JEAN LUCAS POPPE

O gênero *Rhinoleucophenga* Hendel, 1917 (Diptera, Drosophilidae): proposta de estabelecimento de relações evolutivas baseadas em características morfológicas, moleculares e ecologia

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O gênero *Rhinoleucophenga* Hendel, 1917 (Diptera, Drosophilidae): proposta de estabelecimento de relações evolutivas baseadas em características morfológicas, moleculares e ecologia

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O gênero *Rhinoleucophenga* Hendel, 1917 (Diptera, Drosophilidae): proposta de estabelecimento de relações evolutivas baseadas em características morfológicas, moleculares e ecologia

Resumo: O gênero *Rhinoleucophenga* é composto por espécies distribuídas em ambientes abertos nas regiões Neotropical e Neártica, com o bioma Pampa destacando-se pela riqueza de espécies na América do Sul. A ampla distribuição do gênero e a carência de detalhes em muitas descrições parecem esconder uma grande diversidade de espécies. Muitas das espécies conhecidas de *Rhinoleucophenga* foram descritas na primeira metade do século XX, tornando-se evidente a necessidade de redescrever as mesmas nos padrões atuais, dando maior detalhamento às informações morfológicas. Sete espécies foram redescritas e 17 descritas nos atuais padrões de descrição de drosofilídeos (Capítulos II, III, IV, V). As espécies redescritas foram *Rhinoleucophenga brasiliensis* e *R. fluminensis*, originalmente descritas por Lima (1935), além de *R. personata, R. lopesi, R. angustifrons, R. matogrossensis* e *R. nigrescens*, originalmente descritas por Malogolowkin (1946); e as novas descrições para o gênero foram *R. punctata* sp. nov., *R. paraguayensis* sp. nov., *R. ignota* sp. nov., *R. fusca* sp. nov., *R. alata* sp. nov., *R. paulistorum* sp. nov., *R. obscura* sp. nov., *R. fulva* sp. nov., *R. maculosa* sp. nov., *R. nigra* sp. nov. *R. brasilis* sp. nov., *R. punctuloides* sp. nov., *R. trivisualis* sp. nov., *R. flava* sp. nov., *R. grimaldii* sp. nov., *R. exigua* sp. nov. e *R. jacareacanga* sp. nov. Descrições complementares de *Rhinoleucophenga obesa* (Capítulo VI), *R. joaquina* e *R. punctulata* (Capítulo II) também foram realizadas, apontando novos caracteres que facilitam a identificação dessas espécies. A revisão da morfologia de *R. punctulata* revelou variação na forma da espermateca entre diferentes populações da espécie, oriundas dos biomas Pampa, Cerrado e Caatinga, e também da região Amazônica (Capítulo VII); tal variação morfológica foi indicada como intraespecífica por dados moleculares (*COI*). Com base em conjuntos de caracteres morfológicos, relações filogenéticas foram propostas para *Rhinoleucophenga* (Capítulo VIII). Cinco estratégias de combinação e tratamento dos dados morfológicos foram exploradas: (*A*) 58 razões contínuas e 62 caracteres discretos; (*B*) 104 medidas e 62 caracteres discretos; (*C*) 58 razões contínuas *log-transformadas* e 62 caracteres discretos; (*D*) 104 medidas *log-transformadas* e 62 caracteres discretos e (*E*) somente os 62 caracteres discretos. Todas as matrizes foram analisadas no *Software* TNT, com

pesagem igual (tratamentos *A*-*E*) e com pesagem implícita (*K*= 6) (tratamentos *A'*-*E'*). Todos os caracteres contínuos (razões e medidas) foram tratados como aditivos e re-escalonados entre 0-1 para evitar uma pesagem excessiva na transformação dos mesmos – esta é a primeira vez que um grande conjunto de caracteres morfológicos contínuos não é discretizados em estudos filogenéticos com Drosophilidae. *Rhinoleucophenga* apresentou-se como um gênero parafilético em relação à *Pararhinoleucophenga* na maioria das análises realizadas; seis agrupamentos monofiléticos de espécies também foram repetidamente obtidos, principalmente com caracteres discretos associados a caracteres contínuos tratados como razões e *log-transformados* (tratamento C). Os caracteres morfológicos contínuos, tratados como razões ou como medidas absolutas, exercem alta influência sobre a topologia das árvores geradas. Da mesma maneira, foram fundamentais no aprimoramento dos valores de suporte dos principais agrupamentos de espécies obtidos na filogenia proposta para *Rhinoleucophenga*. As árvores geradas com caracteres contínuos *log-transformados* apresentaram melhora nos valores de suporte médio dos clados, porém, a aplicação de pesagem representou maior influência sobre os resultados filogenéticos. Pouco se sabe sobre a ecologia das espécies de *Rhinoleucophenga*, especialmente no bioma Pampa. Buscando suprir a lacuna referente ao conhecimento ecológico deste, e outros gêneros de Drosophilidae, coletas de drosofilídeos foram realizadas no bioma Pampa durante 12 períodos climáticos, considerando áreas naturais e degradadas dentro deste bioma (Capítulo IX). A influência ambiental sobre a estrutura das assembleias foi temporal e espacialmente analisada por meio de nMDS, IndVal e PERMANOVA. O tipo de ambiente amostrado e os componentes climáticos juntos explicaram 56,45% da variação nas assembleias de drosofilídeos. Ambientes Neotropicais abertos, especialmente o Bioma Pampa, têm apresentado alta diversidade de espécies de *Rhinoleucophenga* assim como de Drosophilidae em geral (Capítulo X). Portanto, a descrição de novas espécies é indispensável para melhorar o conhecimento faunístico dessa região. Trabalhos taxonômicos com redescrições e descrições de novas espécies são ferramentas importantes para a correta identificação da fauna de Drosophilidae, gerando dados mais precisos referentes à distribuição dos táxons, e também novos conjuntos dados para estudos com enfoque sistemático e evolutivo, como aqui realizado.

1. CAPÍTULO I

1.1. INTRODUÇÃO

1.1.1. Surgimento e desenvolvimento da Sistemática

As espécies tendem a se modificar ao longo das gerações, transmitindo as "novidades evolutivas" aos seus descendentes, e assim ampliando a diversidade de maneira não-fixista (Wallace 1855; Darwin 1859). Mesmo antes da visão não-fixista de Wallace e Darwin, a crescente observação da diversidade dos seres vivos trouxe a vontade e a necessidade de nomeá-los e organizá-los em grupos de espécies semelhantes; nomeou-se esta ciência classificatória de *Sistemática*, a qual passou por diversas alterações e é o palco de muitas discussões ao longo do tempo.

A tentativa de classificar os seres vivos em grupos vem desde os filósofos gregos, Platão e Aristóteles (Nelson & Platnick 1981; Mishler 2009). No princípio, os sistematas (naturalistas e filósofos) agrupavam as espécies baseando-se nos seus conhecimentos, o que muitas vezes levava à proposição de grupos não naturais, ou seja, baleias e tubarões ou morcegos e pássaros como espécies pertencentes a um mesmo grupo por serem animais aquáticos ou por possuírem asas, respectivamente (Santos 2008; Schuh & Brower 2009). Além disso, deve-se salientar a ausência da visão evolutiva nestes esquemas de classificação, pois, para Aristóteles, as espécies eram eternas e imutáveis, e seus grupos naturais eram estáveis no tempo e no espaço (Nelson & Platnick 1981).

A Sistemática também foi influenciada pelo poder do Cristianismo, tendo como base as interpretações conservacionistas de Platão e Aristóteles para a proposta da criação divina (Santos 2008; Mishler 2009). A força das ideias cristãs começou a diminuir com as observações e propostas do naturalista sueco Carolus Linnaeus. Linnaeus deu o primeiro passo em direção à taxonomia moderna, propondo a padronização da nomenclatura dos seres vivos, elaborando uma extraordinária compilação de informações biológicas referente à diversidade dos seres – *Systema naturae*, uma das obras mais influentes na Biologia. Porém, apesar de Linnaeus não assumir a imutabilidade das espécies como Aristóteles, nos seus pensamentos não se aplicavam propostas transformacionistas das espécies (Schuh & Brower 2009).

A oposição às propostas fixistas foi instigada por Erasmus Darwin e por Jean-Baptiste de Lamarck no final do século XVIII e início do século XIX. Mais tarde, as

ideias evolucionistas ganharam mais força com as observações de Alfred Wallace (1858) e Charles Darwin (1858, 1859), dando pouco espaço para explicações sobrenaturais da biodiversidade – surgia a *Taxonomia Evolutiva*, uma visão da diversidade das espécies como um processo de descendência com modificações, havendo relação entre os seres extintos e os viventes. Nesse contexto não-fixista, o alemão Ernst Haeckel foi o criador do termo "filogenia" em 1866, e o precursor na elaboração de árvores filogenéticas baseadas na similaridade entre os organismos (Santos 2008), embora, na sua obra *A Origem das espécies*, Darwin também tenha apresentado diagramas ramificados para mostrar o surgimento de variações nas espécies. Atualmente, a relação de similaridade entre táxons é apresentada através de *cladogramas*, enquanto que as relações filogenéticas ancestral-descendente são representadas em *árvores filogenéticas* (Schuh & Brower 2009).

A Sistemática baseia-se na inferência de hipóteses de parentesco entre as espécies através de estruturas homólogas (Mayr 1998). O termo *homologia* foi utilizado pela primeira vez em 1843 por Richard Owen, um anatomista inglês que não aceitava a ideia de transformação das espécies. Owen considerava homólogas as estruturas que, mesmo quando diferentes em sua morfologia, faziam parte de uma mesma entidade no desenho geral dos corpos, especialmente quando se referia a arquétipos (Lewin 1997). Algumas décadas mais tarde (1870), Edwin Ray Lankester, um zoológo britânico, definiu o termo *homogenia* para a ocorrência de estruturas similares em espécies com ancestralidade comum (Wheeler 2012). Baseando-se também em caracteres derivados compartilhados entre as espécies, Hennig (1950, 1966) propôs a definição mais amplamente aplicada de *homologia*: características que podem ou não ser semelhantes (devido a modificações sofridas com o passar das gerações), mas que necessariamente surgiram em um ancestral comum às espécies, ou seja, atualmente duas estruturas (morfológicas, comportamentais ou moleculares) são homólogas se suas partes são semelhantes devido a uma origem filogenética comum (Patterson 1982; Titus & Frost 1996). Porém, discussões acerca de homologia e similaridades entre as espécies continuam sendo apresentadas em estudos recentes, uma vez que o enfoque comparativo é fundamental na Sistemática (de Pinna 1991; Mishler 2009; Nixon & Carpenter 2011, Wheeler 2012).

Por outro lado, apesar dos avanços no pensamento evolutivo-sistemático, ainda faltava uma boa base metodológica à Sistemática, uma vez que a aceitação de novas

propostas baseava-se na autoridade social e intelectual dos seus propositores, ao invés de um método passível de repetição (Schuh & Brower 2009), assim era comum o mesmo grupo de organismos possuir diferentes hipóteses evolutivas, contadas por diferentes taxonomistas (Lipscomb 1998).

O reforço metodológico tentou ser alcançado através de uma nova escola da Sistemática – a *Fenética* ou *Taxonomia Numérica*. Os feneticistas propunham a "super amostragem" de caracteres para retirar o efeito da subjetividade da autoridade dos naturalistas clássicos. Portanto, nessa escola o maior ou menor número de semelhanças (caracteres) compartilhadas entre os táxons refletiria seu maior ou menor grau de parentesco (Schuh & Brower 2009). Mas essas observações poderiam gerar agrupamentos não-naturais, em razão de agrupamentos formados com base em caracteres não herdáveis ou não homólogos. Apesar disso, a escola fenética teve grande importância para os avanços na Sistemática, pela priorização do método em detrimento da autoridade do pesquisador.

Embora os pesquisadores mencionados anteriormente tenham contribuído grandemente para o entendimento da evolução e classificação das espécies, ainda faltava à Sistemática a definição de métodos e termos com maior clareza e enfoque evolutivo (Nelson & Platinick 1981; Santos 2008). Então, um entomólogo alemão chamado Willi Hennig propôs a base da Sistemática que praticamos nos dias atuais (Mishler 2009). Em 1950, Hennig publicou uma obra unindo as propostas dos taxonomistas clássicos com os métodos feneticistas, desenvolvendo novos conceitos e procedimentos básicos para a reconstrução da história evolutiva dos organismos; seria o surgimento da *Sistemática Filogenética*, ou *Análise Cladística* (Schuh & Brower 2009). Na proposta de Hennig, os organismos seriam agrupados pelo compartilhamento de caracteres derivados a partir de um ancestral comum, ou seja, pelo compartilhamento de s*inapomorfias* (conjunto de estruturas com características derivadas), e esta constitui a principal diferença entre as propostas dos feneticistas e cladistas. Essa possibilidade de se contar a história evolutiva de um grupo de espécies a partir de algumas características herdadas de um ancestral comum, se tornou o aspecto mais poderoso do sistema filogenético (Mishler 2009; Wheeler 2012).

Além da contribuição para a definição de *homologia* e s*inapomorfia*, como mencionado anteriormente, Hennig (1966) também propôs o termo *plesiomorfia* –

referente a estruturas com características primitivas. Assim surgiu a proposição de parentesco entre as espécies a partir da interpretação de características plesiomórficas e apomórficas. Dessa forma, grupos formados por organismos compartilhando apomorfias e ancestralidade comum exclusiva foram chamados de *monofiléticos* por Hennig, sendo esta a essência da atual Sistemática (Schuh & Brower 2009).

Assim, Hennig revolucionou a Sistemática por permitir a construção de hierarquias sem apoiar-se em arbitrariedades e hipóteses não-testáveis. Hennig deu peso científico às hipóteses evolutivas das espécies e ainda proporcionou uma nova visão à classificação da diversidade, onde espécies extintas passaram da exuberante condição de "*guardas dos segredos das origens"* (ancestral – nó nas filogenias) a coadjuvantes da história evolutiva de seus grupos (táxons terminais das filogenias). Em outras palavras, táxons viventes e extintos estão sujeitos às mesmas interpretações no contexto filogenético (Schuh & Brower 2009).

No entanto, somente algumas décadas mais tarde as propostas de Hennig começaram a ganhar força (Diniz-Filho 2000). A baixa aceitação inicial ocorreu principalmente em razão da resistência por parte dos taxonomistas evolutivos e feneticistas (Mayr 1965, 1974; Sneath 1995), mas também pela obra de Hennig de 1950 ter sido traduzida do alemão para o inglês somente 16 anos mais tarde – *Phylogenetic systematics* (Hennig 1966). Desde então, uma série de adições foram feitas na estrutura conceitual e metodológica inicialmente proposta por Hennig, gerando uma base metodológica bem definida e um grande aperfeiçoamento no campo da Sistemática, dando maior confiabilidade ao método filogenético a partir da congruência entre as hipóteses evolutivas propostas por diferentes taxonomistas (Santos & Klassa 2012).

À medida que a sistemática filogenética se desenvolvia, a Biologia Molecular também estava em ascendência e ganhava novos adeptos (Coutinho 1998). Os avanços na Biologia Molecular e a associação entre os preceitos evolutivos de Darwin com a Genética de Populações, fortaleceram a visão não-fixista das espécies, gerando novas ferramentas para buscar inferir a história evolutiva dos organismos (Wheeler 2012).

Apesar dos avanços na Biologia Molecular e a importante contribuição dessa área para a sistemática filogenética, as informações contidas em conjuntos de dados morfológicos continuam muito importantes para estudos filogenéticos, em função da grande parcela de material depositado em museus que não podem ser incluídos em

trabalhos moleculares. Mesmo em táxons altamente diversos como Drosophilidae, há muitas espécies que não são facilmente encontradas na natureza (Markow & O'Grady 2006), sendo difilmente consideradas em estudos inteiramente voltados à Biologia Molecular. Além disso, Remsen & O'Grady (2002) salientam que dados morfológicos contêm informações que muitos blocos de dados moleculares não apresentam, e por isso devem ser mantidos nas análises. Porém, Wiens (2000) destaca que a questão central deve ser o critério de escolha de cada caracter morfológico, uma vez que a árvore filogenética é apenas uma representação gráfica da matriz de caracteres (Farris 1983; de Pinna 1991; Wiens 2000; Sereno 2007).

Mesmo com os avanços na Sistemática, lacunas no conhecimento evolutivo são encontradas até mesmo em táxons bem estudados, como Drosophilidae. Embora tenham sido desenvolvidos muitos estudos abrangentes com Drosophilidae (Throckmorton 1975; Grimaldi 1990; Van der Linde *et al*. 2010; Yassin 2013), ainda há resultados contraditórios ou apenas parcialmente coerentes (Kwiatowski & Ayala 1999; Bächli *et al*. 2004). As hipóteses evolutivas de um táxon tendem a se alterar à medida que novas informações são adicionadas a tal táxon, por exemplo, a descrição de novas espécies para um gênero; ou ainda à medida que diferentes interpretações ou tratamentos são impostos aos dados (de Pinna 1991; Santos & Klassa 2012). Atualmente, existem diversas metodologias que podem gerar diferentes resultados dentro do mesmo conjunto de dados (Farris 1983; DeSalle 2006; Giribet 2007; Koch *et al*. 2015). Algoritmos heurísticos, mais simples e mais rápidos, podem ser tão ou mais eficientes do que algoritmos mais complexos (Russo *et al*. 1996; Takahashi & Nei 2000; Criscuolo & Gascuel 2008). Assim, as hipóteses evolutivas tendem a flutuar juntamente com o conhecimento referente à diversidade dos organismos (Amorim 1997; Schuh & Brower 2009). Porém, cabe salientar que uma nova hipótese evolutiva não necessariamente invalida hipóteses prévias (Santos & Klassa 2012). Como toda ciência, a classificação dos organismos está em constante alteração (Mishler 2009).

O papel da sistemática filogenética, portanto, além de realizar o trabalho tradicional da taxonomia, de descrever a diversidade biológica, também é de organizar o conhecimento sobre essa diversidade com base nas relações de parentesco entre os grupos. Isso implica considerar o conhecimento da evolução das características morfológicas, comportamentais, ecológicas, fisiológicas, citogenéticas e moleculares dos táxons. Dessa forma, a sistemática e seus métodos de inferência passaram a se

associar a várias outras disciplinas como Zoologia, Botânica, Ecologia e Genética, além de fundamentar a interpretação dos processos evolutivos (de Pinna 1991; Mishler 2009; Santos & Klassa 2012).

A observação da diversidade e da interação dos organismos com o meio ambiente levou os filósofos, naturalistas, estudiosos e cientistas à comparação das diferentes formas de vida, emergindo assim a percepção da evolução dos seres vivos. Desse modo, quaisquer espécies poderiam ser reunidas em um grupo, baseando-se em diferentes atributos. Posteriormente, apenas grupos que refletem monofilia passaram a ser evolutivamente informativos e válidos para a ciência. Sendo assim, a busca pela classificação dos seres vivos passou por Aristóteles, Linnaeus, Lamarck, Wallace, Darwin, Cuvier, Geoffroy, Simpson, Hennig, Patterson, entre outros tantos pesquisadores, durante séculos, e continua até hoje.

1.1.2. A família Drosophilidae

Segundo Throckmorton (1975), a família Drosophilidae teve sua origem na região tropical, há cerca de 50 milhões de anos, migrando posteriormente às demais regiões. Atualmente, drosofilídeos são encontrados ao longo de todo o globo, com exceção das regiões polares (Bächli 2015).

Drosophilidae conta com aproximadamente 4.300 espécies descritas (Bächli 2015). Este número só tende a aumentar, uma vez que estimativas da fauna desconhecida se baseiam nas taxas de novas descrições (Wilson 1999). E no que se refere especificamente à Drosophilidae, no ano de 2006, de acordo com os dados apresentados por Schmitz *et al*. (2007), haviam cerca de 3.800 espécies de drosofilídeos descritas; em 2010 esse número passou para cerca de 4.000 espécies (Mata *et al*. 2010); e atualmente existem aproximadamente 4.300 espécies descritas (Bächli 2015). Ou seja, nos últimos nove anos o número de drosofilídeos descritos aumentou em aproximadamente 56 espécies por ano – durante o desenvolvimento desta Tese foram descritas 17 novas espécies de *Rhinoleucophenga*, cerca de 1/3 da expectativa de novas descrições para Drosophilidae em um ano.

Apesar de os drosofilídeos serem popularmente conhecidos como “moscas das frutas”, a vasta maioria das moscas desta família não se alimenta dos frutos, mas sim

das leveduras que crescem na matéria orgânica em decomposição (Carson 1971). Além disso, essa família se caracteriza por uma grande versatilidade ecológica, apresentando sítios alimentares muito mais amplos, com registros de espécies em flores (revisões em Schmitz & Hofmann 2005; Schmitz *et al*. 2009), guano de morcego (Tosi *et al*. 1990), cladódios de cactos (Carson 1971; Mizuguchi 1978; Vilela *et al*. 1983), carcaças de insetos e carne (Lachaise & Tsacas 1983), fluxos de seiva e material vegetal em decomposição (Carson 1971), podendo ainda exibir comportamento predatório (Lima 1935, 1950; Máca & Otranto 2014), comensal (de caranguejos, aranhas, abelhas), parasitário (de lagartas de lepidópteros) e até de canibalismo no caso de larvas que se encontram em um recurso super povoado (Carson 1971; Ashburner 1981; Lachaise & Tsacas 1983).

Devido a essa grande diversidade morfológica e ecológica, muitos estudos com enfoque evolutivo já foram desenvolvidos com drosofilídeos, principalmente em relação ao gênero *Drosophila*, e com dados moleculares (DeSalle 1992; Russo *et al*. 1995; Markow & O'Grady 2006; Da Lage *et al*. 2007; O'Grady & Markow 2009; Robe *et al*. 2010). São poucos, entretanto, os estudos evolutivos de Drosophilidae baseados em análises de dados morfológicos: Throckmorton (1975), Grimaldi (1988), Okada (1989), Grimaldi (1990) e Sidorenko (2002) – os mesmos serão comentados mais adiante. Essa discrepância entre o número de estudos com dados moleculares e morfológicos pode ser justificada por diversos fatores, entre eles: (i) por *Drosophila* ser, historicamente, um organismo modelo em Genética, e esta área estar fortemente ligada aos avanços da Biologia Molecular; (ii) falta de investimento financeiro a estudos na área de Taxonomia; (iii) falta de valorização científica, uma vez que trabalhos taxonômicos dificilmente são aceitos em periódicos científicos com alto fator de impacto. Tudo isso desestimula os profissionais a atuarem nesta área, refletindo no baixo número de pesquisadores "morfologistas-taxonomistas".

Segundo diversos autores (DeSalle & Grimaldi 1992; O'Grady *et al*. 1998; O'Grady 1999; Remsen & O'Grady 2002; Yassin 2013), existe uma grande carência de estudos evolutivos de Drosophilidae envolvendo dados morfológicos junto de dados moleculares. Os mesmos autores ainda destacam que estas duas classes de dados são complementares, e que juntos os mesmos são capazes, muitas vezes, de revelar relações previamente obscuras. Da mesma maneira, estudos considerando outros gêneros, além

de *Drosophila*, ainda são necessários para a melhor compreensão da evolução da família Drosophilidae (Yassin 2013).

Apesar do interesse em *Drosophila* estar particularmente focado para pesquisas em Genética e Evolução, alguns trabalhos clássicos não relacionados a estas áreas foram desenvolvidos no Brasil nas décadas de 1940 e 1950 a fim de melhor caracterizar a diversidade de drosofilídeos na região. Nesta sentido, Dobzhansky & Pavan (1943), Freire-Maia & Pavan (1949), Dobzhansky & Pavan (1950), Frota-Pessoa (1954) e Pavan (1959) incluem descrições de um grande número de espécies, grandes levantamentos taxonômicos, chaves de identificação e diversas abordagens ecológicas. Porém, estudos com enfoque ecológico se intensificaram significativamente algumas décadas mais tarde, com estudos em áreas de Mata Atlântica (Medeiros & Klaczko 2004; De Toni *et al*. 2007; Gottschalk *et al*. 2007; Döge *et al*. 2008), Cerrado (Tidon *et al*. 2003; Mateus *et al*. 2006; Tidon 2006; Mata *et al*. 2008, 2010), Manguezal (Schmitz *et al*. 2007, 2010), Caatinga (Mizuguchi 1978; Tidon-Sklorz & Sene 1995), Pantanal (Val & Marques 1996), Amazônia (Martins 1987, 1995, 2001), Mata de Araucária (Saavedra *et al*. 1995, Cavasini *et al*. 2014), Restinga (Bizzo & Sene 1982; Bizzo *et al*. 2010) e, mais recentemente, no bioma Pampa ainda mais recentemente (Costa *et al*. 2003; Silva *et al*. 2005; Garcia *et al*. 2012; Poppe *et al*. 2012, 2013, 2014, 2015a, 2015b).

