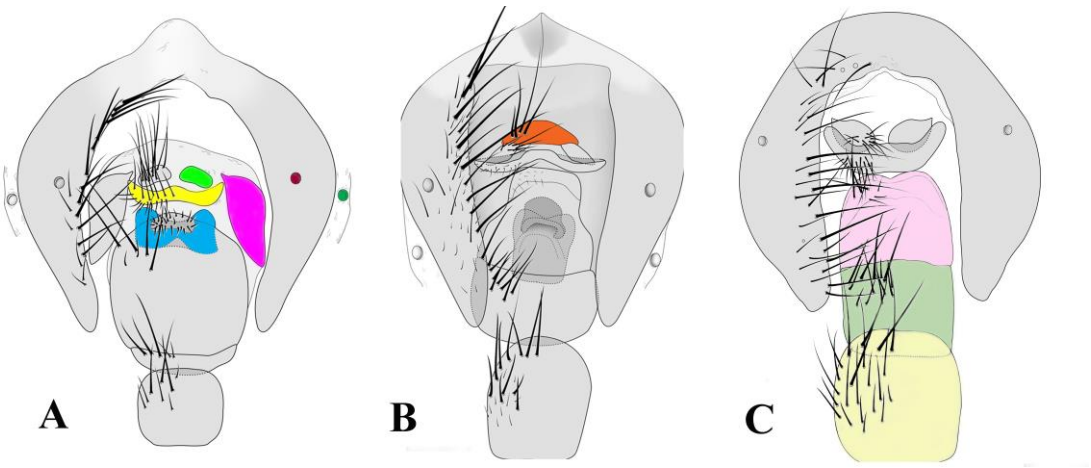

Figure 1. General morphology of female terminalia. A- *Oxysarcodexia paulistanensis* (pink = tergite 8; green= cercu; yellow = hypoproct; blue = vaginal plate; dark red = spiracle 6; dark green = spiracle 7. B- *Peckia* (*Euboettcheria*) *florencioi* (orange= epiproct). C- *Microcerella halli* (light yellow= sternite 5; light green= sternite 6; light pink= sternites 7+8).

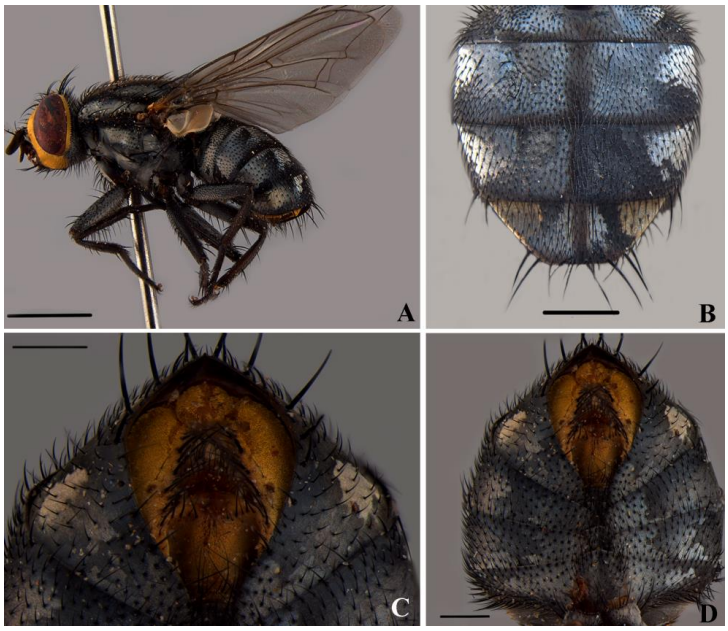

Figure 2. External female morphology of *Oxysarcodexia paulistanensis*. A- *habitus*, lateral view; scale: 2mm; B- abdomen, dorsal view; scale:1mm; C- abdominal terminal segments, ventral view; scale:0,5mm; D- abdomen, ventral view; scale: 1mm.

Figure 3. External female morphology of *Oxysarcodexia riograndensis*. A- *habitus*, lateral view; scale: 2mm B- abdomen, dorsal view; scale:1mm; C- abdominal terminal segments, ventral view; scale:0,5mm; D- abdomen, ventral view; scale:1mm.

Figure 4. External female morphology of *Peckia* (*Pattonella*) *intermutans*. A- *habitus*, lateral view; scale:1mm; B- abdomen, dorsal view; scale: 2mm; C- abdominal terminal segments, ventral view; scale:1mm; D- abdomen, ventral view; scale: 2mm.

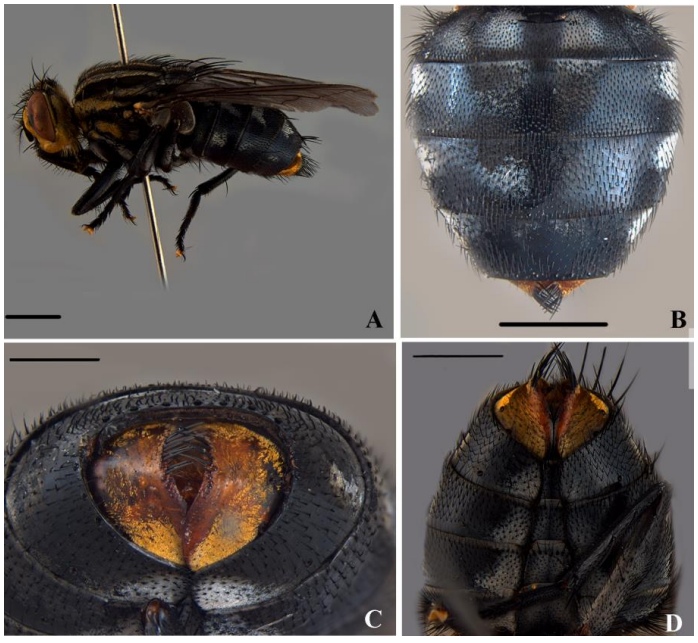

Figure 5. External female morphology of *Peckia* (*Pattonella*) *resona*. A- *habitus*, lateral view; scale: 2 mm; B- abdomen, dorsal view; scale: 2 mm C- abdominal terminal segments, ventral view; scale: 1 mm; D- abdomen, ventral view; scale: 2 mm.

Figure 6. External female morphology of *Peckia* (*Euboettcheria*) *australis*. A- *habitus*, lateral view; scales: 2 mm; B- abdomen, dorsal view; scale: 2 mm C- abdominal terminal segments, ventral view; scale: 0.5 mm; D- abdomen, ventral view; scale: 1 mm.

Figure 7. External female morphology of *Peckia* (*Euboettcheria*) *florencioi*. A- *habitus*, lateral view; scale: 2 mm; B- abdomen, dorsal view; scale: 1 mm C- abdominal terminal segments, ventral view; scale: 0.5 mm D- abdomen, ventral view, scale: 1 mm.

Figure 8. External female morphology of *Peckia* (*Sarcodexia*) *lambens*. A- *habitus*, lateral view; scale: 2 mm; B- abdomen, dorsal view; scale: 1 mm; C- abdominal terminal segments, ventral view; scale: 0.5 mm; D- abdomen, ventral view; scale: 1 mm.

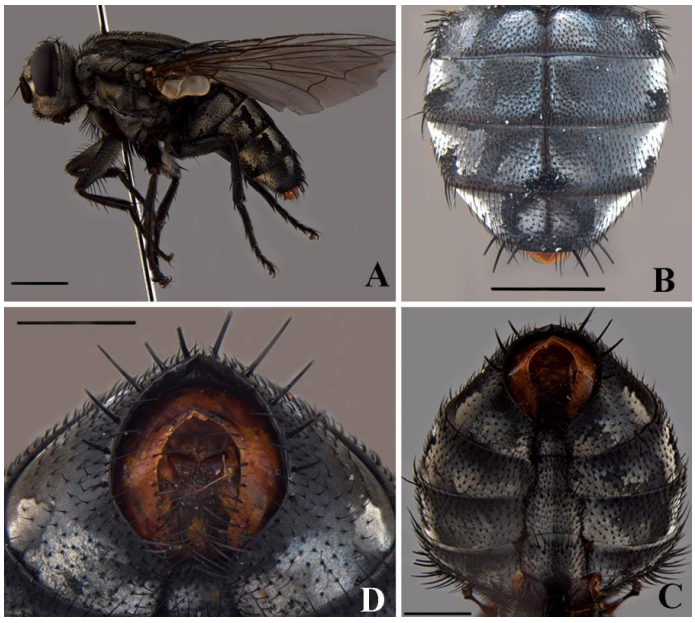

Figure 9. External female morphology of *Microcerella halli*. A- *habitus*, lateral view; scale: 2 mm; B- abdomen, dorsal view; scale: 2 mm; C- abdominal terminal segments, ventral view; scale: 1 mm; D- abdomen, ventral view; scale: 1 mm.

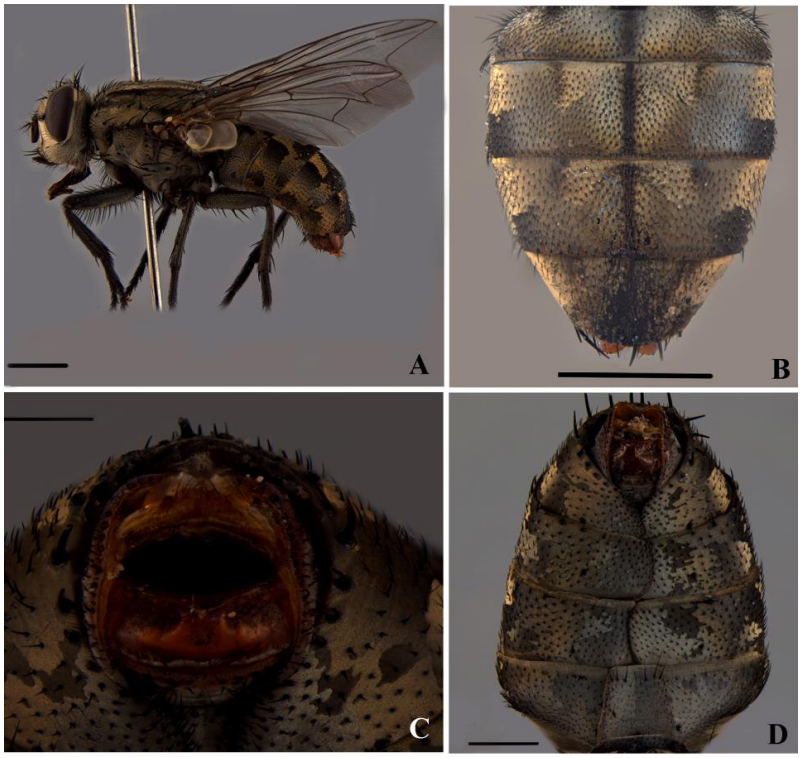

Figure 10. External female morphology of *Sarcophaga* (*Bercaea*) africa. A- *habitus*, lateral view; sacle: 2 mm; B- abdomen, dorsal view; scale: 2 mm; C- abdominal terminal segments, ventral view; scale: 0.5 mm; D- abdomen, ventral view; scale: 1 mm.

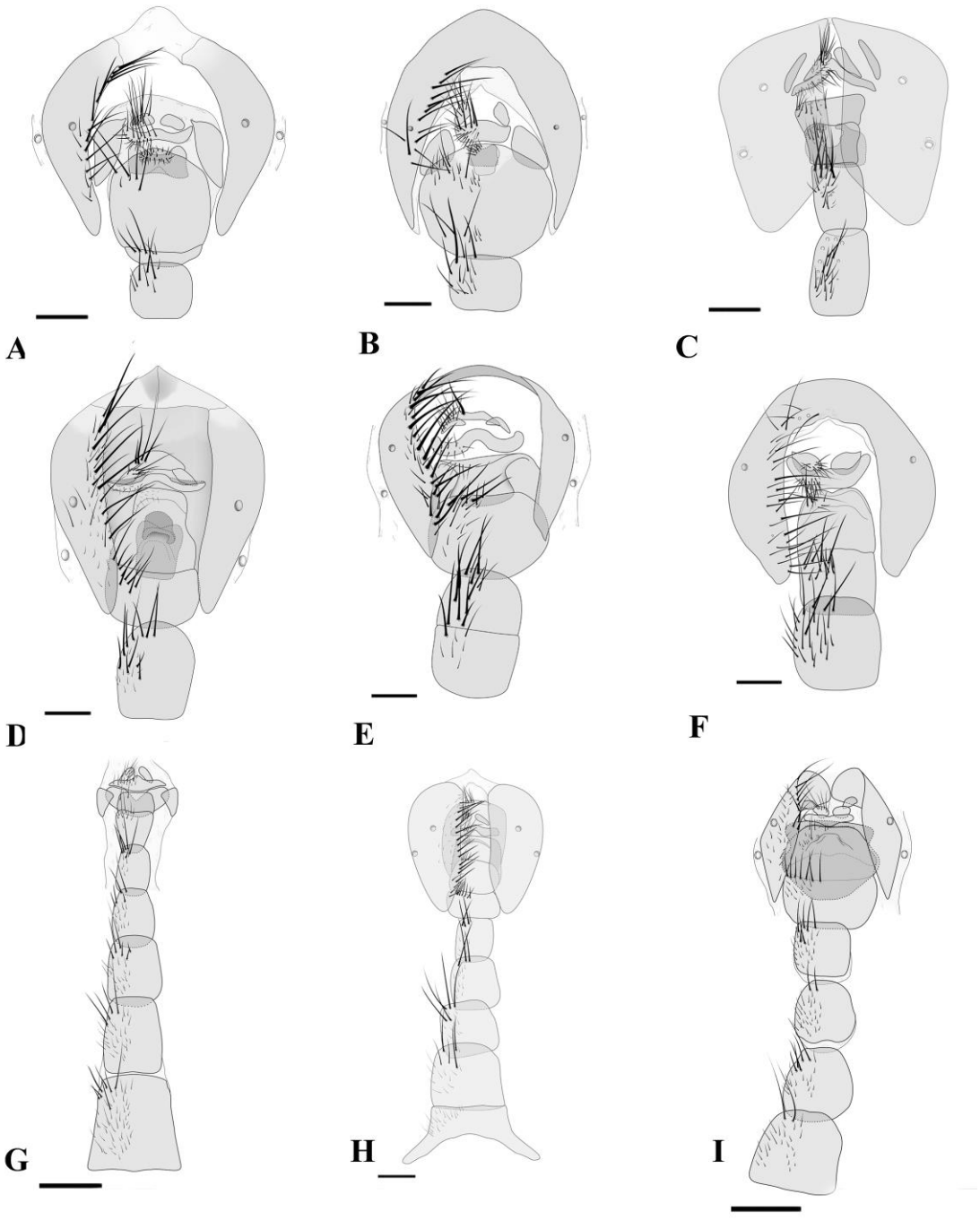

Figure 11. Female terminalia. A- *Oxysarcodexia paulistanensis* (sternites 1-4 ommited); B- *Oxysarcodexia riograndensis* (sternites 1-4 ommited); C- *Peckia* (*Pattonella*) *resona* (sternites 1-4 ommited); D- *Peckia* (*Euboettcheria) florencioi* (sternites 1-4 ommited); E- *Peckia* (*Sarcodexia*) *lambens* (sternites 1-4 ommited); F- *Microcerella halli* (sternites 1-4 ommited); G: *Peckia* (*Pattonella*) *intermutans* (Tergite 6 and sternite 1 ommited); H- *Peckia* (*Euboettcheria*) *australis*; I- *Sarcophaga* (*Bercaea*) *africa* (sternite 1 ommited). Scales: 1 mm.

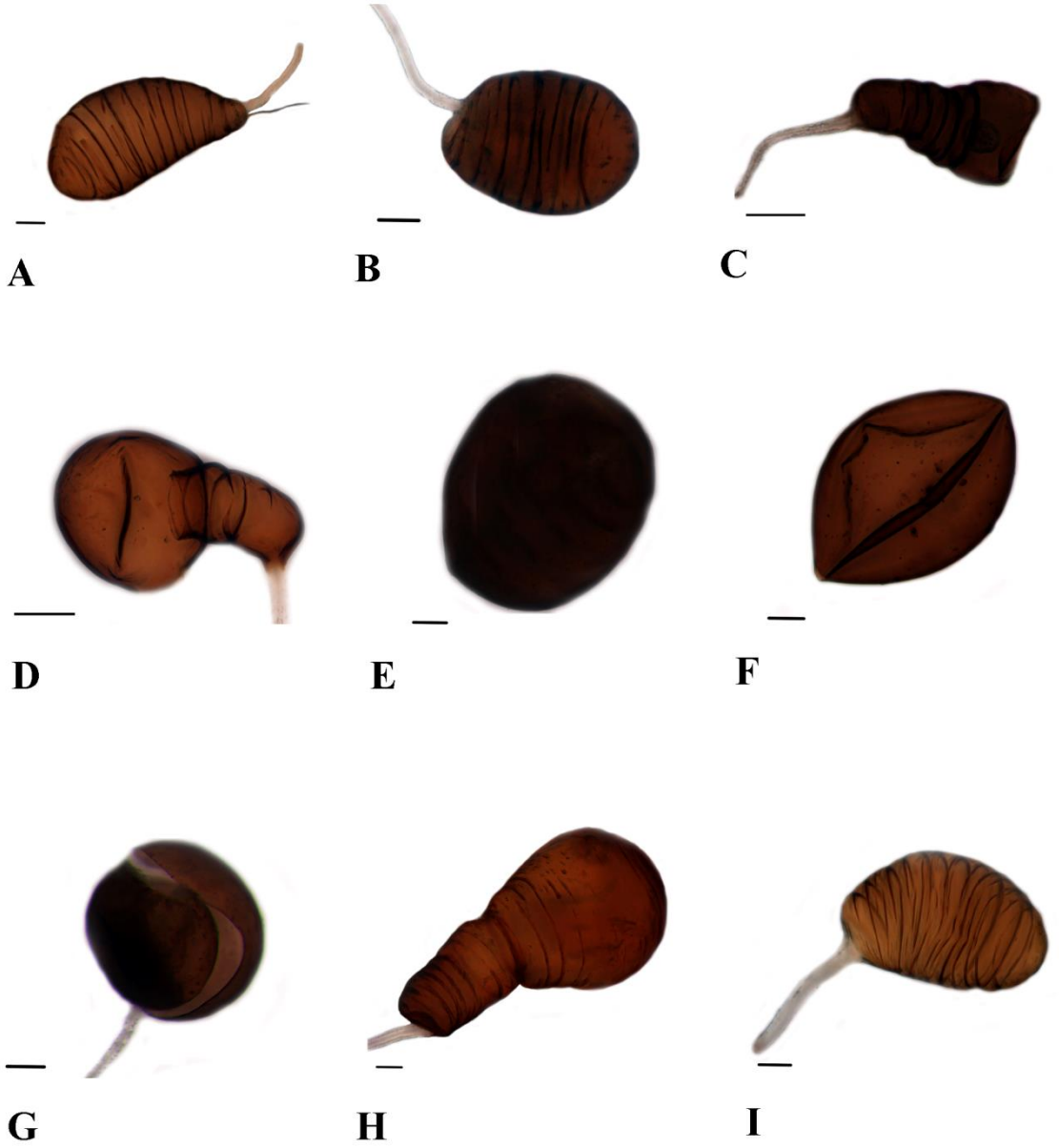

Figure 12. Spermathecae. A- *Oxysarcodexia paulistanensis*, lateral view; scale: 0,05 mm; B- *Oxysarcodexia riograndensis,* lateral view; scale: 0.05 mm; C- *Peckia* (*Pattonella*) intermutans, lateral view; scale: 0.05 mm; D- *Peckia* (*Pattonella*) *resona*, lateral view; scale: 0.05 mm; E- *Peckia* (*Euboettcheria*) *australis*, lateral view; scale: 0.05 mm; F- *Peckia* (*Euboettcheria)* florencioi, ventral view; scale: 0,05 mm; G- *Peckia* (*Sarcodexia*) *lambens*, ventral view; scale: 0.05 mm; H- *Microcerella halli*, lateral view; scale: 0.1 mm; I- *Sarcophaga* (*Bercaea*) *africa*, lateral view; scale: 0.05 mm.

Discussion

Undoubtedly the main female diagnostic characters are in terminalia. However, in a forensic context, where in the most part of the cases fresh material is collected in death scene, the external color could help to identify some species. Color of the gena, postgena, and of the spots in tergites and sternites, for instance, can be very effective in identifying some species, like *Sarcophaga* (*Bercaea*) *africa* and *Microcerella halli.*

On the other hand, other external characters may also be useful, like the presence of long setae in tibia and the size of orbital and postocellar setae. In some cases, these external characters are the mainly differences between males and females of some species, requiring attention in the identification because of this dimorphism.

The characters from the terminalia, such as the microtomentum of tergite 5, can distinguish species even in the same subgenera, as showed in *Pattonella*. The tergite 6 could be divided or undivided. We considered as divided the tergite that has even a narrow or large, sclerotized or not membrane connecting the two plates, as occurs in *Oxysarcodexia paulistanensis*. *Oxysarcodexia* has the three already described states of tergite 6, entire, divided and membranous (Tibana & Mello-Patiu 1985). An undivided tergite 6, but with different degrees of reduction, also occurs in *Nephochaetopteryx* (Mello-Patiu & Santos 2001). In this work, species with tergite 6 undivided were *Oxysarcodexia riograndensis*, *Peckia* (*Sarcodexia*) *lambens,* and *Microcerella halli*.

Concerning the spiracles 6 and 7, all studied species, except those of *Peckia* subgenus *Pattonella*, have the spiracle 6 inside the membrane and the spiracle 7 within the sclerite (tergite 6).

Shewell (1987) considered that the tergite 7 in Sarcophagidae is frequently absent and the tergite 8 is nearly always present, but usually reduced to bare lateral plates. In this work, we used the same interpretation and named as tergite 8 the bare plates in lateral position to the sternite 7 and 8. This sclerite was visible only in *Oxysarcodexia* and in *Peckia* (*Pattonella*) and its presence and shape showed to be an important character to discriminate some females of forensic species in Southern Brazil.

The epiproct, if present, can appear divided and undivided (Camargo 2014). In this work only *Peckia* (*Sarcodexia*) *lambens*, *Peckia* (*Euboettcheria*) *australis, Peckia* (*E*.) *florencioi,* and *Sarcophaga* (*B*.) *africa* have epiproct, entire in the first three species and divided in the last one. The undivided epiproct seems to be the most common state of this character in *Peckia,* but this condiction differs in some species of the same genera or subgenera such as *P.* (*E*.) *collusor* and *P.* (*E*.) *epimelia* (Camargo 2014).

Although the shape of sternites can be a useful character in a general context, an important information to distinguish sarcophagid females comes from the presence (or absence) of fusion of the sternites 6, 7 and 8. These sternites may be considered fused when the posterior margin of preceding sternite is contiguous, main laterally, with the anterior margin of the subsequent one, without a well-marked suture. In *Oxysarcodexia paulistanensis*, *O*. *riograndensis,* and *Sarcophaga (Bercaea) africa* these sternites are fused; in P. (*E.*) *australis*, *P*. (*E*.) *florencioi* and *M*. *halli* only sternites 7 and 8 are fused; and

in *P.* (*P.*) *resona*, *P*. (*P.*) *intermutans* and *P*. (*S*.) *lambens* all sternites are individualized.

Another key character to identify females of these fleshfly species is the presence and shape of vaginal plate. For some genera like *Oxysarcodexia* and *Nephochaetopteryx* the vaginal plate is one of the most important characters to segregate species because it has conspicuous interspecific differences (Tibana & Mello-Patiu 1983; Mello-Patiu & Santos 2001). As previously stated, we found that the shape of vaginal plate is a major character to properly identify *O. paulistanensis*, *O. riograndensis*, *Peckia (E.) florencioi*, *Peckia (P.) resona* and *Sarcophaga (B.) africa*.

The morphology of the spermathecae also can help the differentiation of genera and subgenera. In *Oxysarcodexia* the shape is more elongate (pyriform) and the striations are conspicuous, similar as in *Sarcophaga*. In *P.* (*S*.) *lambens* and *P*. (*E*.) *florencioi* while also rounded it has an opening in ventral view, a characteristic that we are describing for the first time. In *Peckia* (*Pattonella)* and *Microcerella*, the spermathecae are quite different, as it is divided in well defined distal and proximal portions, possessing some constrictions along.

Although Sarcophagidae, in general, and its females, in particular, are considered hard to identify, the key and the descriptions provided makes this task possible to both, taxonomists and non-taxonomists. So, we expected that forensic entomologists could identify the necrophagous fleshfly females in Southern Brazil in a short time and with low cost, broadening the number of species that can be used in crime scene investigations.

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Capítulo II

Imaturos de Sarcophagidae (Diptera) de importância forense

Larvas de Sarcophagidae e a importância da uniformização da terminologia para o estudo de imaturos

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Resumo

Sarcophagidae é um grupo biologicamente diverso que agrupa espécies com diferentes hábitos: coprófagas, necrófagas, predadoras, parasitas e causadoras de miíases em vertebrados. No entanto, estudos com larvas de Sarcophagidae são negligenciados devido à dificuldade na identificação e criação dos espécimes imaturos até a fase adulta. Essa falta de informação acarreta o entrave de pesquisas aplicadas com Sarcophagidae e que são dependentes de um estudo morfológico detalhado prévio. Além disso, a caracterização morfológica de larvas fornece caracteres úteis na delimitação das unidades evolutivas no grupo e de suas relações. Sendo assim, o objetivo desse trabalho foi compilar e discutir informações referentes aos imaturos de Sarcophagidae, revisar a terminologia propondo mudanças e analisar a morfologia de larvas de forma comparada entre as subfamílias.

Palavras-chave: Estágios imaturos. Esqueleto cefálico. Terminologia. Classificação. Identificação.

Abstract

Sarcophagidae is a biologically diverse group comprising species with different habits: coprophagous, necrophagous, predators, parasites and myiasis-inducers in vertebrates. Studies involving Sarcophagidae larvae are often neglected due to difficulties in identification and breeding of immature specimens until the adult stage. This lack of information hinders applied research dependent on a prior detailed morphological study. Moreover, larval morphology has proved important in the classification of this group. The goals of this study were to compile and discuss information regarding the immature stages of Sarcophagidae, to review the terminology suggesting some changes and to compare the larval morphology across the subfamilies.

Key-words: Immature stages. Cephalic skeleton. Terminology. Classification. Identification.

Introdução

Sarcophagidae Hagen, 1881 possui 2510 espécies e aproximadamente 400 gêneros (Mcalpine *et al*.,1987). Embora Sarcophagidae seja distribuída mundialmente, a diversidade é notavelmente concentrada nas regiões de clima tropical e temperado quente (Pape, 1996). A maioria dos Sarcophagidae adultos tem tamanho de médio a grande (8-14 mm) e são, geralmente, acinzentados com três listras pretas no mesonoto, possuem cerdas no mero, subescutelo pouco desenvolvido e abdômen com pontuações ou manchas (Carvalho & Mello-patiu, 2008). A família é dividida em três subfamílias: Miltogramminae, Paramacronychiinae e Sarcophaginae. Os Miltogramminae são geralmente pequenos, acinzentados ou amarelos e as larvas são frequentemente cleptoparasitas em ninhos de himenópteros solitários (Szpila, 2010). Esse grupo é mais diversificado em climas secos como na África e Ásia Central, e são raramente encontrados na região Neotropical (Pape, 1996). Paramacronychiinae é uma subfamília pequena que apresenta maior diversidade nas áreas temperadas do hemisfério norte, incluindo os parasitas e espécies que produzem miíases em vertebrados. Sarcophaginae é a que

possui maior riqueza com maior diversidade na região Neotropical, alocando as espécies de interesse forense e de importância médica (Pape, 1996). Sarcophaginae é monofilética e provavelmente grupo irmão de Paramachronychiinae (Pape, 1992; Giroux *et al*., 2010). Os gêneros de Miltogramminae e Paramacronychiinae são, na sua maioria, monofiléticos (Pape, 1996).

As fêmeas de Sarcophagidae são, geralmente, vivíparas ou ovovivíparas (Mcalpine *et al.* 1987) com desenvolvimento holometábolo. O ciclo de vida é composto de larva de primeiro, segundo e terceiro instar, pupa e adulto sendo estas fases diferenciadas pelo número de fendas no espiráculo, peritrema, tamanho e morfologia dos instares (Gullan & Crunston, 2008). As larvas de Sarcophagidae podem ser distinguidas das demais famílias pelo esqueleto cefálico bem esclerotinizado e pela posição do espiráculo posterior localizado em uma cavidade (Lopes, 1943).

Entre as famílias de dípteros muscoides sinantrópicos, Sarcophagidae é a que possui a menor quantidade de trabalhos de biologia e ecologia, provavelmente devido à dificuldade na identificação das espécies. Nos adultos, os caracteres externos, em sua maioria, não são suficientes para diferenciar as espécies, sendo necessário então, um estudo aprofundado das terminálias masculina e feminina. Nos estágios imaturos, essa dificuldade também ocorre, já que somente um estudo apurado dos caracteres externos e do esqueleto cefálico mostra diferenças interespecíficas. Trabalhos visando à identificação de adultos desta família e principalmente com a subfamília Sarcophaginae são incipientes na literatura e os poucos trabalhos que abordam morfologia usualmente são aplicados à entomologia forense (Carvalho & Mello-Patiu, 2008; Vairo *et al.*, 2011). Estudos morfológicos de larvas desta família são ainda mais escassos, não existindo chaves de identificação de imaturos para a fauna brasileira.

Buscar novas formas de identificar os espécimes desta família tem se mostrado útil, como a utilização da microscopia eletrônica ou biologia molecular como ferramenta para identificação desses imaturos ou para descrições taxonômicas (Leite & Lopes, 1987; Guo *et al*,. 2011) fazendo com que seja possível a identificação a nível específico.

Os estudos morfológicos em Sarcophagidae tem se concentrado na subfamília Miltogramminae, com um viés para o uso dos caracteres em análises filogenéticas (Szpila & Pape, 2007; 2008). No entanto, existem também descrições de espécies de interesse forense, mas que, como não são feitas em um contexto comparativo, não fornecem caracteres úteis para a identificação.

A determinação da espécie tem implicações importantes em áreas aplicadas, como por exemplo, a entomologia forense e médica. Na entomologia forense, o primeiro passo na análise de uma evidência entomológica é a correta identificação da espécie. Se houver erro nessa etapa do trabalho, as informações geradas através dos vestígios entomológicos serão incorretas ocasionando conclusões falsas. De forma similar, na entomologia médica onde os sarcofagídeos podem ser vetores de doenças e causadores de miíases em humanos e animais (Burgess & Spraggs, 1992; Bermúdez *et al.*, 2010; Ahmad *et al*., 2011; Gaglio *et al*., 2011), a falha na identificação da espécie leva o especialista a compreender de maneira incorreta os mecanismos de doença através da biologia do inseto.

É notável que a falta de estudos morfológicos de imaturos e de ferramentas que possam auxiliar na identificação de grupos complexos como Sarcophagidae acarretam um entrave nas ciências aplicadas. Isso ocorre porque a ausência de conhecimento da diversidade e problemas na identificação a nível específico compromete a elaboração de trabalhos aplicados. Sendo assim, os objetivos desse trabalho foram: a) determinar qual o estado atual do conhecimento sobre os estágios imaturos de Sarcophagidae incluindo aspectos da taxonomia e sistemática; b) demonstrar a importância do grupo como modelo biológico para estudos aplicados e c) revisar as terminologias mais utilizadas nas descrições de Sarcophagidae e sugerir uma uniformização. Quando a tradução do inglês para o português não indica o sentido correto do nome, esse foi mantido em sua forma original, porém, entre aspas.

Resultados e Discussão

Terminologias e principais caracteres para a identificação interespecífica

Diversas terminologias foram propostas ao longo dos anos para estágios imaturos de dípteros muscóides. As mais utilizadas para descrição da morfologia interna e externa de larvas estão nos estudos de Townsend (1935 a,b), Teskey (1981), Lopes (1943), Ferrar (1987) e Courtney *et al.* (2000). Dentre esses, o único que aborda exclusivamente a família Sarcophagidae é Lopes (1943). Analisando as chaves de identificação de estágios imaturos para gêneros de Sarcophagidae e descrições da família, os caracteres mais importantes na diferenciação de espécies são: disposição das aberturas espiraculares do espiráculo anterior, tamanho do esqueleto cefálico, morfologia dos escleritos do esqueleto cefálico, tamanho dos espiráculos posteriores, abertura do peritrema, tubérculos anais, morfologia do corno dorsal e ventral, tamanho dos tubérculos do segmento anal, tamanho do arco clipeal, presença e morfologia dos espinhos dos segmentos torácicos e abdominais (Lopes, 1943; Kano & Sato, 1951; Szpila, 2010). Logo, a análise se restringirá as terminologias mais comumente empregadas em relação a esses caracteres larvais, considerados mais informativos.

A terminologia proposta por Townsend (1935a) é complexa e não apresenta ilustrações, sendo de difícil compreensão. Porém, Lopes (1943) baseou-se nessa proposta para seu estudo aprofundado e ilustrado sobre o esqueleto cefálico de larvas de primeiro, segundo e terceiro instar de Sarcophagidae, e por isso, elas serão abordadas conjuntamente nesse trabalho. Quanto ao esqueleto cefálico, Townsend (1935a) considera-o dividido em três partes: labial, hipostomal e faringeal. O setor labial é formado pelos escleritos labial, dentado, supralabial e oral. O setor hipostomal é constituído pelos escleritos hipostomal, infra-hipostomal, sub-hipostomal, supra-hipostomal e mandibular. E, finalmente, o setor faringeal divide-se em faringeal, dorso-faringeal e infra-faringeal. Já Lopes (1943) acrescenta a presença de um anel incompleto situado ventralmente no início do tubo digestivo, logo após a língua.

O sub-hipostomal está situado entre os ramos do esclerito infra-hipostomal. O dentado pode estar incorporado ao labial ou separado por zona de menor pigmentação nas larvas de primeiro instar e separado do labial nas larvas de segundo e terceiro instar. O supra-labial é quase sempre incorporado ao labial. O hipostomal se encontra sempre soldado ao faringeal constituindo-se algumas vezes como longa e estreita barra separada do sub-hipostomal (Fig.1). Em um trabalho posterior, Lopes (1982) sugere ainda, a presença do arco clipeal, que é a parte anterior do esclerito dorso-faringeal.

Quanto à morfologia larval externa, Townsend (1935b) a denomina de exoesqueleto e a divide em: integumento, espiráculos e órgãos sensoriais. O integumento é dividido em 12 segmentos, sendo o primeiro o segmento oral ou pseudocéfalo. Os três segmentos subsequentes representam o tórax e os últimos oito o abdômen. O 12^{o} segmento é a união de três, porém em Muscoidea aparece reduzido a um único segmento. Assim, Towsend (**op. cit**) considera que o espiráculo posterior pertence ao 13^{o} segmento abdominal da larva e o segmento anal ao 14^{o} segmento. O integumento pode ser variável entre os dípteros muscóides, com tubérculos, projeções, coloração diferente, presença de espinhos e de filamentos, todos esses caracteres podem ser importantes na caracterização específica. O espiráculo anterior não é funcional no primeiro instar podendo ser visto somente abaixo do tegumento, caracterizando uma respiração do tipo metapnêustica. Já no segundo e terceiro instares, os espiráculos ocorrem externamente e lateralmente em forma de "leque", caracterizando uma respiração anfipnêustica. Os espiráculos anteriores não diferem muito na forma, mas no número de papilas, podendo apresentar uma variação intraespecífica grande (Perez-Moreno *et al.*, 2006). O espiráculo posterior possui duas placas anais quitinosas contendo estruturas denominadas estigmas anais. Cada estigma possui um número de fendas, aberturas ou fissuras, variando em número e forma dependendo do estágio de desenvolvimento e da espécie. O anel quitinoso que circunda as placas é chamado peritrema podendo-se observar internamente os processos dendríticos por onde o ar entra para o átrio espiracular. O estigma anal possui uma grande variedade de formas, dentre elas: colunar, reticulada, eruciforme e vermiforme (Townsend, 1935b).

Em relação aos órgãos sensoriais, existem estruturas sensoriais externas, como dois pares de tubérculos cônicos localizados na face antero-dorsal dos lobos orais do pseudocéfalo com função ótica, denominados de tubérculos ópticos. Além deles, ocorrem papilas com função tátil. As cerdas com função sensorial podem estar presentes ou ausentes e ainda podem existir outras estruturas, circulares, encontradas na parte ventral dos segmentos torácicos. De maneira geral, existem outros órgãos sensoriais que podem diferir de grupo para grupo sendo esses com função olfatória, acessórias da antena, óticas, gustatória e táteis (sensibilidade à pressão atmosférica e umidade) (Townsend, 1935b).

Para Teskey (1981), os Muscomorpha apresentam um segmento cefálico ou pseudocéfalo compreendendo as antenas e palpos maxilares e internamente um esqueleto cefalofaringeano. O pseudocéfalo é bilobado anteriormente e possui uma antena e um palpo maxilar no ápice de cada lobo. Nota-se também a presença de cristas orais que provavelmente tem função similar a pseudotraquéia nos adultos, ou seja, direcionar os fluidos alimentares para o átrio. A disposição dessas cristas orais pode ser um caráter importante na diferenciação das espécies. O esqueleto cefalofaringeano é dividido em três partes, como em Lopes (1943), mas com nomes diferentes: esclerito tentofaringeal, esclerito hipofaringeal e mandíbulas. O esclerito tentofaringeal consiste de um par de escleritos em forma de “U” denominados de corno dorsal e corno ventral (Teskey, 1981). Em Muscomorpha, o corno ventral aparece expandido para fornecer sustentação para os músculos mandibulares e labiais. Esses escleritos podem ser ligados anterodorsalmente por uma ponte dorsal (Teskey, 1981). A morfologia da ponte dorsal pode ser um caráter importante para a identificação específica e foi considerada por Lopes (1982) como arco clipeal. Segundo Teskey (1981), o esclerito hipofaringeal é assim denominado por estar posicionado abaixo e entre as mandíbulas e o esclerito tentofaringeal possuindo ligação com a hipofaringe. Assim, o nome esclerito hipostomal não seria adequado morfologicamente (Teskey, 1981). Em vista ventral, o esclerito hipofaringeal apresenta forma de “H”. As barras parastomais também podem estar presentes, projetadas na margem anterior do esclerito tentofaringeal e acima do esclerito hipofaringeal. Alguns escleritos labiais podem ocorrer anteriormente, abaixo do esclerito hipofaringeal, apoiando a parede do átrio. As

mandíbulas são esclerotinizadas e curvadas podendo haver diferenças na forma e presença de dentes na margem anterior, que servem como caracteres para diferenciar grupos. Abaixo da base da mandíbula está o esclerito dental (Teskey, 1981). Com relação à morfologia externa, Teskey (1981) difere de Townsend (1935b) somente com relação aos espiráculos posteriores denominando placa espiracular onde estão inseridas as aberturas espiraculares. Além disso, acrescenta que o ânus é localizado ventralmente ou posteriormente no último segmento abdominal dentro ou não de uma fenda transversal ou longitudinal. Essa fenda é usualmente chamada de "perianal pad" apresentando forma, contorno e tamanho que diferem de acordo com o grupo. Existem ainda, as papilas anais que são provenientes do "perianal pad" podendo ter função respiratória e de osmorregulação (Teskey, 1981).

A proposta de Ferrar (1987) não difere muito da terminologia sugerida por Teskey (1981) quanto à morfologia externa e interna, com exceção do esqueleto cefalofaríngeo. Para essa estrutura, Ferrar (1987) sugere que seja adotado o termo "esclerito intermediário" em substituição a "esclerito hipostomal" porque descreve de maneira inequívoca o esclerito, que está localizado entre os principais escleritos do esqueleto. Nesse caso, o esclerito tentofaringeal volta a ser denominado de esclerito faringeal. Ainda, acrescenta que alguns grupos predadores podem apresentar escleritos orais acessórios. Outra modificação é a substituição do termo "anel quitinoso ventral" (Lopes 1943) por "lingulate sclerite", sugerindo a possibilidade de que esse esclerito possa ser homólogo ao esclerito sub-hipostomal. O autor ainda coloca que não se sabe se esse esclerito é homólogo ao esclerito sub-hipostomal.

Courtney *et al.* (2000) afirmam que os termos "segmento cefálico" ou "segmento pseudocefálico" não foram bem empregados considerando que o pseudocéfalo não é composto de um único segmento. Quanto ao esqueleto cefálico (Coutney, **op. cit**) também o divide em três partes: mandíbulas, esclerito intermediário e esclerito basal. Podem existir escleritos dentais, desde que estejam ligados ao apódema abdutor mandibular. Antero-ventralmente ao átrio, os escleritos são usualmente denominados escleritos labiais, podendo haver vários dos quais, somente um é principal. O esclerito intermediário é assim chamado, pois além da origem labial, uma pequena parte poderia pertencer a hipofaringe. Sendo assim, o termo esclerito hipofaringeal não seria

adequado. Em relação ao último segmento abdominal, Courtney *et al.* (2000) sugere também a mudança de terminologia para divisão anal. Isso porque o termo geralmente utilizado por outros autores gera a impressão de que o último segmento é único.

A terminologia mais atual é a melhor?

Para que haja evolução nos estudos e descrições de larvas, o primeiro passo é uniformizar a terminologia existente após a interpretação dos caracteres. Um dos principais caracteres para a identificação de imaturos de Sarcophagidae é o esqueleto cefálico. E é também, o esclerito com maior dificuldade de padronização da terminologia, a começar pelo próprio nome (Fig.1; Tab.1). Atualmente, existe uma tendência em se utilizar a terminologia de Courtney *et al.* (2000). No entanto, com relação à Sarcophagidae, nenhuma das terminologias propostas, com exceção de Ferrar (1987), considerou Lopes (1943;1982), o único que enfocou as particularidades do grupo. Realmente, a terminologia de Lopes (1943), baseada em Townsend (1935a), é complexa e muitas vezes difícil de ser utilizada quando se busca uma padronização. Além disso, a divisão em setores, com repetição de nomes (ex: setor labial, composto por esclerito labial) torna a interpretação de caracteres já complexos ainda mais complicada. Em contrapartida, Lopes (1943) é o único que menciona a presença do anel quitinoso ventral. Esse caráter pode ser conspícuo nas larvas de Sarcophagidae e pode ser outro esclerito labial. A falta de uniformização relacionada a esse esclerito fica clara se observarmos trabalhos de descrição que ignoram tal esclerito, provavelmente por não saberem que nome utilizar (Perez-Moreno *et al.*, 2006; Nandi, 1980). Ferrar (1987) indica a presença de um esclerito acessório denominado “lingulate sclerite” que aparentemente é o “anel quitinoso ventral” definido por Lopes (1943). O “lingulate sclerite” e “anel quitinoso ventral” denominados de escleritos labiais segundo Teskey (1981) e Courtney *et al.* (2000), podendo apresentar morfologias distintas. Nenhum dos dois autores coloca o motivo da sinonimização desses escleritos. Entretanto, os escleritos que formam o esqueleto cefálico variam em forma e tamanho, fornecendo excelentes caracteres para a determinação de táxons em Muscomorpha (Teskey, 1981), e por isso, não podem ser ignorados.

Courtney *et al.* (2000) é a revisão mais completa sobre morfologia de larvas porque sintetiza as ideias anteriores e utiliza outras metodologias, como micrografias de larvas, para identificar as estruturas, facilitando o entendimento das estruturas. Apesar de alguns termos terem sido adotados anteriormente, como por exemplo, o “esclerito intermediário” por Ferrar (1987), o autor justifica detalhadamente a mudança, nesse caso, a pouca ligação com a hipofaringe e não só por ser um esclerito de ligação entre as mandíbulas e esclerito basal. O esclerito faringeal que parecia estar bem determinado, aparentemente não tem origem faringeal. Logo, para não fazer uma inferência incorreta o melhor é utilizar um termo livre de desentendimentos.

Então qual seria a melhor terminologia para as larvas de Sarcophagidae?

O ideal seria mesclar Courtney *et al.* (2000) com a interpretação de Lopes (1943) quanto a um dos escleritos intermediários, resultando então em uma nova terminologia adequada para as larvas de Sarcophagidae.

O esqueleto cefálico pode ser assim denominado por fazer parte do primeiro segmento da larva e por ter origem nos segmentos da cabeça (Courtney *et al.* 2000). Nessa nova proposta, o **esqueleto cefálico** é dividido em um par de **mandíbulas** anteriormente, um **esclerito intermediário** e um **esclerito basal**. Existe também um par de escleritos associados às mandíbulas e localizados abaixo, o **esclerito dentado.** Nessa nova proposta será considerada a terminologia de Lopes (1943) com base principalmente, na diferença de Sarcophagidae em relação a outros grupos, não mencionadas por outros autores e pela morfologia do esclerito dentado em vista ventral analisada em algumas descrições (Lopes, 1943; Vairo, 2011; Perez-Moreno *et al.*, 2006). Sendo assim, é denominado **anel quitinoso ventral** o esclerito localizado posteriormente ao dentado e **esclerito labial** o subsequente, podendo estes estar ausentes dependendo da espécie. Já o **esclerito basal** possui diversas estruturas como: **barra parastomal**, **ponte dorsal**, **braço dorsal, corno dorsal** e **ventral** e **placa vertical** (Fig. 1). Usualmente esse esclerito é considerado como faringeal, porém a origem do esclerito é proveniente do cibário e não da faringe tornando o termo “faringeal” não adequado (Snodgrass, 1953). Lopes (1982) nomeia a “dorsal bridge” como arco clipeal, termo incorreto já que provavelmente, essa estrutura tem origem

no labro. Em relação à morfologia externa, a larva se divide em 12 segmentos, um **pseudocéfalo**, **três torácicos, sete abdominais e** uma **divisão anal.** O termo divisão anal foi adotado, pois, "último segmento abdominal" traz a ideia de um único segmento, mas, embriologicamente, esse segmento é uma fusão de segmentos (ainda há divergências em relação à quantidade), resultando em uma divisão anal única com função de respiração e excreção (Snodgrass, 1924; Courtney *et al.*, 2010). Além dos espiráculos e ânus, na divisão anal também podem ser encontradas as **papilas anais** (raramente encontradas) e o "**anal pad**".

Adaptações morfológicas nas subfamílias

As larvas da maioria dos Miltogramminae são deixadas na entrada, ou próximas à entrada de ninhos de himenópteros, se desenvolvendo como inquilinas e muitas vezes destruindo ovos e larvas dos hospedeiros. Quando não os destroem, tendem a ingerir todos os recursos, deixando as larvas dos hospedeiros sem alimento até a morte (Szpila, 2010). Além disso, podem parasitar outros insetos como Orthoptera e Tabanidae e ainda, tartarugas e ovos de lagartos, predar aranhas e cupins e praticar necrofagia (Shewell, 1987; Schwendinger & Pape, 2000; Szpila, 2010).

Como se pode observar nas descrições, as principais diferenças em relação às outras subfamílias é o labro bem desenvolvido (Fig.2), o tegumento ornamentado e a rara presença de "dorsal bridge" no esclerito basal (Szpila, 2010). A morfologia do labro é um caráter importante na separação das espécies, podendo ser gradualmente reduzido da base para o ápice ou de uma base muito larga que abruptamente se torna afilada no ápice. A parte basal do labro é fusionada com as extremidades das barras parastomais. Já as mandíbulas raramente aparecem descritas, provavelmente pelo tamanho e pouca esclerotinização (Szpila, 2010). Porém, a parte apical das mandíbulas pode apresentar um único dente ou uma fileira. A "dorsal bridge" é ausente ou raramente presente, sendo pouco desenvolvida e fracamente esclerotinizada (Szpila, 2010). Em relação à morfologia externa, a principal diferença dos Miltogramminae são duas modificações na forma do primeiro segmento torácico. A primeira é um alongamento da porção antero-ventral do segmento, formando duas longas protuberâncias. A segunda é uma estrutura esférica na

superfície dorsal que ocupa mais de 50% da superfície do primeiro segmento torácico (Szpila & Pape, 2008). Não se conhece a função dessas estruturas, provavelmente, essas modificações no esqueleto cefálico são adaptações para a predação. Em alguns casos as diferenças morfológicas no aparelho bucal e a cutícula ornamentada com cristas e ranhuras podem indicar uma adaptação. Isso porque, a evolução dessas características pode estar associada com o hábito cleptoparasita da subfamília, possibilitando uma melhora no sistema sensorial para localizar as presas e/ou hospedeiros, facilitar a degradação do alimento e ainda, enfrentar o ambiente seco em que vivem (Szpila & Pape, 2005).