Por outro lado, apesar dos esforços dos pesquisadores brasileiros para amostrar e descrever a diversidade de drosofilídeos, mesmo em biomas bem estudados, como a Mata Atlântica, ainda há muitas espécies para serem descritas (Medeiros & Klaczko 2004). Além disso, ainda existem muitas regiões no território nacional cuja diversidade de drosofilídeos é praticamente desconhecida (Gottschalk *et al*. 2008). Inevitavelmente, com o estado de degradação de alguns biomas, muita informação já se perdeu com a devastação de ambientes e a extinção de espécies (Klink & Machado 2005; MMA 2007; Paes & Dias 2008). Amplas áreas endêmicas estão sumindo tão depressa que os pesquisadores não têm mais a oportunidade de estudá-las satisfatoriamente (Döge *et al*. 2004; Blauth & Gottschalk 2007; Paes & Dias 2008; Poppe *et al*. 2014).

O aumento no número de estudos com enfoque ecológico revelou os drosofilídeos como bons bioindicadores ambientais, ajudando no monitoramento de áreas degradadas. Mata *et al*. (2008) reforçaram a importância ecológica de

drosofilídeos como organismos bioindicadores, em função da Taxonomia bem definida, Genética e ciclo de vida bem conhecidos, fácil amostragem, grande abundância e distribuição na natureza, fácil estocagem e cultivo em laboratório, além do baixo custo para as pesquisas. Essa importante característica bioindicadora já foi aplicada por muitos pesquisadores tanto em ambientes naturais (Tidon-Sklorz & Sene 1999; Martins 2001; Tidon *et al*. 2003; Mata *et al*. 2008) quanto em ambientes urbanos (Lucchese *et al*. 2002; Gottschalk *et al*. 2007).

Além de indicadores de alteração ambiental (espacial), os drosofilídeos também são bastante sensíveis à variação sazonal (temporal), com muitos estudos apontando para a preferência sazonal de algumas espécies (Döge *et al*. 2003; Silva *et al*. 2005; Tidon 2006; Torres & Madi-Ravazzi 2006; De Toni *et al*. 2007; Penariol 2007; Gottschalk *et al*. 2009; Bizzo *et al*. 2010; Schmitz *et al*. 2007, 2010; Poppe *et al.* 2013, 2015a) e também associando essas preferências a fatores genéticos (Partridge 1988; Hoffmann & Harshman 1999; Brisson *et al*. 2005; Kellermann *et al*. 2009; Zivanovic & Mestres 2011). Isso tudo reforça a importância científica de Drosophilidae, como ferramenta ecológica, dentro do crescente cenário de alteração ambiental e climática do planeta Terra.

Apesar de Drosophilidae ser um grupo de organismos amplamente estudado, ainda existem vários aspectos biológicos para se conhecer sobre esta família, especialmente em ambientes pouco explorados, como o bioma Pampa, e em relação a outros gêneros além de *Drosophila*, como o gênero *Rhinoleucophenga*, pertencente à subfamília Steganinae.

1.1.3. Subfamília Steganinae Duda

Drosophilidae divide-se em duas subfamílias: Drosophilinae e Steganinae. Alguns autores mencionam Steganinae como basalmente divergente em relação à Drosophilinae (Grimaldi 1988; Bächli *et al*. 2004; Markow & O'Grady 2006). Van der Linde *et al*. (2010) mencionam que Steganinae seria parafilético em relação à Drosophilinae, porém, os mesmos autores chamam a atenção para o reduzido número de estudos envolvendo espécies de ambas as subfamílias.

Steganinae é composta por cerca de 500 espécies descritas em 28 gêneros (Markow & O'Grady 2006; Bächli 2015), dentre os quais *Stegana* Meigen é o gênero tipo da subfamília. Segundo Bächli *et al*. (2004), as moscas dessa subfamília apresentam os seguintes caracteres diagnósticos: de 2.0-8.0 mm de comprimento; aristas com ramos micropubescentes a longos; cerdas orbitais de tamanhos muito próximos; cerda orbital posterior reclinada normalmente mais próxima da cerda mediana vertical do que orbital proclinada; cerdas pré-escutelares longas; duas cerdas esternopleurais de tamanhos próximos; veia costal normalmente terminando na veia R_{4+5}; epândrio e surstilo completamente fusionados, ou pelo menos fusionados por uma membrana; fêmeas com ovipositor dificilmente esclerotinizado e cercos presentes. Grimaldi (1990) ainda menciona a presença de apódemas tentoriais dorsolaterais paralelos e a ausência do VII par de espiráculos nos machos. Porém, os mesmos autores mencionam que este conjunto de características pode não ser comum a todos os membros da subfamília e nem mesmo exclusivo da mesma.

O número de estudos envolvendo Steganinae ainda é reduzido se comparado com Drosophilinae (Markow & O' Grady 2006; Otranto *et al*. 2008), em função, principalmente, da grande diversidade de *Drosophila* na maioria dos ambientes e da sua aplicabilidade em todas as áreas da Biologia. Considerando apenas a subfamília Steganinae, os dois estudos evolutivos mais abrangentes são de Sidorenko (2002) e Otranto *et al*. (2008). Sidorenko (2002) analisou 34 espécies de nove gêneros (não incluiu *Rhinoleucophenga*) e 78 caracteres discretizados, propondo as tribos Steganini e Gitonini. O autor desenvolveu uma análise de parcimônia com pesos iguais e, com a ordenação de alguns caracteres. Porém, alguns caracteres apresentam certa ambiguidade na sua elaboração, por exemplo, no caracter #10 o autor menciona juntamente comprimento da arista e número de ramos na estrutura. Otranto *et al*. (2008) analisaram sequências de *Citocromo oxidase I* (COI) para 13 espécies pertencentes a oito gêneros, sendo que, o gênero *Rhinoleucophenga* também não foi incluído neste estudo.

Na região Neotropical, historicamente, existem poucos estudos relacionados às espécies de Steganinae (Lima 1935, 1937, 1950; Malogolowkin 1946; Jiménez *et al*. 1993), provavelmente, devido à baixa abundância das espécies desta subfamília em armadilhas com isca de banana fermentada, principal recurso utilizados em coletas de drosofilídeos (Goñi *et al*. 2002; Medeiros & Klaczco 2004; Tidon 2006; Schmitz *et al*. 2007; De Toni *et al*. 2007; Gottschalk *et al*. 2009; Bizzo *et al*. 2010; Poppe *et al*. 2014).

Outro fator limitante mencionado por alguns especialistas seria a grande dificuldade de identificação das espécies de Steganinae, pois alguns gêneros apresentam espécies crípticas com terminálias altamente complexas (D. Grimaldi e M.S. Gottschalk, comunicação pessoal).

Apesar disso, no Brasil, alguns gêneros da subfamília como *Leucophenga*, *Amiota* e *Rhinoleucophenga*, têm sido encontrados em estudos de levantamento de fauna utilizando iscas de banana (Blauth & Gottschalk 2007; Roque & Tidon 2008, 2013; Mata *et al*. 2008; Hochmüller *et al*. 2010; Roque *et al*. 2013; Poppe *et al*. 2012, 2014). Poppe *et al*. (2014) encontraram uma grande diversidade de *Rhinoleucophenga* no bioma Pampa unicamente através de coletas com iscas de banana, desencadeando o desenvolvimento de outros estudos envolvendo o gênero (Poppe *et al*. 2015b) e, inclusive, a presente Tese. Ainda assim, a escassez de estudos com Steganinae na região Neotropical contrasta com a ampla variedade de estudos taxonômicos, ecológicos e evolutivos envolvendo a subfamília (em especial os gêneros *Leucophenga*, *Amiota*, *Stegana*, *Phortica*) realizados na região Asiática (Chen & Toda 2001; Máca 2003; Chen *et al*. 2004, 2005a, 2005b; Otranto *et al*. 2006a, 2006b; Cheng *et al*. 2008, 2009; Prigent & Chen 2008; Cao & Chen 2009; Lu *et al*. 2011; Cao *et al*. 2008, 2011; Wang *et al*. 2011; Zhang *et al*. 2012; Li *et al*. 2013; Shao *et al*. 2014; Gao 2014; Huang *et al*. 2013, 2014; Zhang & Chen 2015).

1.1.4. *Rhinoleucophenga* **Hendel**

Rhinoleucophenga é um dos 77 gêneros, além de *Drosophila*, que compõem Drosophilidae, estabelecido por Hendel em 1917 com base na descrição de *R. pallida*, coletada no Peru. Este gênero pertence à subfamília Steganinae e é composto atualmente por 29 espécies descritas (Poppe *et al*. 2015b; Vidal & Vilela 2015), distribuídas nas regiões Neotropical e Neártica. Esse limite distribucional tornou-se fator determinante para a diferenciação entre *Rhinoleucophenga* e *Gitona*. Brake & Bächli (2008) transferiram cinco espécies de *Gitona* (*G. bivisualis* (Patterson), *G. americana* (Patterson), *G. fluminensis* (Lima), *G. brasiliensis* (Lima) e *G. sonoita* (Wheeler)) para *Rhinoleucophenga* por serem as únicas com registros no Novo Mundo; enquanto as demais espécies de *Gitona* apresentam registros de ocorrência na África, Europa, Ásia e Austrália (Máca 1988; Bächli 2015).

A maioria das espécies de *Rhinoleucophenga* foi descrita na primeira metade do século XX (Duda 1927, 1929; Lima 1935, 1950; Malogolowkin 1946). Porém, recentemente, muito se tem investido na descrição de novas espécies (Junges & Gottschalk 2014; Poppe *et al*. 2014, 2015b; Vidal & Vilela 2015) e em revisões de descrições antigas (Vilela 1990; Vilela & Bächli 2009). A contribuição dos trabalhos de Malogolowkin (1946) e Lima (1935, 1937, 1950) foi muito importante para o conhecimento faunístico e comportamental de *Rhinoleucophenga* na região Neotropical, especialmente no Brasil. Porém, a carência de informações morfológicas detalhadas nas descrições, associado ao "Massacre de Manguinhos" – quando uma parcela do material das séries tipo se perdeu (Costa *et al*. 2008), tornou a redescrição dessas espécies fundamental para a viabilidade de estudos futuros. Deste modo, essa Tese apresenta, em seus capítulos II, III, IV, V e VI, descrições e redescrições detalhadas de espécies de *Rhinoleucophenga* sob uma metodologia taxonômica e de análise morfológica atualizada, buscando o refinamento da definição das características morfológicas de algumas espécies e, ao mesmo tempo, visando minimizar os erros de identificação e a geração de dados espúrios quanto à distribuição das mesmas.

Embora existam alguns poucos registros de *Rhinoleucophenga* em áreas de mata (De Toni *et al*. 2007; Hochmüller *et al*. 2010; Poppe *et al*. 2015a), este gênero parece ter preferência por ambientes abertos. Recentemente Poppe *et al*. (2014, 2015b) destacaram a alta diversidade de *Rhinoleucophenga* no bioma Pampa, assim como em ambientes de Caatinga (G.F. Oliveira, comunicação pessoal; Poppe *et al*. 2015b), Cerrado (Blauth & Gottschalk 2007; Gottschalk *et al*. 2007; Mata *et al*. 2008; Roque & Tidon 2008, 2013; Roque *et al*. 2013), Restinga (Schmitz *et al*. 2009) e ainda diversos registros em áreas abertas entre as latitudes 37°N (Texas, Estados Unidos) (Malloch & McAtee 1924; Vilela 1990) e 34°S (Argentina) (Thomson 1869; Vilela 1990).

Mesmo com o crescente número de novos registros e descrições de espécies nos últimos anos (Culik & Ventura 2009; Schmitz *et al*. 2009; Junges & Gottschalk 2014; Poppe *et al*. 2014, 2015b), a ecologia de *Rhinoleucophenga* ainda é pouco conhecida. O pouco que se sabe é referente a larvas parasitando os coccídeos *Dysmicoccus brevipes* (Cockerell) (Culik & Ventura 2009), *Orthezia praelonga* Douglas e *Aclerda campinensis* Hempel (Lima 1935, 1950), predando formigas (Vidal & Vilela 2015), associadas a outros coccídeos (Ashburner 1981; D. Grimaldi, comunicação pessoal) e

um único registro de *Rhinoleucophenga* em flores de *Dyckia encholirioides* (Bromeliaceae) (Schmitz *et al*. 2009).

Quanto à distribuição, *Rhinoleucophenga obesa* parece ser a espécie mais amplamente distribuída do gênero, com registros desde os Estados Unidos (Patterson 1943; Throckmorton 1962; Grimaldi 1990) até o Sul do Brasil (Hochmüller *et al*. 2010). Porém, ainda existem muitas dúvidas acerca da identidade dessa espécie (Hsu 1949; Wheeler 1952; Wheeler & Takada 1971; Vilela 1990; Poppe *et al*. 2015b) e, consequentemente, em relação aos seus limites distribucionais. Alguns autores comentam a possibilidade de que sob o nome de *R. obesa* (Loew), exista um grupo críptico de espécies, incluindo *R. gigantea* (Thomson) (Vilela 1990). Na presente Tese, damos um importante passo em direção à elucidação desse caso, redescrevendo os espécimes de *R. obesa* identificados por Malogolowkin (1946) e Lima (1935) e comparando-os com espécimes de diversas localidades, apresentando semelhanças entre os mesmos e diferenças marcantes destes com relação a *R. gigantea* redescrita por Vilela (1990) – para mais detalhes ver o capítulo VI. Apesar disso, para contribuir ainda mais para a questão taxonômica de *R. obesa*, é necessário observar espécimes coletados na região Neártica, e também o holótipo que provavelmente está depositado na Coleção Entomológica do Museu de Zoologia Comparada da Universidade de Harvard (Vilela 1990).

Outra espécie com registros de ampla distribuição é *Rhinoleucophenga punctulata* Duda. Recentemente, Poppe *et al*. (2015b) descreveram *R. punctuloides*, espécie críptica com *R. punctulata* e com registros nos biomas Pampa e Cerrado, o que levantou suspeitas em relação à identidade de *R. punctulata*, que é frequentemente mencionada em listas de inventariamentos na região Neotropical (Roque & Tidon 2008; Vilela & Bächli 2009; Roque & Tidon 2013; Poppe *et al*. 2014; G.F. Oliveira, comunicação pessoal). Baseados no edeago dos machos e nos ramos das aristas das fêmeas, Poppe e colaboradores confirmaram a identidade de *R. punctulata* ao longo do território brasileiro. Após a análise de espermatecas das fêmeas, os mesmos pesquisadores perceberam uma variação na morfologia dessas estruturas, possivelmente relacionada com fatores ambientais aos quais as populações estavam expostas. Com o auxílio de dados moleculares, foi verificada uma variação intraespecífica entre as populações, com o Nordeste brasileiro sendo provavelmente o centro de origem da espécie, tal como apresentado no capítulo VII desta Tese. Além disso, existe uma

suspeita de que *R. bivisualis* (Patterson) tenha sido erroneamente mencionada em algumas localidades em função da sua semelhança com *R. punctulata* (Vilela 1990; Poppe *et al*. 2015b).

Através de novas descrições e também de revisões tanto de descrições antigas quanto de dados de ocorrência de espécies, as questões taxonômicas de *Rhinoleucophenga* tendem a ser melhor esclarecidas. Além disso, uma base taxonômica mais sólida deve fornecer suporte a estudos de caráter evolutivo e ecológico que levem a melhor compreensão do gênero como um todo.

1.1.5. Relações filogenéticas de *Rhinoleucophenga*

Inicialmente, a sistemática de Drosophilidae foi objeto de estudo de apenas alguns dipterologistas, mas passou a receber forte interesse após este táxon se tornar organismo modelo de estudos genéticos, especialmente com Sturtevant (1921, 1942), que considerou caracteres morfológicos e citogenéticos em suas inferências. Os principais estudos filogenéticos baseados em caracteres morfológicos de espécies da família Drosophilidae são: Throckmorton (1975), Okada (1989), Grimaldi (1990) e Sidorenko (2002), mas o último não incluiu espécies de *Rhinoleucophenga*.

Throckmorton (1975) considerou um grande conjunto de caracteres de morfologia externa e interna dos drosofilídeos. Porém, os principais resultados do estudo são em relação à *Drosophila*. Okada (1989) fez a proposição de subtribos para Drosophilidae, com base em 14 caracteres discretos binários para 62 gêneros. Propôs *Rhinoleucophenga* em Leucophengini. Porém, não menciona as espécies que representam cada gênero (incluindo *Rhinoleucophenga*) e também não apresenta um detalhamento metodológico das análises. Grimaldi (1990) analisou filogeneticamente a família Drosophilidae com base em 217 caracteres morfológicos discretizados e 120 espécies, incluindo *Rhinoleucophenga obesa* e *R. pallida*. Propôs as tribos Steganini e Gitonini, incluindo *Rhinoleucophenga* nesta última. Mas Grimaldi (1990) não especificou alguns parâmetros das suas análises, como o número de réplicas, iterações e o número total de árvores geradas, assim como alguns detalhes de pesagem. Apesar disso, este é o trabalho sistemático de maior abrangência de dados morfológicos para Drosophilidae.

Além desses trabalhos, Grimaldi (1988), em uma análise biogeográfica de alguns grupos de Drosophilidae, propôs uma pequena filogenia baseada em 18 caracteres morfológicos de indivíduos adultos e sete caracteres de larvas, todos discretizados. *Rhinoleucophanga obesa*, *R. pallida* e *R.* sp., foram consideradas neste estudo. O clado de *Rhinoleucophenga* foi suportado pela presença de mais de dois pares de cerdas pré-escutelares bem desenvolvidas e fronte altamente pilosa, já o comportamento predatório das larvas foi apontado como homoplásico em Steganinae. Porém, a matriz de caracteres e os parâmetros de análise para a elaboração da árvore não foram apresentados pelo autor.

Posteriormente, outros poucos estudos filogenéticos, com dados moleculares, consideraram *Rhinoleucophenga*. Remsen & O'Grady (2002) incluíram *R. obesa* em uma análise de sequências de três genes nucleares, dois mitocondriais e alguns caracteres morfológicos de Grimaldi (1990). Van der Linde *et al*. (2010), com *R. obesa* e *R. bivisualis*, desempenharam o maior esforço amostral com dados moleculares, analisando sequências de nove genes nucleares e quatro mitocondriais. E, finalmente, Yassin (2013), com *R. obesa, R. subradiata* Duda e *R. bivisualis*, analisou sete genes nucleares e um mitocondrial. Além disso, este autor plotou caracteres de genitália dos machos de algumas espécies sobre as árvores de dados moleculares. É importante mencionar que nos três estudos os dados não foram igualmente levantados para todas as espécies, principalmente com relação à *Rhinoleucophenga* que teve muita informação faltante.

Destaca-se aqui o fato de que a diversidade morfológica de *Rhinoleucophenga* é muito ampla, com espécies de 2.0 mm até 7.0 mm, de coloração amarelo até preto. Assim, *R. obesa*, principal representante do gênero nos trabalhos mencionados anteriormente, não seria capaz de representar significativamente o gênero como um todo. Esse pode ser o motivo para o diferente posicionamento de *Rhinoleucophenga* nas tribos propostas por Okada (1989) e Grimaldi (1990). Neste sentido, embora os gêneros *Gitona* e *Rhinoleucophenga* tenham sido apresentados como irmãos por Grimaldi (1990) e Remsen & O'Grady (2002), as relações filogenéticas entre as espécies que compõem a tribo Gitonini e até mesmo a exata relação entre *Gitona* e *Rhinoleucophenga* ainda são consideradas obscuras (Otranto *et al*. 2008; Yassin 2013). Portanto, de modo geral, pouco se sabe a respeito das relações evolutivas de

Rhinoleucophenga com outros gêneros de Drosophilidae; e praticamente nada se sabe quanto às relações entre as espécies deste gênero.

Logo, é visível a necessidade de estudos envolvendo *Rhinoleucophenga*, tanto para a geração de uma primeira proposta evolutiva para o gênero, como para a proposição de uma hipótese mais consistente quanto a seu posicionamento dentro de Drosophilidae. Para isso é fundamental a utilização de dados morfológicos, pois a maioria das espécies de *Rhinoleucophenga* é pouco abundante, e/ou representadas por somente alguns indivíduos em coleções entomológicas. Talvez esse seja o principal motivo da utilização predominante de *R. obesa* em estudos filogenéticos: além de seu tamanho avantajado (5-6 mm), esta espécie é, normalmente, mais abundante do que as demais espécies do gênero em estudos de levantamento de fauna. Por outro lado, como mencionado anteriormente, existem alguns problemas taxonômicos em relação a esta espécie.

Este enfoque evolutivo ganha ainda mais importância quando lembramos que a Sistemática Filogenética fornece subsídios para a análise, interpretação e resolução de uma série de indagações características da Biologia (Powell & DeSalle 1995; Futuyma 1997; Grimaldi & Engel 2005). Assim, o conhecimento das relações evolutivas de *Rhinoleucophenga* dentro da subfamília Steganinae é importante não apenas para estimar a origem deste táxon e propor sua história evolutiva, mas também para melhorar a compreensão da evolução de Drosophilidae como um todo.

Uma vez que o Brasil apresenta ¾ dos registros das espécies de *Rhinoleucophenga* descritas no mundo e o bioma Pampa revelou-se bastante rico em espécies deste gênero (Poppe *et al*. 2014, 2015b), a investigação das assembleias de Drosophilidae neste bioma tornou-se particularmente relevante.

1.1.6. O Pampa

O bioma Pampa apresenta condições ideais para o desenvolvimento de estudos ecológicos, devido a sua heterogeneidade ambiental e climática. As temperaturas oscilam de valores negativos no inverno, até mais de 36°C no verão, e estas encontram-se associadas a diferentes fitofisionomias campestres entremeadas por manchas de mata

de florestas Estacional Decidual e Ombrófila Densa (MMA 2007; Boldrini *et al*. 2010; Poppe *et al*. 2013, 2014, 2015a).

Este bioma tem sofrido grande perda de biodiversidade e de hábitats devido ao acelerado processo de expansão agrícola iniciado nos anos 1970, e agravado recentemente pelos planos para conversão de extensas áreas de campos em monoculturas florestais, de acordo com o Censo Agropecuário (MMA 2007). De fato, muitas vezes, restam apenas pequenos fragmentos de Pampa em uma paisagem predominantemente agrícola (Risser 1997; Porto 2002; Bencke 2003). Em geral, apenas 11,7% do Pampa permanecem sem nenhum tipo de influência antrópica no Rio Grande do Sul (PROBIO 2007). Por esses motivos, este bioma vem recebendo atenção especial do Ministério do Meio Ambiente, com propostas de áreas prioritárias para a conservação da biodiversidade (Hasenack 2007) e para o desenvolvimento de práticas de inventariamento da fauna (MMA 2007). Por outro lado, apesar do bioma Pampa se estender por aproximadamente 63% do território gaúcho (MMA 2007), o Código Estadual do Meio Ambiente (instituído pela Lei 11.520/ 2000) do estado do Rio Grande do Sul não menciona em nenhum trecho do seu texto a palavra "pampa", tristemente evidenciando o descaso das autoridades com este bioma.

Embora o estado do Rio Grande do Sul seja um dos mais bem estudados do Brasil em relação à fauna de drosofilídeos, grande parte dos estudos concentra-se em localidades pertencentes ao bioma Mata Atlântica (Petersen 1960; Franck & Valente 1985; entre outros), enquanto que o Pampa tem sido grandemente negligenciado, sendo um dos mais inexplorados do Brasil, como notado por Gottschalk *et al*. (2008). Apenas recentemente, a região noroeste do Rio Grande do Sul tem sido melhor estudada em relação a fauna de Drosophilidae por Poppe *et al*. (2012, 2013, 2014, 2015a, 2015b). Em relação ao gênero *Rhinoleucophenga*, Poppe *et al*. (2014, 2015a) coletaram dez espécies em uma área natural de Pampa, descrevendo cinco dessas como novas espécies, e ampliando os pontos de ocorrência de outras.

Outro fator que salienta a importância da realização de inventários nesse bioma é a pouca representatividade dos Campos Sulinos no Sistema de Unidades de Conservação, de modo que apenas 2,58% da área total de campos naturais ainda existentes no Estado do Rio Grande do Sul encontram-se protegidos por UCs, sendo insuficiente para a proteção do patrimônio ecológico e genético do Pampa (Brandão *et*

al. 2007). Assim, inventários da fauna e da flora podem desempenhar um papel importante na proposta de ampliação das áreas de preservação deste bioma.

1.2. Objetivos

1.2.1. Objetivo geral

Ampliar as informações referentes à diversidade das assembleias de *Rhinoleucophenga* na região Neotropical. A partir disso, testar a hipótese de monofilia de *Rhinoleucophenga* com base principalmente em dados morfológicos, expandindo o conhecimento quanto às relações filogenéticas entre as espécies deste gênero, e destas com espécies de outros gêneros de Steganinae.