Paramachronychiinae e Sarcophaginae envolvem espécies saprófagas com uma tendência a se tornarem parasitas facultativos ou obrigatórios, causando miíases em vertebrados ou ainda, parasitando invertebrados (Greene, 1925; Hilton, 1973; Ferrar, 1987; Shewell, 1987; Pape, 1996). Pelas duas subfamílias possuirem espécies com hábitos alimentares semelhantes, serão tratadas em conjunto em relação às adaptações morfológicas. As informações acerca da morfologia de representantes de Paramachronychiinae são raras, com exceção do gênero *Wohlfahrtia* (Brauer & Bergenstamm), intensamente estudado por serem causadores de miíases em vertebrados (Hall, 1995). O esclerito intermediário pode ser muito largo, sendo difícil encontrar essa característica em Sarcophaginae (James & Gassner, 1947). A principal diferença em relação às outras subfamílias é que algumas espécies de *Wohlfahrtia* apresentam três ganchos orais (um central e dois laterais) formando o que chamamos de mandíbula. Ainda, através da microscopia eletrônica de varredura, pode-se observar canalículos na superfície do gancho central nas larvas de primeiro e segundo instar de algumas espécies. Além disso, existem espinhos modificados perto das cristas orais, principalmente em larvas de segundo e terceiro instar (Hilton, 1973; Khedre, 1999). Essas características se devem, provavelmente, a uma adaptação ao parasitismo, já que nas larvas de primeiro instar, os três ganchos orais e os canalículos facilitariam a fixação e a penetração na superfície do hospedeiro (Khedre, 1999; Ruiz-Martinez *et al.*, 1987; 1989). A presença desses espinhos modificados proeminentes produz, além do suporte da mandíbula durante a

locomoção, um efeito abrasivo direcionando o alimento para as cristas orais (Ruiz-Martinez *et al.*, 1990).

As larvas da subfamília Sarcophaginae em sua maioria estão diretamente relacionadas ao homem sendo saprófagas e causadoras de miíases. Por isso um grande número de espécies de importância médica já foi descrita, principalmente do gênero *Sarcophaga* (Greene, 1925; Zumpt, 1965). Analisando as descrições e dando importância a análise de diferentes gêneros, não se observa nenhuma modificação morfológica em relação ao hábito alimentar, como presença de labro desenvolvido (Fig. 2) ou um terceiro gancho oral, nem mesmo nas espécies causadoras de miíases em vertebrados (Greene, 1925; Lopes, 1943; Kano, 1951; Kano & Sato, 1951; Newhouse *et al.*, 1955; Sanjean, 1957; Ishijima, 1967; Yates, 1967; Lopes, 1978; Nandi, 1980; Cantrell, 1981; Khan & Khan, 1984; Lopes & Leite 1986; Leite & Lopes, 1987; Lopes & Leite, 1987;Leite & Lopes, 1989; Aspoas, 1991, Kirk-Spriggs, 1999; Kirk-Spriggs, 2000; Mendéz & Pape, 2002; Perez-Moreno *et al.*, 2006; Draber-Monko *et al.*, 2009; Singh *et al.*, 2012). A exceção está nos genêros *Panava* (Dodge) e *Titanogrypa* (Townsend) que possuem uma esclerotinização na superfície do pseudocéfalo, provavelmente uma adaptação a predação (Lopes, 1978). As variações interespecíficas existem e são muitas vezes facilmente observadas, porém, mesmo gêneros com a morfologia amplamente estudada e com hábitos alimentares variados, como *Sarcophaga* (Meigen), não apresentam modificações morfológicas associadas ao tipo alimentar (Perez-Moreno *et al.*, 2006).

A importância do estudo dos imaturos de Sarcophagidae

Como foi citado anteriormente, Sarcophagidae está diretamente relacionada ao homem, principalmente, devido ao seu hábito coprófago e necrófago. Podem ainda, auxiliar na polinização de alguns grupos vegetais (Machado *et al.*, 2010; Sousa *et al.*, 2010; Reichert *et al.*, 2010). Apesar de não existirem muitas pesquisas aplicadas com Sarcophagidae, podemos extrapolar para o grupo alguns dados referentes a dípteros muscoides que possuem biologia e hábitos similares. Hipotetiza-se, que essa falta de pesquisas aplicadas utilizando como modelo biológico espécies de Sarcophagidae seja

principalmente pela dificuldade de identificação do grupo em comparação com outros Muscoidea como Calliphoridae e Muscidae.

Sarcophagidae é atraída e se alimenta de matéria orgânica em decomposição, como fezes de animais e humanos e carcaças. Logo, a presença de larvas no ambiente em que vivemos podem indicar condições de higiene inapropriadas, oferecendo problemas do ponto de vista da saúde pública (Ishijima, 1967). Clinicamente essas larvas podem causar miíases em vertebrados (Greenberg, 1971, 1973) e na área forense podem ser importantes indicadores de intervalo pós-morte.

Diversos estudos demonstram que os dípteros muscóides apresentam alto índice de sinantropia, sendo potenciais disseminadores de formas infestantes e infectantes de bioagentes patogênicos nas diversas doenças de importância epidemiológica e epizoótica (Cordeiro-de-Azevedo,1960; Greenberg, 1971; Monzon *et al.*, 1991; Oliveira *et al*., 2002). Esses dípteros podem transmitir agentes patogênicos a seres humanos e animais, já que frequentam matéria orgânica em decomposição, fezes e urina. Essa transmissão pode ocorrer principalmente de três formas: mecânica, através das pernas (nas quais agentes possivelmente patogênicos como cistos de protozoários, bactérias, vírus e ovos de helmintos se prendem nas cerdas), através das peças bucais (perante a ingestão de alimentos sólidos, eliminam saliva para liquefazê-los) e através da defecação (Marcondes, 2001). Inclusive, algumas espécies apresentam cerdas modificadas nos pulvilos que auxiliam no carreamento de patógenos (Sukontason *et al.*, 2006). Algumas pesquisas relacionam dípteros muscóides como transmissores de rotavirus e *Escherichia coli* (Tan *et al.*, 1997; Kobayiashi *et al*., 1999).

Além de serem potenciais transmissores de patógenos, podem produzir miíases em seres humanos e animais. Miíases são afecções causadas por larvas de moscas em órgão e tecidos do homem ou de outros animais vertebrados, onde elas se nutrem e desenvolvem-se como parasitos (Rey,2008; Hall, 1995) e são frequentes nos trópicos e em países subdesenvolvidos, onde as condições de saúde pública são precárias (Torruella, 1997).

As espécies com hábitos ectoparasitas podem ser divididas em três grupos, baseados no seu hábito alimentar. Podem ser saprófagos, que se

alimentam de tecido em decomposição; ectoparasitas facultativos que vivem como saprófagos ou que podem iniciar miíases e viver como ectoparasitas; e parasitas obrigatórios que se alimentam apenas de tecidos vivos (Zumpt,1965). Há alguns trabalhos que relatam casos de míiases e levantamento de lesões em humanos e animais causadas por moscas da família Sarcophagidae (Panu *et al.*, 2000; Ahmad *et al.,* 2011; Gaglio *et al.*,2011).

Por outro lado, larvas necrófagas podem auxiliar na cicatrização de tecidos, principalmente em lesões de pacientes que sofrem de diabetes (Jarczyc *et al*,. 2008). Essa metodologia de inserir larvas nos tecidos danificados com a finalidade de diminuir a inflamação buscando a limpeza da ferida é denominada terapia larval (Wolff *et al.,* 2010). A família Calliphoridae é mais amplamente utilizada, porém larvas de Muscidae e Sarcophagidae também podem ser empregadas (Wolff *et al.*, 2010). A digestão extracorpórea através de enzimas proteolíticas é o principal mecanismo de limpeza das lesões (Sherman *et al.*, 2000), se tornando um tratamento de baixo custo para hospitais e clínicas (Sherman & Whyle, 1996).

Outra aplicação do estudo de imaturos e talvez a mais evidente nos dias atuais é a entomologia forense, o campo em que a pesquisa de artrópodes interage com a justiça (Byrd & Castner, 2001). Os insetos da ordem Diptera em especial os muscóides, são os primeiros a chegarem aos cadáveres, podendo ovipor logo após encontrá-lo (Carvalho *et al.*, 2000; Smith,1986). As larvas destas famílias podem se desenvolver em tecido em decomposição, sendo responsáveis por 90% da degradação da massa corpórea (Salviano *et al.* 1996), e Sarcophagidae pode estar presente durante todo o período de decomposição (Vairo *et al.* 2011). Quando um corpo é encontrado com mais de 72 horas do óbito, análises morfológicas e temperatura corpórea podem não ser suficientes para saber quando a morte ocorreu (Amendt *et al.* 2004). Assim, nestes casos, os insetos podem ajudar na datação do intervalo pós-morte, ou seja, determinar há quanto tempo o corpo está exposto ao ambiente. A idade dos estágios imaturos encontrados em um cadáver pode estimar a data da morte de um dia até meses, dependendo das espécies envolvidas e das condições climáticas do local (Amendt *et al.* 2004; Turcheto & Vanin, 2004).

Utilizando a entomologia forense, pode-se também, fazer uma análise toxicológica das larvas necrófagas para identificar a presença de drogas ou

outro agente tóxico que a pessoa ingeriu ainda viva (Amendt *et al*.2004), pode indicar o deslocamento do cadáver (Goff, 1991) e até mesmo negligência a seres humanos e animais (Benecke & Lessing, 2001; Benecke *et al.* 2004; Anderson & Huitson, 2004).

Para obter êxito na utilização dos sarcofagídeos como vestígio entomológico, a identificação correta do espécime é essencial. Determinar a espécie envolvida em uma investigação criminal ou cível é o fator mais importante e fornece a base sólida para todas as inferências posteriores a análise. Com descrições e chaves taxonômicas o processo de identificação se torna mais rápido, característica essa de extrema importância em um contexto forense. É importante ressaltar que para se estimar o intervalo pós-morte é necessária uma pesquisa prévia detalhada acerca do desenvolvimento da espécie, e isso só é feito depois da coleta de espécimes, identificação e criação em laboratório por um especialista.

Em casos envolvendo transmissão de patógenos e miíases, determinar a espécie carreadora ou responsável diretamente pela doença ou lesão, é imprescindível para que possam ser traçadas metas de controle da espécie e pesquisas para o entendimento do ciclo da doença. Não podemos esquecer também, da importância da descrição de larvas para o conhecimento da biodiversidade e como já foi relatado aqui, para auxiliar em estudos evolutivos.

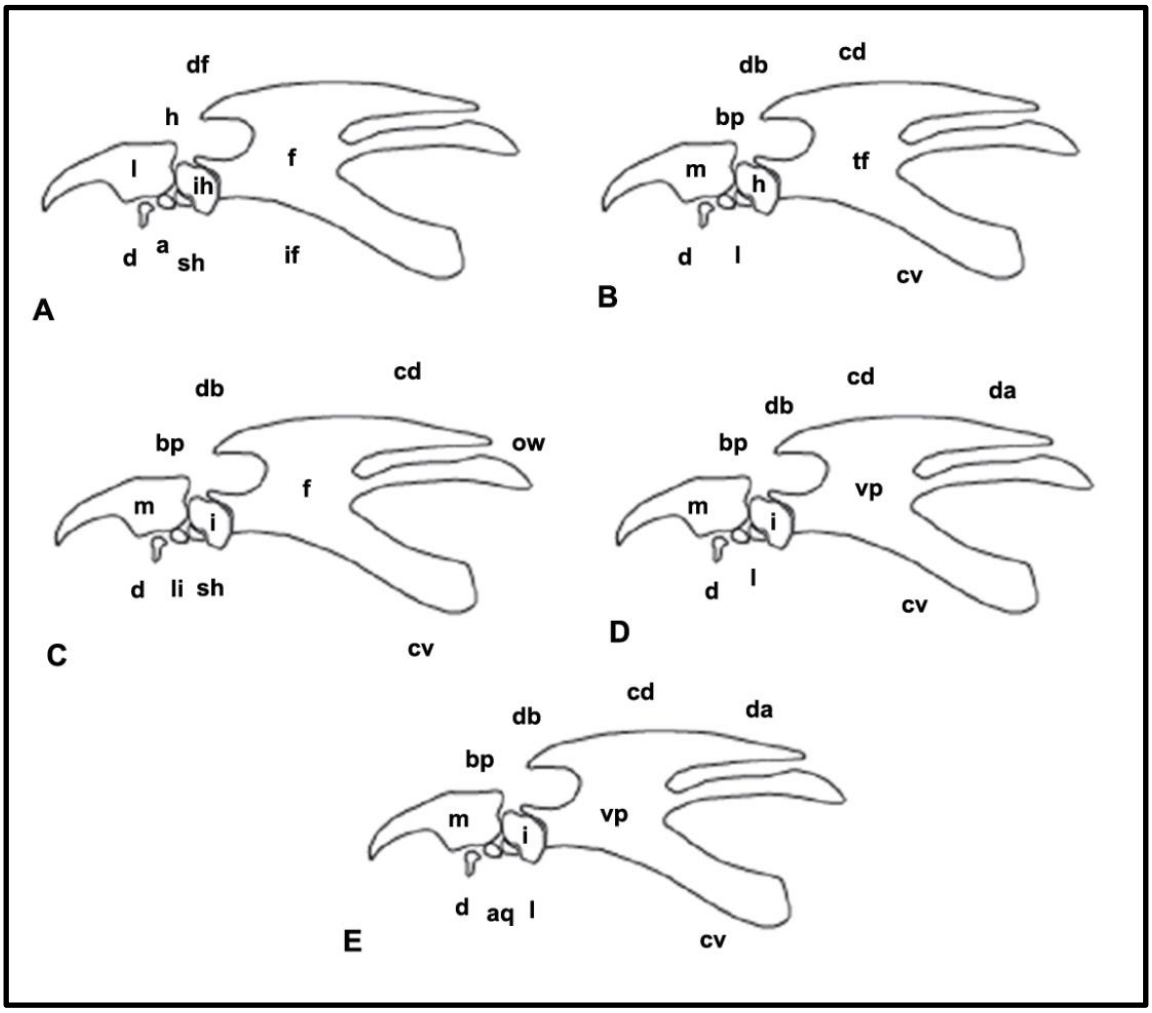

Figura 1**.** Desenhos esquemáticos utilizando como modelo o esqueleto cefálico de larvas de terceiro instar da espécie *Sarcodexia lambens* (Wiedemann) representando a terminologia de cada um dos autores. A- Terminologia de TOWSEND (1935) + LOPES (1943); B- Terminologia de TUSKEY (1981); C- Terminologia de FERRAR (1987); D- Terminologia de COURTNEY et al. (2000); E- Terminologia nova proposta pela autora. Abreviaturas: bp- barra parastomal; cd- corno dorsal; cv- corno ventral; d- dentado; da- braço dorsal; df- dorso-faringeal; f- faringeal; tf: “tentorial phragma”; h- hipostomal; ih- infra-hipostomal; if- infra-hipostomal; l- labial; li- “lingulate sclerite”; m- mandíbulas; ow- “open window”; sh- sub-hipostomal; vp- “vertical plate”.

NOVA PROPOSTA (AUTORA)	TOWNSEND (1935a) - LOPES (1943,1982)	TESKEY (1981)	FERRAR (1987)	COURTNEY *et al.* (2000)
Esqueleto cefálico	Esqueleto cefálico	Esqueleto cefalofaringeano	Esqueleto cefalofaringeo	Esqueleto cefálico
Mandíbulas	Labial	Mandíbulas	Mandíbulas	Mandíbulas
Esclerito dentado	Esclerito dentado	Esclerito dentado	Esclerito dentado	Esclerito dentado
Anel quitinoso ventral	Anel quitinoso ventral	Labial	"lingulate sclerite"	Labial
Intermediário	Infra-hipostomal	Hipostomal	Intermediário	Intermediário
Basal	Faringeal	Esclerito tentofaringeal	Faringeal	Basal
Labial	Sub-hipostomal	Labial	Sub-hipostomal	Labial
"dorsal bridge"	Dorso faringeal/ arco clipeal	"dorsal bridge"	"dorsal bridge"	"dorsal bridge"
Barra parastomal	Hipostomal	Barra parastomal	Barra parastomal	Barra parastomal
Corno dorsal	Dorso faringeal	Corno dorsal	Corno dorsal	Corno dorsal
Corno ventral	Infra faringeal	Corno ventral	Corno ventral	Corno ventral

Tabela 1. Nomes dos principais escleritos do esqueleto cefálico segundo os autores.

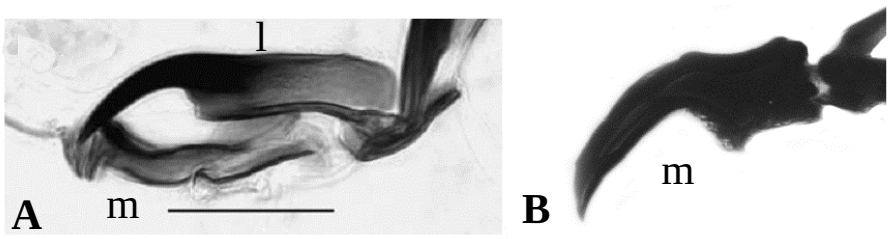

Figura 2. Comparação de larvas de primeiro instar de Miltogramminae (labro desenvolvido) e Sarcophaginae (mandíbula desenvolvida). A: *Metopia campestris* (Fallén) adaptado de Szpila & Pape, (2005); B: *Peckia* (*Sarcodexia*) *lambens* adaptado de (Vairo, 2011). Abreviaturas- l: labro; m: mandíbulas.

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Comparative morphology of third instar fleshflies larvae (Diptera: Sarcophagidae) of forensic importance in Southern Brazil

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Abstract

Sarcophagidae is a diverse group having species that rear on corpses, myiasis inducers and vector of diseases, being important for forensic and medical entomology. To understand the disease mechanism or to use the insects information for forensic entomology the first step is identify the species. In places where richness is evident like Brazil, regional identification keys are the best solution because is cheaper and faster than other methodologies. For this reason, we present here the first key for third instar larvae of flesh flies with medical and forensic importance in Southern Brazil. The species were collected and analysed by optical and scanning electron microscopy. The main characters used to differentiate the species were larvae surface smooth or with spines, hairs or warts, distribution of spines, morphology of cephaloskeleton mainly mounthooks and intermediate sclerites and size of anal papilla.

Key-Words: *Oxysarcodexia*, *Peckia*, *Microcerella*, *Sarcophaga*, descriptions, key, legal medicine, medical importance, myiasis

Introduction

Sarcophagidae is a biologically diverse group comprising species with different habits: coprophagous, necrophagous, predators, parasites and myiasis-inducers in vertebrates (Pape, 1996). This breadth of resource use prompts a series of possible ways Sarcophagids interacts with humans. For instance, the presence of flesh flies immature stages could be associated with bad sanitary conditions being a risk for public health (Ishijima, 1967). Also, some species are disease vectors, as others produces myiasis in humans and animals and some are related to corpses (Cordeiro-de-Azevedo, 1960; Burgess & Spraggs, 1992; Bermúdez *et al.*, 2010; Oliveira &Vasconcelos, 2010; Ahmad *et al*., 2011; Gaglio *et al*., 2011). In all cases, the species identification is extremely important to understand the mechanism of disease, identify the cause of injury or properly analyze a forensic case.

Regarding forensic entomology, the flesh fly subfamily Sarcophagine are directly associated with decaying process around the world with species related to carcasses (Velazquez, 2008; Wang *et al*.; 2008; Vairo *et al.*; 2011). In veterinary entomology, the study of myiasis inducers species could even estimate the time of a possible neglect or death in animals (Anderson & Huitson, 2004).

After collect the oldest specimen found on the death place or in the wound, the first step for a forensic entomologist is species identification. Commonly, the most important material collected in a death scene is immature stages of flies, mainly third instar larvae. Unfortunately, the descriptions and keys available are biased toward Calliphoridae (Szpila & Villet, 2011; Szpila *et al*., 2012; Szpila *et al*., 2013; Szpila *et al*., 2014) letting Sarcophagidae with the label of difficult to identify using morphology. As a consequence, there is a growing use of molecular methods to identify fleshflies (Zehner *et al*., 2004; Meiklejohn *et al.*, 2011). However, morphological studies is still faster and cheaper (Szpila and Villet 2011).

In last years there was a progress in immature identification with consistent descriptions using classic morphology and scanning electron microscopy of Miltogramminae morphology (Szpila & Pape, 2005a; Szpila & Pape, 2005b). However, for Sarcophaginae the descriptions do not provide enough information to allow species delimitation (Mendonça *et al.*, 2013; Buenaventura, 2013).

In Brazil, compared to other countries, the richness of flesh flies collected on carcasses is high and greatly differs between regions (Barros *et al*., 2008; Rosa *et al*., 2011; Vairo *et al*., 2011). This makes the identification process difficult since there is no key for immature stages of Sarcophagidae species with forensic importance.

In regions where forensic entomology is well established, like Southern Brazil (Vairo *et al*., 2014), there is an urgency to identify the entomological evidence as soon as possible to proceed the analyze of the minimum time since death because it is the most important information requested by Scientific Police to forensic entomologists. Thus, the best way is to focus the research on species that rear on corpses and can be helpful to estimate the minimum *post mortem* interval. In this sense, based on previously literature and samplings in Southern Brazil, the most important forensic species are *Oxysarcodexia paulistanensis* (Mattos, 1919), *Oxysarcodexia riograndensis* (Lopes, 1946), *Microcerella halli* (Prado & Fonseca 1932), *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830), *Peckia* (*Pattonella*) *resona* (Lopes, 1935) and *Peckia* (*Pattonella*) *intermutans* (Walker 1861) (Wiedemann 1830), *Peckia* (*Euboettcheria*) *australis* (Fabricius, 1805), *Peckia* (*Euboettcheria*) *florencioi* (Mattos, 1919) and *Sarcophaga* (*Bercaea*) *africa* (Wiedemann, 1824). As correct species identification is fundamental to forensic entomology we provide descriptions of third instar larvae for the above mentioned species of Sarcophagidae, excluding *P*. (*S*.) *lambens* that was already described (Vairo *et al.* submitted). Also, we provide a key for all species. All species we describe here have a large geographic range that makes our key to be useful on a broad geographical scale. (Pape 1996; Moura *et al.,* 1997; Vairo *et al*., 2011).

Material and Methods

Third instar larvae of *O. paulistanensis*, *O. riograndensis*, *P*. (*P*.) *intermutans*, *P*. (*P*.) *resona*, *P.* (*E.*) *australis, P.* (*E*.) *florencioi, M. halli* and *S.* (*B.*) *africa* were obtained from laboratory colonies. Females were collected at Curitiba, Paraná (25°27'16"S 49°14'9"W) except *S.* (*B.*) *Africa*, that were collected in Bombas, Santa Catarina (27^{o}8’10”S, 48^{o}30’54”W). Females were captured using a butterfly bait trap that allows the researcher to choose flies in the field or actively. All females were reared individually in small cages, with sugar, powder milk (1:1), water and bovine meat until the larviposition occurs. The larvae were reared in diet (Estrada *et al*., 2009) until the emergence of adults. After the emergence, males were identified based on Vairo *et al.* (2011) ensuring the identification. The second, third and fourth generation were used for morphological studies. After leave the diet, third instar larvae were killed in hot water (approximately 95^{o}C) to avoid deformations and fixed in 70% alcohol. To optical analysis, 10 larvae of each species were cleared in KOH 10%, immersed in acetic acid and washed few times in distilled water. After these procedures, larvae were slide-mounted in Hoyer’s medium for light microscopy (Szpila & Pape, 2005). The pictures were taken in Leica M205 dissecting scope and the draws edited using GIMP 2.8.