1.2.2. Objetivos específicos

1. Descrever novas espécies pertencentes ao gênero *Rhinoleucophenga* (Capítulos II, III, IV e V);
2. Contribuir para a elucidação de problemas taxonômicos referentes à carência de detalhamento em descrições antigas de espécies de *Rhinoleucophenga*, através de redescrições (Capítulos III, IV e VI);
3. Verificar a variabilidade de caracteres morfológicos e moleculares entre populações de *Rhinoleucophenga punctulata* Duda de diferentes localidades na região Neotropical (Capítulo VII);
4. Testar a monofilia do gênero *Rhinoleucophenga* com base em caracteres morfológicos, propondo uma primeira hipótese evolutiva referente às relações filogenéticas entre as espécies do gênero (Capítulo VIII);
5. Investigar a influência de caracteres morfológicos contínuos sob diferentes tratamentos metodológicos para a proposição de hipóteses filogenéticas para *Rhinoleucophenga* (Capítulo VIII);
6. Investigar a flutuação da estrutura das assembleias de Drosophilidae através da interação das espécies com variáveis abióticas em uma área de bioma Pampa (Capítulo IX);

7. Atualizar a lista de espécies de Drosophilidae com registros no bioma Pampa proposta por Poppe *et al*. (2014) (Capítulo X).

1.3. Resultados Gerais

1.3.1. Redescrições e novas espécies de *Rhinoleucophenga*

O gênero *Rhinoleucophenga* Hendel é composto por 29 espécies formalmente descritas, com distribuição exclusiva nas regiões Neotropical e Neártica. O crescente número de espécies coletadas em trabalhos de levantamento de fauna associado a grande quantidade de espécies mal identificadas, em função da precária riqueza de detalhes em descrições antigas, nos fez revisar e redescrever *Rhinoleucophenga brasiliensis* e *R. fluminensis* originalmente descritas por Lima (1935) (Capítulo III), e *R. personata*, *R. lopesi*, *R. angustifrons*, *R. matogrossensis* e *R. nigrescens* originalmente descritas por Malogolowkin (1946) (Capítulo IV). A revisão morfológica e taxonômica do gênero motivou o desenvolvimento de pesquisas com enfoque evolutivo – um dos objetivos desta Tese.

Descrições complementares de *Rhinoleucophenga obesa* (Loew) (Capítulo VI), *R. joaquina* Schmitz, Gottschalk & Valente (Capítulo II) e *R. punctulata* Duda (Capítulo VII) também foram realizadas, apontando novos caracteres que facilitam a identificação dessas espécies.

Dezessete novas espécies foram propostas para o gênero (Capítulos II, III, IV e V): *Rhinoleucophenga punctata* sp. nov., *R. paraguayensis* sp. nov., *R. ignota* sp. nov., *R. fusca* sp. nov., *R. alata* sp. nov., *R. paulistorum* sp. nov., *R. obscura* sp. nov., *R. fulva* sp. nov., *R. maculosa* sp. nov., *R. nigra* sp. nov. *R. brasilis* sp. nov., *R. punctuloides* sp. nov., *R. trivisualis* sp. nov., *R. flava* sp. nov., *R. grimaldii* sp. nov., *R. exigua* sp. nov. e *R. jacareacanga* sp. nov.

1.3.2. Revisão de *Rhinoleucophenga obesa* sensu Malogolowkin (1946) e Lima (1935)

Espécimes identificados e descritos como *R. obesa* por Malogolowkin (1946) e Lima (1935) foram revisados e redescritos nos atuais padrões taxonômicos para Drosophilidae (Capítulo VI).

Características morfológicas relacionadas ao número e a forma de inserção das preensisetas no epândrio dos machos, e a ausência de "espículas" na cápsula da

espermateca das fêmeas de *R. obesa* sensu Malogolowkin (1946) e sensu Lima (1935) foram indicados como bons caracteres para a separação entre aquela espécie e *R. gigantea* (Thomson). Esses caracteres foram eficientes para a identificação de espécimes coletados em diferentes localidades do sul ao norte do Brasil. Adicionalmente, a separação entre *R. obesa* sensu Malogolowkin (1946) e *R. gigantea* foi confirmada através da comparação de sequências de *COI* e análise filogenética.

Rhinoleucophenga gigantea foi registrada pela primeira vez no Bioma Cerrado. Os espécimes identificados no Bioma Pampa como *R. obesa* por Poppe *et al*. (2014) foram transferidos para *R. gigantea*. Ainda, *R. obesa* sensu Malogolowkin (1946) foi registrada pela primeira vez no estado de Pernambuco.

1.3.3. Variação morfológica e molecular entre populações de *Rhinoleucophenga punctulata* na região Neotropical

Rhinoleucophenga punctulata Duda parece ser uma das espécies mais amplamente distribuídas do gênero, sendo comumente e abundantemente encontrada em ambientes abertos e quentes na região Neotropical. Populações de *R. punctulata* dos biomas Caatinga, Cerrado, Pampa e também da região Amazônica revelaram variação morfológica referente à forma das espermatecas das fêmeas (Capítulo VII). Em nível molecular, a média de divergência entre as populações do Pampa, Cerrado e Amazônia para o gene *COI* foi de 0,7-1,0%, enquanto que a variação entre a população da Caatinga e as demais foi de 2,0-2,4%; porém, a variação máxima dentro da população da Caatinga superou esses valores, apontando para altos níveis de variação intraespecífica em *R. punctulata*.

Através de análises filogenéticas um clado exclusivo de espécimes da Caatinga foi obtido. A associação entre os resultados filogenéticos e os valores de divergência entre as sequencias de *COI* indicam uma possível origem de *R. punctulata* na região da Caatinga, migrando posteriormente para o Cerrado e região Amazônica, em seguida expandindo sua distribuição ao Sul, no bioma Pampa.

1.3.4. Relações filogenéticas de *Rhinoleucophenga* com base em dados morfológicos.

Caracteres morfológicos contínuos, tratados como razões ou como medidas absolutas, exercem alta influência sobre a topologia das árvores geradas (Capítulo VIII). Da mesma maneira, foram fundamentais para a elevação dos valores de suporte dos principais agrupamentos de espécies obtidos na filogenia proposta para *Rhinoleucophenga*.

Independente do tratamento aplicado aos dados contínuos, *Rhinoleucophenga* mostrou-se um gênero parafilético em relação ao gênero *Pararhinoleucophenga*. Além disso, seis agrupamentos monofiléticos de espécies foram repetidamente obtidos nas análises desenvolvidas, principalmente quando os caracteres contínuos foram tratados como razões e *log-transformados*, sendo esta, teoricamente, a melhor hipótese filogenética para *Rhinoleucophenga* até o momento.

1.3.5. Influência de fatores ambientais sobre as assembleias de drosofilídeos

O bioma Pampa apresentou-se como um ambiente muito diverso em relação à fauna de Drosophilidae. Em um total de 55.860 drosofilídeos coletados, foram encontradas 62 espécies de *Drosophila*, 13 de *Rhinoleucophenga*, oito de *Zygothrica*, duas de *Amiota*, duas de *Leucophenga* e uma de *Zaprionus*; deste total, 26 morfotipos não foram relacionados a espécies já descritas. Cabe destacar a riqueza de *Rhinoleucophenga* no Pampa. De fato, este passou a ser o ambiente Neotropical com maior número de espécies registradas do gênero.

Drosophila trapeza Heed & Wheeler, *D. senei* Vilela, *D. suzukii* Matsumura e *Zy. dispar* Wiedemann foram encontradas pela primeira vez no Pampa, sendo este o ponto de registro mais ao sul para *D. trapeza* e *Zy. dispar*.

A heterogeneidade ambiental foi um fator importante na estrutura das assembleias, explicando 8,86% da composição das mesmas. No entanto, a interação entre temperatura e os níveis de umidade foi indicada como o componente ambiental mais influente sobre as assembleias de Drosophilidae, explicando 37,28% de toda a

variação percebida. Mais interessante foi a interação entre o tipo de ambiente amostrado e os componentes climáticos; juntos estes elementos explicaram 56,45% da variação percebida nas assembleias de drosofilídeos (Capítulo IX).

As assembleias do interior das manchas de mata são menos afetadas pela adversidade climática, sendo um ambiente mais estável e dominado por espécies neotropicais (62,3%). No entanto, ambientes abertos e fechados, campos e matas, respectivamente, apresentaram composição peculiar das assembleias de Drosophilidae e, devem ser igualmente considerados em estratégias de preservação do bioma Pampa.

1.3.6. Atualização da lista de espécies de Drosophilidae com registros no Pampa

Treze espécies foram incluídas na lista de registros originalmente proposta por Poppe *et al*. (2014) para o bioma Pampa (Capítulo X), são elas: *Drosophila senei* Vilela, *D. suzukii* Matsumura, *D. trapeza* Heed and Wheeler, *Hirtodrosophila levigata* (Burla), *H. mendeli* (Mourão, Gallo and Bicudo), *H. morgani* (Mourão, Gallo and Bicudo), *Mycodrosophila projectans* (Sturtevanti), *Paraliodrosophila antennata* (Wheeler), *Rhinoleucophenga joaquina* Schmitz, Gottschalk and Valente, *R. punctuloides* Poppe, Schmitz and Valente, *Zygothrica poeyi* (Sturtevanti), *Zy. prodispar* Duda e *Zy. dispar* Wiedemann. Para quase todas, exceto *Drosophila senei* e *D. suzukii*, o Pampa representa a localidade de registro mais ao sul.

Novas áreas amostradas também foram incluídas: São Gabriel (30°20'44"S, 54°19'32"O), Santiago (Robe *et al*. 2014) (29°11′09″S, 54°53′50″O), Pelotas (Robe *et al*. 2014) (31°48′58″S, 52°25′55″O) e Rio Grande (Robe *et al*. 2014) (32°32′25″S, 52°32′34″O).

Até o momento, totaliza-se 108 espécies registradas neste bioma compartilhado por Brasil, Uruguai e Argentina.

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2. CAPÍTULO II

(Manuscrito publicado no periódico *Zootaxa*)

Poppe, J.L., Schmitz, H.J. & Valente, V.L.S. (2015) The New World genus *Rhinoleucophenga* (Diptera: Drosophilidae): new species and notes on occurrence records. *Zootaxa*, 3955, 349–370.

2.1. The New World genus *Rhinoleucophenga* (Diptera: Drosophilidae): new species and notes on occurrence records

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2.1.1. ABSTRACT

The genus *Rhinoleucophenga* Hendel comprises 26 nominal species with New World distribution. In the present study, two new species are described from samples in the Pampa and Caatinga biomes in Brazil, *R. punctuloides* sp. nov. and *R. trivisualis* sp. nov., respectively. *Rhinoleucophenga punctuloides* sp. nov. is a sibling species of *R. punctulata* Duda. Furthermore, two females of *R. joaquina* Schmitz, Gottschalk & Valente were found for the first time and a description is presented. A taxonomic dichotomous key with pictures is given for the *Rhinoleucophenga* species recorded in the Caatinga and Pampa biomes. The Neotropical open environments are areas of high diversity for *Rhinoleucophenga*. The description of new species and review of some older descriptions can change the area of species distribution and improve the faunistic knowledge of other localities in which previous studies have shown unidentified or misidentified *Rhinoleucophenga* species.

KEY WORDS: Taxonomy, Steganinae, neotropics, Pampas, Caatinga, biodiversity.

2.1.2. RESUMO

O gênero *Rhinoleucophenga* Hendel compreende 26 espécies descritas com distribuição no Novo Mundo. No presente estudo, duas novas espécies são descritas a partir de coletas realizadas nos biomas Pampa e Caatinga, *R. punctuloides* sp. nov. e *R. trivisualis* sp. nov., respectivamente. *Rhinoleucophenga punctuloides* sp. nov. é uma espécie críptica de *R. punctulata* Duda. Além disso, duas fêmeas de *R. joaquina* Schmitz, Gottschalk & Valente foram encontradas pela primeira vez e a descrição para a espécie é apresentada. Uma chave taxonômica dicotômica com imagens é apresentada para espécies de *Rhinoleucophenga* com registros nos biomas Pampa e Caatinga. Os ambientes neotropicais de vegetação aberta têm apresentado alta diversidade de espécies de *Rhinoleucophenga*. Porém, a descrição de novas espécies e a revisão de algumas descrições antigas pode mudar o cenário de distribuição das espécies, e também melhorar o conhecimento faunístico de algumas localidades, para as quais existem muitos estudos prévios com espécies de *Rhinoleucophenga* não identificadas ou identificadas incorretamente.

PALAVRAS-CHAVE: Taxonomia, Steganinae, neotrópico, Pampa, Caatinga, biodiversidade.

2.1.3. INTRODUCTION

Rhinoleucophenga Hendel is a genus of Drosophilidae with Neotropical and Nearctic distribution. It was established by Hendel (1917) with *R. pallida* Hendel from Peru as the type-species. Currently, *Rhinoleucophenga* comprises 26 nominal species (Bächli 2014), which were mostly described in the first half of the 20th century (Duda 1927, 1929; Malogolowkin 1946). Recently Poppe *et al*. (2014) highlighted the diversity of this genus in the South American Pampa biome, in which ten out of the 51 Drosophilidae species collected belonged to *Rhinoleucophenga*, representing the greatest richness found in the genus.

However, some taxonomic problems associated with the genus remain, especially concerning species identities and the existence of several undescribed species, resulting in misidentification and uncertainties in the geographical distribution of some species, e.g., *R. obesa* Loew. This would be the most widespread species of *Rhinoleucophenga*, including records from Canada to the south of Brazil. However, substantial doubt regarding the identity and, consequently, the distribution of the species

remain, and some authors suggest that the name *R. obesa* may indeed comprise a group of sibling species (Vilela 1990). Other widespread species include *R. punctulata* Duda, which is broadly found in open environments in South America (Vilela & Bächli 2009; Roque & Tidon 2013; Poppe *et al*. 2014). No sibling species of *R. punctulata* has been found until now, despite confusion regarding the Nearctic species *R. bivisualis* (Patterson), which also has a spotted thorax. On the other hand, the genus is very poorly known, several *Rhinoleucophenga* species are known only from types or specimens collected at the type-locality (Duda 1927; Malogolowkin 1946; Lima 1950; Poppe *et al*. 2014).

In the present study, two new species are described, *R. trivisualis* sp. nov. and *R. punctuloides* sp. nov. The latter is a sibling species of *R. punctulata*; thus, a review of many *R. punctulata* specimens collected in different areas was performed to elucidate its intraspecific variability. The description of the female of *R. joaquina* Schmitz, Gottschalk & Valente is also presented, together with new distribution records. An illustrated dichotomous key is given for the *Rhinoleucophenga* species recorded in the Pampa and Caatinga biomes based on the available literature and collected specimens.

2.1.4. MATERIALS AND METHODS

Specimens of *Rhinoleucophenga* were collected in banana-baited traps (Tidon & Sene 1988) in the Pampa (28°45’01”S 54°56’55”W, 200 m) and Caatinga (9°30'39"S 38°32'12"W, 500 m) biomes. Both areas predominantly consist of open vegetation with a mosaic of forest patches and have been rapidly degraded as a consequence of the unsustainable exploration (MMA 2007; Paes & Dias 2008) (Fig. 1).

Descriptions are based on measures and indices given by Bächli et al. (2004); measurements represent averages followed by the ranges in parentheses, which were measured with an ocular reticle inserted into a stereomicroscope. Male and female terminalia were disarticulated in glycerol after treatment with 10% potassium hydroxide (KOH) and acid fuchsine (Bächli et al. 2004). The genitalia were mounted in a piece of glycerine jelly (ca. 2 x 2 x 2 mm) on a clean slide (Grimaldi 1987). Photos of the specimens were taken with a digital camera coupled to an optical stereomicroscope after the specimens were dried with hexamethyldisilazane (HMDS) (Brown 1993) and pinned. Drawings of the genitalia were made with a *camera lucida* system attached to a compound microscope with 40× and 10× objective lenses and a 10× ocular lens. The terminology follows Grimaldi (1990), Vilela (1990) and Bächli *et al*. (2004). All

holotypes and paratypes are deposited in the Entomological Collection of the Institute Oswaldo Cruz (CEIOC), at Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil. The paratype specimens are stored in microvials with 96% alcohol. The disarticulated terminalia are stored in microvials with glycerol and pinned with the respective specimens.

The type series of *R. joaquina* was examined to establish the identity of females. Samples of *R. punctulata* were obtained from the Cerrado biome (Roque & Tidon 2008), southern Amazonian savanna enclave (6°13'36"S 48°27'55"W), and the Pampa (Poppe *et al*. 2014) and Caatinga biomes (Oliveira GF, personal communication). The specimens were obtained by collection with banana baited traps. Additional specimens from Chaco (Asunción, Paraguay) deposited in the Entomological Collection of the Institute Oswaldo Cruz (CEIOC) at Fundação Oswaldo Cruz (Fiocruz), Brazil, were also analyzed. The collecting methods and coordinate information of the verified specimens from Paraguay are unknown.

To check the identity of *R. punctulata* specimens, the same characteristics of general morphology and terminalia used in the descriptions were observed. Male and female terminalia were disarticulated as above.

A dichotomous key is given for the *Rhinoleucophenga* species recorded in the Pampa and Caatinga biomes, with illustrations of all species. This is an update of the dichotomous key for species from the Pampas proposed by Poppe *et al*. (2014) and presents the main characteristics used to differentiate the species.

2.1.5. RESULTS

New ***Rhinoleucophenga*** **species**

Rhinoleucophenga Hendel

Rhinoleucophenga Hendel, 1917: 44-45

Pseudophortica Sturtevant, 1918: 37

Gitona (in New world) Brake & Bächli, 2008: 291

Rhinoleucophenga trivisualis sp. nov.

(Figures 2a-e; 3a-d; 4a-c)

Type series. Holotype: 1m# labelled "*Rhinoleucophenga trivisualis*; HOLOTYPE m#; Brazil, Bahia, Estação Ecológica Raso da Catarina/ Município de Paulo Afonso. 9°30'39"S 38°32'12"W, 22.iv.2012 col.: GF Oliveira; banana bait". Postabdomen of holotype dissected, stored in microvial with glycerin, stored on the same pin with the respective specimen. Paratypes: 2m# and 2f# labelled "*Rhinoleucophenga trivisualis*; PARATYPE; Brazil, Bahia, Estação Ecológica Raso da Catarina/ Município de Paulo Afonso. 9°30'39"S 38°32'12"W, 22.iv.2012 col.: GF Oliveira; banana bait". Holotype and paratypes are deposited at CEIOC/Fiocruz.

Diagnosis. Head covered with ca. 26 scattered interfrontal setulae, eyes with three transverse light stripes (well noted when the specimen is preserved in alcohol). Scutum and scutellum brownish covered with small dark brown spots (Fig. 2b), pleura yellowish with two large dark brown longitudinal stripes, abdomen yellowish with brown stripes in each tergite medially widely interrupted and laterally connected (Fig. 2a-b). Wings hyaline (Fig. 2c). Male terminalia as in Figure 3a-d.

Description. m#. Head (Fig. 2a; 2d-e). Front brownish, covered with ca. 26 scattered interfrontal setulae, frontal length 0.57mm (0.56-0.58); frontal index = 1.14 (1.12-1.16); top-to-bottom width ratio = 1.09 (1.06-1.12); ocellar triangle to front length ratio = 0.42 (0.38-0.46); or1/or3 ratio = 1.08 (1.07-1.09); or2/or1 ratio = 0.61 (0.58-0.64); vibrissal index = 0.43 (0.36-0.50). Carina prominent. Cheek index = 8.10 (6.7-9.50). Eye index = 1.31 (1.27-1.35). Antenna with the tip of flagellomeres darker brown, arista with short branches, 12 dorsal branches and 10 ventral branches plus terminal fork. Palpus yellowish with ca. 15 setae on lower part. Eyes with three transverse light stripes (Fig. 2d) (well noted when the specimen is preserved in alcohol).

Thorax (Fig. 2a-b). Thorax length 1.60mm (1.52-1.68). Scutum and scutellum brownish; scutum covered with many small dark brown spots. 10 rows of acrostichal setulae. 2 pairs of prescutellar acrostichal setae, the central pair strongest, about 56% (48-64) of posterior dorsocentral setae. Postpronotum with one setae. Transverse distance between dorsocentral setae 3.32x (2.80-3.85) longitudinal distance. Basal scutellar setae divergent. Sterno index = 1; median katepisternal setae absent. Halteres whitish. Legs yellow with brown annuli subdistally on femora and basally on tibiae.

Wings (Fig. 2c). Hyaline. Length 2,92mm (2.85-3.00); width 1.22mm (1.20-1.25). Indices: C = 3.72 (3.36-4.09); hb = 0.49 (0.40-0.59); Ac = 1.26 (1.15-1.38); 4c = 1.12

(1.00-1.25); 4v = 3.10 (2.95-3.25); 5x = 1.46 (1.42-1.50); M = 1.02 (1.00-1.05); prox.x = 1.40 (1.36-1.45).

Abdomen (Fig. 2a-b). Yellowish, each tergite with broad brown posterior stripes widely interrupted dorsomedially and expanded and connecting laterally.

Body length: 3.62mm (3.50-3.75).

Terminalia m# (Fig. 3a-d). Epandrium microtrichose, fused with surstyli. Approximately 9 prensisetae. About 6 upper and 20 lower setae on each side of epandrium. Cerci elongate, with ca. 25 setae each, 3-4 longer setae in the apical portion. Aedeagus ring-shaped, with squared aspect in frontal and dorsal view, the apical portion slightly wider than the base. Aedeagus apodeme long and bifurcate in the posterior region.

f#. Head. Same color pattern and setation as in male. Frontal length = 0.51mm (0.50-0.52); frontal index = 1.04; top-to-bottom width ratio = 1.10 (1.08-1.12); ocellar triangle to front length ratio = 0.43 (0.42-0.44); or1/or3 ratio = 0.93 (0.86-1.00); or2/or1 ratio = 0.86 (0.81-0.91). vibrissal index = 0.35 (0.33-0.38). Cheek index = 9.12 (8.75-9.50). Eye index = 1.28 (1.25-1.31). Other characters as in male.

Thorax. Same color pattern as in male. Thorax length 1.52mm (1.50-1.54). 10 rows of acrostichal setulae. 1 pair of prescutellar setae, about 67% (64-71) of posterior dorsocentral setae. Transverse distance between dorsocentral setae 3.6x (3.40-3.80) the longitudinal distance. Sterno index =1. Other characters as in male.

Wings. Hyaline. Length 2.77mm (2.75-2.78); width 1.25mm. Indices: C = 3.05 (2.73-3.38); hb = 0.48 (0.46-0.50); Ac = 1.60 (1.44-1.76); 4c = 1.12 (1.04-1.20); 4v = 2.48 (2.40-2.56); 5x = 1.23 (1.13-1.33); M = 0.74 (0.68-0.80); prox.x = 1.06 (1.00-1.12).

Abdomen. Same color pattern as in male.

Body length: 3.25mm (3.10-3.40).

Terminalia f# (Fig. 4a-c). Cerci long and well sclerotized with 4 longer apical setae on each one. Epiproct with ca. 7 setae, two longer apical ones. Hypoproct with many subequal setae and ca. 6 longer apical setae. Spermathecal capsule slightly elongated, with basal introvert reaching ca. ¾ of inner capsule.

Etymology. The species name refers to its three light stripes on the eyes, which is a peculiar characteristic of this species.

Type locality. Brazil, Bahia, Estação Ecológica Raso da Catarina/ Município de Paulo Afonso (9°30'39"S 38°32'12"W).

Distribution. Known from the type locality, and from Parque Nacional Serra da Capivara, municipality of São Raimundo Nonato, Piauí state, Brazil (Oliveira GF, personal communication).

Biology. Collected in fermented-banana traps, in the Caatinga *sensu strictu*.

Rhinoleucophenga punctuloides sp. nov.

(Figures 5a-d; 6a-d; 7a-c)

R. lp1 Poppe *et al*., 2014: 219, 221, 230 (key)

Type series. Holotype: 1m# labelled "*Rhinoleucophenga punctuloides*; HOLOTYPE m#; Brazil, Rio Grande do Sul, Bossoroca. 28°45'01"S 54°56'55"W, 20.xii.2011 col.: JL Poppe; banana bait". Postabdomen of holotype dissected, stored in microvial with glycerin, stored on the same pin with the respective specimen. Paratypes: 4m# and 2f# labelled "*Rhinoleucophenga punctuloides*; PARATYPE; Brazil, Rio Grande do Sul, Bossoroca. 28°45'01"S 54°56'55"W, 20.xii.2012 col.: JL Poppe; banana bait". Holotype and paratypes are deposited in CEIOC/Fiocruz.

Diagnosis. Scutum brown covered with many small dark brown spots at bases of setae and setulae, two diffuse longitudinal dark brown stripes (Fig. 5a). Head covered with ca. 40 scattered interfrontal setulae (Fig. 5c), abdomen yellow with dark brown band which is medially interrupted and laterally broadened (Fig. 5a). Wings hyaline, C-index= 2.77 (2.4-3.14) in male (Fig. 5b). Male terminalia as in Figure 6a-d. The females' spermathecal capsule presents an invagination that reaches the basal introvert (Fig. 7c).

Description. m#. Head (Fig. 5c). Front brownish-yellow, covered with ca. 40 scattered interfrontal setulae, frontal length 0.46mm (0.43-0.50); frontal index = 1.28 (1.23-1.33); top-to-bottom width ratio = 1.15 (1.10-1.20); ocellar triangle to front length ratio = 0.45 (0.40-0.50); or1/or3 ratio = 1.04 (1.00-1.08); or2/or1 ratio = 0.78 (0.70-0.87), each orbital setae with a brown patch around base; vibrissal index = 0.36 (0.25-0.47). Carina narrow, slightly nose-like and sulcate. Cheek index = 8.01 (7.2-9). Eye index = 1.29 (1.24-1.34). Antenna with flagellomeres of the same color as front, arista pubescent, with 6 dorsal branches and 4 ventral branches plus terminal fork. Palpus yellowish with ca. 15 setae on lower part.

Thorax (Fig. 5a). Thorax length 1.31mm (1.21-1.41). Scutum and scutellum brown; scutum covered with many small dark brown spots at bases of setae and setulae, with two diffuse longitudinal dark brown stripes. 8 rows of acrostichal setulae. 3 pairs of prescutellar acrostichal setae, the central pair strongest, about 60% (59-62) of posterior

dorsocentral setae. Postpronotum with one setae. Transverse distance between dorsocentral setae 3.86x (3.57-4.16) longitudinal distance. Basal scutellar setae divergent. Sterno index = 0.91 (0.88-0.94); median katepisternal setae absent; pleura yellowish with a diffuse brownish median stripe. Halteres whitish yellow. Legs yellow.

Wings (Fig. 5b). Hyaline. Length 2.43mm (2.33-2.54); width 0.97mm (0.86-1.08). Indices: C = 2.77 (2.40-3.14); hb = 0.52 (0.48-0.57); Ac = 1.68 (1.45-1.92); 4c = 1.27 (1.06-1.48); 4v = 2.81 (2.60-3.02); 5x = 1.62 (1.25-2.00); M = 0.60 (0.50-0.71); prox.x = 1.14 (0.95-1.33).

Abdomen (Fig. 5a). Abdomen with yellow ground color, tergite II with a dark brown stripe widely interrupted medially, tergites III to VI each with a broad, dark brown stripe which is medially interrupted and laterally broadened; the stripes are gradually enlarged towards tip of abdomen.

Body length: 2.65mm (2.50-2.80).

Terminalia m# (Fig. 6a-d). Epandrium microtrichose, fused with surstyli. Approximately 14 prensisetae and about 7 inner setae and 17 outer setae on each surstylus. About 7 upper and 8 lower setae on each side of epandrium. Cerci elongated presenting a peculiar curved shape, with ca. 40 setae each, 15-20 longer setae in the apical portion; among the cerci there are two elongated structures microtrichose similar to finger tips. Aedeagus elongate, compound by two parallel structures wider in the base, apical portion is pointed; dorsal side with a medially pointed elongation, ventral side with about seven small pointed elongations like spines, both structures can be seen in lateral view. Apodeme long and bifurcate in the posterior region.

f#. Head. Same color pattern and setation as in male. Frontal length = 0.52mm (0.48-0.56); frontal index = 1.21 (1.19-1.24); top-to-bottom width ratio = 1.11 (1.07-1.14); ocellar triangle to frontal length ratio = 0.55 (0.50-0.60); or1/or3 ratio = 1.18 (1.11-1.25); or2/or1 ratio = 0.77 (0.70-0.83). vibrissal index = 0.29 (0.25-0.32). Cheek index = 6.3 (5.00-7.60). Eye index = 1.25 (1.20-1.30). Other characters as in male.

Thorax. Same color pattern as in male. Thorax length 1.46mm (1.32-1.60). 6 rows of acrostichal setulae. 1 pair of prescutellar setae, about 65% (61-68) of posterior dorsocentral setae. Transverse distance between dorsocentral setae 4x (3.85-4.16) the longitudinal distance. Sterno index =0.95 (0.90-1.00). Other characters as in male.

Wings. Hyaline. Length 2.55mm (2.35-2.75); width 1.1mm (1.00-1.20). Indices: C = 3.26 (3.11-3.41); hb = 0.65 (0.58-0.72); Ac = 1.52 (1.38-1.67); 4c = 1.04 (0.95-1.12); 4v

= 2.55 (2.35-2.75); 5x = 1.50 (1.29-1.70); M = 1.52 (0.67-0.85); prox.x = 1.05 (1.00-1.10).

Abdomen. Same color pattern as in male.

Body length: 2.85mm (2.70-3.00).

Terminalia f# (Fig. 7a-c). Cerci long with ca. three longer apical setae on each. Epiproct short with few subequal setae. Hypoproct large with many setae including few longer ones. Spermathecal capsule elongate. Basal introvert reaching almost the top if inner capsule. The top of the spermathecal capsule presents an invagination that reaches the basal introvert.

Etymology. The species name refers to its spotted thorax and its strong similarity with *R. punctulata* based on external morphology.

Type locality. Brazil, Rio Grande do Sul, Bossoroca (28°45'01"S 54°56'55"W).

Distribution. Known only from the type locality.

Biology. Collected in fermented-banana traps, along the edges of forest patches of Pampa biome.

Rhinoleucophenga joaquina Schmitz, Gottschalk & Valente

(Figures 8a-c; 9a-c)

R. joaquina Schmitz *et al*., 2009: 786-790

Type series. 2f# labelled "*Rhinoleucophenga joaquina*; Specimen 01 f#; Brazil, Rio Grande do Sul, Bossoroca. 28°45'01"S 54°56'55"W, 12.x.2013 col.: JL Poppe; banana bait". Specimen 02 f#; Brazil, Bahia, Estação Ecológica Raso da Catarina/ Município de Paulo Afonso. 9°30'39"S 38°32'12"W, 22.iv.2012 col.: GF Oliveira; banana bait". Holotype and paratypes are deposited in CEIOC/Fiocruz.

Diagnosis. Head covered with ca. 55 (50-60) scattered interfrontal setulae, arista microtrichose, with ca. 10 very short dorsal branches and 6 ventral branches (Fig. 8a). One strong pair of prescutellar acrostichal setae (Fig. 8b). Legs yellow, wings hyaline (Fig. 8c). The abdominal color pattern yellow, with black, medially interrupted marginal bands and a medial black stripe extending from the tip of the abdomen to tergite III or IV (Fig. 8b).

f#. Head (Fig. 8a). Frons yellowish covered with ca. 55 (50-60) scattered interfrontal setulae. Frontal length = 0.61mm (0.60-0.62); frontal index = 1.27 (1.24-1.30); top-to-bottom width ratio = 1.00 (0.92-1.08); ocellar triangle to frontal length ratio = 0.36 (0.33-0.38); or1/or3 ratio = 1.53; or2/or1 ratio = 0.35. Carina prominent, nose-like.

Cheek index = 5.25 (4.24-6.26). Eye index = 1.30 (1.27-1.32). Antenna with flagellomeres of the same color as front, arista microtrichose with ca. 10 dorsal branches and 6 ventral branches plus terminal fork. Palpus yellowish with ca. 20 setae on lower part.

Thorax (Fig. 8b). Scutum homogeneously brownish or with three faint longitudinal stripes slightly darker. Thorax length 1.38mm (1.33-1.44). 14 rows of acrostichal setulae. 1 pair of prescutellar setae, about 57% of posterior dorsocentral setae. Postpronotum with one setae. Transverse distance between dorsocentral setae 4.71x (4.42-5.00) the longitudinal distance. Basal scutellar setae divergent. Sterno index = 0.89; median katepisternal setae absent; pleura brownish. Halteres whitish yellow. Legs yellow.

Wings (Fig. 8c). Hyaline. Length 3.3mm (3.1-3.5); width 1.47mm (1.4-1.55). Indices: C = 3.25 (3-3.5); hb = 0.41 (0.40-0.43); Ac = 1.24 (1.11-1.36); 4c = 1.00 (1.00-1.00); 4v = 2.45 (2.23-2.66); 5x = 1.57 (1.46-1.68); M = 0.81 (0.73-0.90); prox.x = 0.98 (0.96-1.00).

Abdomen (Fig. 8b). Yellow, with black, medially interrupted marginal bands on tergites and a medial black stripe extending from the tip of the abdomen to tergite III or IV.

Body length: 3.62mm (3.50-3.75).

Terminalia f# (Fig. 9a-c). Cerci long with many longer apical setae on each one. Epiproct short with few subequal setae. Hypoproct wider than long. Spermathecal capsule rounded, basal introvert reaching ca. ¾ of inner capsule.

Type locality. Brazil, Rio Grande do Sul, Bossoroca (28°45’01”S 54°56’55”W).

Distribution. Males known previously only from the type-locality (Joaquina, Florianópolis, Santa Catarina, Brazil). Now the distribution is extended southwards to Pampa (Bossoroca, Rio Grande do Sul) and northwards to Caatinga (Raso da Catarina, Paulo Afonso, Bahia).

Biology. Previously this species has been found breeding in *Dyckia encholirioides* (Bromeliaceae) flowers in coastal dunes (Schmitz *et al.* 2009). Collected in fermented-banana traps, along the edges of forest patches in pampas, and in the Caatinga *sensu strictu*.

Rhinoleucophenga punctulata Duda

(Figures 10a-e; 11a-c)

R. punctulata Duda, 1929: 43-44; *R. punctulata* Malogolowkin, 1946: 417, 422; *R. punctulata* Roque & Tidon, 2008: 97; *R. punctulata* Vilela & Bächli, 2009: 186-191; *R. punctulata* Roque & Tidon, 2013: 119; *R. punctulata* Poppe *et al*., 2014: 220, 230, 235.

The identity of all *R. punctulata* specimens was confirmed through the male terminalia according to the redescription performed by Vilela & Bächli (2009) and through the comparison of the arista branch pattern with *R. punctuloides* sp nov. The arista branches are longer and curved (s-shaped) in *R. punctulata* (Fig. 10c), while branches are short and straight in *R. punctuloides* sp. nov. (Fig. 5d). Because both species occur sympatrically in the Pampas, knowledge of the terminalia of *R. punctulata* female from the Pampas (Fig. 11a-c) is also important to the species determination. The females can be differentiated through comparisons of the spermathecal capsule; *R. punctuloides* sp. nov. presents an invagination in the tip of the spermathecal capsule (Fig. 7c) that is not seen in *R. punctulata* (Fig. 11c).

Key to *Rhinoleucophenga* species recorded in the Pampa and Caatinga biomes

1. Wings clouded at least on cross veins and on the end of veins R_{2+3} and R_{4+5}; body length 5.5 mm or larger…….……..…...2
- Wings hyaline; body length less than 5.5 mm ……..……..…..4

2. End of M vein not clouded, costal cell not clouded; R_{2+3} vein without supernumerary veins; body color mainly yellow ……..……...3

- End of M vein strongly clouded, costal cell clouded; R_{2+3} vein with ca. 2-4 clouded supernumerary veins; body color brownish…… *R. pampeana* Poppe *et al*. (Fig. 12a-d)

3. Arista with 9-10 dorsal branches; aedeagus somewhat oval-shaped, rounded on top, with a slight elongation (Fig. 11a in Poppe *et al*. 2014)…….....*R. obesa* Loew (sensu Malogolowkin 1946) (Fig. 13a-d)

- Arista with 7-8 dorsal branches; aedeagus somewhat D-shaped, with an elongation medially on top (Fig. 11b in Poppe *et al*. 2014)…….…….*R. gigantea* Thomson (sensu Vilela 1990) (Fig.14a-d)

4. Scutum unicolorous, without spots or stripes……..…… 5

- Scutum covered with small dark brown spots and with two diffuse longitudinal brown stripes between the dorsocentral setae ……..…..... 6

5. Arista with short branches or microtrichose; yellowish fly; abdomen yellowish with interrupted brown stripes…….……..…….. 8

- Arista with long branches; brownish fly; abdomen brownish with terminal portion darker brown, stripes continuous or interrupted ...……...…….... 9

6. Body length 3.5 mm or larger; eyes with three transverse light stripes; pleura yellowish with two large dark brown longitudinal stripes .....….*R. trivisualis* sp. nov. (Fig. 2a-d)

- Body length less than 3.0 mm; eyes without transverse stripes pleura yellowish with one or without large dark brown longitudinal stripes …….....….7

7. Arista with long curved branches; the top of the spermathecal capsule without any invagination…..…….....……...*R. punctulata* Duda (Fig. 10a-e)

- Arista with short straight branches; the top of the spermathecal capsule presents an invagination that reaches the basal introvert……...*R. punctuloides* sp. nov. (Fig. 5a-d)

8. Arista with short branches; abdomen yellowish with interrupted brown stripes on all tergites without medial stripe extending from the tip of abdomen ……..…*R. subradiata* Duda (Fig. 15a-c)

- Arista microtrichose; abdomen yellowish with interrupted brown stripes on tergite II and with medial stripe extending from the tip of abdomen to tergite III or IV…….....….*R. joaquina* Schmitz, Gottschalk & Valente (Fig. 8a-c)

9. Arista with 5 or 6 ventral branches; pleura brownish; coxa with more than 20 setae; aedeagus basal portion as wide as the apice; aedeagus presents a dorsal projection ……....…..…….…….…….. 10

- Arista with 4 ventral branches; pleura dark brown; coxa with less than 20 setae; aedeagus basally wider than the apical portion, aedeagus presents a ventral projection.....*R. capixabensis* Culik & Ventura (Fig. 16a-d)

10. Front covered with ca. 60 scattered interfrontal setulae, frontal index approximately 1.0; costal index ca. 3.5, the abdomen tip darker..... *R. missionera* Poppe *et al*. (Fig. 17a-d)

- Front covered with ca. 50 scattered interfrontal setulae, frontal index approximately 1.3; costal index ca. 2.5, abdomen tip not darker….. *R. sulina* Poppe *et al*. (Fig. 18a-d).

2.1.6. DISCUSSION

The species described here belong to *Rhinoleucophenga* based on the following features: strong prescutellar acrostichal setae, frons densely covered with scattered interfrontal setulae, surstyli fused to epandrium bearing small peg-like prensisetae, simple aedeagus (Vilela & Bächli 2009), only two katepisternal setae, postpronotum with one setae and a pair of divergent basal scutellar setae (Malogolowkin 1946).

Rhinoleucophenga trivisualis sp. nov. resembles *R. bivisualis* (Patterson) because it has a scutum covered with small brownish spots and spotted eyes. It also resembles *R. punctulata* because it has a scutum covered with spots, hyaline wings and similar body color pattern, but it is larger than *R. punctulata*. However, it clearly differs from these two species and from all known species of the genus due to its distinctive pleura, striped pattern, and eye color, with three transverse light stripes, in addition to its aedeagal morphology.

Rhinoleucophenga punctuloides sp. nov. resembles *R. punctulata* because all of the external morphological characteristics are identical, except for the length of the aristal branches and the form of the spermathecal capsule. *R. punctuloides* sp. nov. clearly differs from *R. punctulata* and from all known species of the genus in the morphology of the epandrium and aedeagus.

Rhinoleucophenga punctulata has been widely recorded in the South American continent (Duda 1929; Bächli 1990; Vilela & Bächli 2009; Roque & Tidon 2008, 2013; Poppe *et al*. 2014), ranging from subtropical-temperate to tropical climates. Vilela & Bächli (2009) recorded *R. punctulata* in the Cerrado and Chaco regions and suggested

that it could be a widespread species in these biomes. It may be a widespread species in Pampa and Caatinga as well (Poppe *et al*. 2014; Oliveira GF, personal communication). At this stage, among the *R. punctulata* species analyzed, no *R. punctuloides* sp. nov. were found.

The existence of other sibling species in the *Rhinoleucophenga* genus should not be discounted. Sibling species are well documented in widespread and well-studied groups of *Drosophila*, e.g., *D. willistoni* Sturtevant (Burla *et al*. 1949; Ehrman & Powell 1982), *D. repleta* Sturtevant (Tidon-Sklorz & Sene 2001) and the *D. melanogaster* Sturtevant species group (Tsacas *et al*. 1971; Bock & Wheeler 1972; Moreteau *et al*. 1995). Most species of these sibling sets are widely distributed, but some are geographically restricted and discriminated only by genetic or chromosomal markers or subtle morphological differences, such as the *D. willistoni* subgroup (Cordeiro & Winge 1995; Malogolowkin 1952). Thus, more studies and samples are necessary to determine if *R. punctuloides* sp. nov. is restricted to the Pampas or has a wider distribution, if *R. trivisualis* is restricted to Caatinga, and if other sibling species of *R. punctulata* or other *Rhinoleucophenga* species exist as well.

The first species of *Rhinoleucophenga* collected in flowers in a subtropical area on the south coast of Brazil was *R. joaquina* (Schmitz *et al*. 2009); the present study was conducted with banana traps in the southern subtropical-temperate Pampas, as well as a previous study performed in the northern tropical Caatinga region (Oliveira GF, personal communication). These studies showed that *R. joaquina* is a widely distributed species. Although these environments are climatically different, both are a mosaic of open vegetation with forest patches; these forest patches have more humidity and a more stable temperature, and can act as refugia to flies in hostile environments as shown by Poppe *et al*. (2015) in drosophilids in the Pampas. Consequently the environmental conditions provided by these "refugia" enable the existence of the same species in environments with distinct climatic conditions, allowing a broader distribution of the species, possibly broader than suggested here. Unfortunately no specimen of *Dyckia* was found during field work, and no *Rhinoleucophenga* specimens emerged from other flower samples brought to the laboratory during a previous study (Poppe *et al*. 2014).

The Neotropical open environments are areas of high diversity for *Rhinoleucophenga* (Poppe *et al*. 2014; Oliveira GF, personal communication). However, the description of new species and a review of earlier descriptions can change

the area of species distribution and improve the faunistic knowledge of other localities in which previous studies have shown unidentified or misidentified *Rhinoleucophenga* species.

2.1.7. ACKNOWLEDGEMENTS

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2.1.8. REFERENCES

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2.1.9. FIGURES

Figure 1: Map of South America showing the collection points (Pampa: #1; Caatinga: #2). a: Geopolitical map of South America; b: satellite visualization of South Brazil with the Pampas boundaries (black line); c: satellite visualization of Northeast Brazil with the Caatinga boundaries (black line).

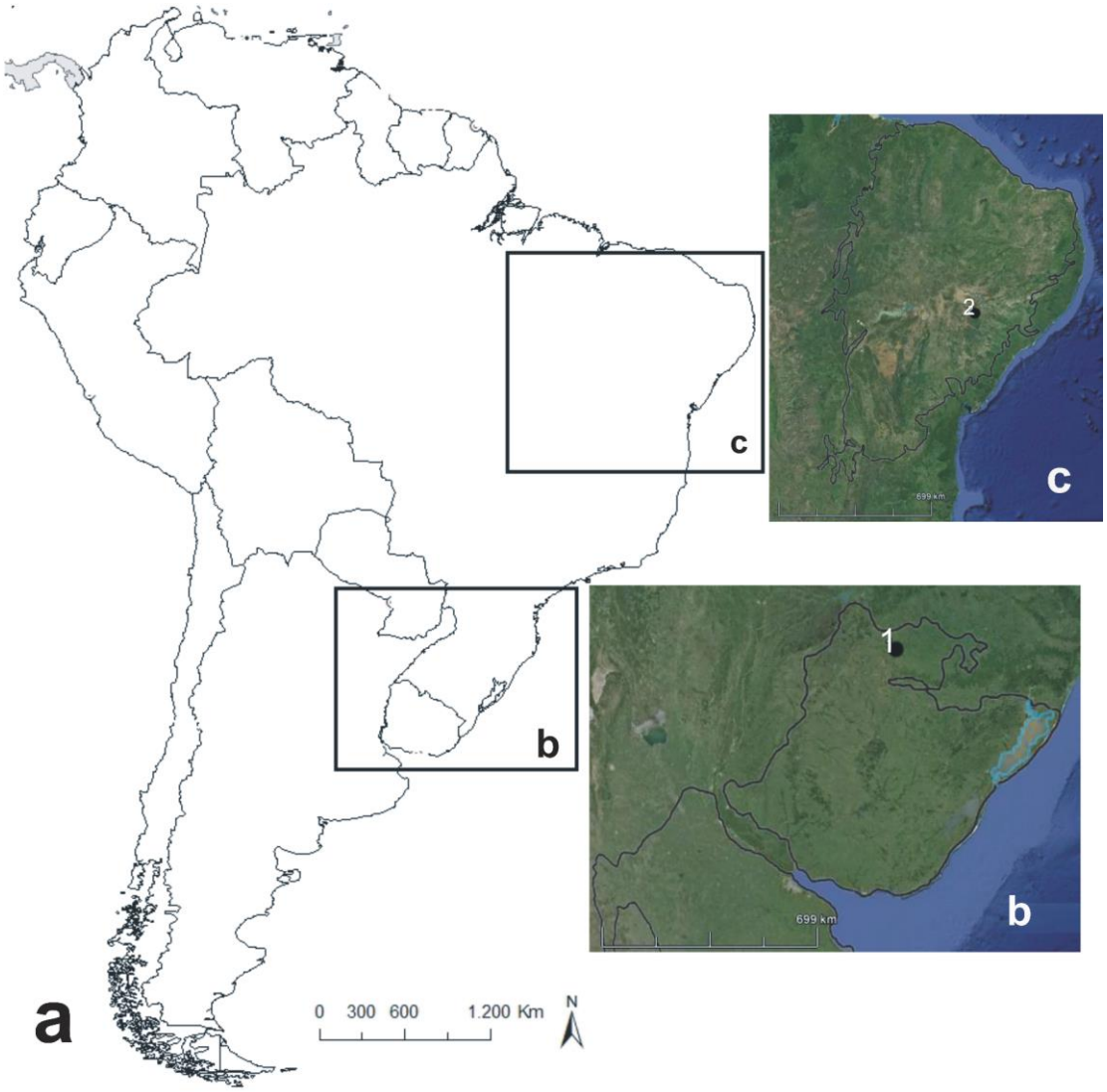


Figure 2: Holotype of *R. trivisualis* sp. nov. a: general habitus, lateral view; b: general habitus, dorsal view; c: wing; d: eyes with three light stripes (indicated by the arrows, in alcohol); e: head, frontal view (scale bar 1.0 mm in a, b and c; 0.5 mm in e; 0.1 mm in d).

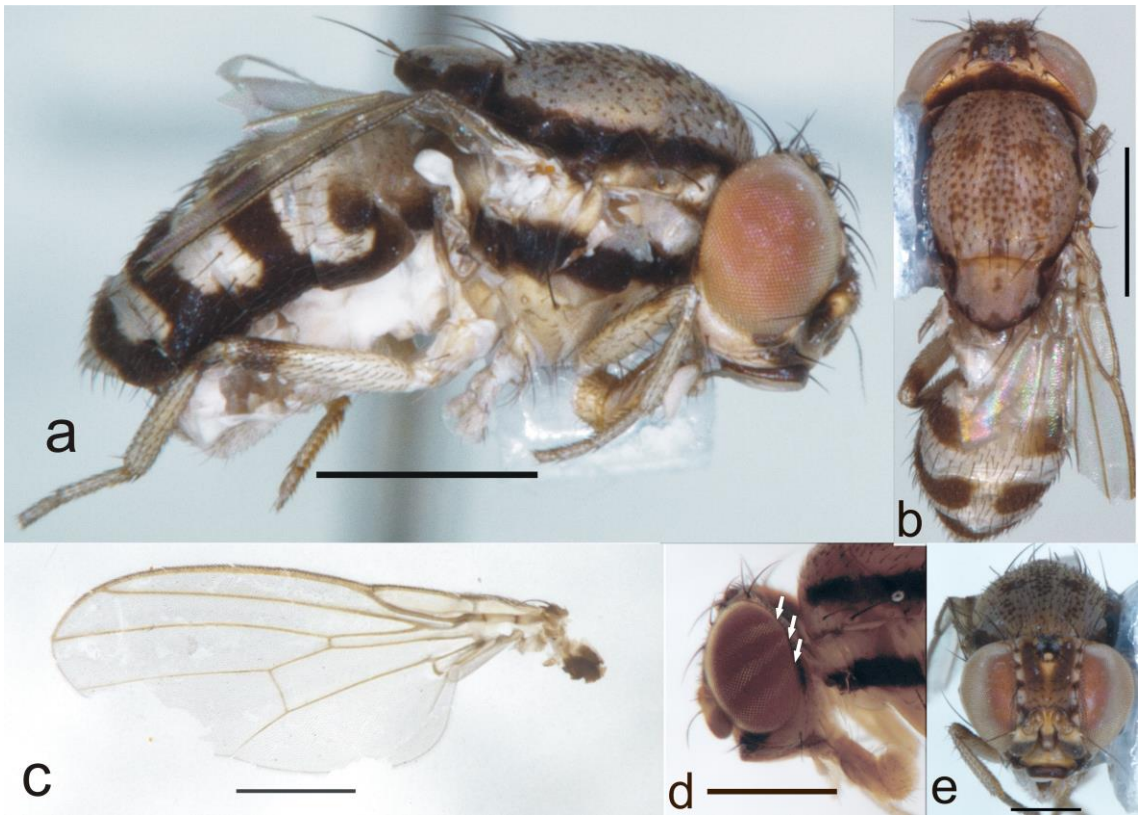


Figure 3: Male terminalia of the holotype of *R. trivisualis* sp. nov. a-c: aedeagus and aedeagal apodeme. a: dorsal view; b: ventral view; c: lateral view; d: epandrium, cerci and surstyli, caudal view (scale bar: 0.05 mm).