To scanning electron microscope (SEM), larvae were killed and fixed as described before, dehydrated in 80, 90 and 99,5% ethanol, critical point dried in CO2. Some larvae were coated with gold and some with platinum. The pictures were taken using SEM at the Zoological Museum of Denmark, JSM-6335F Field Emission and SEM at the Geological Museum of Denmark, FEI Inspect S.

The terminology adopted for general morphology is Courtney *et al.* (2000) and for the oral sclerites we followed Lopes (1943).

Results

All third instar larvae have the same general morphology as others Calyptrata. It has a bilobed pseudocephalon, three thoracic segments (T1-T3),

seven abdominal segments (A1-A7) and the anal division (Szpila, 2010). Usually larvae are elongated and slender in anterior part.

Oxysarcodexia paulistanensis (Mattos, 1919)
(Figures 1A, 2A, 3 and 11)

Pseudocephalon: Pseudocephalon is bilobed with an antennal complex and a maxillary palpus. Antennal complex have a short antennal dome and a high basal ring. Maxillary palpus are located in the anterior part of pseudocephalon lobe and are clearly distinguished from the surface of pseudocephalon by several cuticular folds, it possess three sensilla coeloconica and two sensilla basiconica located in the central cluster, additionally to central cluster of sensilla two additional sensilla are present; around the central cluster there are a few conspicuous sensilla ampullacea. Some sensilla coeloconica are also present in the dorsal part of pseudocephalon. The labial lobe is triangular with sensilla in the labial organ. The facial mask has two kinds of structures, just behind maxillary palpus there are three-four rows of broad scale-like structures clearly differentiated from the conspicuous oral ridges situated posteriorly. The oral ridges cover most the ventral and latero-ventral surface of pseudocephalon.

Cephaloskeleton: all sclerites are well sclerotised and have well-defined shape. Mouthhooks are massive, strongly sclerotised with the apical part of each mouthhook in the form of a down-curved, pointed hook. The basal part of each hook is considerably thicker than the anterior part and has one dorsal and one ventral apodeme. The dental sclerite is broad comparing to the “anel quitinoso ventral” and the labial sclerites in lateral view. The intermediate sclerite is very thick and has a ventral rectangular expansion. The basal sclerite consists of prominent parastomal bars, a complete but short dorsal bridge, a very broad vertical plate which the width is two times longer than the length of ventral cornua. The dorsal cornua are about two times longer than the ventral cornua

Thoracic segments: The thoracic segments have the anterior spinose bands complete, the spines are uniform on all surfaces, narrow, broad at the base and tapering to the apex and arranged densely. T1 without hairs on interband

surface. T2 is covered with hairs except on the posterior part of the segment. T3 is covered with cuticular hairs, more dense in the dorsal part but more sparsely arranged in the end of the segments. Keilin's organ visible. The anterior spiracle has 14-15 lobes. The surface of all segments does not have ridges, tubercles or processes.

Abdominal segments: almost the whole surface of the segments A1-A7 is covered with hairs. The posterior edges of all segments also have hairs but more sparsely arranged than on the remaining surface of all segments. Spines of anterior and posterior spinose bands are uniform in shape and similar to the hairs of interband surfaces. The spines in the bands gradually changes to form the cuticular hairs which makes impossible a precise delimitation between spines of bands and hairs on interband surface not possible. Surface of all segments with series of papilla arranged in lines perpendicular to the anterior-posterior body axis, surface of papilla also with cuticular long hairs.

Anal division: Anal pads large, strongly protruding, conical and covered with long cuticular hairs. The anal tuft has abundant spines/hairs. The perianal pads are very large. Six pairs of papillae (P1-P6) of conical shape protruded covered with hairs, P3 and P5 longer than the other papillae. The spiracular cavity is surrounded by a dense hair-like spines. Peritreme is dark brown, incomplete but with well-developed ventral arc, button is not visible. Each posterior spiracle has 3 linear slits, the central slit is straight whereas the outher and inner slits are slightly curved; the shortest distance between the central and inner slit is almost two times the distance between the outher and central slits.

Oxysarcodexia riograndensis (Lopes, 1946)

(Figures 1B, 2B, 4 and 12)

The description of *O. riograndensis* is identical to *O. paulistanensis* except that the hairs on segments seems to be more sparsely arranged than in *O. paulistanensis*. This character is not conspicuous and must be carefully analysed to be used in identification.

Peckia (*Pattonella*) *intermutans* (Walker, 1861)

(Figures 1C, 2C, 5 and 13)

Pseudocephalon: Identical to *O. paulistanensis* in most elements but scale-like structures of facial mask are arranged in 1-2 rows.

Cephaloskeleton: all sclerites are well sclerotised and have well-defined shape. Mouthhooks are massive, strongly sclerotised with apical part of each mouthhook in the form of a down-curved, pointed hook. The curvature is less apparent than in *Oxysarcodexia* spp. or *Microcerella halli*. The basal part of each mouthhook is massive, with almost the same length and height in lateral view. It has a dorsal and ventral apodeme not conspicuous. The intermediate sclerites is triangular in lateral view. The basal sclerite consists of prominent parastomal bars. The dorsal bridge is short and straight. The vertical plate is broad and it width is almost equal to length of ventral cornua. The dorsal cornua is two times longer than ventral cornua.

Thoracic segments: The thoracic segments have complete anterior spinose bands. The spines are sclerotised, brownish, broad at the base and tapering to the apex, uniformly arranged and disposed alone or in groups of 3,4, 7 or 10 on ventral surface. The inter-band area is smooth, without hairs, spines or warts. Keilin's organ visible. The anterior spiracle has 18-23 lobes (n=10), arranged in two irregular rows. Some series of papilla arranged in lines perpendicular to long body axis with smooth surface are present in all thoracic segments in lateral view. T2-T3 have papilla in all views, but dorsally it is more apparent than laterally and ventrally it is less apparent than laterally.

Abdominal segments: The anterior spinose bands A1-A5 are complete. A6 has the band of spines interrupted dorsally. A7 has a complete band only on ventral and lateral surfaces. A1 has a posterior spinose band forming two groups of spines on ventro-lateral surfaces. A2 has posterior spinose band restricted to ventral and ventro-lateral surfaces presenting a small group of spines on dorso-lateral surface. A3 band of spines is interrupted on lateral surface. A4-A7 has complete bands of spines. A6-A7 has bands of spines broad, larger than others. Other morphological details like in *P. (E.) australis*. Some series of papilla arranged in lines perpendicular to long body axis with smooth surface are present in all abdominal segments in all views.

Anal division: The anterior spinose band is developed on ventral and ventro-lateral surfaces with additional groups of small spines on lateral surface of anal division and posteriorly to anal pads and anal opening. The anal pads are large, protruding, conical, covered with short spines except of apical part. The perianal pads are not present. The anal papilla has apical part smooth and with short spines at the base. The anal tuft has abundant spines. It has six pairs of papillae (P1-P6) protruded with conical shape and without spines. P6 smaller than other papilla. The spiracular cavity is surrounded by fine hair-like spines. The peritreme is dark brown, incomplete, without a button. Each spiracle has 3 linear slits, situated in similar distance to each other.

Peckia (*Pattonella*) *resona* (Lopes, 1935)

(Figures 1D, 2D, 6 and 14)

Pseudocephalon: Identical to *O. paulistanensis* in most elements but scale-like structures of facial mask are arranged in 1-2 rows.

Cephaloskeleton: All sclerites are well sclerotised with well-defined shape. Mouthhooks are massive, strongly sclerotised, with apical part forming a down-curved pointed hook. The curvature is less apparent than in *Oxysarcodexia* spp. or *Microcerella halli*. The basal part of each mouthhook is massive, with almost the same length and height in lateral view. Dorsal and ventral apodeme is not conspicuous. The intermediate sclerite with a small rectangular expansion in lateral view. The basal sclerite has prominent parastomal bars. The dorsal bridge is short and anteriorly slightly curved down. The vertical plate is broad, it width is slightly shorter than the length of ventral cornua. The dorsal cornua is almost two times longer than ventral cornua.

Thoracic segments: The thoracic segments have complete anterior spinose bands. The spines are sclerotised, brownish, broad at the base and tapering to the apex, uniformly arranged and disposed alone or in groups of 3,4, 7 or 10 on ventral surface. The inter-band area are smooth without hairs, spines or warts. Pairs of some papilla as described in *P. intermutans.* Keilin's organ visible. The anterior spiracle has 18-24 lobes (n=10), arranged in two irregular rows.

Abdominal segments: The anterior spinose bands of A1-A6 complete. The spines on dorsal surface of A5 and A6 are not sclerotised, almost transparent. A7 with an interrupted band dorsally. A1 posterior spinose band forming two groups of spines on ventro-lateral surfaces. A2 posterior spinose band restricted to ventral and ventro-lateral surfaces. A3 and A4 bands are interrupted on lateral surface. A5-A7 have complete posterior spinose bands, being A6-A7 bands broad comparing to the others. Other morphological details like in *P. (E.) australis*. The same papilla as described in *P. (P.) intermutans.*

Anal division: The anterior spinose band developed on ventral and ventro-lateral surfaces with additional groups of small spines on lateral surface of anal division and posteriorly to anal pads and anal opening. The anal pads are large, protruding, conical and covered with short spines except for the apical part. The perianal pads are not present. The anal papilla has apical part smooth and short spines at the base. The anal tuft has abundant spines. Six pairs of papillae (P1-P6) protruded with conical shape and without spines are present. P6 is small comparing to others; The spiracular cavity is surrounded by robust spines. The peritreme is dark brown, incomplete, with a button. Each spiracle has 3 linear slits, situated in similar distance to each other.

Peckia (*Euboettcheria*) *australis* (Townsend, 1927)

(Figures 1G, 2G, 7 and 15)

Pseudocephalon: Identical to *O. paulistanensis* in most elements but scale-like structures of facial mask are arranged in 1-2 rows.

Cephaloskeleton: All sclerites are well sclerotised with well-defined shape. The mouthhooks are massive, strongly sclerotised, with apical part of each mouthhook with a down-curved pointed hook shape. The curvature is less apparent than in *Oxysarcodexia* spp. The basal part of each mouthhook relatively elongated, it length larger than height in lateral view. The mouthook dorsal apodeme conspicuous directed posteriorly. The intermediate sclerite like an inverted triangle in lateral view. The basal sclerite has prominent parastomal bars. The dorsal bridge is of mid length only slightly curved down. The distance between the dorsal bridge and parastomal bars is similar as the distance between the dorsal apodema of mouthhook and paratomal bars. The vertical

plate is narrow and their width is two times shorter than the length of ventral cornua. The dorsal cornua with long window, slightly longer than ventral cornua.

Thoracic segments: The thoracic segments have complete anterior spinose bands. The spines are broad at the base and tapering to the apex, uniformly arranged and disposed alone or in groups of 3,4, 7 or 10 on ventral surface. The interband area smooth without hairs, spines or warts. Pairs of Keilin's organ visible. Anterior spiracle with 13-14 lobes (n=10) arranged in one row.

Abdominal segments: The interband area of A1-A7 has smooth surface, without spines or warts. The lateral creeping welt covered with spines. The spines with uniform shape broad at the base and tapering to the apex. The spines on ventral surface more massive and hook-like. The anterior spinose bands A1-A5 complete. A6 has the band of spines interrupted dorsally and laterally. A7 has a large band only on ventral and ventro-lateral surfaces, with additional small group of spines on dorso-lateral surface. A1 posterior spinose band forming two small groups of spines on ventro-lateral surfaces. A2 band is restricted to ventral and ventro-lateral surfaces. A3 band is very broadly interrupted on dorsal surface. A4-A7 have complete bands of spines. A6-A7 bands of spines broad. The surface of all segments with series of papilla arranged in lines perpendicular to long body axis.

Anal division: The anterior spinose band developed on ventral and ventro-lateral surfaces with additional groups of small spines on lateral surface of anal division and posteriorly to anal pads and anal opening. The anal pads are large, protruding, conical and covered with short spines except of apical part. Perianal pads are developed. The anal papilla has apical part smooth and short spines at the base. The anal tuft has abundant spines around. Six pairs of papillae (P1-P6) protruded with conical shape and without spines. P6 is smaller than the others. The spiracular cavity is surrounded by fine hair-like spines. The peritreme is brownish, incomplete, with a button. Each spiracle has 3 linear slits. The slits are slightly bent or curved, the distance between the central and inner slit is almost two times the distance between the outher and central slits.

Peckia (*Euboettcheria*) *florencioi* (Prado & Fonseca, 1932)

(Figures 1H, 2H, 8 and 16)

Pseudocephalon: Identical to *O. paulistanensis* in most elements but scale-like structures of facial mask are arranged in 1-2 rows.

Cephaloskeleton: All sclerites are well sclerotised with well-defined shape. The mouthhooks are massive and strongly sclerotised. The apical part of each mouth hook has the form of a down-curved pointed hook and curvature is less apparent than in *Oxysarcodexia* spp. The basal part of each mouthhook is relatively elongated and their length is larger than height in lateral view. Dorsal apodeme is conspicuous and directed posteriorly. The intermediate sclerite with a large ventral apodema, almost half the sclerite size in lateral view. The basal sclerite with prominent parastomal bars. The dorsal bridge is of mid length and slightly curved down. The vertical plate is large, it width is two times the width of ventral cornua. The dorsal arm is slightly longer than ventral cornua.

Thoracic segments: T1 and T3 have complete anterior spinose bands. T2 has anterior spinose band broadly interrupted on lateral surface, just behind anterior spriracle. All inter-band area is smooth without cuticular warts, spines or hairs. T1- T3 anterior bands has fine spines disposed alone or in groups of 2 or 3 dorsally. The spines are broad at the base and tapering to the pointed apex, on ventral surface and arranged densely. Pairs of Keilin's organ are visible. The anterior spiracle with 13-15 lobes (n=10) arranged in an arcuate row.

Abdominal segments: The interband area of A1-A7 has smooth surface, without spines or warts. The lateral creeping welts covered with spines. A1-A5 anterior spinose bands are complete. A6 has anterior spinose band interrupted dorsally. A7 has anterior spinose band interrupted laterally and dorsally. A1 posterior spinose band forming two small groups of spines on ventro-lateral surfaces. A2-A3 bands are restricted to ventro-lateral surfaces. A4-A5 bands are interrupted laterally. A6 and A7 have broad and complete bands of spines. The spines are slender and sparsely distributed dorsally, with uniform shape broad at the base and tapering to the apex, hook-like, ventrally. The surface of all segments has series of papilla arranged in lines perpendicular to body axis.

Anal division: The anterior spinose band is developed on ventral and ventro-lateral surfaces and additional groups of small spines are present on lateral surface of anal division and posteriorly to anal pads and anal opening. The anal pads are large, protruding, conical, covered with short spines except of apical part. The perianal pads are very large and yellowish. The anal papilla has a smooth apical part and short spines at the base. The anal tuft has abundant spines around. Six pairs of conical shape papillae (P1-P6) protruded and without spines. P6 smaller than the others segments. The spiracular cavity is surrounded by fine hair-like spines. The peritreme is brown, incomplete and do not have button. Each spiracle has 3 linear slits whereas the distance between the inner and central slit is almost 2,5 times the distance between the outher and central slit.

Microcerella halli (Engel, 1931)

(Figures 1G, 2G, 9 and 17)

Pseudocephalon: The general form of pseudocephalon resembles *Oxysarcodexia*. The Oral ridges are conspicuous and cover most of the ventral and latero-ventral surface of pseudocephalon.

Cephaloskeleton: All sclerites are sclerotised with well-defined shape. The mouthhooks are massive, strongly sclerotised and short. The apical part of each mouthhook strongly down-curved, the medium part bearing an apodema and the basal part massive with prominent dorsal and ventral apodema. The dental sclerite is widest than the “anel quitinoso ventral” and labial sclerites in lateral view. The “anel quitinoso ventral” and labial sclerites are elongated. The intermediate sclerite is very thick and triangular in lateral view. The basal sclerite has a paired parastomal bars extended more than half of the length of intermediate sclerites. The dorsal bridge is short and straight in lateral view. The vertical plate is broad, the width is larger than length of ventral cornua. The dorsal arm more than two times longer than ventral one.

Thoracic segments: Thoracic segments with complete anterior spinose bands. The spines of spinose bands are massive, broad at the base and tapering to the apex, slightly curved. T2-T3 except of anterior spinose bands are covered with

warts. Pairs of Keilin's organ are visible. The anterior spiracle with 12-15 lobes (n=10) distributed in one row. T2-T3 have some series of papilla arranged in lines perpendicular to body axis with smooth surface in lateral and dorsal view. T1-T3, ventrally, without these papilla.

Abdominal segments: whole surface of segments A1-A7 are covered with spines and warts. The spines of anterior and posterior bands are uniform in shape and similar to the spines of interband surfaces. The spines on interband area on ventral surface are replaced by massive warts. Some series of papilla arranged in lines perpendicular to the long body axis with a smooth surface in all abdominal segments in all views. Ventrally, these papillae are sparser.

Anal division: Almost whole surface of anal divison covered with spines except of small area anteriorly to anal opening covered with warts. The anal pads are not conspicuous. The perianal pads are not developed. Six pairs of papillae (P1-P6) protruded with conical shape and covered with small spines, all papillae have similar size. The spiracular cavity is surrounded by dense spines, some of them are bi- or tricuspid. The peritreme is thick, dark brown, incomplete, with slightly visible button. Each spiracle has 3 linear slits, the outher and central ones are straight but the inner slit is slightly bent. Slits are situated in similar distance to each other.

Sarcophaga (*Bercaea*) *africa* (Wiedemann, 1824)

(Figures 1H, 2H, 10 and 18)

Pseudocephalon: Identical to *O. paulistanensis*.

Cephaloskeleton: all sclerites are well sclerotised and have well-defined shape. The mouthhooks are massive and strongly sclerotised. The apical part of each mouthhook has the form of a down-curved pointed hook but this curvature is less apparent than in *Oxysarcodexia* spp. or *Microcerella halli*. The basal part of each mouthhook is massive, with almost the same length and height in lateral view. Dorsal and ventral apodeme are not conspicuous. The intermediate sclerites is massive with a ventral apodema almost half of the size of the entire sclerite in lateral view. The basal sclerite has a prominent parastomal bars. The dorsal bridge is short and slightly curved down. The

vertical plate is broad, it width is the same like the length of ventral cornua. The dorsal arm is broad with arched upper edge almost two times longer than ventral cornua.

Thoracic segments: Thoracic segments have complete anterior spinose bands and uniform spines on all surfaces. The spines are narrow, broad at the base and tapering to the apex and arranged densely. The interband surface of T1 is smooth, without spines/hairs/warts. T2- T3 are covered with cuticular spines. These spines gradually transform to warts toward posterior edge of segments. The most posterior surface of T2 and areas around papillae on segments T2 and T3 without spines/warts. Pairs of Keilin's organ visible. The anterior spiracle with 15-16 lobes (n=10) in one row. Some series of papilla arranged in lines perpendicular to long body axis with smooth surface are present in all thoracic segments in lateral view. T1-T3 without these papillae dorsally and T1-T2 without these papilla ventrally.

Abdominal segments: the whole surface of segments A1-A7 covered with small spines. Lateral creeping velts covered with small spines. The spines of anterior and posterior spinose bands uniform in shape and similar to spines on interband surface. Precise definition of border between spines of bands and spines of surface on interband surface is not possible. The anterior surface of all segments with series of papilla arranged in lines perpendicular to long body axis but not too apparent as thoracic segments because of the warts and spines.

Anal division: Almost whole surface covered with spines except for a longitudinal area on posterior surface between spiracular cavity and anal complex. The anal pads are large, protruding, conical, covered with spines in all extension. The perianal pads are very large. The anal tuft has abundant spines. Six pairs of papillae (P1-P6) protruded with conical shape and covered with spines. P5 longer comparing to others, almost 2 times the size of P1. The spiracular cavity is surrounded by dense hair like spines. The peritreme is brownish, incomplete and has a button. Each spiracle has 3 linear slits curved. Inner slit is slightly bent; central and outher slits are straight and the distance between the inner and central slit is almost two size the distance between central and outher slit.

Identification key for third instar larvae of fleshflies forensic species of Southern Brazil

1. Larvae with smooth surface..... 2

1'. Larvae with hairs, warts or spines on surface 6

2. Antero -ventral part of mouthooks forming a right angle, dorsal bridge long, almost touching the intermediate sclerite 3

2'. Antero-ventral part of mouthhooks forming a different angle, dorsal bridge short..... 4

3. T2 with anterior spinose band broadly interrupted on lateral surface, just behind anterior spriracle. Mouthook with a dorsal proeminent apodema ..... *Peckia* (*Euboettcheria*) *florencioi*

3'. T2 anterior spinose band, just behind anterior spriracle, not interrupted on lateral surface, just behind anterior spriracle. Mouthook without a dorsal proeminent apodema..... *Peckia* (*Euboettcheria*) *australis*

4. Anterior spiracle with more than 18 openings, larvae usually bigger than 1 cm 5

4'. Anterior spiracle with less than 18 openings, larvae usually small than 1 cm ..... *Peckia* (*Sarcodexia*) *lambens*

5. Intermediate sclerite with a conspicuous ventral apodema, dorsal arm longer than ventral arm, anal segment with spines laterally ..... *Peckia* (*Pattonella*) *intermutans*

5'. Intermediate sclerites without a conspicuous ventral apodema, dorsal and ventral arm with almost the same length, anal segment without spines laterally ..... *Peckia* (*Pattonella*) *resona*

6. Surface covered with hairs, vertical plate large, intermediate sclerites with a ventral conspicuous projection in lateral view, no series of papilla arranged in lines perpendicular to long body axis..... *Oxysarcodexia spp*.

6' Surface covered with spines or warts, vertical plate not large, intermediate sclerites without a conspicuous projection in lateral view, series of papilla arranged in lines perpendicular to long body axis 7

7. Anal papilla 5 (P5) almost two times the length of other papilla and elongated, surface covered of spines, peritreme not well sclerotized, ventral creep velts not conspicous..... *Sarcophaga* (*Bercaea*) *africa*

7'. Anal papilla 5 (P5) has the same size and lenght as the other papilla, surface covered with warts, peritreme well sclerotized, conspicous ventral creep velts..... *Microcerella halli*

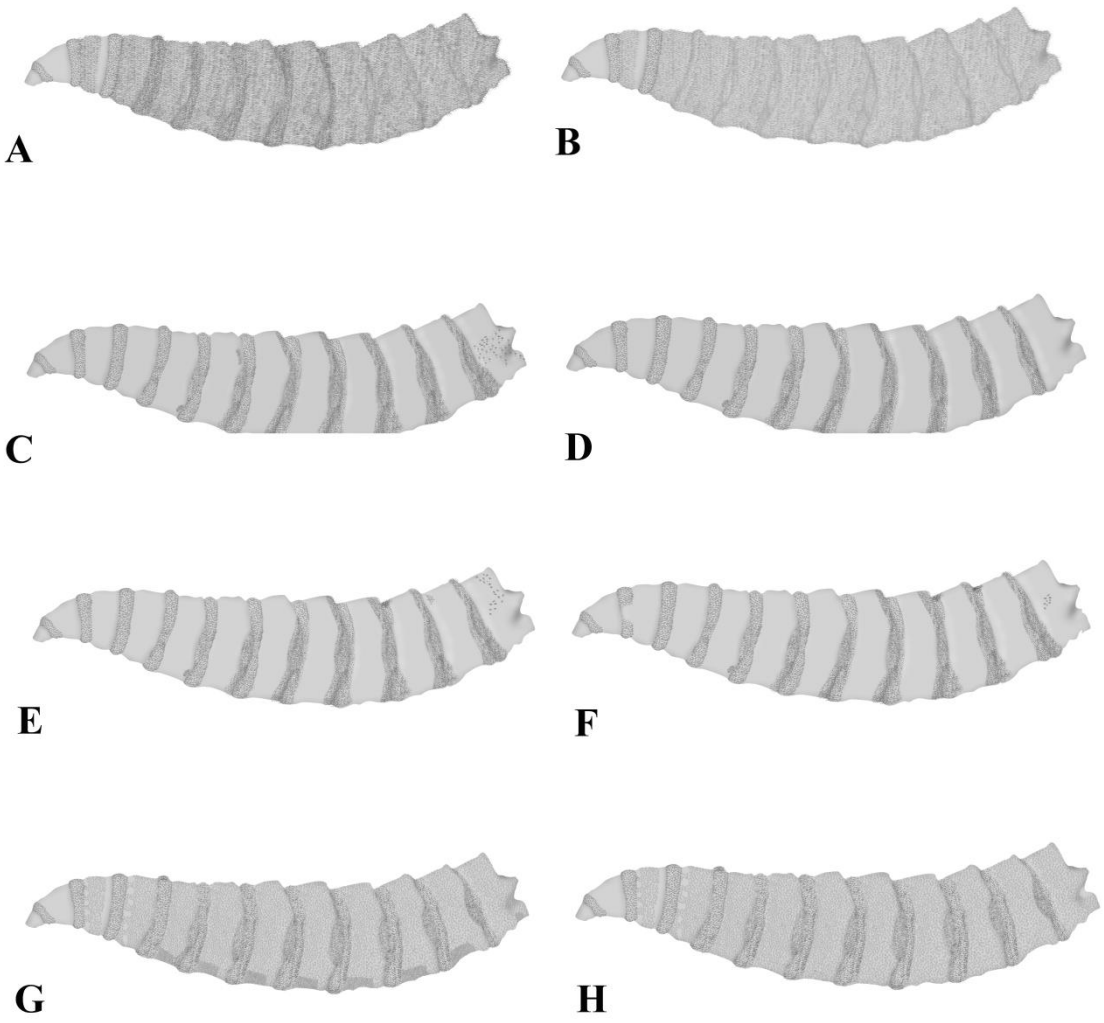

Figure 1. Distribution of spines. A: *Oxysarcodexia paulistanensis*; B: *Oxysarcodexia riograndensis*; C: *Peckia* (*Pattonella*) *intermutans*; D: *Peckia* (*Pattonella*) *resona*; E: *Peckia* (*Euboettcheria*) *australis*; F: *Peckia* (*Euboettcheria*) *florencioi*; G: *Microcerella halli*; H*: Sarcophaga* (*Bercaea*) *africa*.