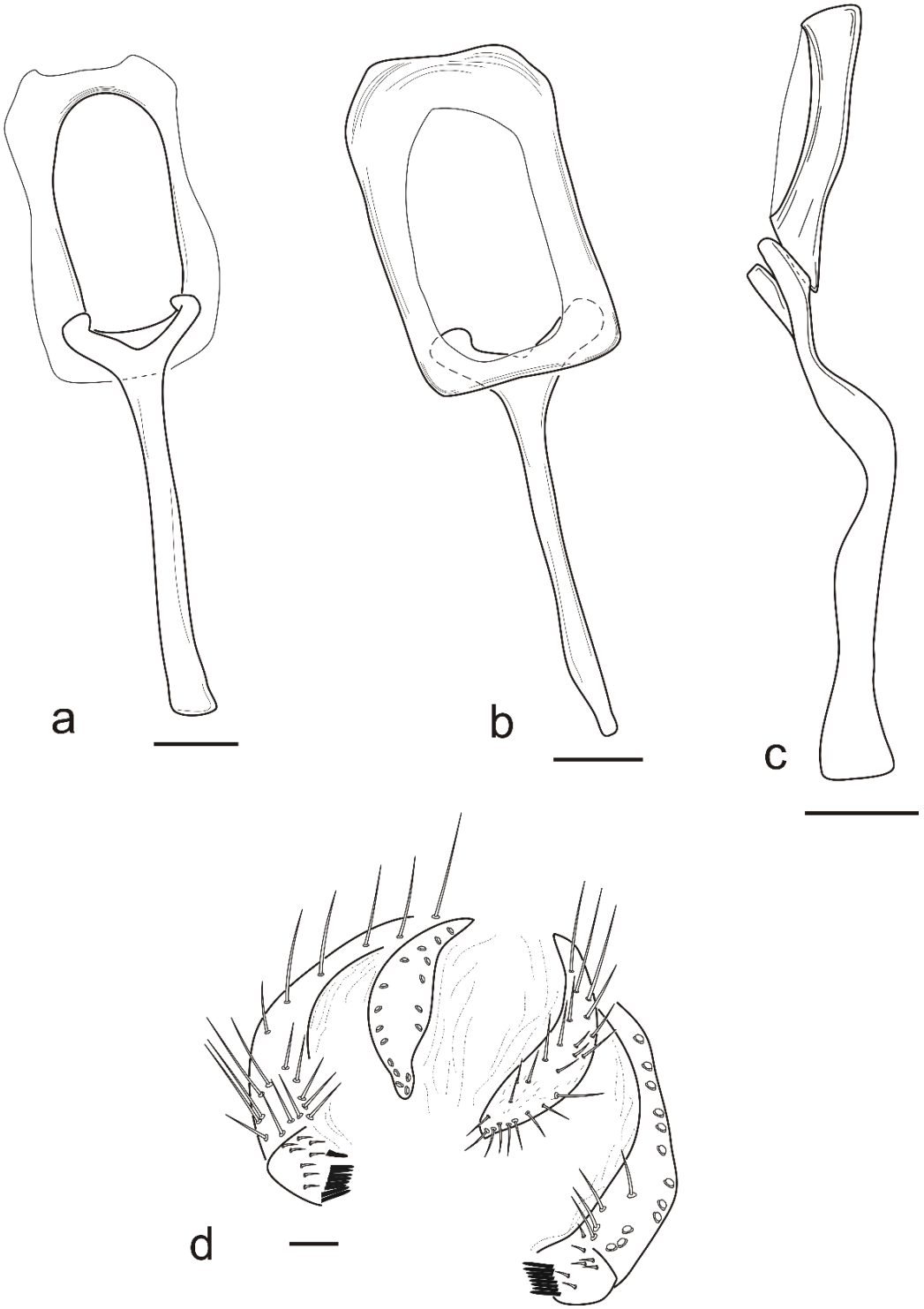


Figure 4: Female terminalia of the paratype of *R. trivisualis* sp. nov. a: ventral view; b: dorsal view; c: spermathecal capsule (scale bar: 0.1 mm).

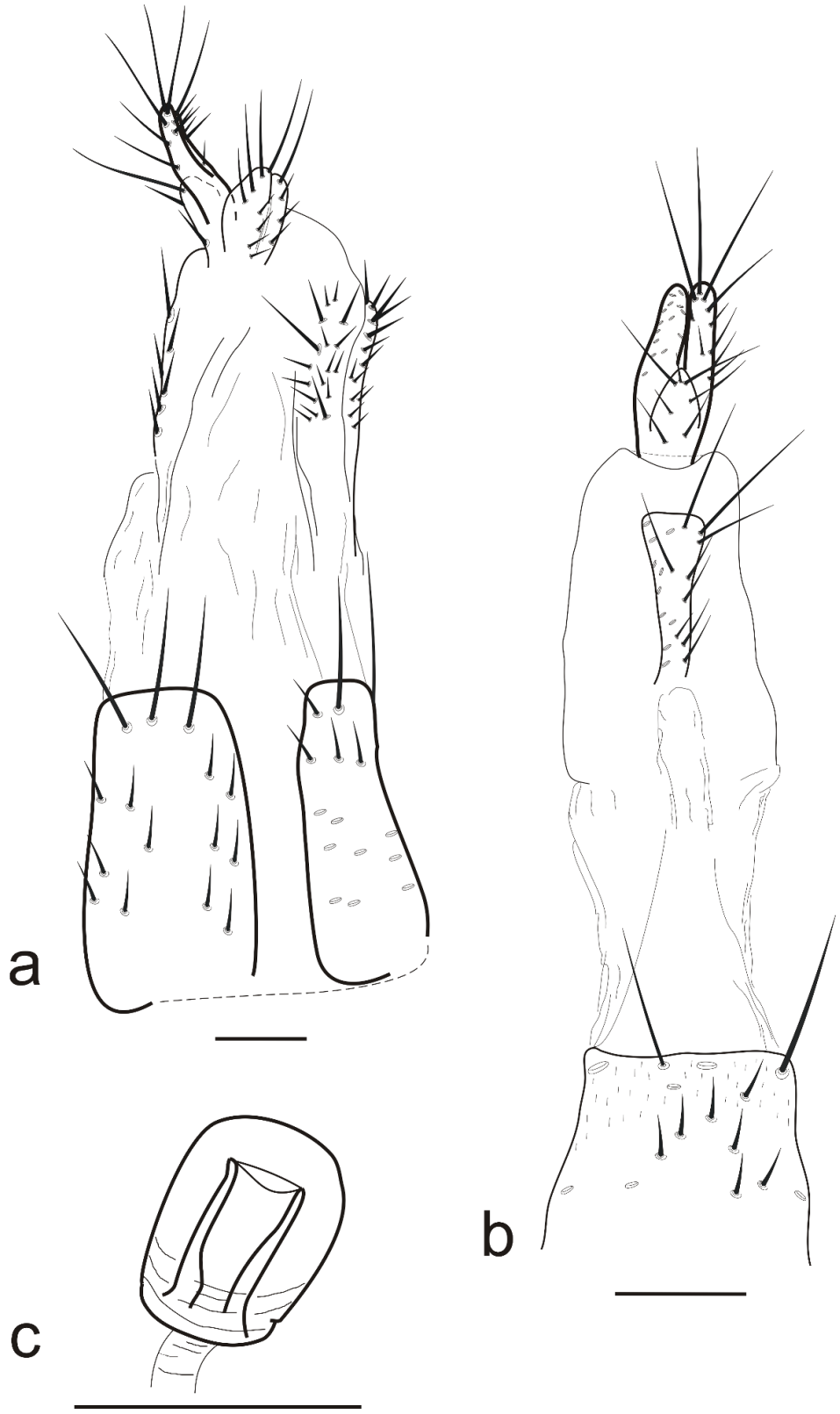


Figure 5: Holotype of *R. punctuloides* sp. nov. a: general habitus, dorsal view; b: wing; c: head, frontal view; d: antennae (scale bar 1.0 mm in a and b; 0.5 mm in c; 0.1 mm in d).

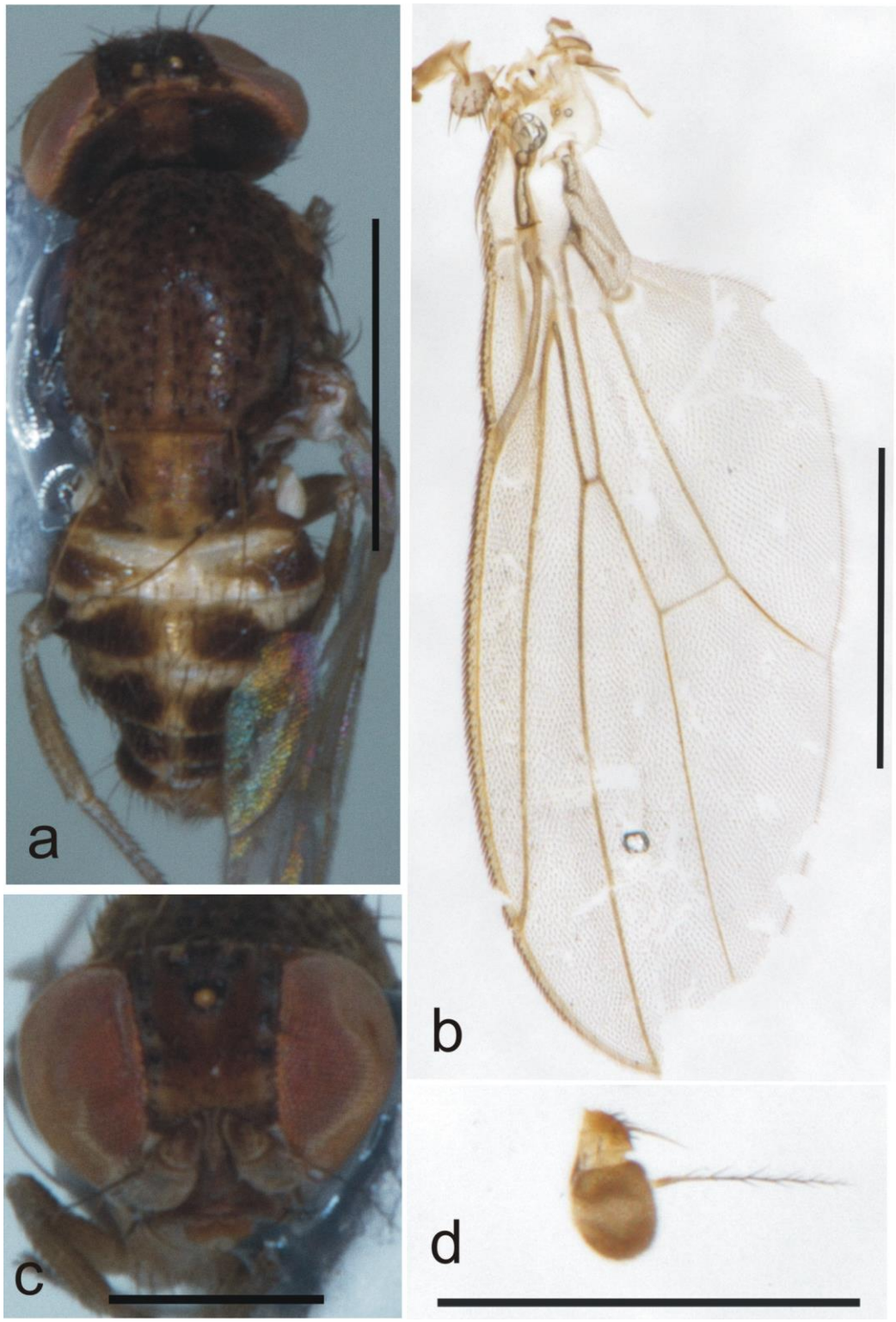


Figure 6: Male terminalia of holotype of *R. punctuloides* sp. nov. a-c: aedeagus and aedeagal apodeme. a: ventral view; b: dorsal view; c: lateral view; d: epandrium, cerci and surstyli, posterior view (scale bar: 0.05 mm).

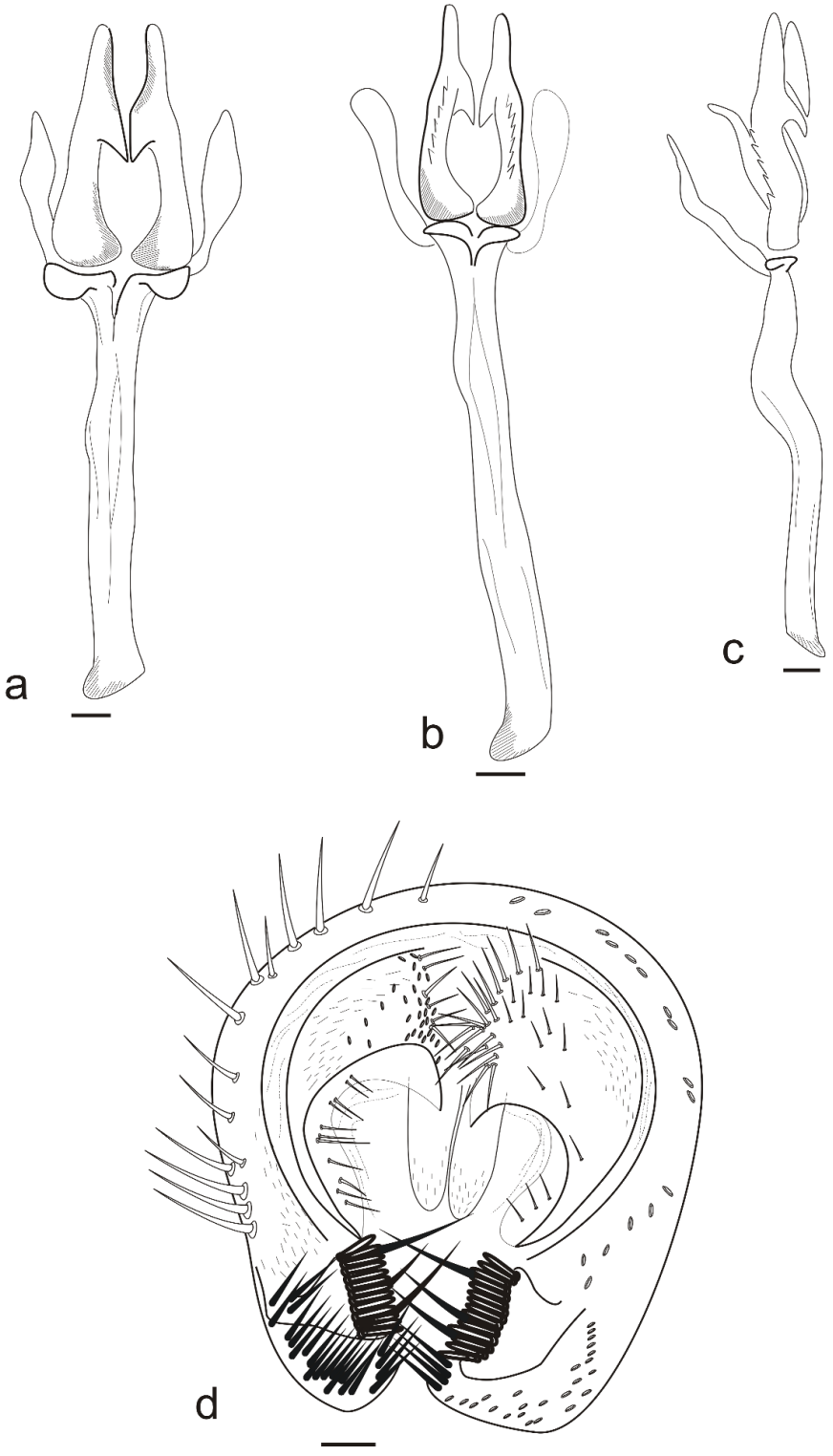


Figure 7: Female terminalia of the paratype of *R. punctuloides* sp. nov. a: dorsal view; b: latero-ventral view; c: spermathecal capsule (scale bar: 0.1 mm).

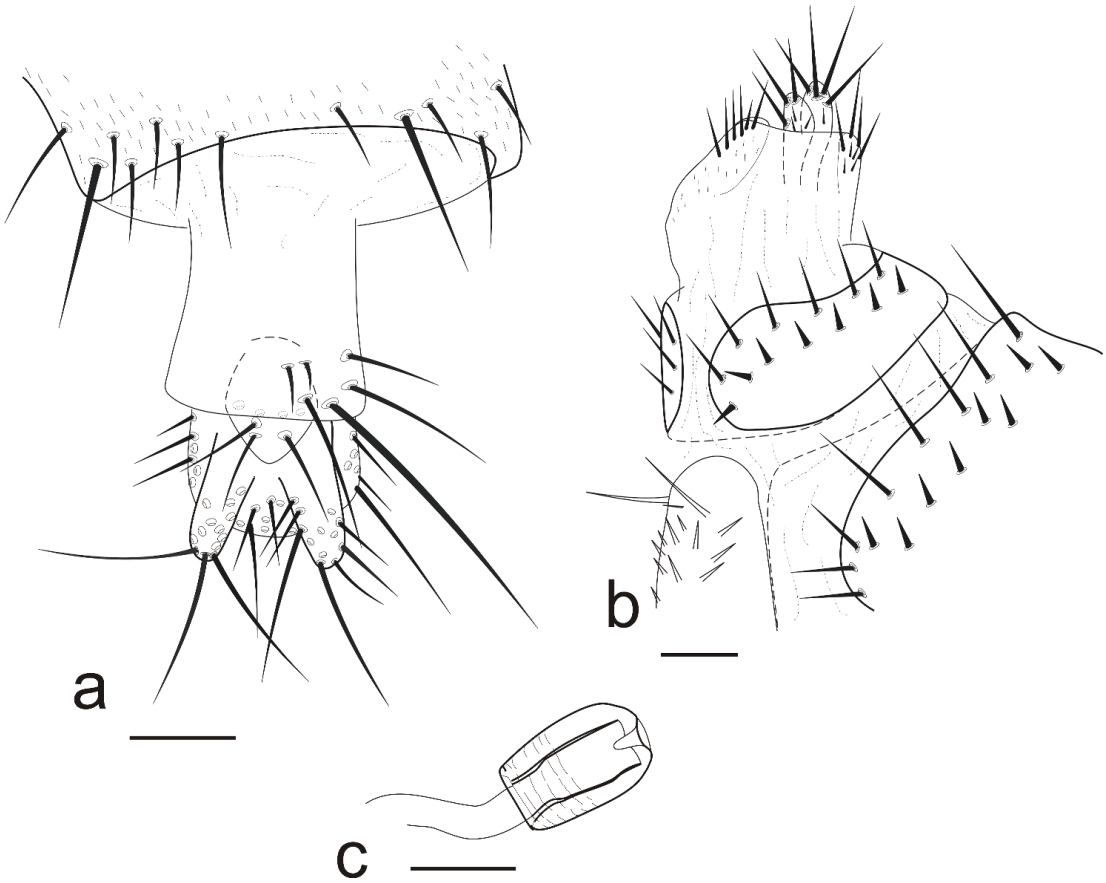


Figure 8: Female of *R. joaquina* collected in Bossoroca, Rio Grande do Sul. a: head, frontal view; b: thorax and abdomen, dorsal view; c: wing (scale bar 0.1 mm).

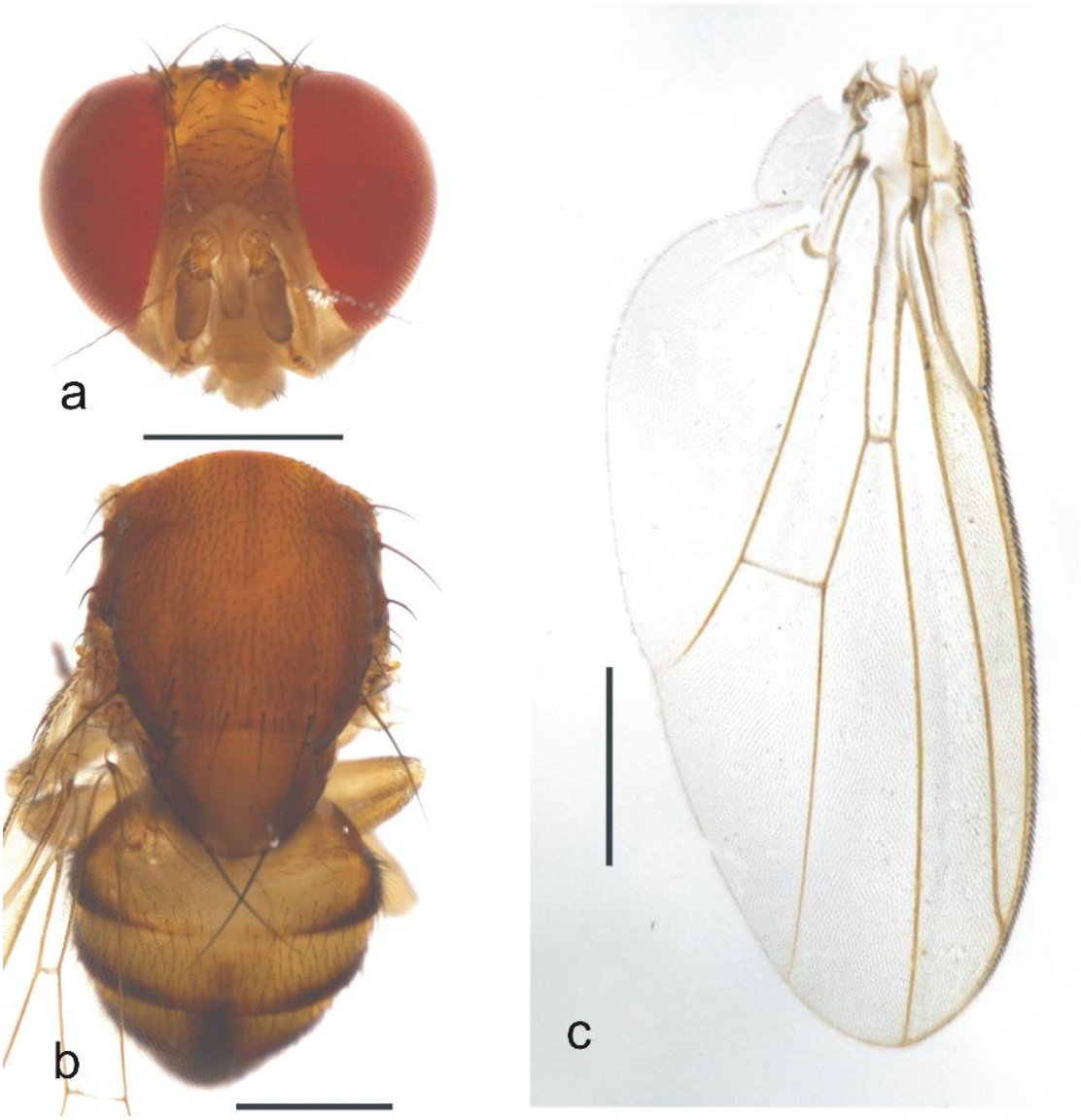


Figure 9: Female terminalia of *R. joaquina* collected in Bossoroca, Rio Grande do Sul. a: ventral view; b: spermathecal capsule; c: dorsal view (scale bar: 0.1 mm).