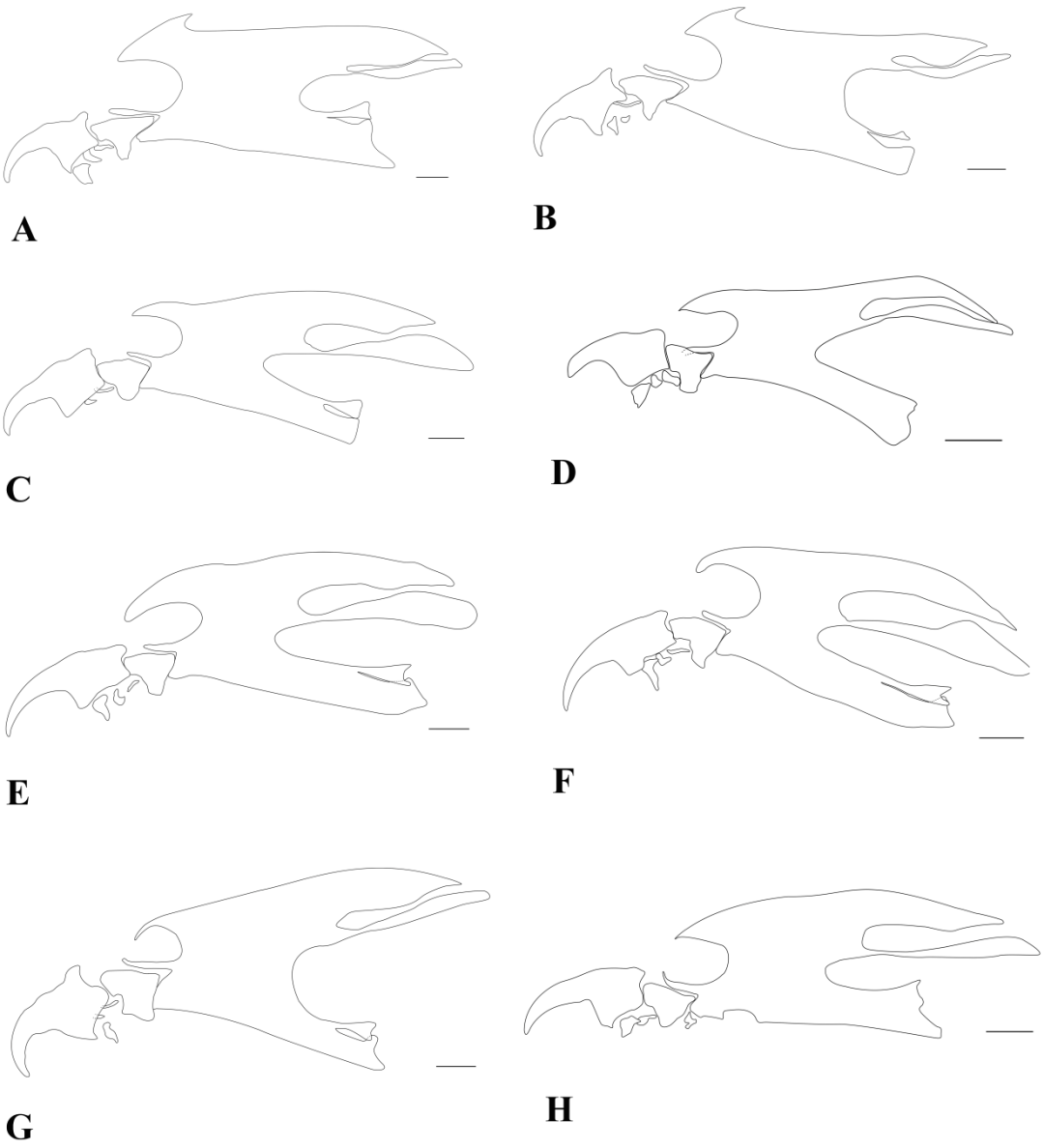

Figure 2. Cephaloskeleton. A: *Oxysarcodexia paulistanensis*; B: *Oxysarcodexia riograndensis*; C: *Peckia* (*Pattonella*) *intermutans*; D: *Peckia* (*Pattonella*) *resona*; E: *Peckia* (*Euboettcheria*) *australis*; F: *Peckia* (*Euboettcheria*) *florencioi*; G: *Microcerella halli*; H*: Sarcophaga* (*Bercaea*) *africa*. Scales: 0,5mm.

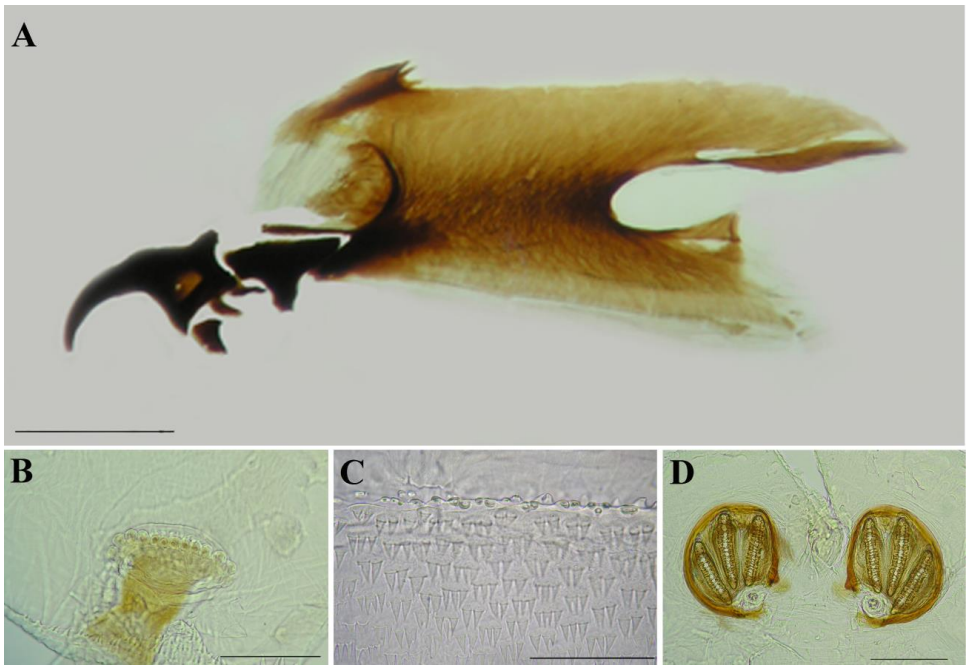

Figure 3. *Oxysarcodexia paulistanensis*. A: Cephaloskeleton, lateral view; scale: 1mm. B: anterior spiracle; scale: 0,5mm; C: abdominal spines; scale: 1mm. D: posterior spiracles; scale: 1 mm.

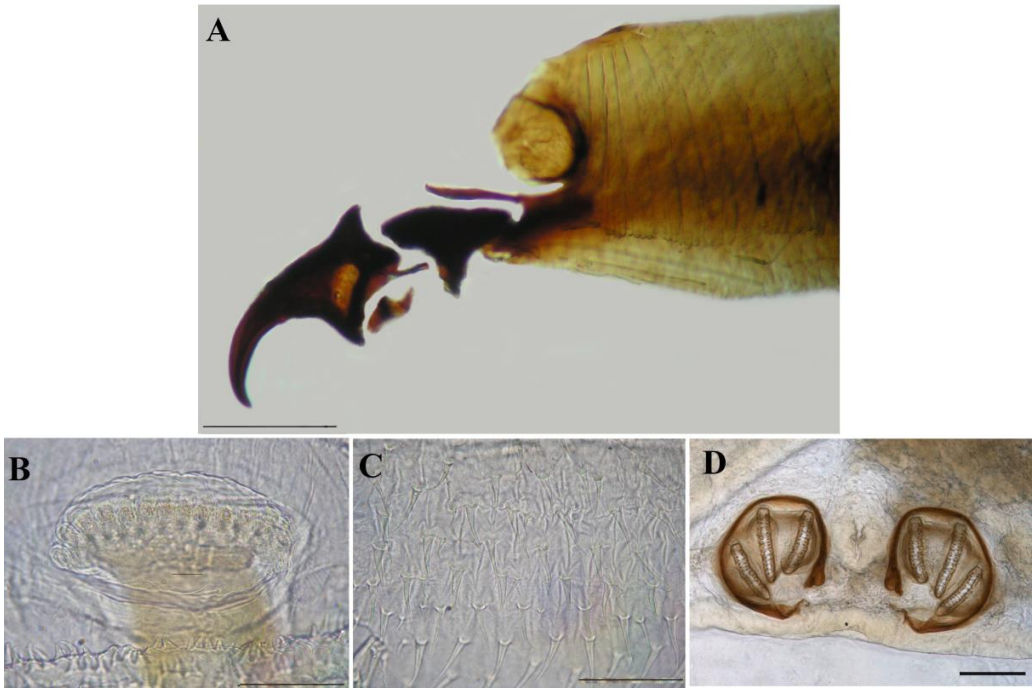

Figure 4. *Oxysarcodexia riograndensis*. A: cephaloskeleton, lateral view; scale: 1mm. B: anterior spiracle; scale: 0,5mm. C: abdominal spines; scale: 1mm. D: posterior spiracles; scale: 1mm.

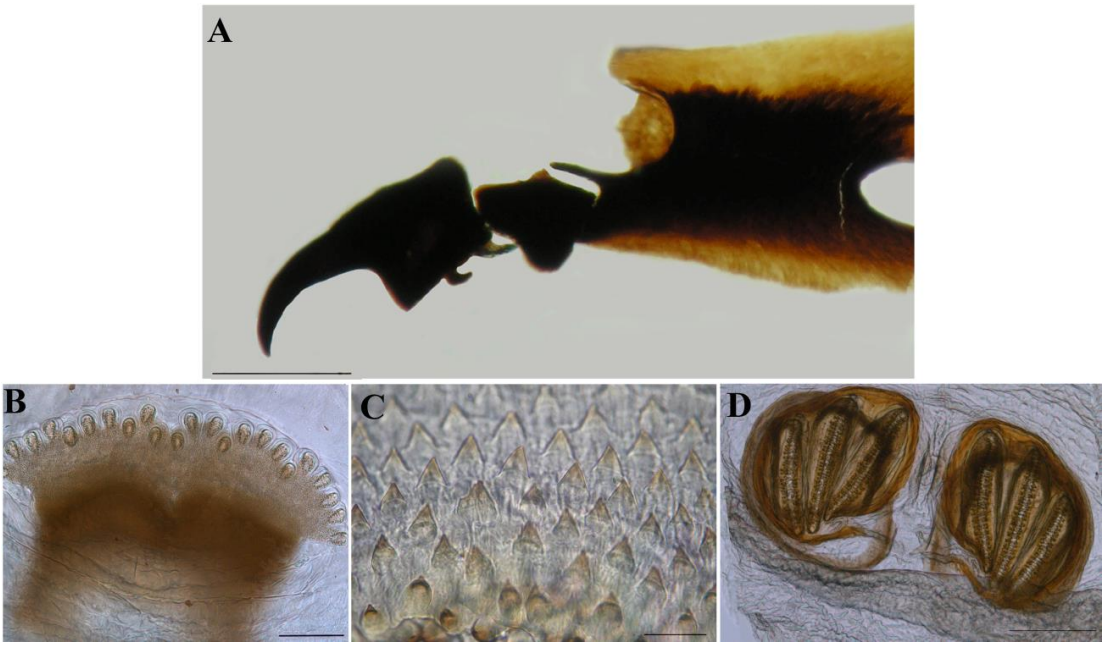

Figure 5. *Peckia* (*Pattonella*) *intermutans*. A: cephaloskeleton, lateral view; scale: 1mm. B: anterior spiracle; scale: 0,5mm. C: abdominal spines; scale: 1mm. D: posterior spiracles; scale: 1mm.

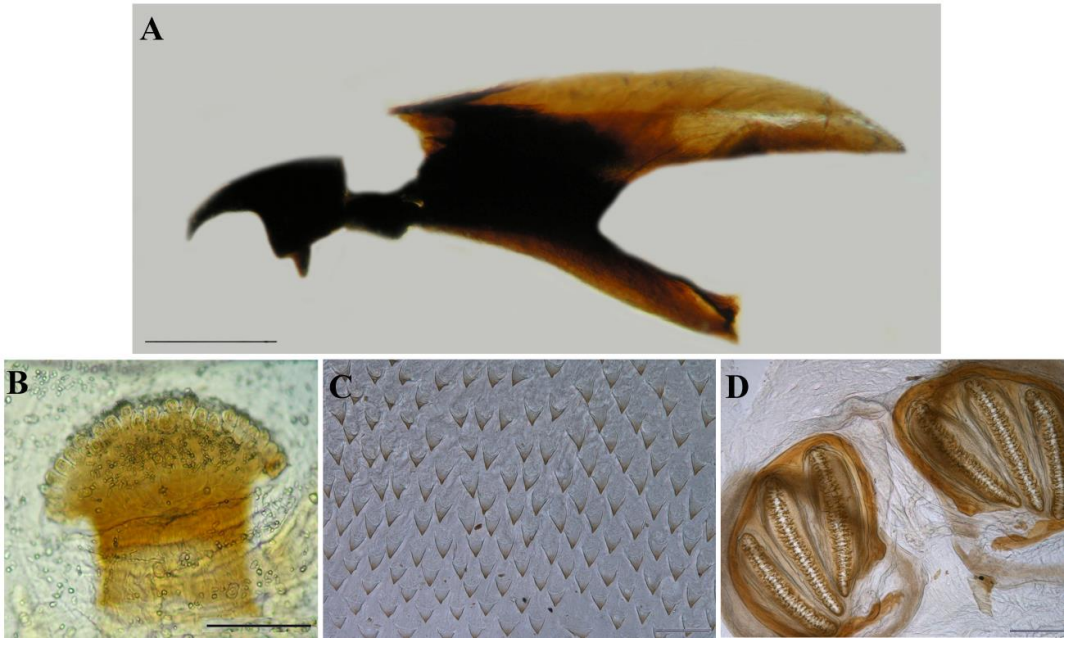

Figure 6. *Peckia* (*Pattonella*) *resona*. A: cephaloskeleton, lateral view; scale: 1mm. B: anterior spiracle; scale: 0,5mm. C: abdominal spines; scale: 1mm. D: posterior spiracles; scale: 1mm.

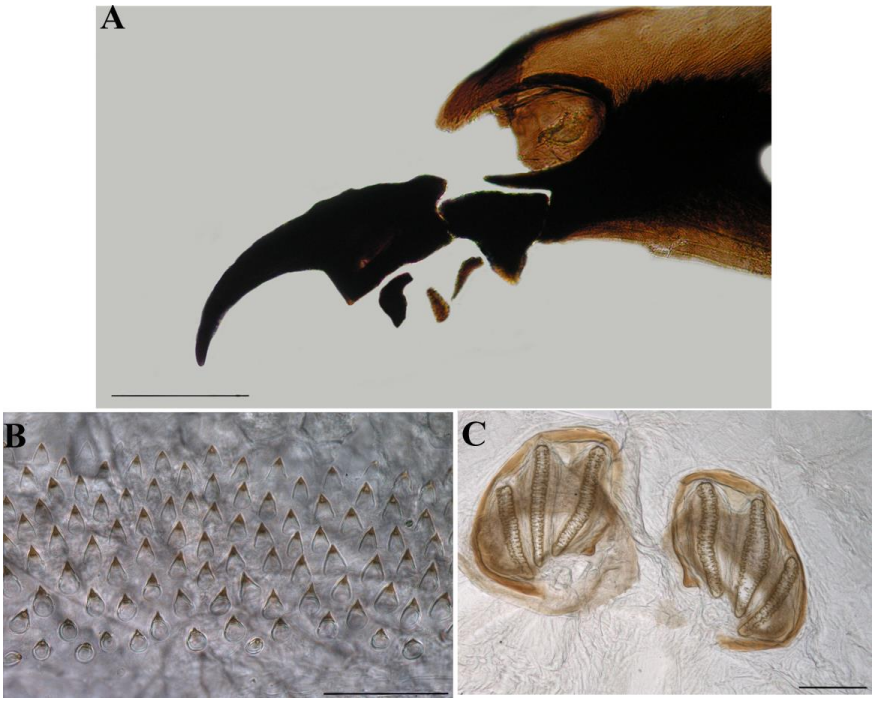

Figure 7. *Peckia* (*Euboettcheria*) *australis*. A: cephaloskeleton, lateral view; scale: 1mm. B: abdominal spines; scale: 1mm. C: posterior spiracles; scale: 1mm.

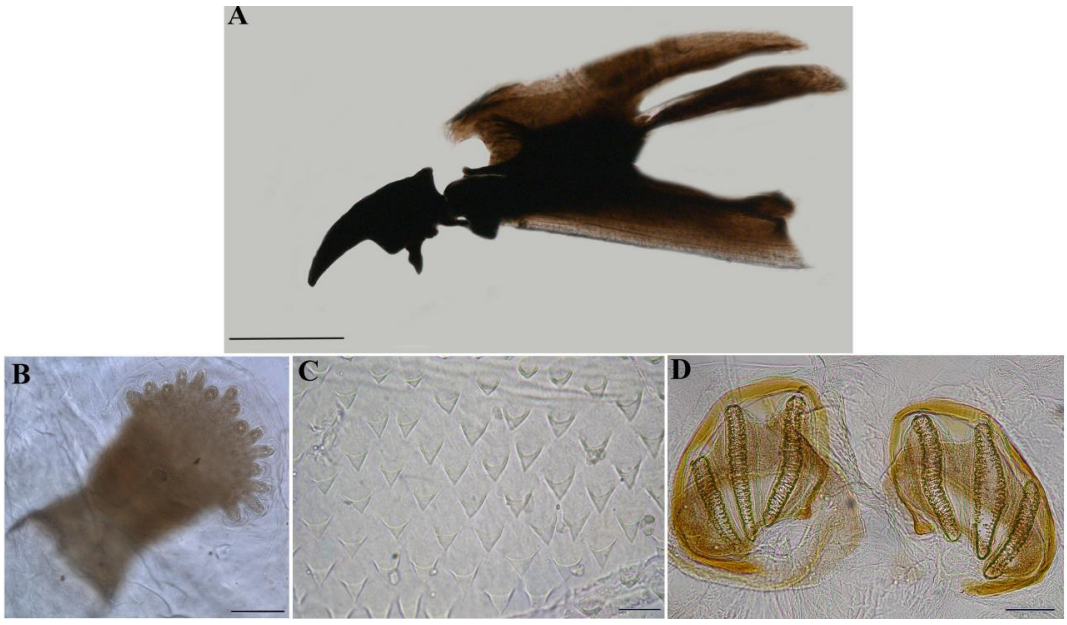

Figure 8. *Peckia* (*Euboettcheria*) *florencioi*. A: cephaloskeleton, lateral view; scale: 1 mm. B: anterior spiracle; scale: 0,5mm. C: abdominal spines; scale: 1mm. D: posterior spiracles; scale: 1mm.

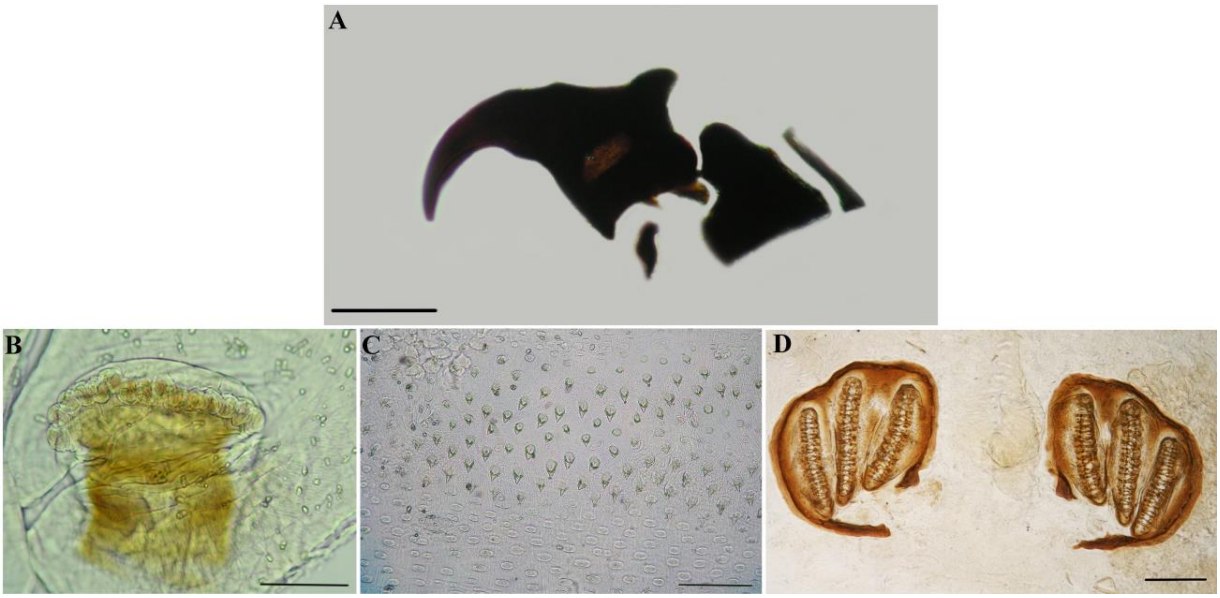

Figure 9. *Microcerella halli*. A: cephaloskeleton, lateral view; scale: 1mm. B: anterior spiracle; scale: 0,5mm. C: abdominal spines; scale: 1mm. D: posterior spiracles, scale: 1 mm.