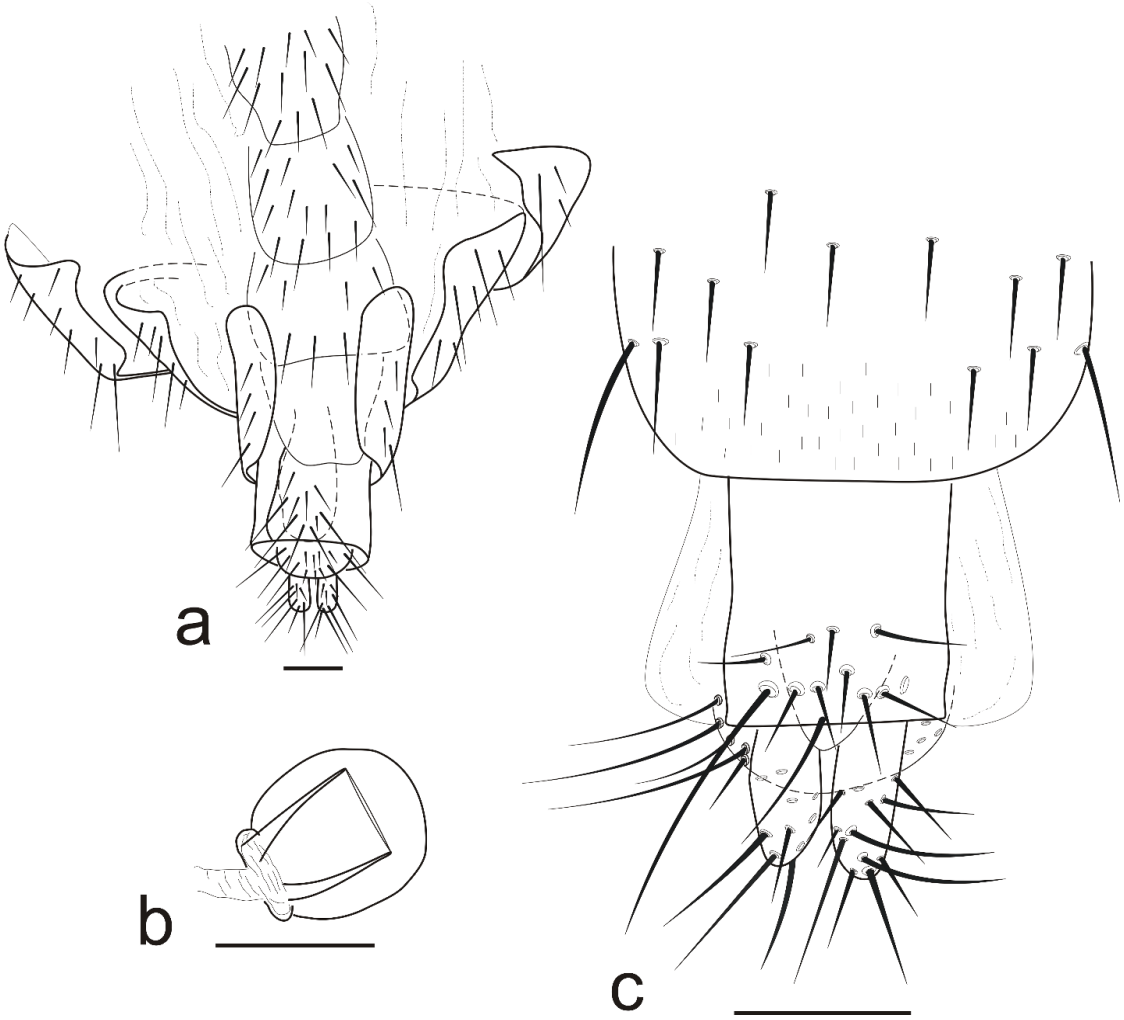


Figure 10: Specimen of *R. punctulata* collected in the Caatinga biome. a: general habitus, dorsal view; b: wing; c: antenna; d: head, frontal view; e: general habitus, lateral view (scale bar 0.5 mm, except in c: 0.1 mm).

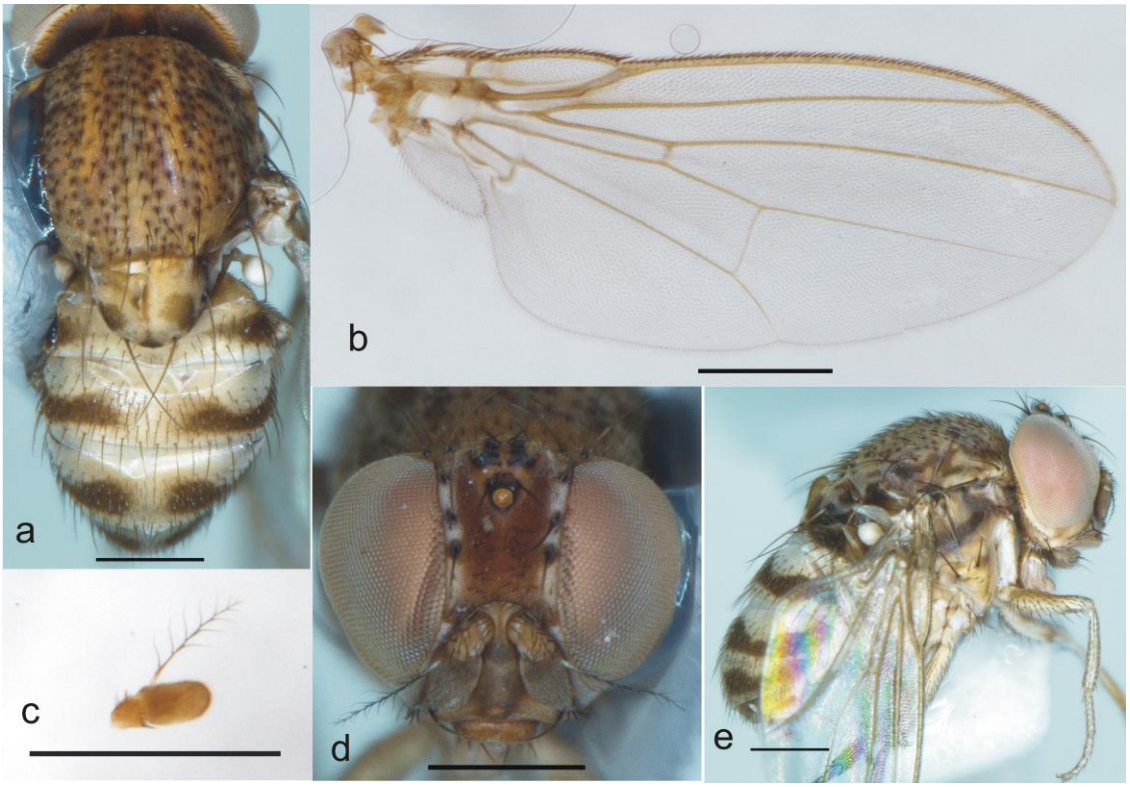


Figure 11: Female terminalia of *R. punctulata* (specimen collected in Bossoroca, Rio Grande do Sul). a: ventral view; b: dorsal view; c: spermathecal capsule (scale bar: 0.1 mm).

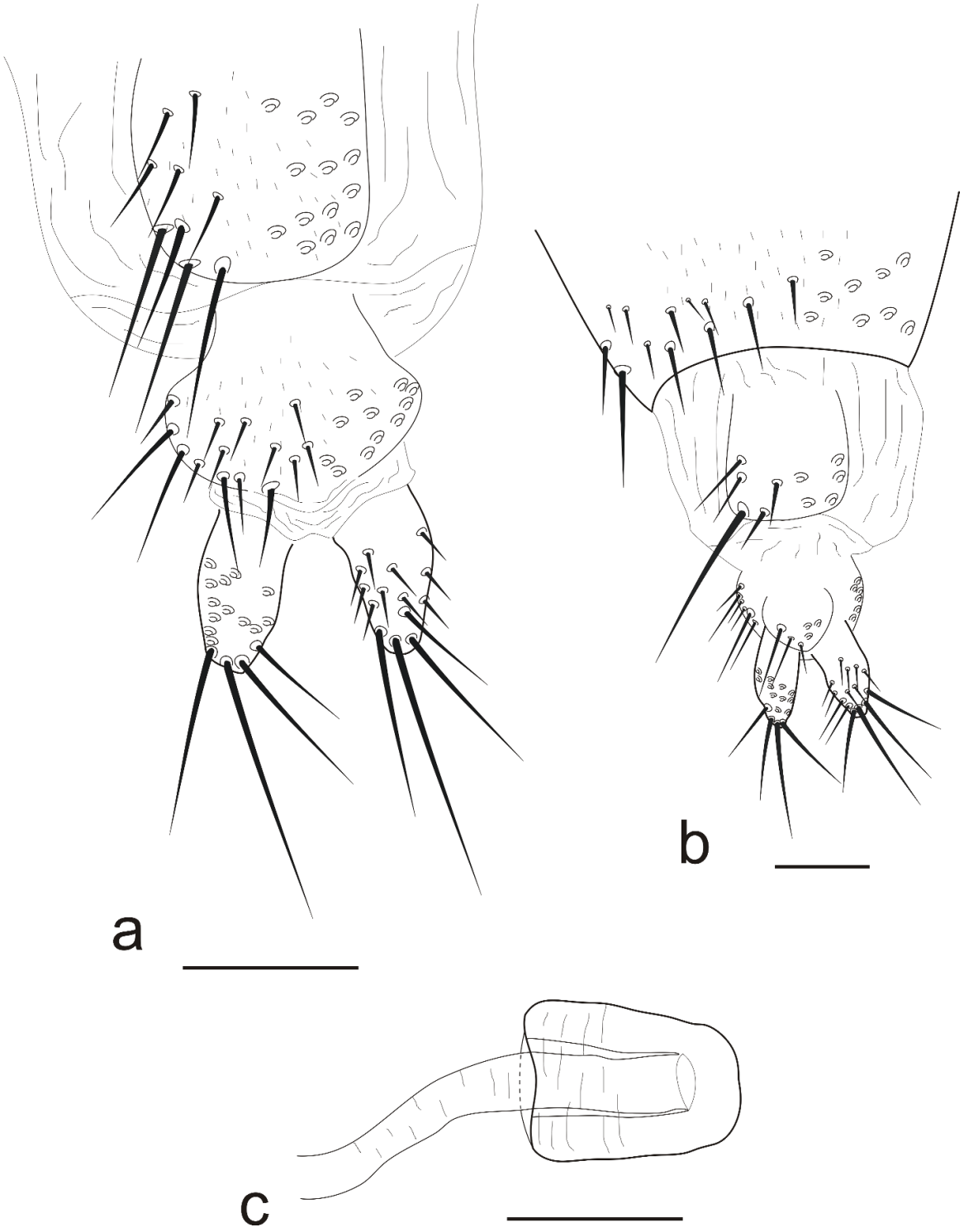


Figure 12: Holotype of *R. pampeana*. a: thorax, dorsal view; b: head, frontal view; c: abdomen, dorsal view; d: wing (scale bar 0.5 mm).

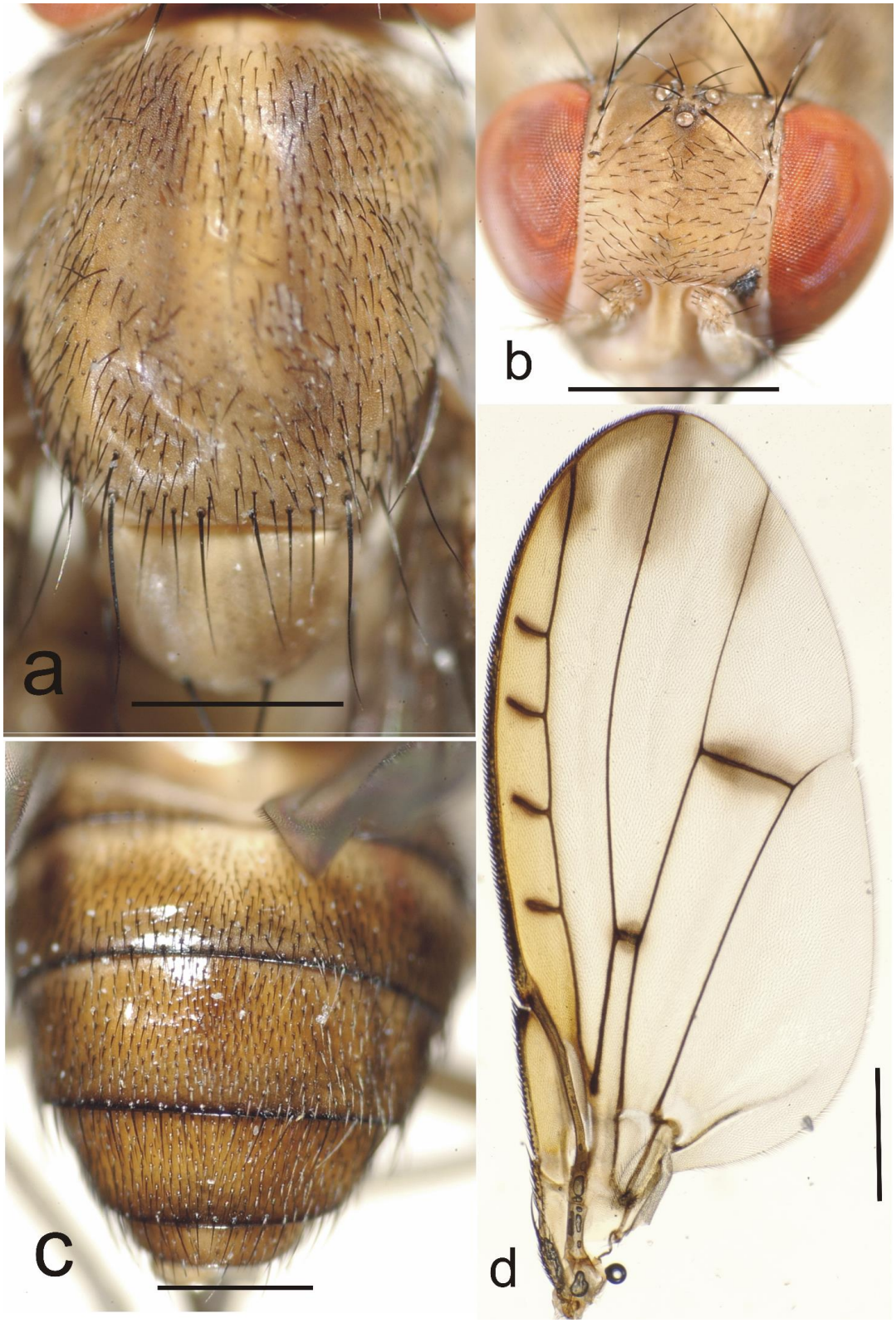


Figure 13: Ordinary specimen of *R. obesa* collected in the Pampa biome. a: head, frontal view; b: thorax, dorsal view; c: abdomen, dorsal view; d: wing (scale bar 0.5 mm).

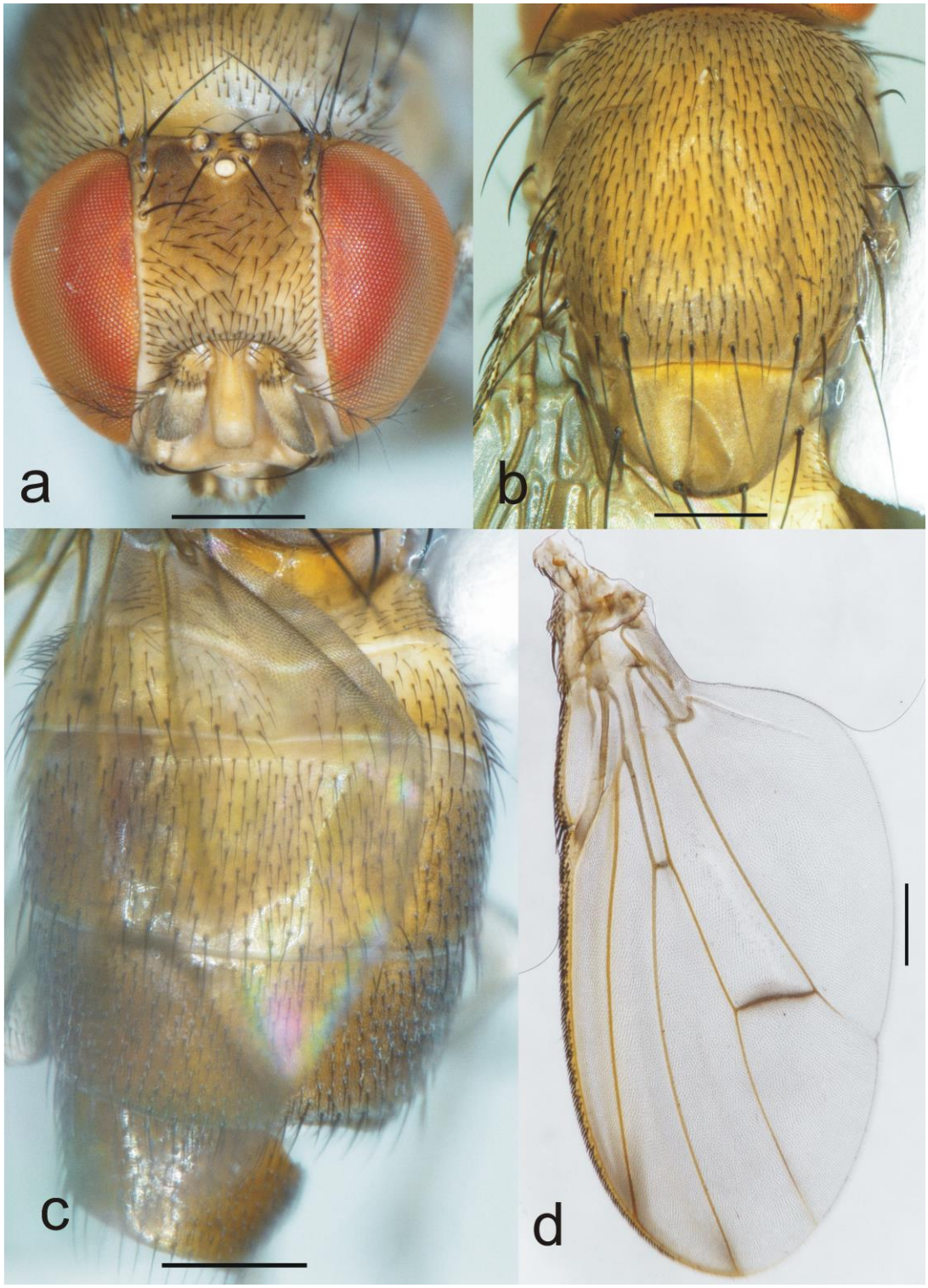


Figure 14: Ordinary specimen of *R. gigantea* collected in the Pampa biome. a: thorax, dorsal view; b: head, frontal view; c: abdomen, dorsal view; d: wing (scale bar 0.5 mm).

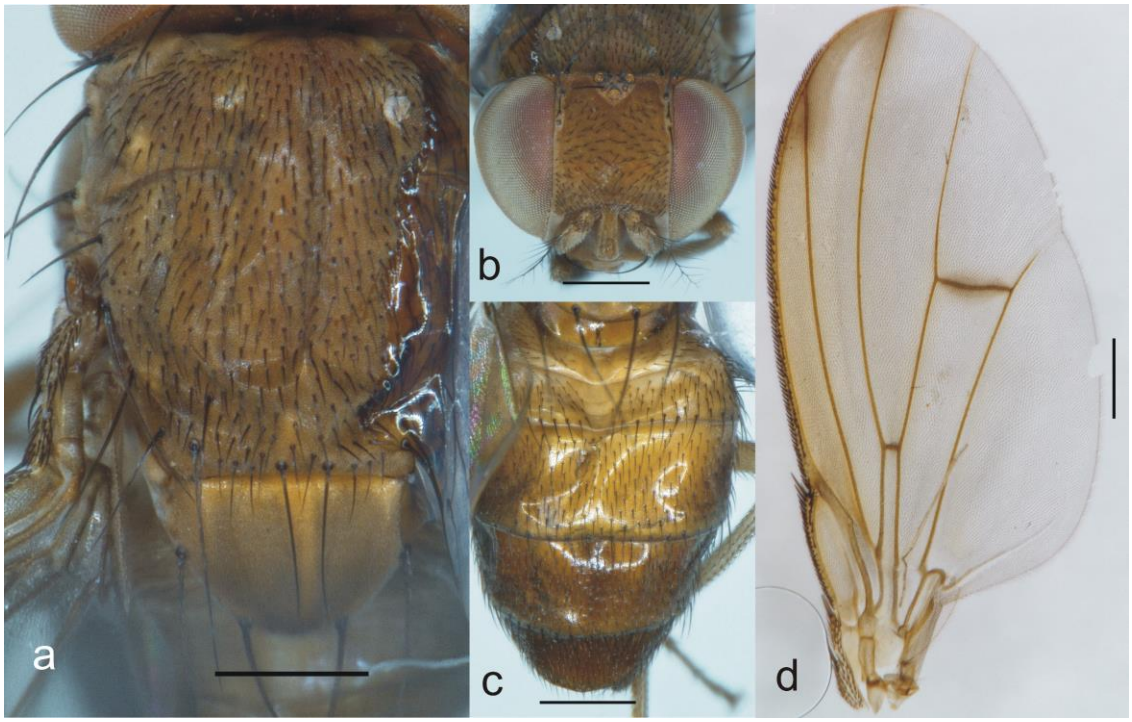


Figure 15: Ordinary specimen of *R. subradiata* collected in the Pampa biome. a: wing; b: general habitus, dorsal view; c: head, frontal view (scale bar 0.5 mm).

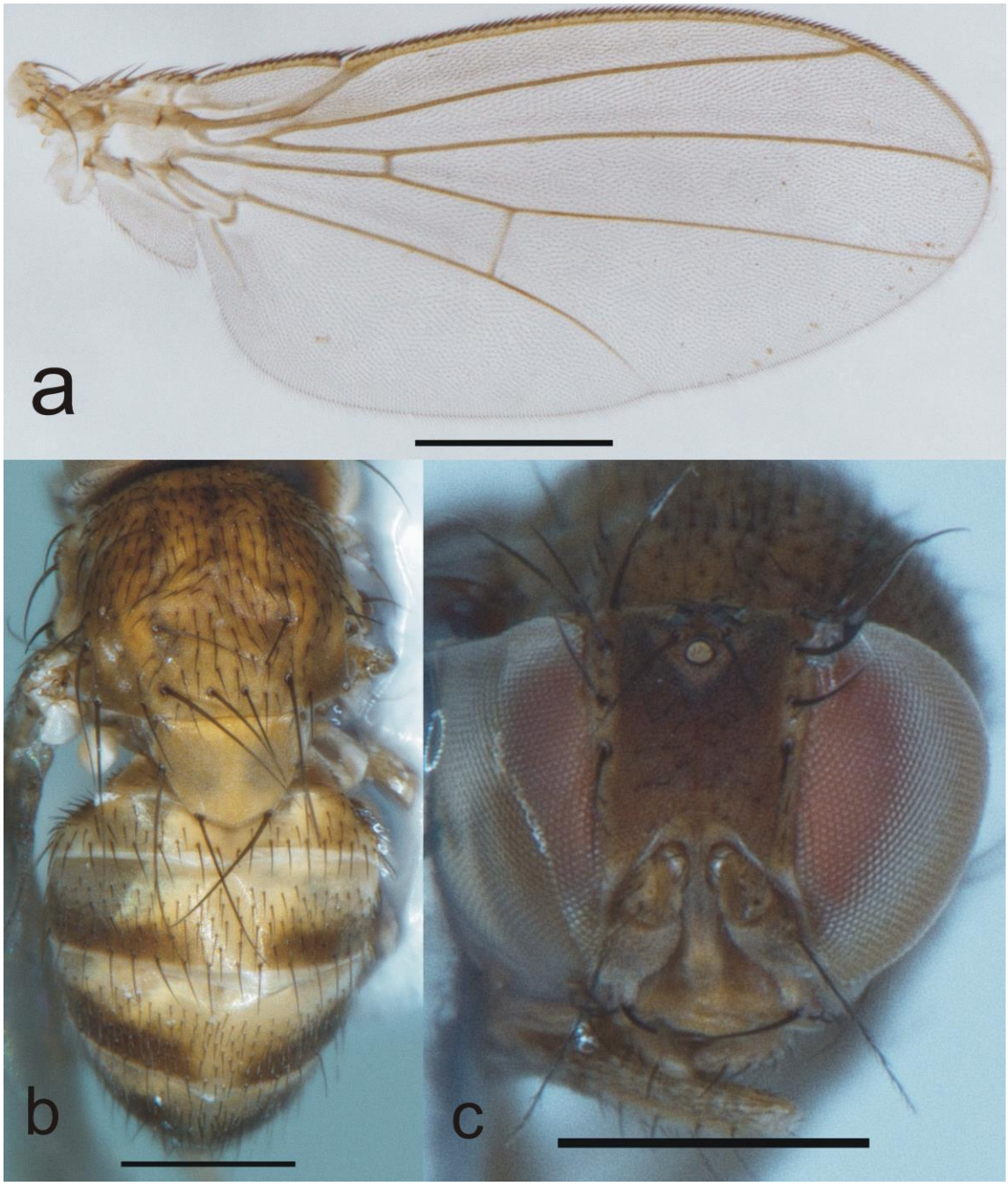


Figure 16: Ordinary specimen of *R. capixabensis* collected in the Caatinga biome. a: wing, b: head, frontal view; c: abdomen, dorsal view; d: thorax, dorsal view (scale bar 0.5 mm).

Figure 17: Holotype of *R. missionera*. a: thorax, dorsal view; b: head, frontal view; c: abdomen, dorsal view; d: wing (scale bar 0.5 mm).