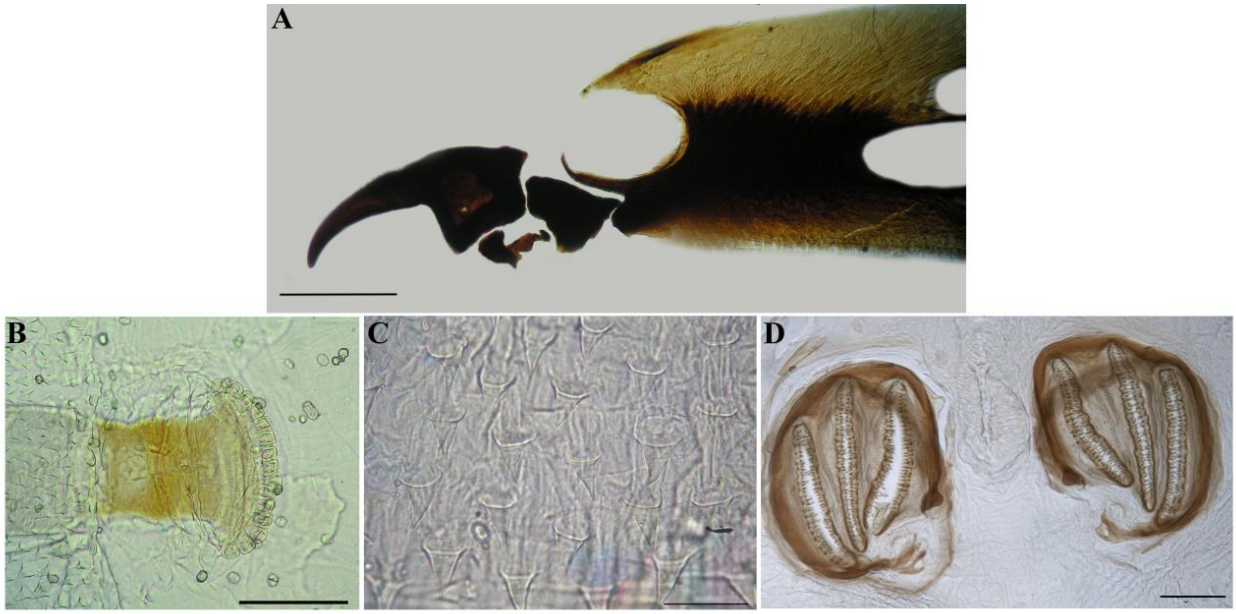

Figure 10. *Sarcophaga* (*Bercaea*) *africa*. A: cephaloskeleton, lateral view; scale: 1mm. B: anterior spiracle; scale: 0,5mm. C: abdominal spines; scale: 1mm. D: posterior spiracles, scale: 1mm.

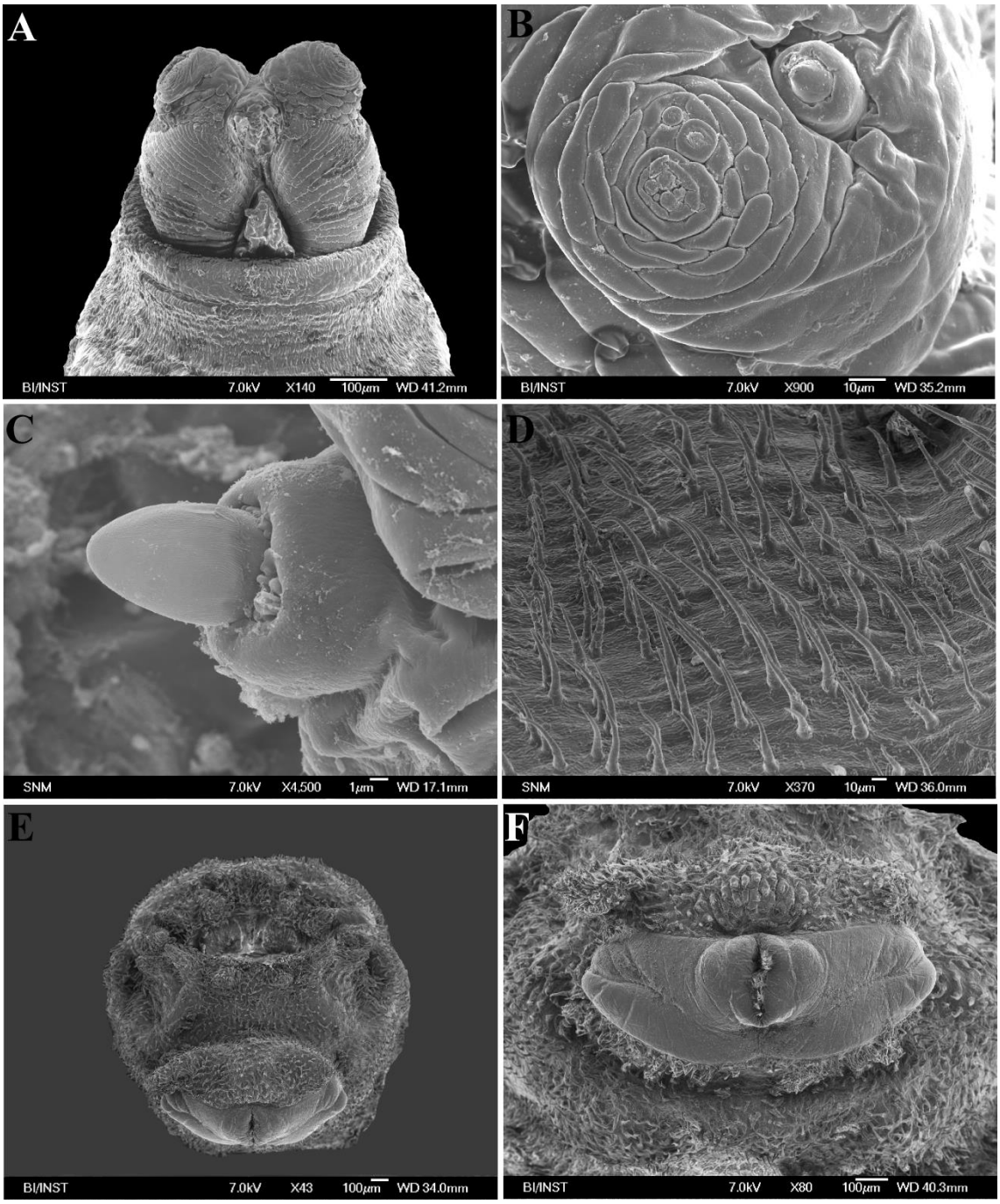

Figure 11. SEM of *Oxysarcodexia paulistanensis*. A: pseudocephalon; B: antenna and maxillary palpus; C: antenna; D: ventral spines (A3); E: anal division; F: perianal pads.

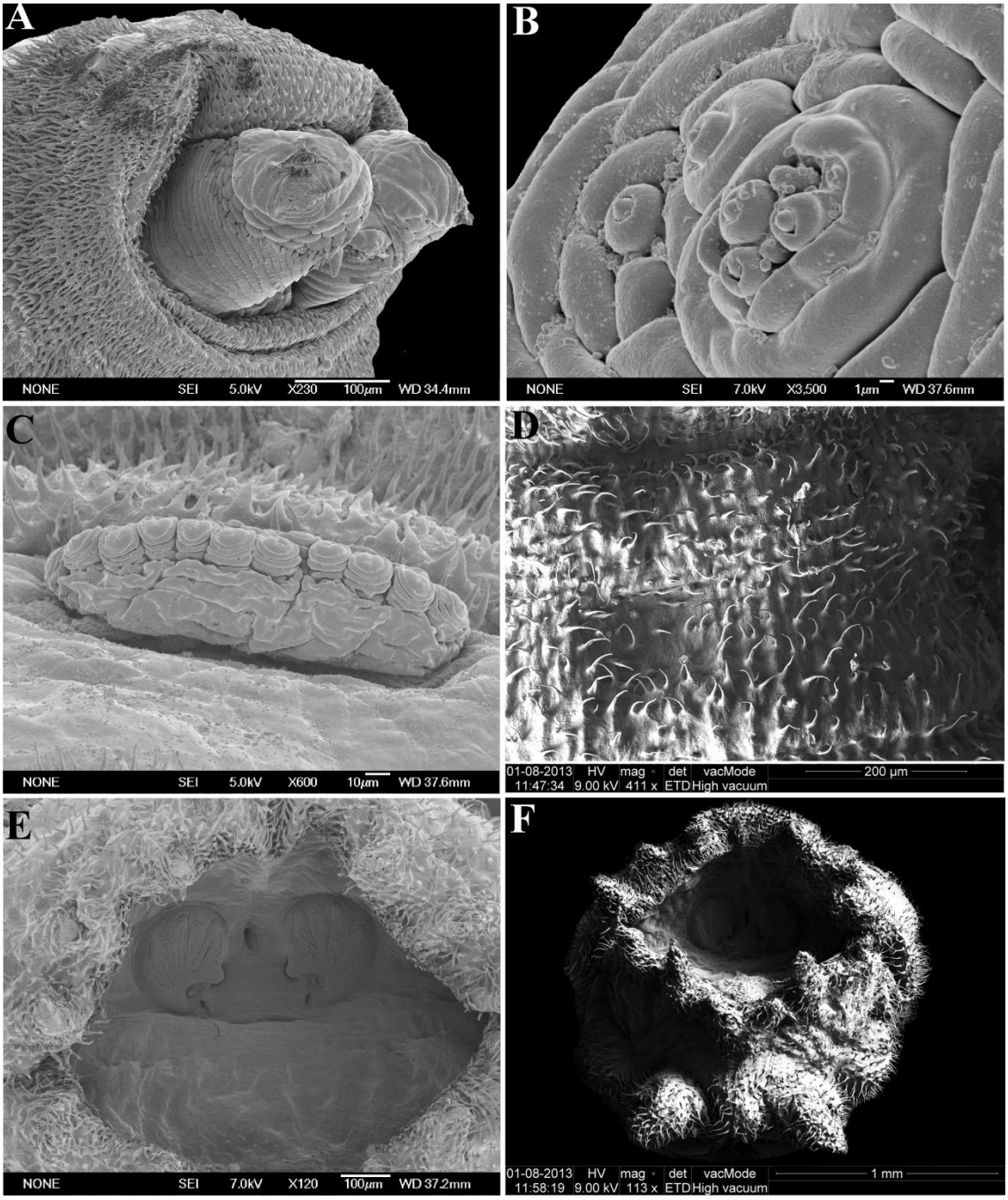

Figure 12. SEM of *Oxysarcodexia riograndensis*. A: pseudocephalon; B: maxillary palpus; C: anterior spiracle; D: dorsal spines (A7); E: posterior spiracle; F: anal division.

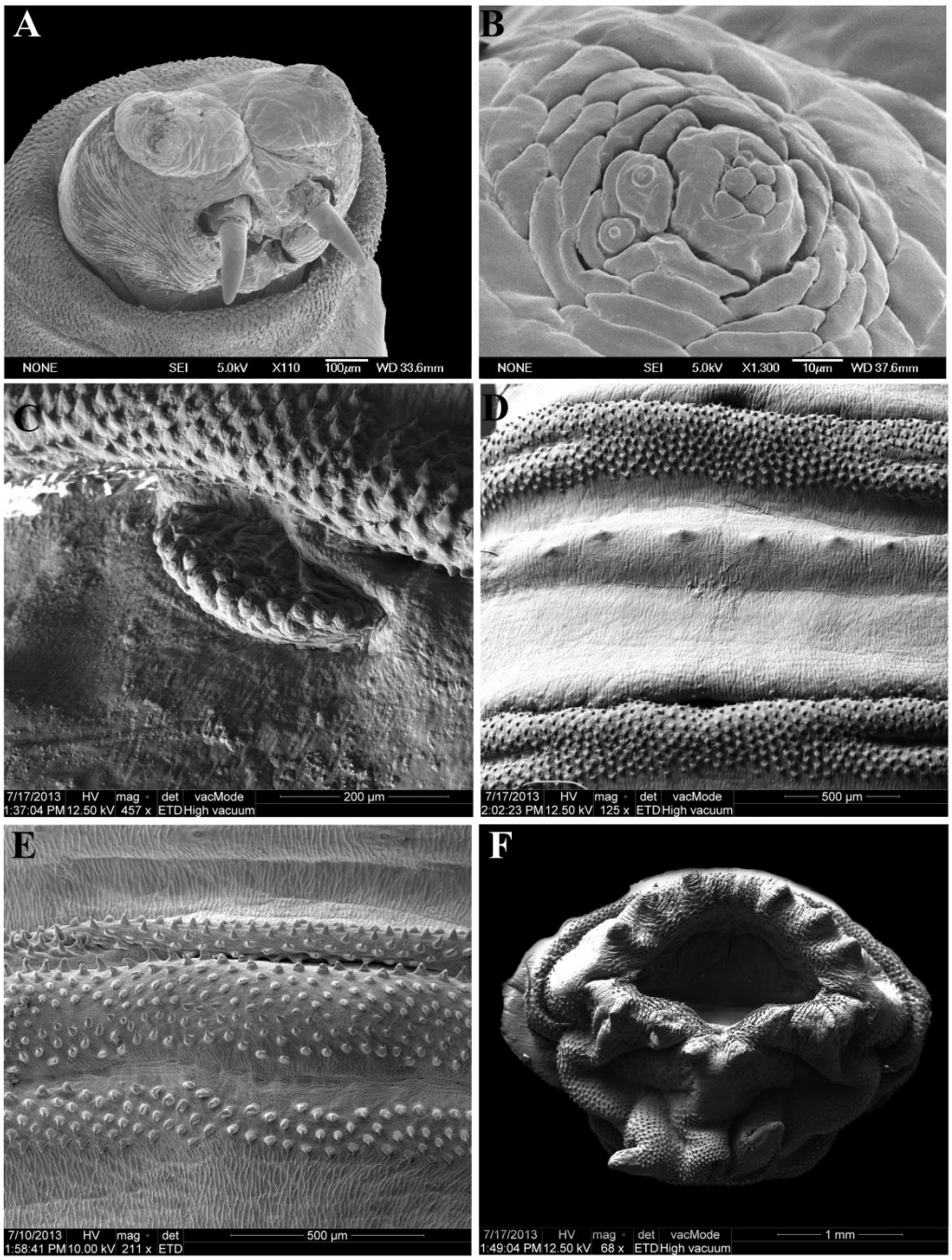

Figure 13. SEM of *Peckia* (*Pattonella*) *intermutans*. A: pseudocephalon; B: maxillary palpus; C: anterior spiracle; D: dorsal papilla (A6); E: ventral spines (A4); F: anal division.

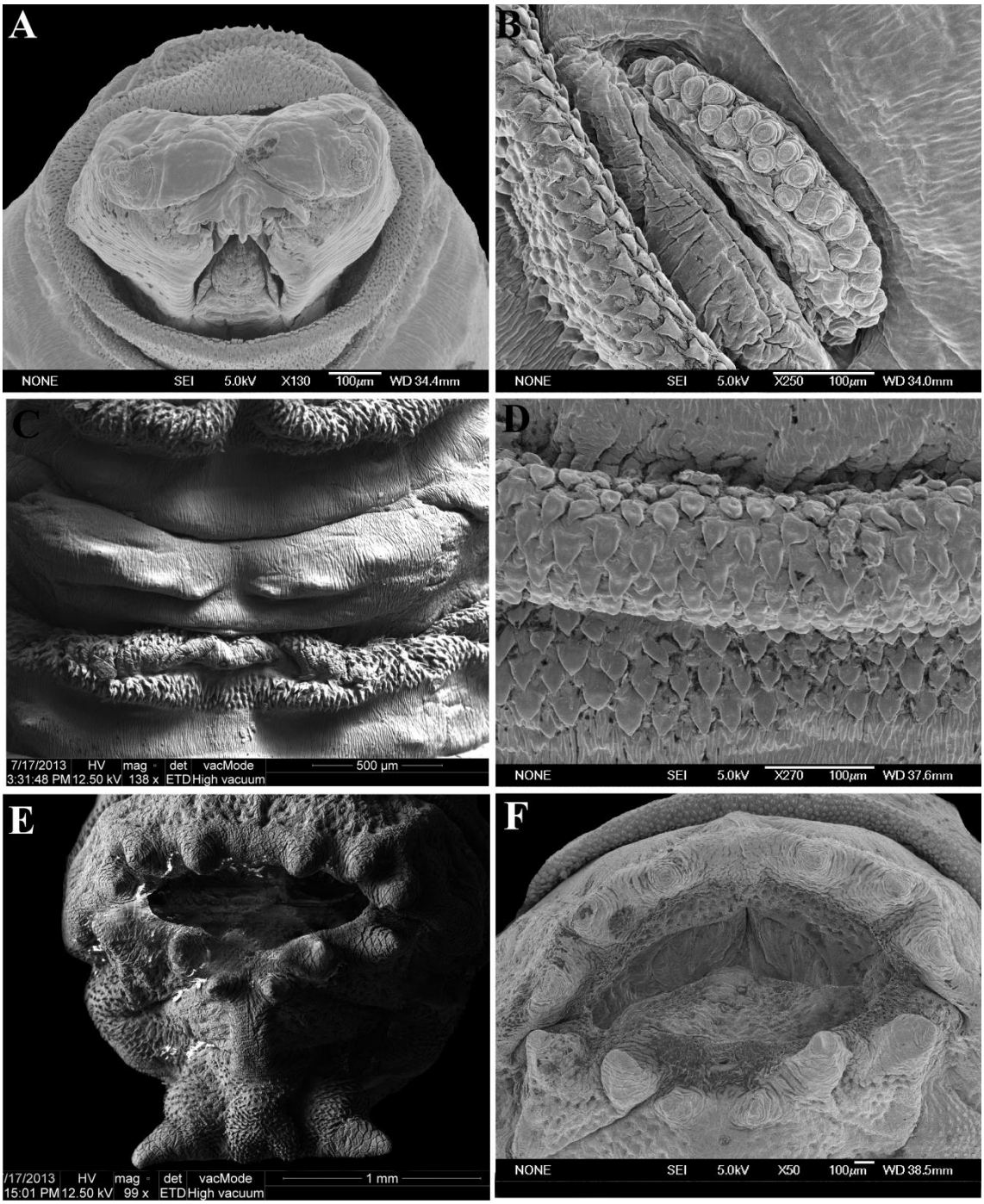

Figure 14. SEM of *Peckia* (*Pattonella*) *resona*. A: pseudocephalon; B:anterior spiracles; C: ventral papilla (A1); D: dorsal spines (A3); E: anal division F: anal papilla.

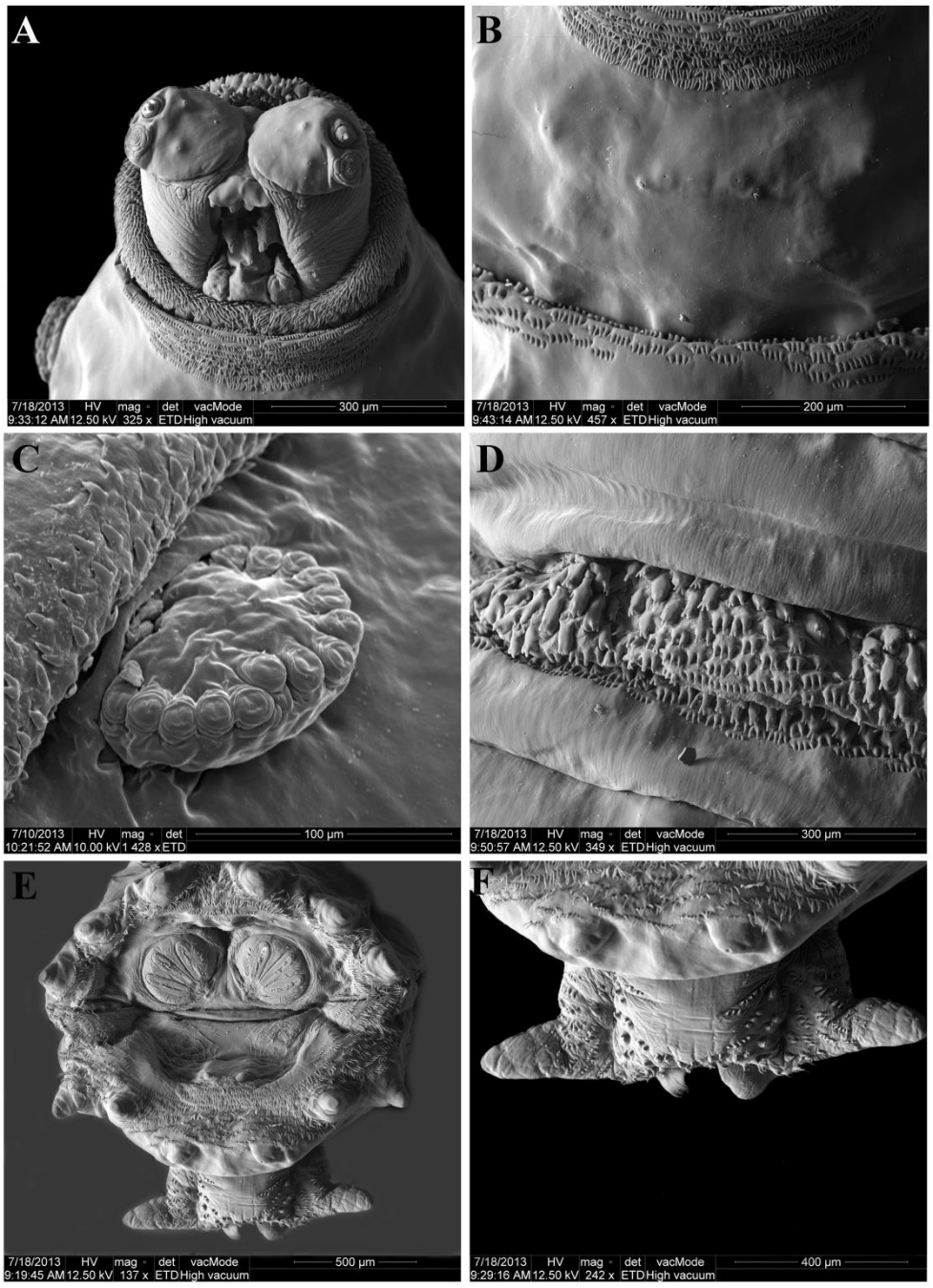

Figure 15. SEM of *Peckia* (*Euboettcheria*) *australis*. A: pseudocephalon; B: sensilla (T2), ventral; C: anterior spiracle; D: ventral spines (A5); E: anal division; F: anal pads.

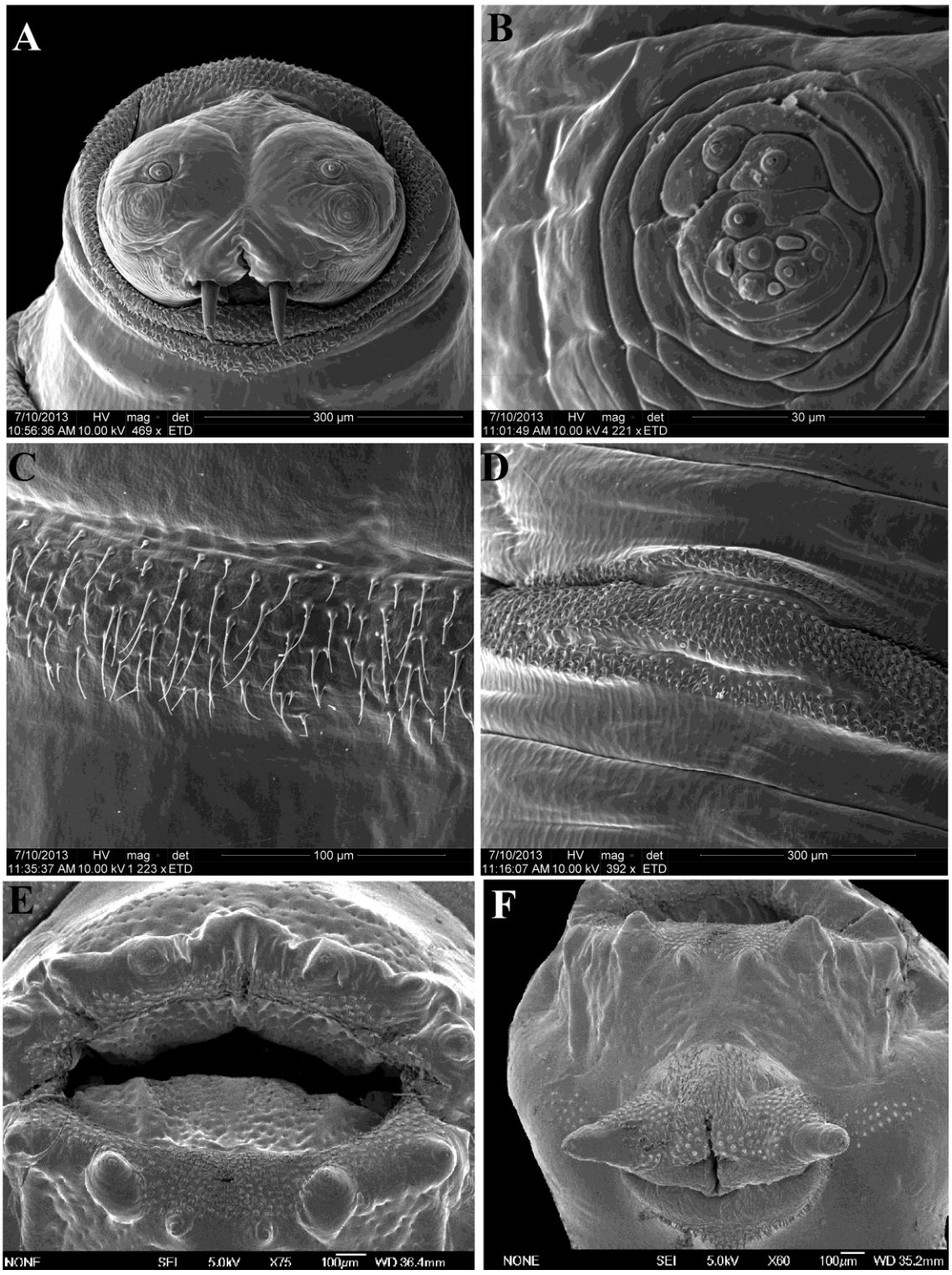

Figure 16. SEM of *Peckia* (*Euboettcheria*) *florencioi*. A: pseudocephalon; B:maxillary palpus; C: dorsal spines (A3); D: ventral spines (A4); E: anal division; F: anal pads.

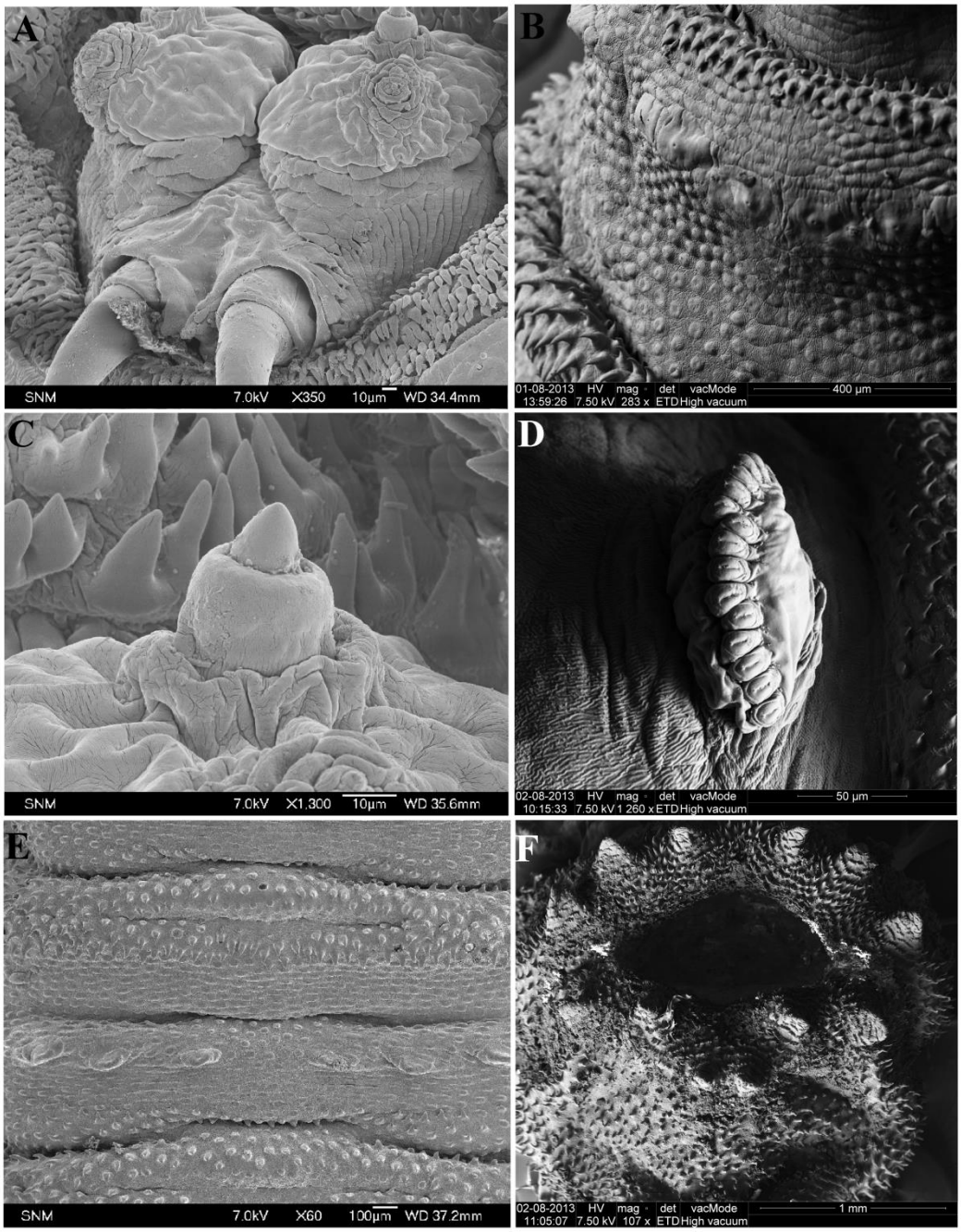

Figure 17. SEM of *Microcerella halli*. A: pseudocephalon; B: warts (A2); C: antenna; D: anterior spiracle; E: ventral spines and papilla (A5); F: anal division.

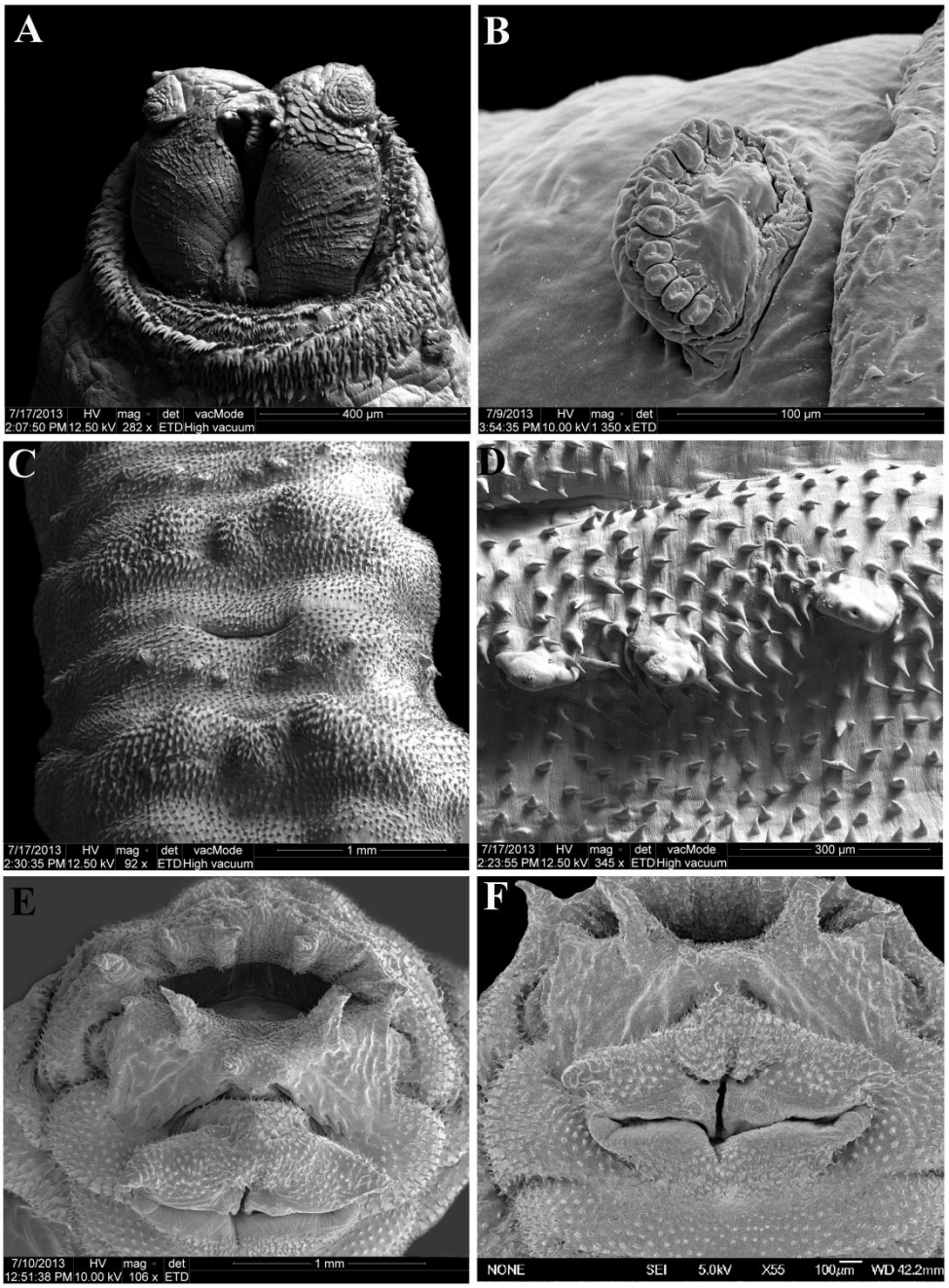

Figure 18. SEM of *Sarcophaga* (*Bercaea*) *africa*. A: pseudocephalon; B:anterior spiracle; C: dorsal spines (A5); D: papilla, ventral(A5); E: anal division; F: anal opening.

Discussion

The identification of fleshflies larvae is considered a difficult task comparing to other Diptera families (Mendonça *et al.*, 2013). The most recent studies about Sarcophagidae larvae are mainly restricted to Miltogramminae (Szpila & Pape, 2005a; Szpila & Pape, 2005b; Szpila & Pape, 2007; Szpila & Pape, 2008) which have many characters used for identification as opposed to Sarcophaginae, a subfamily that are morphologically much more homogeneous.

In Neotropical region, where the fauna is diverse and medical and forensic entomology studies are growing, there is a need to methodological improvements toward flies immature stages identification. One possibility of methodological improvement is to use molecular biology (Amorim *et al.*, 2014), but the drawbacks of this methodology for forensic entomology are that it is not widespread used in Police Departments and the costs to use molecular markers in every case would be prohibitive. For this reason, traditional taxonomic work shows to be the best investment to identify material from a death scene or from a myiasis wound.

The studies addressing flesh fly species from Neotropical Region usually fall between two extremes, SEM analysis or optical microscopy. However, neither of these approaches alone are enough to find diagnostic characters (Lopes, 1943; Lopes, 1982; Mendonça *et al.*, 2013, Buenaventura, 2013). So, the best way to find inter specific diagnostics characters is to combine different techniques like SEM and optical analysis.

This work describes, based in external and internal morphology, eight forensic important species. The key characters for species identification were the cephaloskeleton, the distribution of spines, the anterior spiracle and the presence of hairs, spines and warts on surface, a pattern similar to other Sarcophagidae larvae (Kano & Sato, 1951; Lopes, 1943; Aspoas, 1991; Szpila, 2010).

The pseudocephalon did not have any diagnostic characters for the species we study because the antenna, maxillary palpus and sensilla are similar. Usually, in the first instar larvae the ridges (or festoons) on pseudocephalon are important for classification (Lopes, 1982), which did not occurs for third instar because even with small differences in some cuticular

folds it is not diagnostic for any species or groups. It contrasts with other fleshflies subfamilies such as Miltogramminae, where the antenna is specie specific (Szpila & Pape, 2008; Szpila, 2010).

The spinulation on thoracic and abdominal segments is a strong character even in lower taxonomic scale, such as within subgenera, like *Euboettcheria*. In *Microcerella*, *Pattonella* and *Bercaea* the presence and distribution of papilla on interband segments are a diagnostic character. The number and disposition of anterior spiracle papilla can be used to identify species, although there are many intraspecific variations.

In the anal segment ithe key characters are the distribution of spines, the size and length of anal papilla and the development of perianal pads. In addition, the posterior spiracles can show some differences in the peritreme thickness, distance between slits and the presence of the button. All these characters showed to be important to species level identification.

Undoubtfully, the key characters to identify species based on larval morphology are in the cephaloskeleton. The shape of mouthooks and accessory sclerites like “anel quitinoso ventral”, “dentado” and labial showed to be important inter and intrageneric characters (Lopes, 1943). The shape of the intermediate sclerite, the dorsal bridge, the dorsal and ventral arm length and the vertical plate width are important characters to identify third instar larvae.

Oxysarcodexia is morphologycally a homogeneous group, which makes very difficult to find specific diagnostic characters. This lack of external diagnostic characters occurs in larvae and adults, where even males and females are so similar that only details in the terminalia makes possible to distinguish (Vairo *et al.*, 2011). The third instar external morphology of *Oxysarcodexia paulistanensis* was already described by Lopes & Leite (1987) using SEM but not as detailed as in this work. This species rear and breeds on carcasses (Moura *et al.*, 2005; Barros *et al.*, 2008; Rosa *et al.,* 2011; Vairo *et al*., 2011) and is one of the most common and abundant in forensic studies. *Oxysarcodexia riograndensis* is associated to carcasses and corpses (Rosa *et al.,* 2011; Oliveira & Vasconcelos, 2010; Vairo *et al*., 2011) but did not had the larval morphology described. In third instar larvae, both species are hairy, making difficult to find external characters. Even the distribution of spines could not be described in details because it was impossible to check, by optical

analysis, the limit between the hairs and the spinose bands. For this reason even after a deeply study we could not find robust characters to discriminate these two *Oxysarcodexia* species.

Peckia (*Pattonella*) *intermutans* and *Peckia* (*Pattonella*) *resona* third instar are described for the first time and such as the adults the larvae are morphologically similar. These species are associated to corpses and forensic surveys (Moura *et al*., 1997, Moura *et al*., 2005, Barros *et al*., 2008, Vairo *et al*., 2011; Rosa *et al*., 2009; Oliveira & Vasconcelos, 2010). The cephaloskeleton sclerites and the distribution of spines in the anal segment are the main characters to identify then. Although the larval size is not a good diagnostic character because is related to the amount of food available, these two species together with *Microcerella halli* are twice the size of *Oxysarcodexia spp.* and *Euboettcheria spp*.

The distribution of spines easily distinguish *Peckia* (*Euboettcheria*) *australis* and *P*. (*E*.) *florencioi*, which has T2 with the anterior spinose band interrupted on lateral surface. This character has never been described before in other species. These species were already collected in decaying carcasses (Rosa *et al*., 2011; Vairo *et al*., 2011).

Microcerella halli is associated to decaying process in animals (Moretti *et al*., 2009, Vairo *et al*., 2011). The cephaloskeleton of *M. halli* was already described in details by Lopes (1943) and our description did not differ from this previous description in any significant way. The most important character of *M*. *halli* is the presence of warts in whole surface of the body which is a kind of tissue modification present in other Sarcophagidae subfamilies (Szpila & Pape, 2005).

Sarcophaga (*Bercaea*) *africa* is a well studied species because of its broad geographic distribution and its medical and forensic importance (Villet *et al*., 2011; Medina *et al.*, 2011). The third instar larvae were already described (Augel, 2008) but we found some new characters not already took into account. This species showed a different aspect in anal papilla 5 (P5) that is more elongated and longer than the other papillae. In addition, it has spines covering all its surface making almost impossible the observation of spine distribution on the body.

As *Peckia* (*Sarcodexia*) *lambens* was already described and deeply discussed before (Vairo *et al.* submitted) we will not discuss further. Nevertheless, is important to mention that this species is broadly distributed in Neotropical Region and has forensic and medical importance (Guimarães *et al*., 1983; Fernandes *et al*., 2009; Hagman *et al*., 2005; Oliveira &Vasconcelos, 2010).

A comparative morphological framework allows to determine if is possible or not to identify Sarcophagidae third instar larvae. This work showed that it is possible for entomologists to identify Sarcophagidae larvae. However, not all larval characters are conspicuous, which highlights the need of a deep morphological analysis using a large sample. Larger samples allow disentangling intra and interspecific variation on characters, such as the number of papilla in anterior spiracle and some rows of abdominal spines and so, to better define character states. Although we provide ways to forensic experts to identify these Sarcophagidae species, without a basic knowledge in entomology this process may be not achievable.

In Southern Brazil, the major material received by forensic entomologists to species identification is third instar fly larvae. The key we provide make the identification easier, without the need of rearing until the adult emergence, saving time and money. As some of these species have broad geographic ranges the descriptions and pictures are of broad interest.

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CAPÍTULO III

Flies and Decay: The role of Acetophenone and Indole for *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830) attractiveness

Flies and Decay: The role of Acetophenone and Indole for *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830) attractiveness

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Abstract

Sarcophagidae flies are one of the most important insects for forensic entomology. The decaying process of a cadaver releases volatile organic compounds (VOCs) that can be perceived by necrophagous flies. Although there are profiles for death compounds in literature there is still a gap in the understanding of insect-VOCs interplay. Therefore, to fill this gap, we tested the attraction of VOCs released by rat carcasses in *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830) (Diptera: Sarcophagidae). We used an array of chemical ecology methods such as headspace collection of volatiles, coupled Gas Chromatography-mass Spectrometry (GC-MS), Gas Chromatography with Electroantennographic detection (GC-EAD) and bioassays to identify the compounds responsible for fly attraction. After analysis, indol and acetophenone were identified as the compounds responsible for electrophysiological responses in the species tested. Also, these compounds play a key role in attractiveness of *Peckia* (*S*.) *lambens*.
These results show that a qualitative analysis is important but to understand the entomological succession a quantitative analysis regarding concentration can reveal differences in attractiveness between species.

Key-Words: Sarcophagidae, *Peckia* (*Sarcodexia*) *lambens*, tanatochemistry, forensic entomology.

Introduction

The human decomposition starts after death with autholysis progressing to *livor mortis*, *algor mortis*, *rigor mortis* and putrefaction [1]. The chemical reactions resulting from these processes produce volatile organic compounds (VOCs) that are liberated from the death body [2-4]. These VOCs are a chemical trail to the corpses and also a signal of the ongoing decomposition process. These decompositional odors are used for training dogs to detect human remains and to determine the *post mortem* interval through the pattern of compounds released [5-7]. Recently, there is a growing interest to understand the role of these compounds in fly attraction to corpses, and applying this knowledge in forensic entomology.

The cadaveric VOCs are probably responsible for the attraction of necrophagous beetles and flies to corpses [8]. The olfaction is a major source of information for insects in such a way that morphological changes in specific organs, such as antennae, can be associated with increased capacity of odour discrimination [9]. Insects use olfaction for major tasks such as finding resources and mates [10-11]. Since odours emanating from carcasses follow a pattern corresponding to the decomposition process, some of those compounds could be used as cues for necrophagous insects. Therefore, the characterization of such compounds can enhance our compreenhension on insect activity in corpses and its forensic utility. Also the association between compound identity and decision associated with ovipostion patterns will broaden our knowledge of mechanism driving successional patterns. Thus, characterizing odors sources can help understanding their activity on insects biology and behavior [12-13]. For example, in blowflies, the compounds emmited from carcasses help identify the best decaying stage for larval development [14].

Researchs addressing flies behavior and VOC's are rare and usually correlate previously described compounds and insects, without comparison of behavior between species of different families. This comparison between species that occur during the decay process in the same region could help to understand if there is an expected successional pattern.

There are many Coleoptera and Diptera species involved in the decomposition process around the world and adults and immature stages of flies are the most common entomological evidence collected in a death scene. Calliphoridae, Muscidae and Sarcophagidae have adults and larvae consistently recorded on death bodies, which made all biological aspects related to these fly families extremely important for forensic entomology [15-17]. Flies may be useful to estimate the time since death by analyzing the period of insect activity on the body or by entomofauna succession [18]. The successional pattern is dependent of the nutritional preferences of each species [19] and can be understood through the VOC's released during decomposition.

Considering that the relation between thanatochemistry and necrophagous flies is poorly explored, the goal of this study was to identify which chemicals trigger the attractiveness of *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830) to carcasses. *Peckia* (*Sarcodexia*) *lambens* is well distributed in the Neotropical Region and was registered as mysiasis inducers in vertebrates and rearing on corpses [20-22]. This species have adults registered for the bloated stage and dry remains in pig carcasses [30] and advanced decay on rabbit carcasses (Vairo, personal observation).

This species was selected by it attested importance to forensic entomology representing one of the most important Diptera families attracted by corpses.

Material and Methods

Rat carcasses and volatile collection

The volatile collection was made using rat carcasses inoculated with fly larvae. Two rat carcasses (*Mus musculus* L.) weighing approximately 30g were sacrified and inoculated with 20 first instar larvae of *Peckia* (*Pattonella*) *intermutans* (Walker 1861). The larvae were inoculated in oral cavities, ears and anus to simulate usual larviposition sites. Then, the carcasses were individually placed in plastic bags which allowed volatile collection in an experimental setup adapted from Zarbin *et al*. and Runyon *et al.* [23-25] (Fig.1). Each chamber (plastic bags) was submitted to a continuous flow (0,75 L.min $^{-1}$) of humidified

charcoal-filtered air. The volatiles were adsorbed in a Porous Polymer HayeSep® (matrix HayeSep D, 80-100 mesh, Sigma-Aldrich). The desorptions were performed using 300µL of dichlorometane (DCM) followed by 300µL of hexane.

The decaying process of the rat carcasses was determined by external characteristics of these carcasses and it was divided in: fresh (9 hours), bloated stage/decay/ advanced decay (09- 120 hours) and dry remais (120-168 hours) (Fig. 2). The distinction between bloated, decay and advanced decay was not possible because of the small size of the carcass. In addition, we decided not to include the fresh stage in the analysis because of low volatile concentration and reduced forensic importance regarding the attraction of insects [29-30].

The experiments were run in constant temperature (25^{o}C) and photoperiod (12L: 12D hours). Vollatile sampling was done based on decompositional stages (fresh, advanced decay and dry remains): each 3 hours (during the first 15 hours); each 6 hours (15-27 hours) and each 12 hours until the decaying process ended. Each carcass was considered one replica but since the compounds profile were similar between extracts of the same decomposition stage, after GC analysis we mixed the extracts from each stage of the decomposition for posterior analysis.

Chemical analysis

Extracted volatiles were analyzed with a Shimadzu GC2010 Gas Chromatograph equipped with an FID detector, a SLB^{TM} –5ms (Supelco, 30 m×0.25 mm×0.25 µm film thickness) capillary column, and helium as the carrier gas. The GC was operated in splitless mode (injector temperature: 250°C). The oven temperature began at 50 °C for 1 min and increased by 7 °C/min until reaching 270°C, which was maintained for 10 min. To determine the retention indexes [31] we used a solution containing the straight chain hydrocarbons C10-C26 (concentration: 10 ppm each). Gas chromatography–mass spectrometry (GC/MS) data was acquired using a Shimadzu QP2010-Plus electron ionization mass detector operating in electron impact mode (70 eV) with an SLB^{TM} –5ms (Supelco, 30 m×0.25 m×0.25 µm) capillary column. The

compounds which presented eletrophisiollogical responses were identified by mass spectrometry comparison with comercial libraries NIST27 and NIST147, retention indexes and coinjected with reference compounds to confirm identification.

Rearing of flies

Peckia (*Sarcodexia*) *lambens* used in GC-EAD and bioassays was reared at constant temperature (25^{o}C) and photoperiod (12L: 12D hours) in a rearing room. Flies were captured in Curitiba (Paraná) (25°27'16"S 49°14'9"W) and adults fed with a diet composed of sugar and powder milk (1:1), water and fresh bovine minced meat until the larviposition occured. First instar larvae of *Peckia* (*Pattonella*) *intermutans* (Walker, 1861) (Diptera: Sarcophagidae) were inoculated on carcasses just after eclosion.

Colonies were established and maintained during all bioassays and larvae were reared using an appropriated diet [32].

Freshly emerged males and females of all species were kept together to copulate cages with fresh meat available. Only mated females of 10-15 days of age were tested because they promptly search for a substract for oviposition, an important characteristic for a forensic experiment.