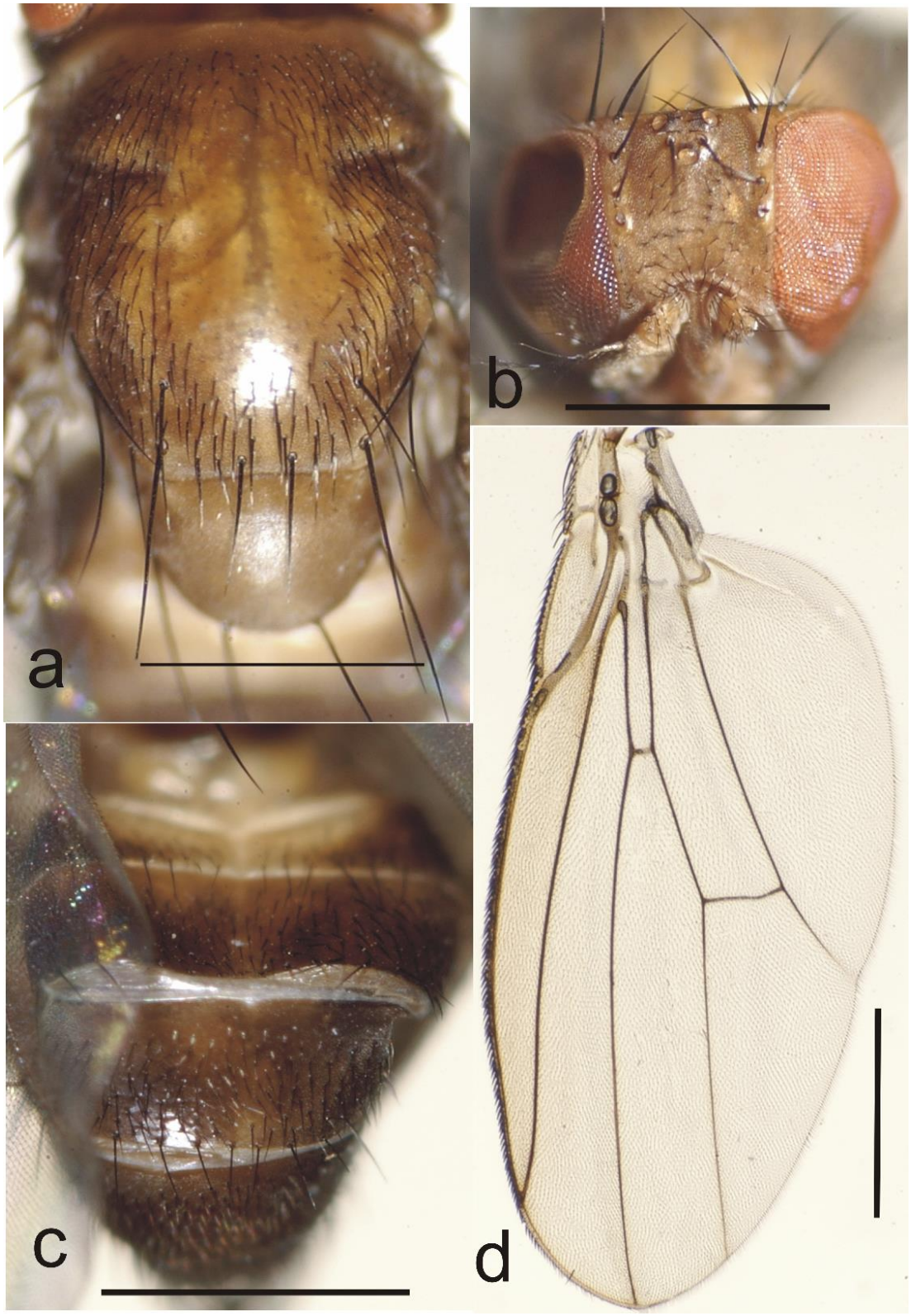
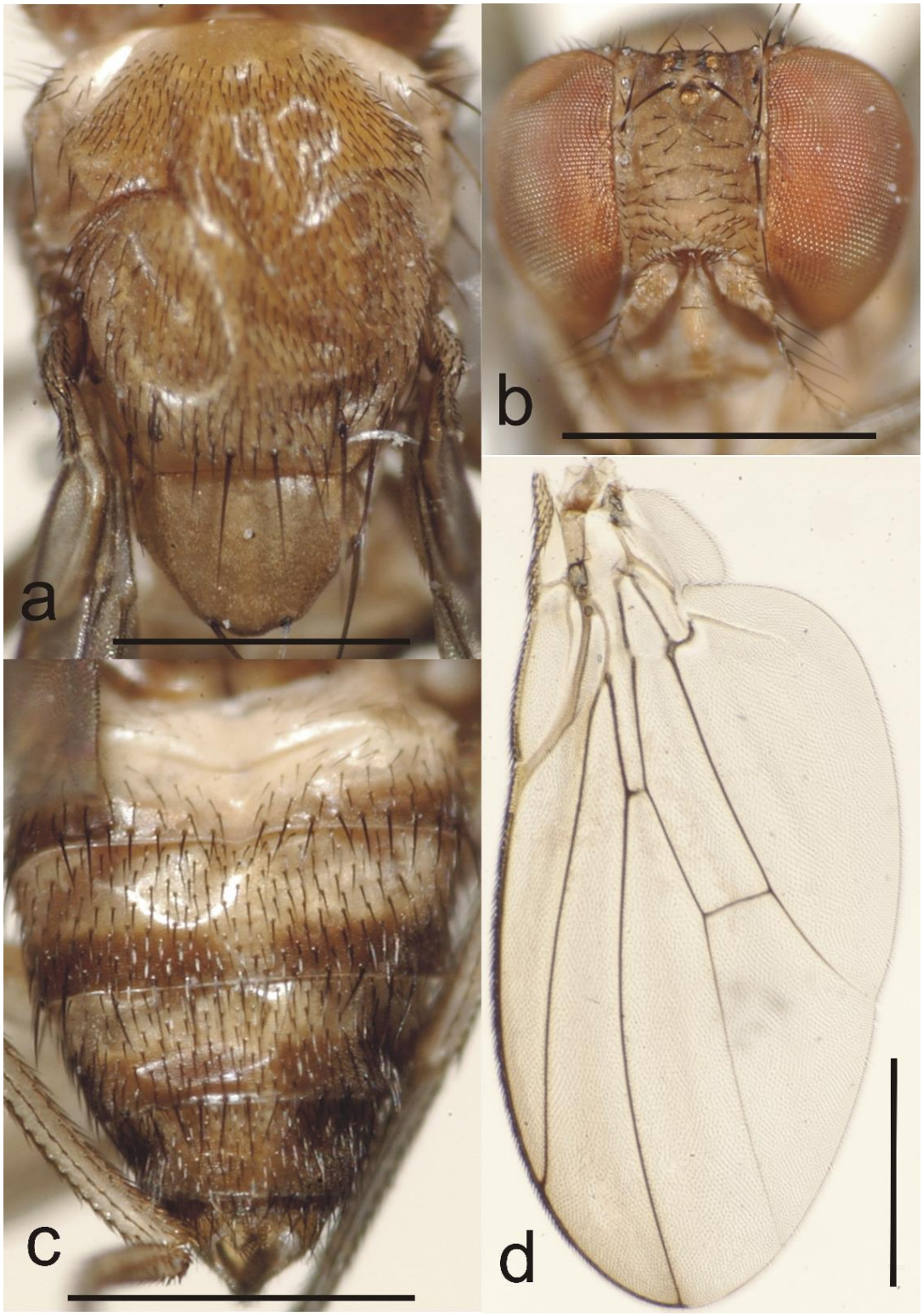


Figure 18: Holotype of *R. sulina*. a: thorax, dorsal view; b: head, frontal view; c: abdomen, dorsal view; d: wing (scale bar 0.5 mm).



3. CAPÍTULO III

(Manuscrito em revisão no periódico *Zootaxa*)

3.1. Redescription of *Rhinoleucophenga* species (Diptera: Drosophilidae) originally described by Lima (1950) and description of three new yellow species of *Rhinoleucophenga* from Neotropical region

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3.1.1. ABSTRACT

The genus *Rhinoleucophenga* Hendel comprises 28 nominal species with New World distribution. In the present study two species are redescribed: *Rhinoleucophenga brasiliensis* (Lima) and *R. fluminensis* (Lima). Other species, *R. flava* sp. nov. and *R. grimaldii* sp. nov. are described from collections performed with banana-baited traps in northeast region of Brazil, States of Pernambuco and Bahia, respectively. And a third species, *R. exigua* sp. nov. is from the specimens deposited at CEIOC/Fiocruz; it is probably from Trinidad and Tobago, but its collecting method is unknown. The description of new species and review of some older descriptions of *Rhinoleucophenga* species is important to improve the faunistic knowledge of Neotropical areas, in which previous studies have shown unidentified or misidentified *Rhinoleucophenga* species.

Key words: *Gitona*, drosophilids, Steganinae, biodiversity.

3.1.2. RESUMO

O gênero *Rhinoleucophenga* Hendel é composto por 29 espécies formalmente descritas, com distribuição no Novo Mundo. No presente estudo, duas espécies são redescritas: *Rhinoleucophenga brasiliensis* (Lima) e *R. fluminensis* (Lima). Outras espécies, *R. flava* sp. nov. e *R. grimaldii* sp. nov. são descritas a partir de indivíduos coletados em armadilhas com banana fermentada na região Nordeste do Brasil, nos estados de Pernambuco e Bahia, respectivamente. Uma terceira espécie, *R. exigua* sp. nov. é descrita a partir de espécimes depositados na Coleção Entomológica do Instituto Oswaldo Cruz no Brasil (CEIOC/Fiocruz); esta espécie é provavelmente oriunda de Trinidad e Tobago, mas seu método de coleta é desconhecido. A descrição de novas espécies e a revisão de descrições antigas de *Rhinoleucophenga* é fundamental para o aprimoramento do conhecimento faunístico das áreas Neotropicais, nas quais estudos prévios têm mostrado espécies de *Rhinoleucophenga* não identificadas ou erroneamente identificadas.

Palavras-chave: *Gitona*, drosofilídeos, Steganinae, biodiversidade.

3.1.3. INTRODUCTION

Rhinoleucophenga Hendel is a genus of Drosophilidae with Neotropical and Nearctic distribution. In the last years, some species have been described (Junges & Gottschalk 2014; Poppe *et al*. 2014, 2015) and new records of *Rhinoleucophenga* species have been done in Neotropical areas (Mata *et al*. 2008; Roque & Tidon 2008, 2013; Vilela & Bächli 2009; Roque *et al*. 2013; Poppe *et al*. 2015). This increase in the records of *Rhinoleucophenga* species has highlighted the need for reviews of old description studies and the complementary description of some species (Vilela & Bächli 2009), since most of species were described in the first half of the 20th century (Duda 1927, 1929; Malogolowkin 1946; Lima 1950).

Important ecological aspects of some species were described by Lima (1937, 1950), including the predatory behavior of *R. brasiliensis* (Lima) and *R. fluminensis* (Lima) in *Orthezia praelonga* Douglas (Lima 1950). These species are known as small yellow flies, but more details about their morphology are important, mainly due to the increasing number of records in areas until then poorly explored (Roque & Tidon 2008, 2013; Poppe *et al*. 2014, 2015), including the first records of other small *Rhinoleucophenga* yellow species.

Thus, the review of some species description is fundamental to avoid some taxonomic problems in *Rhinoleucophenga*, which are due the lack of well detailed descriptions, resulting in some species misidentification and uncertainties about the geographical distribution data. In this sense, considering the current patterns of species description, the present study presents the redescription of *Rhinoleucophenga brasiliensis* and *R. fluminensis*, and the description of three new yellow species of *Rhinoleucophega*: *R. flava* sp. nov., *R. grimaldii* sp. nov. and *R. exigua* sp. nov.

3.1.4. MATERIALS AND METHODS

Descriptions are based on measures and indices given by Bächli *et al*. (2004), which were done with an ocular reticle inserted into a stereomicroscope. Measurements in the text represent averages followed by the ranges in parentheses. Male and female terminalia were disarticulated in glycerol after treatment with 10% potassium hydroxide (KOH) and acid fuchsine (Bächli *et al*. 2004). The genitalia were mounted in a piece of glycerine jelly (ca. 2 x 2 x 2 mm) (Grimaldi 1987), stored in microvials with glycerol and pinned with the respective specimen. Photos of the specimens were taken with a digital camera coupled to an optical stereomicroscope. Drawings of the genitalia were made with a *camera lucida* system attached to a compound microscope with 40× and 10× objective lenses and a 10× ocular lens. The terminology follows Grimaldi (1990), Vilela (1990) and Bächli *et al*. (2004).

3.1.5. RESULTS

Rhinoleucophenga Hendel

Rhinoleucophenga Hendel, 1917: 44-45

Pseudophortica Sturtevant, 1918: 37

Gitona (in New world) Brake & Bächli, 2008: 291

Rhinoleucophenga brasiliensis (Lima)

(Figures 1a-e, 2a-c; Table 1)

Gitona brasiliensis Lima, 1950: 251-252 (figure 3); *Rhinoleucophenga brasiliensis* Roque & Tidon, 2013: 118,119 (table); *Rhinoleucophenga brasiliensis* Roque *et al*., 2013: 661, 663 (table)

Material examined: Seven dried specimens glued on a pinned card point and labeled "*Gitona brasiliensis* n. sp. / Recife 4-v-1936 J. Alves Albu. / 2858 / PARATYPO". Four specimens had the terminalia disarticulated and stored in a microvial with glycerin and attached with the pinned exemplar. Three slides with wings labeled "Inst. Osw. Cruz, N. 3079, Divisão 19, Caixa 103 / Inst. Osw. Cruz, No. 2858, *Gitona fluminensis* n. sp., predador de, *Orthezia* no. 2857, C. L. prep. xii-1936"; "Inst. Osw. Cruz, N. 3080, Divisão 20, Caixa 103 / Inst. Osw. Cruz, No. 2858, *Gitona brasiliensis* n. sp., predador de, *Orthezia* no. 2857, C. L. prep. xii.1936, C. L. det. 1. 1948"; "Inst. Osw. Cruz, N. 3081, Divisão 21, Caixa 103 / Inst. Osw. Cruz, No. 2858, *Gitona brasiliensis* n. sp., predador de, *Orthezia* no. 2857, C. L. prep. xii.1936, C. L. det. 1. 1948". 01 slide with a puparium labeled "Inst. Osw. Cruz, N. 3082, Divisão 22, Caixa 103 / Inst. Osw. Cruz, No. 2858, *Gitona brasiliensis* n. sp., predador de, *Orthezia* no. 2857, C. L. det. 1. 1948, C. L. prep. 12.xii.1936". 01 slide with two specimens labeled "Inst. Osw. Cruz, N. 3082, Divisão 18, Caixa 103 / Inst. Osw. Cruz, No. 2858, *Gitona fluminensis* [scratched] *brasiliensis* n. sp., predador de, *Orthezia* no. 2857, C. L. det. 1948, C. L. prep. xii.1936". All specimens were deposited in the Coleção Entomológica do Instituto Oswaldo Cruz (CEIOC)/ Fiocruz.

Diagnosis. Body yellow (Fig 1a-b); front covered with ca. 20 scattered interfrontal setulae. Top-to-bottom frontal width ratio 1.18 (1.08-1.30). Scutum covered with a diffuse longitudinal light yellow stripe; hyaline wings; tergites with brown stripes interrupted medially. Body length ca. 1.53 mm (1.44-1.70). Female terminalia as in figure 2a-c.

Description. Male and female present the same follow characteristics:

Head (Fig 1b, d). Front homogeneously yellow, covered with ca. 20 scattered interfrontal setulae; ocellar triangle yellow (or brownish in some specimens) with brown ocelli. Carina nose-like and ca. 50% sulcated. Face and gena yellowish; antenna with flagellomere and pedicel homogeneously yellow; arista micropubescent with 6 dorsal branches and 5 ventral branches plus terminal fork. Palpus yellowish with ca. 20 (15-20) setae on lower part.

Thorax (Fig 1a-b). Scutum and scutellum yellow, scutum covered with a diffuse longitudinal light yellow stripe. Six irregular rows of acrostichal setulae. Two pairs of

prescutellar acrostichal setae, the central one is the longest. Pleura, halteres and legs yellowish.

Wings (Fig 1c). Hyaline, without spots.

Abdomen. Abdomen proximally yellowish and distally brownish; tergites with brown stripes interrupted medially.

Female terminalia (Fig 2a-b). Epiproct microtrichose with ca. 10 setae. Hypoproct microtrichose with ca. 20 setae. Cerci with ca. four longer apical setae on each one. Spermathecal capsule (Fig 2c) with basal introvert reaching ca. ¾ of inner capsule, length to width ratio = 1.14.

Puparia (Fig 1e). Length 2.3 mm. Barrel-shaped, narrowed anteriorly and posteriorly, without protuberances, ornaments or constrictions at the segmental borders. Anterior spiracles inconspicuous, positioned at the front end. Intersegmental spines predominantly single pointed and disposed in 4-5 rows. Caudal segment with an elongated projection directed upward, where are placed the posterior spiracles.

For more measures and indices see Table 1.

Distribution. Known from the type locality (State of Rio de Janeiro, Brazil), Recife (State of Pernambuco, Brazil) and from the Brazilian Institute of Geography and Statistics (IBGE) Ecological Reserve (State of Goiás, Brazil) (Roque & Tidon 2013; Roque *et al*. 2013).

Note. This species was recorded in the Cerrado biome by Roque & Tidon (2013) and Roque *et al*. (2013). However, we are not sure of the identity of these individuals. Since this species belongs to a sibling species complex, it is necessary to analyze the male or female terminalia of each specimen to perform the species recognition.

Rhinoleucophenga fluminensis (Lima)

(Figures 3a-e, 4a-d, 5a-d, 6; Table 1)

Gitona fluminensis Lima, 1950: 249, 250 (figure 1), 251 (figure 2); *Rhinoleucophenga fluminensis* Roque & Tidon, 2008: 97; *Rhinoleucophenga fluminensis* Roque & Tidon, 2013: 119 (table); Roque *et al*., 2013: 661, 663 (table).

Material examined: LECTOTYPE (here designed): 01m# labeled "*Gitona fluminensis* n. sp. / COTIPO / Theresopolis 21-3-37 C. L. col. / 3056 / #04m# / LECTOTYPE". Terminalia disarticulated and stored in a microvial with glycerin and attached with the pinned exemplar. PARALECTOTYPES (here designed): 05 labeled "*Gitona fluminensis* n. sp. / COTIPO / Theresopolis 21-3-37 C. L. col. / 3056 / PARALECTOTYPE". Some specimens have the terminalia disarticulated and stored in a microvial with glycerin and attached with the pinned exemplar. 01 slide with three puparia labeled "Inst. Osw. Cruz, N. 3322, Divisão 22, Caixa 111 / Inst. Osw. Cruz, No. 3056, *Leucophenga* [scratched]*Gitona brasiliensis* [scratched] C. H. *fluminensis*, C. L. col. Therezopolis, 21.ii.937, C. H. prep. 18.iii.937, C.L. det. 1-1948". 01 slide with three puparia labeled "Inst. Osw. Cruz, N. 3323, Divisão 23, Caixa 111 / Inst. Osw. Cruz, No. 3056, *Leucophenga* [scratched] *Gitona brasiliensis* [scratched] *fluminensis*, C. L. col. Therezopolis, 21.ii.937, C. H. prep. 18.iii.937, C.L. det. 1-1948". ". 01 slide with one puparium labeled "Inst. Osw. Cruz, N. 3324, Divisão 24, Caixa 111 / Inst. Osw. Cruz, No. 3056, *Leucophenga* [scratched] *Gitona brasiliensis* [scratched] *fluminensis*, C. L. col. Therezopolis, 21.ii.937, C. H. prep. 18.iii.937, C.L. det. 1-1948". 01 slide with f# terminalia labeled "Inst. Osw. Cruz, N. 3325, Divisão 25, Caixa 111 / Inst. Osw. Cruz, No. 3056, *Gitona brasiliensis* [scratched] *fluminensis*, Spermateca, C. H. prep. 28.ii.937, C.L. det. 1-1948". 01 slide with wing labeled "Instituto Oswaldo Cruz, N. 4651, Divisão 1, Caixa 156 / Instituto Oswaldo Cruz, No. 3056, *Gitona brasiliensis* [scratched] *fluminensis* n. sp., s/ *Orthezia*, Terezopolis (E. Rio), 21.ii.1937, C. L. prep. 1948, C.L. det. 1-1948". 01 slide with head and f# terminalia labeled "Instituto Oswaldo Cruz, N. 4652, Divisão 2, Caixa 156 / Instituto Oswaldo Cruz, No. 3056, *Gitona brasiliensis* [scratched] *fluminensis* n. sp., s/ *Orthezia*, Terezopolis (E. Rio), 21.ii.1937, C. L. prep. 1948, C.L. det. 1-1948". 01 slide with f# terminalia labeled "Instituto Oswaldo Cruz, N. 4653, Divisão 3, Caixa 156 / Instituto Oswaldo Cruz, No. 3056, *Gitona brasiliensis* [scratched] *fluminensis* n. sp., s/ *Orthezia*, Terezopolis (E. Rio), 21.ii.1937, C. L. prep. 1948, C.L. det. 1-1948". All specimens were deposited in the Coleção Entomológica do Instituto Oswaldo Cruz (CEIOC)/ Fiocruz.

Diagnosis. Body yellowish; front covered with ca. 30 (28-30) scattered interfrontal setulae. Top-to-bottom frontal width ratio 1.29 (1.20-1.38). Scutum covered with a diffuse longitudinal light yellow stripe; hyaline wings; tergites with brown stripes

interrupted medially. Body length 2.05 mm (2.00-2.20). Male aedeagus as in figure 4a-d. Female terminalia as in figure 5a-d.

Description. Male and female present the same follow characteristics:

Head (Fig 3b, d-e). Front homogeneously yellow, covered with ca. 30 (28-30) scattered interfrontal setulae; ocellar triangle yellow (or brownish in some specimens) with brown ocelli. Carina nose-like and ca. 90% sulcated. Face and gena yellowish; antenna with flagellomere and pedicel homogeneously yellow; arista pubescent with 6 dorsal branches and 5 ventral branches plus terminal fork. Palpus yellowish with ca. 20 (15-20) setae on lower part.

Thorax (Fig 3a-b). Scutum and scutellum yellow-brownish, scutum covered with a diffuse longitudinal light yellow stripe. Eight irregular rows of acrostichal setulae. Two pairs of prescutellar acrostichal setae, the central one is the longest. Pleura, halteres and legs yellowish.

Wings (Fig 3c). Hyaline, without spots.

Abdomen. Abdomen proximally yellowish and distally brownish; tergites with brown stripes interrupted medially.

Male terminalia (Fig 4a-d). Aedeagus curved ventral-dorsally with a membranous projection dorsally; medially wider than the base and top. Epandrium microtrichose, fused with surstyli with ca. 9 prensisetae. Cerci elongate, with ca. 26 setae each one.

Female terminalia (Fig 5a-d). Cerci long and well sclerotized with 4 longer apical setae on each one. Epiproct with ca. 8 setae. Hypoproct with ca. 50 subequal setae. Spermathecal capsule elongated with basal introvert reaching ca. 2/3 of inner capsule, length to width ratio = 2.50.

Puparia (Fig 6). Length 2.0 mm. Barrel-shaped, narrowed anteriorly, without protuberances, ornaments or constrictions at the segmental borders. Anterior spiracles inconspicuous, positioned at the front end. Intersegmental spines predominantly single pointed and disposed in 4-5 rows. Caudal segment rounded.

For more measures and indices see Table 1.

Distribution. Known from the type locality (Teresópolis, State of Rio de Janeiro, Brazil), and from the Brazilian Institute of Geography and Statistics (IBGE) Ecological Reserve (State of Goiás, Brazil) (Roque & Tidon 2008, 2013; Roque *et al*. 2013).

Note. As to *R. brasiliensis*, this species was recorded in the State of Goiás by Roque & Tidon (2008, 2013) and Roque *et al*. (2013). However, we are not sure of the identity of these individuals. Since this species belongs to a sibling species complex, it is necessary to analyze the male or female terminalia of each specimen to perform the species recognition.

R. flava sp. nov.

(Figures 7a-d, 8a-d; Table 2)

Type series. Holotype: 1m# labelled "*Rhinoleucophenga flava*; HOLOTYPE m#; Brazil, Recife, Reserva Ecológica de Dois Irmãos. 8°0'25"S 34°56'49"W, 2010 col.: J Gomes; banana bait". Postabdomen disarticulated stored in a microvial with glycerin and attached with the respective specimen. The Holotype is stored in alcohol 100% and deposited in the Coleção Entomológica do Instituto Oswaldo Cruz (CEIOC)/ Fiocruz.

Diagnosis. Body yellow; front covered with ca. 30 scattered interfrontal setulae. Frontal index 1.40. Carina nose-like not sulcated. Arista with long branches. Hyaline wings; tergites with dark brown stripes interrupted medially. Body length ca. 2.30 mm. Male terminalia as in figure 7a-d.

Description. Head (Fig 7c). Front homogeneously yellow, covered with ca. 30 scattered interfrontal setulae; ocellar triangle yellow with brown ocelli. Carina nose-like not sulcated. Face and gena yellowish; antenna with flagellomere and pedicel homogeneously yellow; arista with 5 long dorsal branches and 4 long ventral branches plus terminal fork. Palpus yellowish.

Thorax (Fig 7a-b). Scutum and scutellum homogeneously yellow. Six irregular rows of acrostichal setulae. One pair of prescutellar acrostichal setae. Pleura, halteres and legs yellowish.

Wings (Fig 7d). Hyaline, without spots.

Abdomen (Fig 7a-b). Abdomen proximally yellowish and distally brownish; tergites with ca. ¼ covered by dark brown stripes interrupted medially.