Electroantennography

The identification of eletrophysiollogical active compounds to *P.* (*S.*) *lambens* was perfomed using a Shimadzu GC2010 Gas Chromatograph equipped with an electroantenography system Syntech 35 (Hilversum, Netherlands) and using mated females antennae. The oven temperature began at 50 °C for 1 min and increased by 7 °C/min until reaching 250 °C, and maintained at this temperature for 10 min.

After one minute in approximately 3^{o}C, each fly had its head removed and immediately mounted in electrodes using a conductive gel (Sigma gel, Parker Labs., EUA) (Fig. 4). The chromatograms were viewed with Syntech GC-EAD32 (4.6 version).

Olfactometer Bioassays

The attractiveness of *P.* (*S.*) *lambens* by different decaying stages was tested at the same concentrations as the samples collected from rat carcasses. The experiments were conducted in 25°C and between 08:30 hours and 14:00 hours. Before and after this period the flies did not present the same behavior.

Behavioral responses were tested in a Y-tube olfactometer using humidified, charcoal-filtered air flowing at 1.5 L/min. The olfactometer consisted of a Y-shaped glass tube (4×40 cm) with two 20 cm arms. The odor sources were placed at the ends of the arms and the tube inclinated at 45° after previous tests were conducted (Fig. 3). Each odor source consisted of a piece of filter paper (2×2 cm) impregnated with 2 µl of extracts or hexane (control). To record the atractiveness response a fly was introduced into the olfactometer's base and its behavior observed for 5 minutes. A positive response implied that the fly walked against the airflow more than 5 cm into an arm towards the odour source. Each insect was considered one replica (n=50) and was tested only once. The odour source was replaced after every test and the olfactometer was inverted after every 5 tests to exclude any external influences. The same protocol was used to test for the attraction of flies to synthetic EAD responding compounds.

The data on choice experiments was analysed using a chi-square test with R (R Core Team, 2013). The conducted bioassays are described in Table 1.

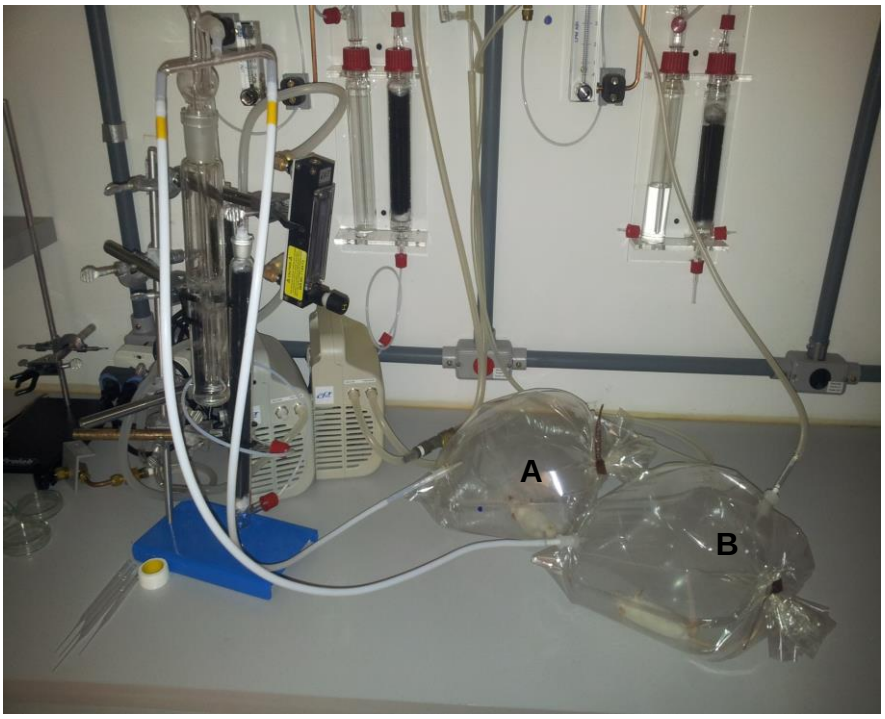

Figure 1. Headspace volatile collection system adapted from Zarbin et al. and Runyon et al. [23-25]. A, B: rat carcass placed in a plastic bag for headspace volatile collection.

Dual Choice Experiments (2µl)	
Bioassays (A)	Advanced decay *against* Hexane Dry remains *against* Hexane
Bioassays (B)	Advanced decay *against* Dry remains
Bioassays (C)	Acetophenone + Indole *against* Hexane (9: 1) (100 ppm)

Table 1. The logical structure of dual- choice experiments. All bioassays were performed with mated females (10-15 days old). (A): extracts against control (B) extract against extract (C) identified electrophisiollogy active compounds against control.

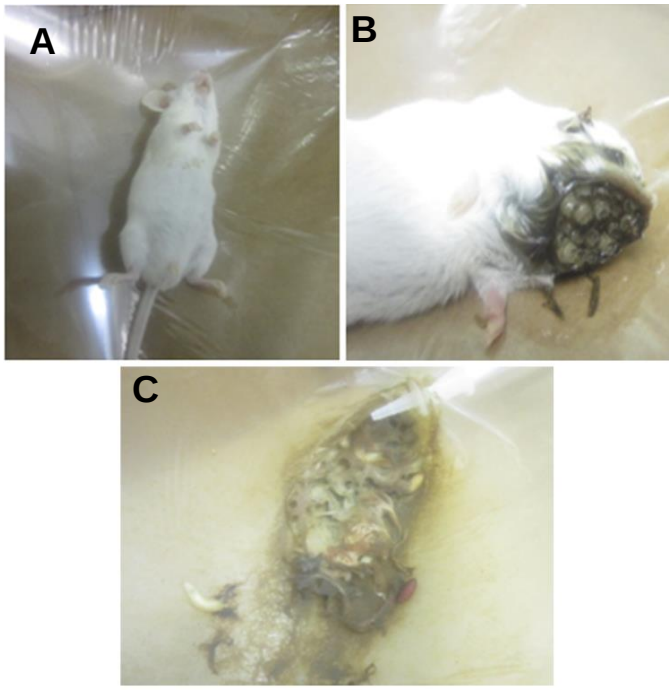

Figure 2. Rat carcasses indicating morphological changes that define our classification of decaying stages. A: fresh stage, B: advanced decay, C: dry remains

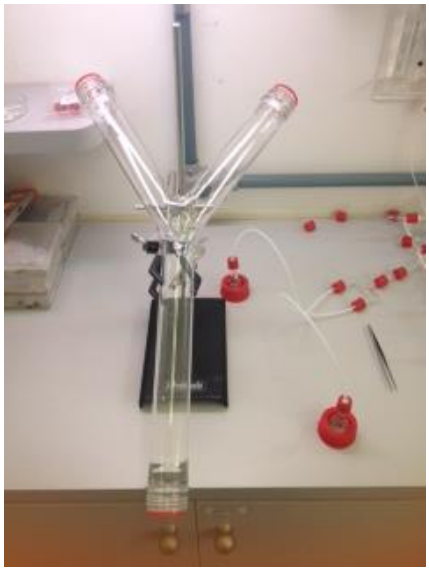

Figure 3. Y-tube inclinated device used for dual-choice experiments with mated females of *Peckia* (*S*.) *lambens*.

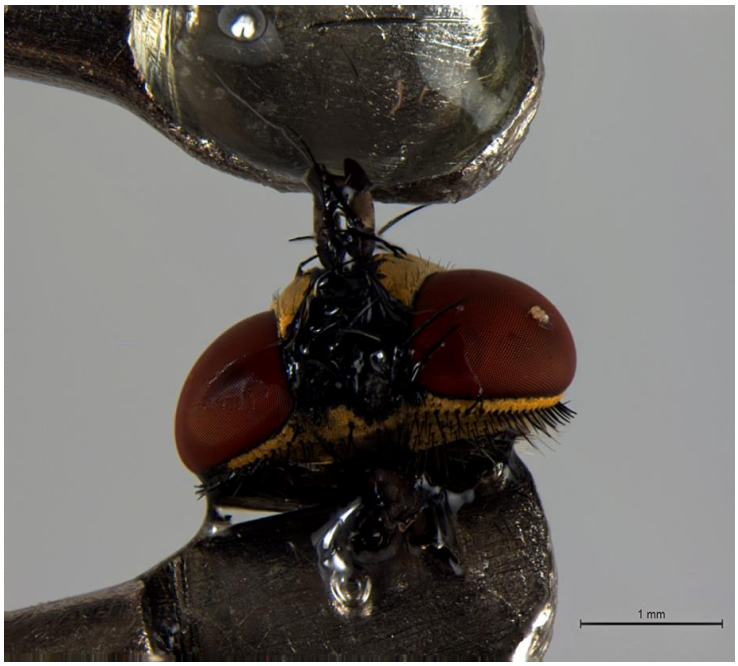

Figure 4. Head of *Peckia* (*Sarcodexia*) *lambens* mounted to perform GC-EAD. The head is mounted on eletrodes and the conductive gel is distributed between the base and the apex of the antenna.

Results

Bioassays – headspace collection and active compounds

When confronted with extracts and control, all species responded moving towards the headspace samples of advanced decay and dry remains. In advanced decay against control, *Peckia* (*S*.) *lambens* (Chi^2 = 8, p =0.004678 , df=1); *S.chrologaster* (Chi^2 = 11,52, p =0.0006885, df=1) and *S. nudiseta* (Chi^2 = 3,92, p =0.04771, df=1). In dry remains against control *Peckia* (*S*.) *lambens* (Chi^2 = 5,12, p =0.02365, df=1); *S.chrologaster* (Chi^2 =5,12, p =0.02365, df=1) and *S. nudiseta* (Chi^2 =2, 0.1573, df=1). However, when flies were allowed to choose between the two decomposition stages, species presented different behaviours, *Peckia* (*S*.) *lambens* (Chi^2 = 5,12, p =0.02365, df=1); *S.chrologaster* (Chi^2 = 0,72, p =0.3961, df=1) and *S. nudiseta* (Chi^2 = 2,88, p =0.08969, df=1).

The synthetic compounds 1 and 2 were tested (9:1) against control for *Peckia* (*S*.) *lambens* (Chi^2 = 6,48, p =0.010, df=1) demonstrating the attractiveness of this species for both compounds.

Electroantennography

The GC-EAD analysis of advanced decay compounds showed that all three species had the same two eletrophysiology active compounds (compound 1 and 2) (Figures 5-7). However, none of the dry remains compounds trigered a response for any species tested in this work.

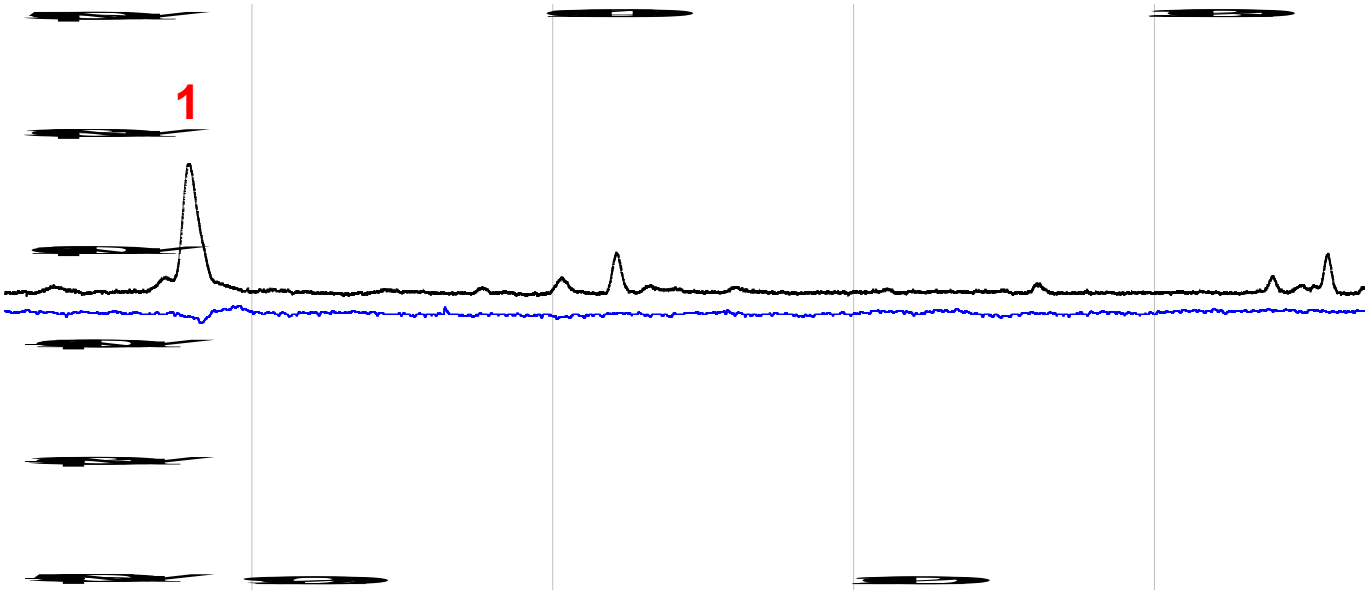

Figure 5. Electroantenogram of *Peckia* (*Sarcodexia*) *lambens* showing the activity for compound 1 and 2.

Identification of compounds

The compound 1 showed a retention time of 8,8 minutes and a retention index of 1070. Through the analysis of fragmentation patterns [*m*/*z* (%)120 (34), 105 (100), 77 (79), 51 (25), 43 (11)], NIST library comparison and retention index we suggest that this compound is acetophenone (Fig. 6). The compound 2 has a retention time of 13,58 and a retention index of 1305. Through the analysis of fragmentation pattern [*m*/*z* (%)117 (100), 90 (48), 89 (31), 63 (10), 58 (10)], NIST library comparison and retention index we suggest that this compound is indole (Fig. 7). The identification was confirmed by co-injection with synthetic compounds of acetophenone and indole (Figs. 8 and 9). The synthetic standards of indole and acetophenone were additionally tested with GC-EAD and lead to positive results.

Acetophenone was also found in dry remains extracts but with a lower concentration compared to the advanced decay. Indole was also recorded in fresh stage but with a lower concentration compared to advanced decay.

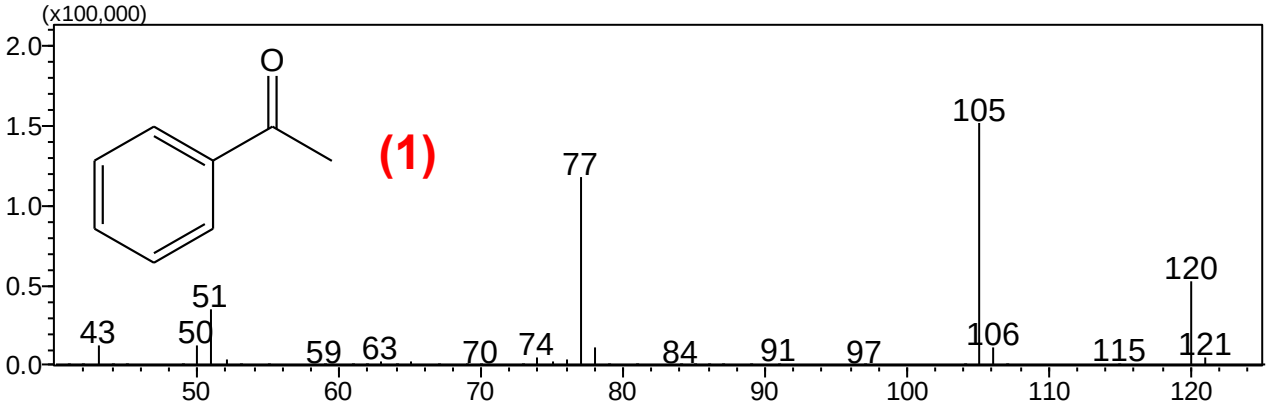

Figure 6. Spectra and structure of acetophenone (compound 1).

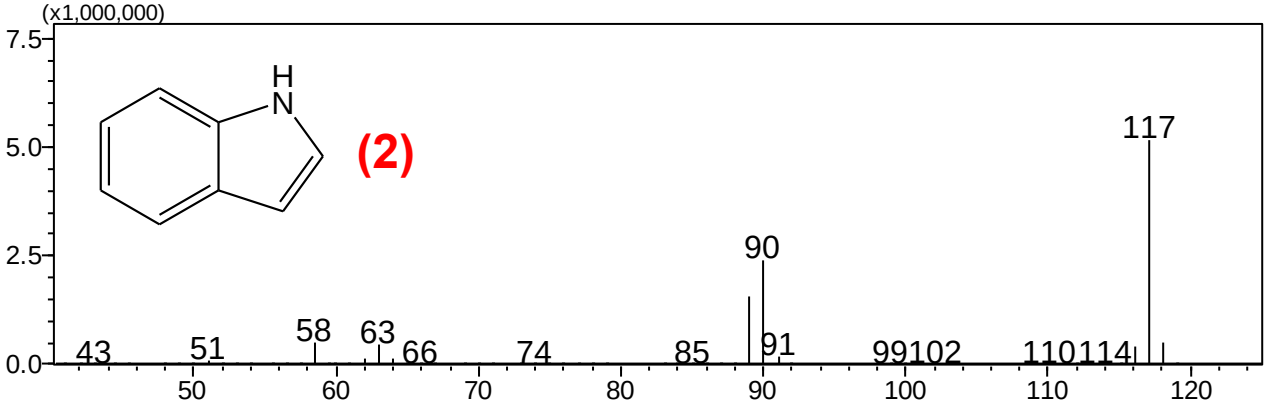

Figure 7. Spectra and structure of indole (compound 2).

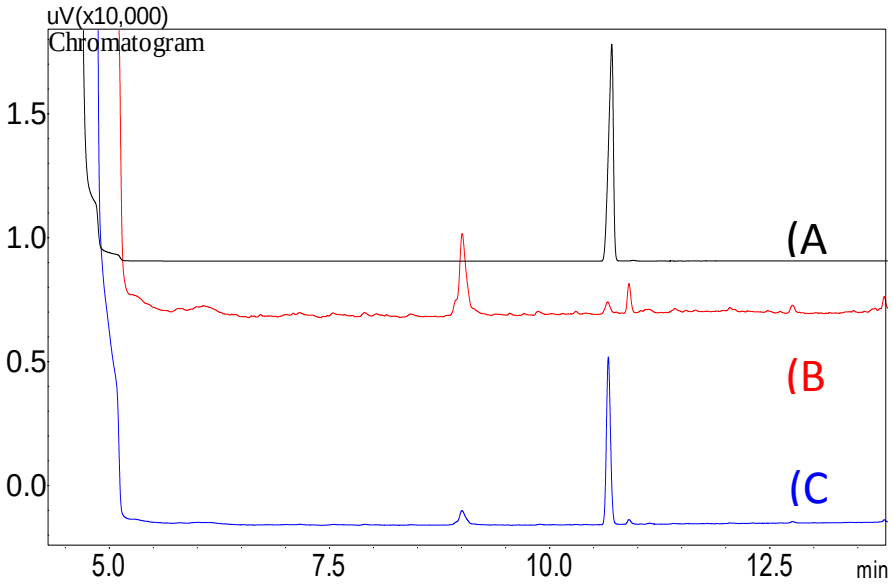

Figure 8. Co-injection of acetophenone. A= acetophenone (synthetic), B= extract from carcasses, C= co-injection

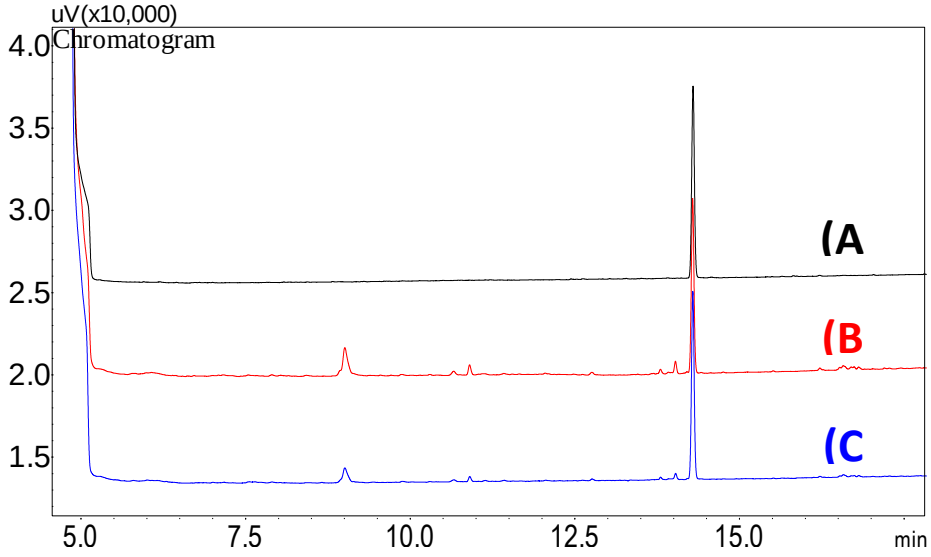

Figure 9. Co-injection of indole. A= indole (synthetic), B= extract from carcasses, C= co-injection.

Discussion

After the analysis we found that indole and acetophenone are responsible for attractiveness in *P.* (*S.*) *lambens*. The results showed that the behavior of these flies previously described in literature have a chemical explanation.

Peckia (*Sarcodexia*) *lambens* adults were already registered in bloated stage and dry remains in pig carcasses [30] and in advanced decay on rabbit carcasses (Vairo, personal observation), corroborating the information provided herein.

This result suggests that a successional pattern could be attributed not just to different attractive compounds but to differences in concentration that could stimulates or not the insect. One example is the presence of acetophenone in low concentration in dry remains that did not register any eletrophysiological response. However, *Peckia* (*Sarcodexia*) *lambens* is attracted by indole and acetophenone showing that if these compounds are registered in decay processes, mature females will probably be present looking for a reproduction source.

Understanding the interaction between the decomposition process and necrophagous insects is one of the most fundamental aspects when building a theoretical framework for forensic entomology. In this context, two questions emerge: which cues insects use to locate the corpse and if these cues change along the sucession. Our analysis showed that indole and acetophenone are potentially the main VOC's responsabile for attraction in the three species tested.

Numerous researches has been made to gather information regarding the pattern of decaying compounds considering many variables such as temperature, soil, air and body conditions [4,8,34]. The problem is that few compounds were recorded consistently, mainly due to differences in methodologies and techniques [26]. At the same time, some classes of compounds always appear such as alcohols, thiolesters, hydrocarbons, aldehydes and sulfides [2, 5, 8, 35, 36].

Indole is one of the dominant VOCs detected in human decomposition [30]. Although it occurs in all stages of human decomposition it has higher concentration in advanced decay [34, 37], the same pattern was found in rats. As indole attracts and stimulates the oviposition of some blowflies [38-39] it served to *P.* (*S.*) *lambens* as a chemical cue to locate an oviposition site since all females were mature.

Acetophenone is a compound that has not been previously recorded in human remains or animals carcasses, except mouse carcass [39]. Nevertheless, all species tested responded to acetophenone which suggets that it is a key compound for attraction of these flies to the advanced decay stage of decomposition. We also registered acetophenone in dry remains in low concentration but without a electrophysiological stimuly in the flies tested. This suggests that, as in other compounds, the flies response could only be triggered when a minimum threshold is achieved [27].

Other aspect to be considered is the age of flies tested. Young males or females that breed on carcasses may be important for succesional studies considering that even newly emerged flies can identify compounds concentrations [40]. However to understand the attraction of flies that rear on corpses and consequently are important to estimate the time since death, mature males and pregnant females must be tested in order to identify which stage is considered better to oviposition. In addition, other aspects as the pattern of compounds released by carcasses, bacteria, soil, larvae and pheromones should be considered. Previous studies mention that some beetle species are not attracted only to carcasses but to other species releasing pheromones that are present breeding and rearing on a carcass [3,41]. Therefore, the other chemicals released by the decay associated fauna could be an important factor for the attraction of flies by corpses.

This work shows that indole and acetophenone are responsible for attractiveness of *P*. (*S*.) *lambens.* Our results emphasize that a qualitative approach is important to determine which compounds are important in the decay process. On the other hand, a quantitative analysis should be made and tested since different concentrations showed to affect fly behavior directly.

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