Male terminalia (Fig 8a-d). Aedeagus with a triangular shape, wider in the base, dorsal-ventrally curved. There is a thin apical projection ventrally, like a spicule. Epandrium microtrichose, fused with surstyli with 20 prensisetae. Cerci round shaped, with ca. 20 setae each one, four larger ones.

For more measures and indices see Table 2.

Etymology. The species name refers to the word *flavo* that means yellow in Latin.

Type locality. Brazil, Recife, Reserva Ecológica de Dois Irmãos (8°00'25"S 34°56'49"W).

Distribution. Known only from the type locality.

Biology. Collected in banana-baited traps, in the Atlantic rainforest biome.

R. grimaldii sp. nov.

(Figures 9a-d, 10a-d; Table 2)

Type series. Holotype: 1m# labelled "*Rhinoleucophenga grimaldii*; HOLOTYPE m#; Brazil, Bahia, Estação Ecológica Raso da Catarina/ Município de Paulo Afonso. 9°30'39"S 38°32'12"W, 22.iv.2012 col.: GF Oliveira; banana bait". Postabdomen disarticulated stored in a microvial with glycerin and attached with the respective specimen. The Holotype is stored in alcohol 100% and deposited in the Coleção Entomológica do Instituto Oswaldo Cruz (CEIOC)/ Fiocruz.

D**iagnosis**. Body yellow; front covered with ca. 40 scattered interfrontal setulae. Frontal index 1.10. Carina nose-like not sulcated. Arista with long branches. Ten irregular rows of acrostichal setulae. Hyaline wings; tergites with dark brown stripes widely interrupted medially. Body length ca. 2.85 mm. Male terminalia as in figure 10a-d.

Description. Head (Fig 9c). Front homogeneously yellow, covered with ca. 40 scattered interfrontal setulae; ocellar triangle and ocelli homogeneously yellow. Carina nose-like not sulcated. Face and gena yellowish; antenna with flagellomere and pedicel

homogeneously yellow; arista with 6 long dorsal branches and 5 long ventral branches plus terminal fork. Palpus yellowish.

Thorax (Fig 9a-b). Scutum and scutellum homogeneously yellow. Ten irregular rows of acrostichal setulae. Pleura, halteres and legs yellowish.

Wings (Fig 9d). Hyaline, without spots.

Abdomen (Fig 9a). Abdomen proximally yellowish and distally brownish; tergites with large dark brown stripes widely interrupted medially.

Male terminalia (Fig 10a-d). Aedeagus with an oval shape, with a triangular membranous projection on dorsal side. Epandrium ventrally thin, fused with surstyli with seven prensisetae. Cerci oval shaped, wider ventrally, with ca. 30 setae each one, four larger ones.

For more measures and indices see Table 2.

Etymology. The species name is homage to the Professor Dr. David Grimaldi, who developed important studies with Drosophilidae, contributing to the better comprehension of the family diversity, taxonomy and evolution.

Type locality. Brazil, Bahia, Estação Ecológica Raso da Catarina/ Município de Paulo Afonso (9°30'39"S 38°32'12"W).

Distribution. Known only from the type locality.

Biology. Collected in fermented-banana traps, in the Caatinga *sensu strictu*.

R. exigua sp. nov.

(Figures 11a-d, 12a-c; Table 2)

Material examined: Six dried specimens labeled "*Rhinoleucophenga* sp. Trinidad". Deposited in the Coleção Entomológica do Instituto Oswaldo Cruz (CEIOC)/ Fiocruz.

Type series. Holotype: 1f# labelled "*Rhinoleucophenga exigua*; HOLOTYPE f#04; Locality unknown. Det.: JL Poppe and MS Gottschalk; v.2014". Postabdomen of holotype and paratype #03 female disarticulated, stored in microvial with glycerin, on the same pin with the respective specimens. 05 Paratypes labeled "*Rhinoleucophenga*

exigua; PARATYPE #01 (up to five), sex (when was possible to determine it); Locality unknown. Det.: JL Poppe and MS Gottschalk; v.2014". All specimens were deposited in the Coleção Entomológica do Instituto Oswaldo Cruz (CEIOC)/ Fiocruz.

Diagnosis. Body yellow; front covered with ca. 32 scattered interfrontal setulae. Frontal index 1.41 (1.38-1.48). Carina nose-like ca. 80% sulcated. Arista pubescent. Eight irregular rows of acrostichal setulae. Hyaline wings; tergites with brown stripes interrupted medially. Body length ca. 1.97 (1.70-2.16) mm. Female terminalia as in figure 12a-c.

Description. Head (Fig 11b-c). Front homogeneously yellow, covered with ca. 32 (30-34) scattered interfrontal setulae; ocellar triangle yellow with brown ocelli. Carina nose-like and ca. 80% sulcated. Face and gena yellowish; antenna with flagellomere and pedicel homogeneously yellow; arista pubescent with 5 dorsal branches and 4 ventral branches plus terminal fork. Palpus yellowish with ca. 16 (15-16) setae on lower part.

Thorax (Fig 11a, c). Scutum yellow, scutellum brownish. Eight irregular rows of acrostichal setulae. Two pairs of prescutellar acrostichal setae, the central one is the longest. Pleura, halteres and legs yellowish.

Wings (Fig 11d). Hyaline, without spots.

Abdomen. Abdomen proximally yellowish and distally brownish; tergites with brown stripes interrupted medially.

Female terminalia (Fig 12a-c). Cerci long and well sclerotized with 4 longer apical setae on each one. Epiproct with ca. 8 setae. Hypoproct with ca. 20 subequal setae and 10 apical longer ones. Spermathecal capsule round shaped with basal introvert reaching ca. 2/3 of inner capsule, length to width ratio = 1.00.

For more measures and indices see Table 2.

Etymology. The species name refers to the word *exiguus* that means small in Latin.

Type locality. Unknown. However, we believe it is from Trinidad Island, from Trinidad and Tobago, next to the Northeast coast of Venezuela. This is because the specimens' original label "*Rhinoleucophenga* sp. Trinidad".

Distribution. Probably Trinidad Island, Trinidad and Tobago.

Biology. Collect method unknown.

3.1.6. DISCUSSION

The high number of new species recently proposed to *Rhinoleucophenga* (Junges & Gottschalk 2014; Poppe *et al*. 2014, 2015) anticipated the need of reviewing old descriptions, mainly to avoid future taxonomic problems of spurious data of new species or occurrence records.

Rhinoleucophenga brasiliensis and *R. fluminensis* are very small yellow species. They are similar in general body color with *R. subradiata* Duda and *R. joaquina* Schmitz, Gottschalk & Valente. *Rhinoleucophenga brasiliensis* presents arista with micropubescent branches as *R. joaquina*, while *R. fluminensis* presents arista with pubescent branches as *R. subradiata*. But *R. brasiliensis* and *R. fluminensis* differ from these species by their body size, number of arista branches, number of rows of acrostichal setulae and terminalia characteristics.

Lima (1950) presented in the figure 3 of his manuscript two different spermathecal capsules as belonging to *R. brasiliensis*. The first pair (left) he mentioned as from Rio de Janeiro, and it is in accordance with the spermathecal capsule found in the specimen disarticulated by us (figure 2c). However, we were allowed to dissect only one specimen, precluding us to reject the presence of a sibling species in the type series, which would be represented by the right pair of spermathecal capsules in the Lima's manuscript (mentioned as from Recife).

Regarding the type series of *R. brasiliensis*, Lima (1950) designed six cotypes from Rio de Janeiro. However, the specimens studied here present paratype labels, even being the material collected in Recife and originally not mentioned as type series. Unfortunately, we could not access the material type mentioned by Lima (1950), since it is probably placed in the Coleção Costa Lima of the Universidade Federal Rural do Rio de Janeiro.

The dissected female of *R. fluminensis* presented an elongated spermathecal capsule (figure 5c). But in the same series of *R. fluminensis* there was a slide with a pair of shorter spermathecal capsules (figure 5d); both spermathecal capsules present the same internal introvert length and basal capsule width. It could represent a technical

artifact of the slide preparation due to specimens conditions (probably newly emerging) or even the presence of other misidentified species in the series type.

The *Rhinoleucophenga* yellow species are all similar to each other. *Rhinoleucophenga flava* sp nov. resembles *R. brasiliensis* and *R. fluminensis* by its small size and general yellow coloration. But it differs from them by its carina, which not sulcated and by the arista with long branches. Furthermore, it differs of all other know species of *Rhinoleucophenga* for its aedeagus and epandrium morphology.

Rhinoleucophenga grimaldii sp nov. is similar to *R. flava* sp nov. due its carina not sulcated. But it differs from the other yellow *Rhinoleucophenga* species for its large and widely interrupted tergites stripes, its larger body size, higher number of rows of acrostichal setulae and aedeagus and epandrium morphology.

Rhinoleucophenga exigua sp nov. is very similar to *R. fluminensis*, but differs from this for presenting a smaller number of arista branches, scutum not covered with a longitudinal stripe and spermathecal capsule not elongated. The length of arista branches (pubescent) is also important to differentiate *R. exigua* sp nov. from *R. brasiliensis*, *R. flava* sp nov. and *R. grimaldii* sp nov.

Therefore, a set of very similar small yellow species of *Rhinoleucophenga* has been found in the Neotropical region. Thus, the new species presented here and those redescribed imposed us the further need of reviewing some distributional data. The Brazilian savannah is an environment of high diversity of *Rhinoleucophenga*, but the records of *R. brasiliensis* and *R. fluminensis* (Roque & Tidon 2008, 2013; Roque *et al*. 2013) should be reanalyzed now, since Cerrado is the only biome with records of both species out of their type locality, and also due the present description of a set of very similar species.

3.1.7. ACKNOWLEDGEMENTS

We thank Dr[a] Jane Costa, Dr. Márcio Felix and Danielle Cerri from the Entomological Collection of the Institute Oswaldo Cruz (CEIOC) for allowing us to access the many specimens deposited there; Dr[a] Georgia F. Oliveira for the specimens kindly provided; the National Council of Technological and Scientific Development (CNPq), PRONEX-FAPERGS (10/0028-7) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing grants and fellowships.

3.1.8. REFERENCES

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3.1.9. FIGURES

Figure 1: *Rhinoleucophanga brasiliensis* (Lima 1950), paratype. a: general habitus, dorsal view; b: head and thorax, frontal-lateral view; c: wing; d: head of the specimen on slide nº 3078, lateral view; e: puparium of the slide nº 3058, dorsal view (scale bar 1.0 mm, except in d: 0.5 mm).

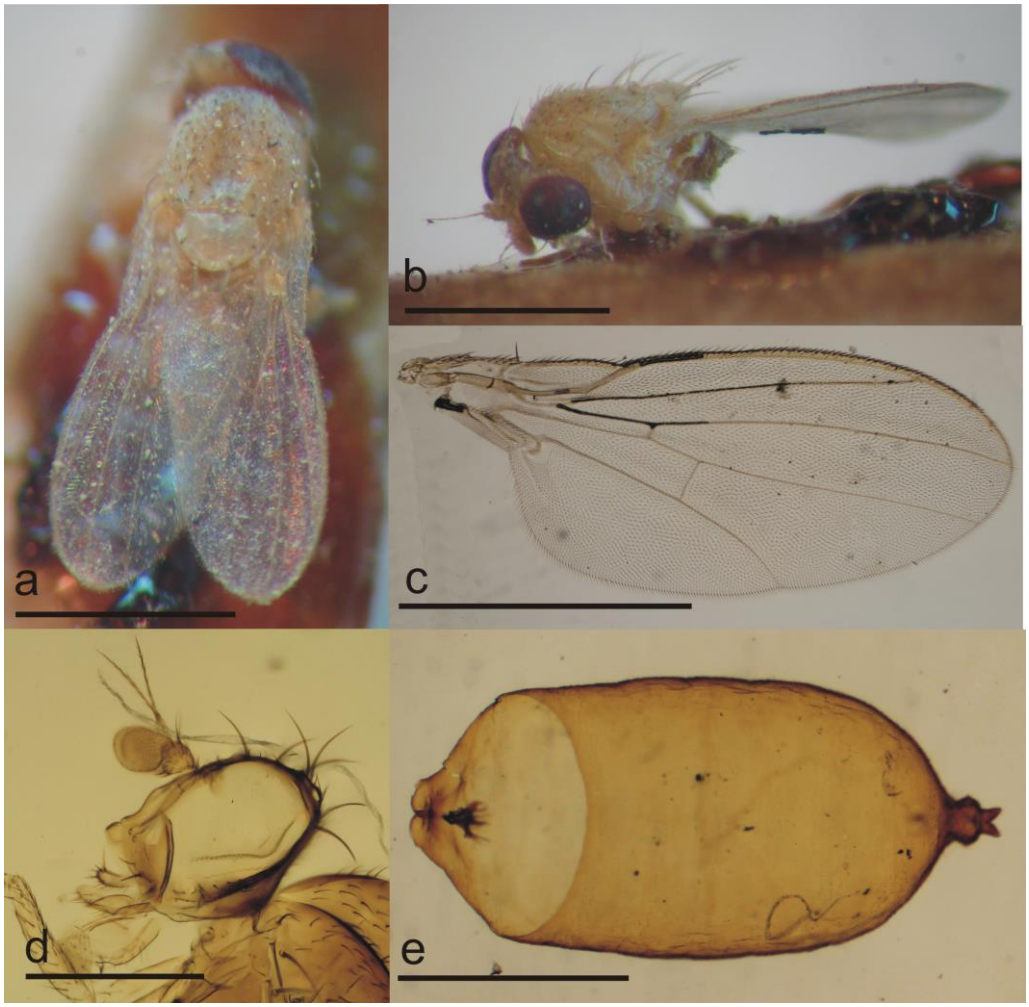


Figure 2: *Rhinoleucophenga brasiliensis* (Lima 1950). Female terminalia, a: posterior view; b: lateral view; c: spermathecal capsule (scale bar 0.1 mm).

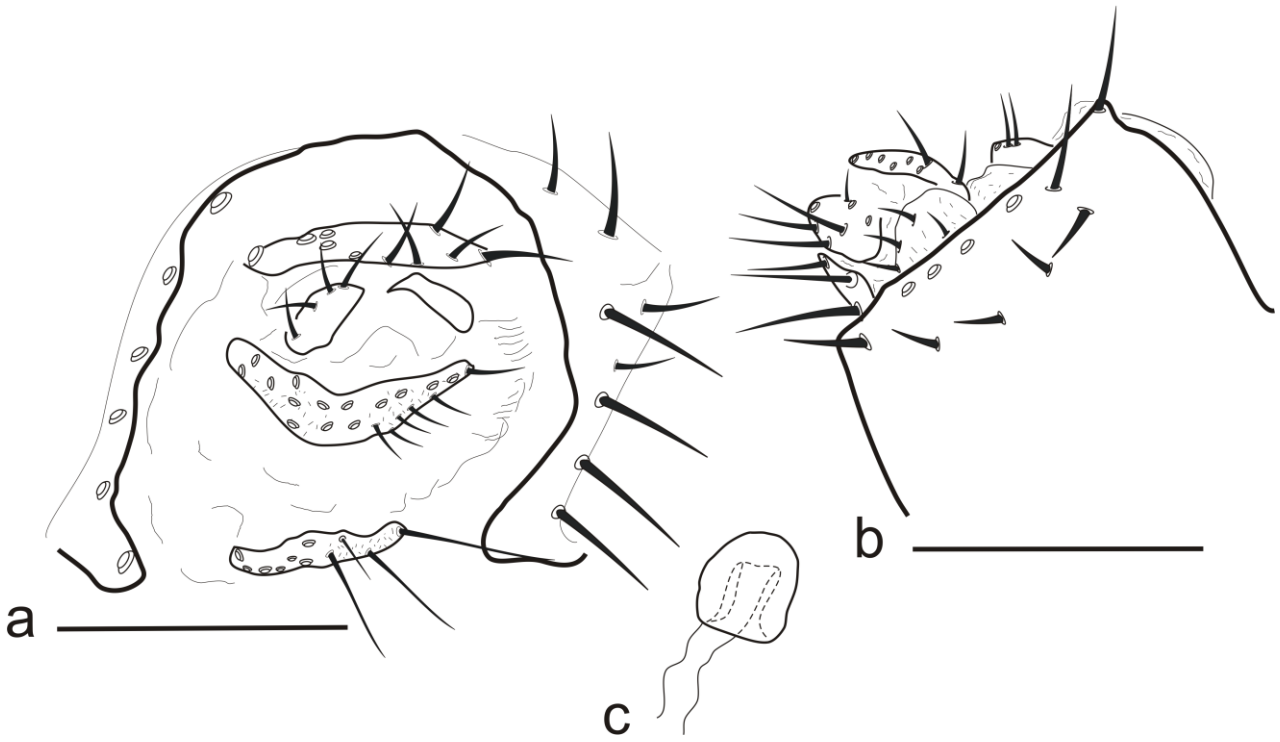


Figure 3: *Rhinoleucophenga fluminensis* (Lima 1950). Paralectotype, a: general habitus, dorsal view; b: general habitus, lateral view; c: wing; d: head, frontal view; e: antennae of the slide nº 4652, lateral view (scale bar 1.0 mm, except in e: 0.2 mm).

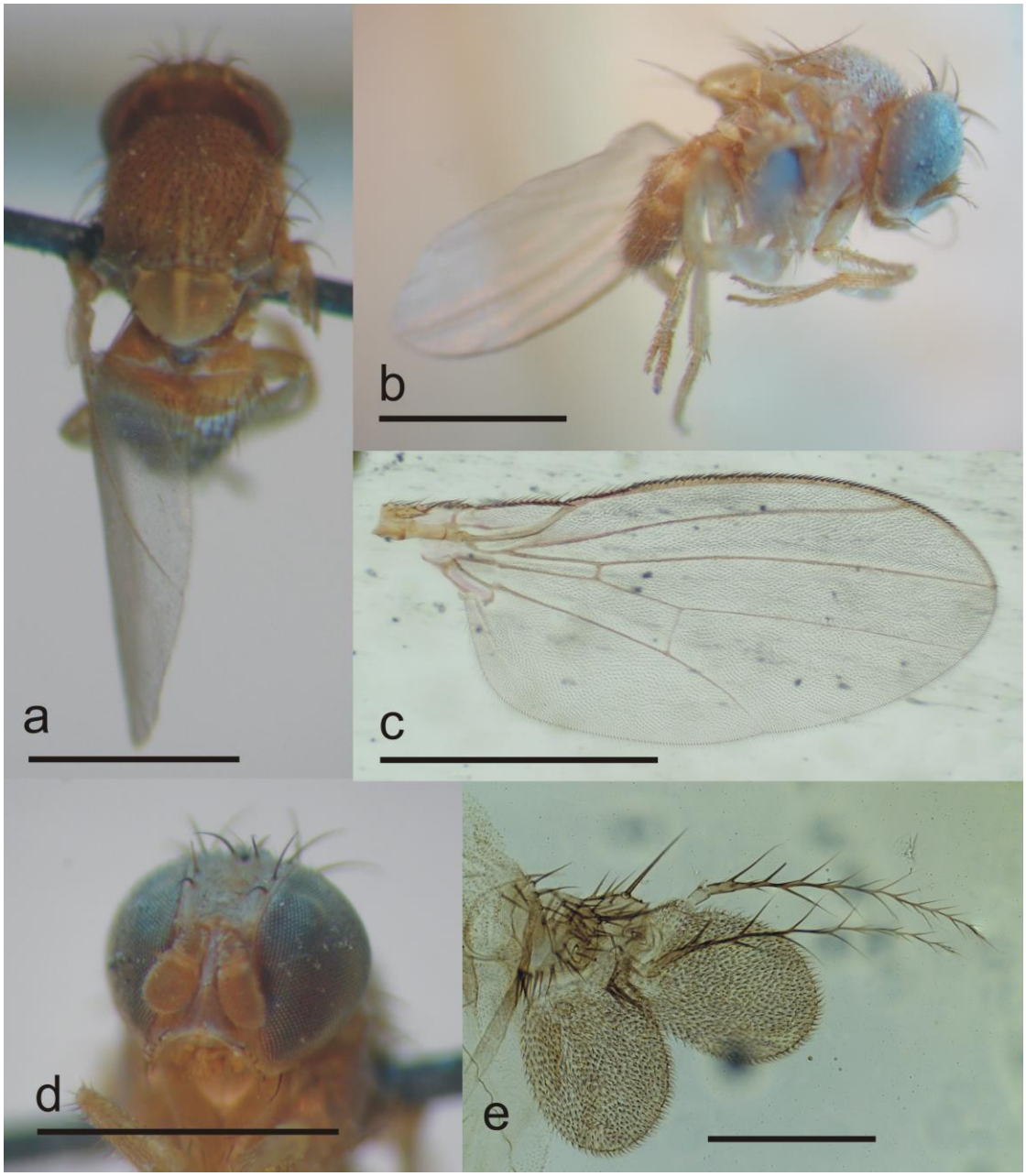


Figure 4: *Rhinoleucophenga fluminensis* (Lima 1950). Male terminalia, a: aedeagus, dorsal view; b: aedeagus, lateral view; c: aedeagus, ventral view; d: epandrium, posterior view (scale bar 0.1 mm).

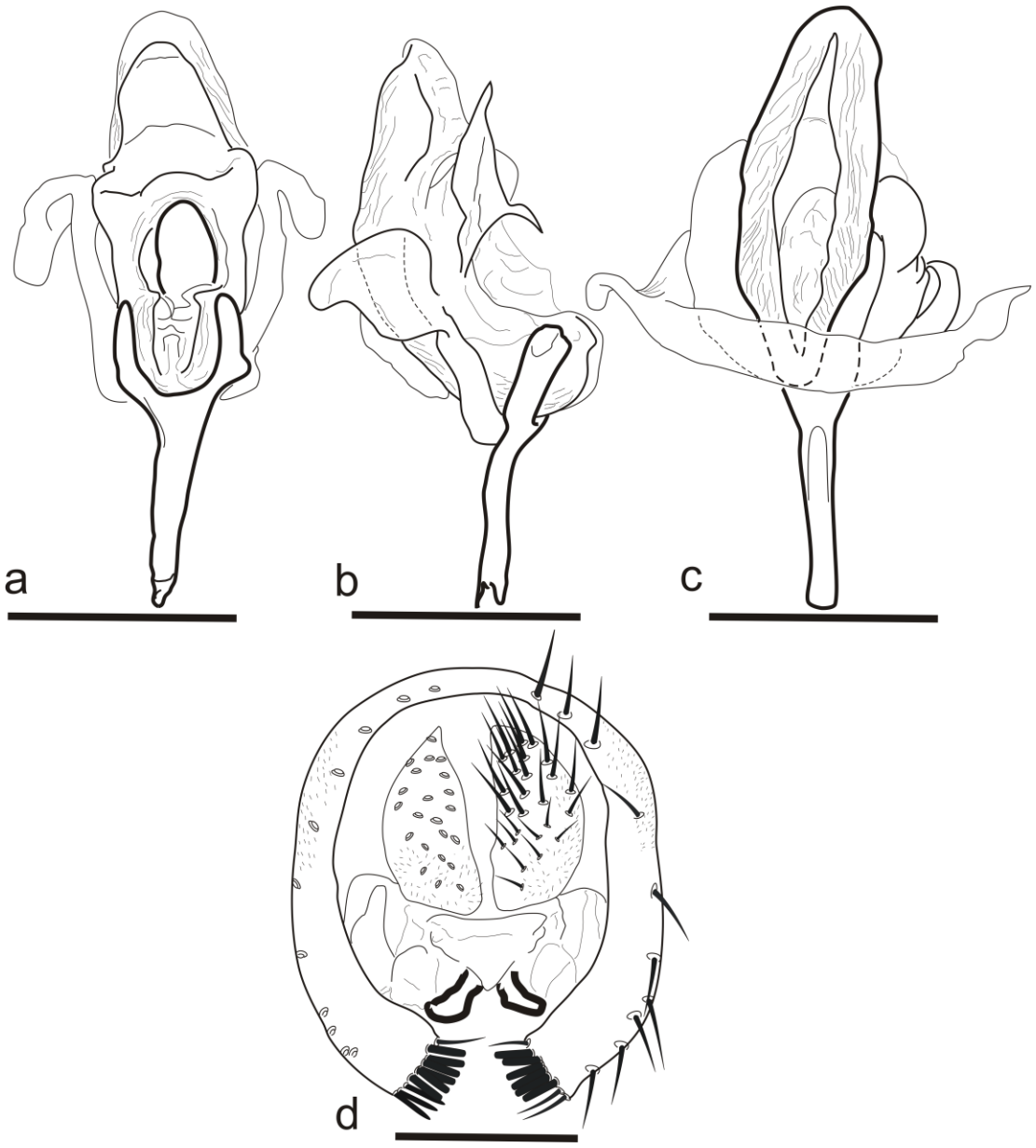


Figure 5: *Rhinoleucophenga fluminensis* (Lima 1950). Female terminalia, a: ventral view; b: dorsal view; c: spermathecal capsule; d: spermathecal capsule, from the storage slide labeled "*R. fluminensis*. 4652/ 3056" (scale bar 0.1 mm).

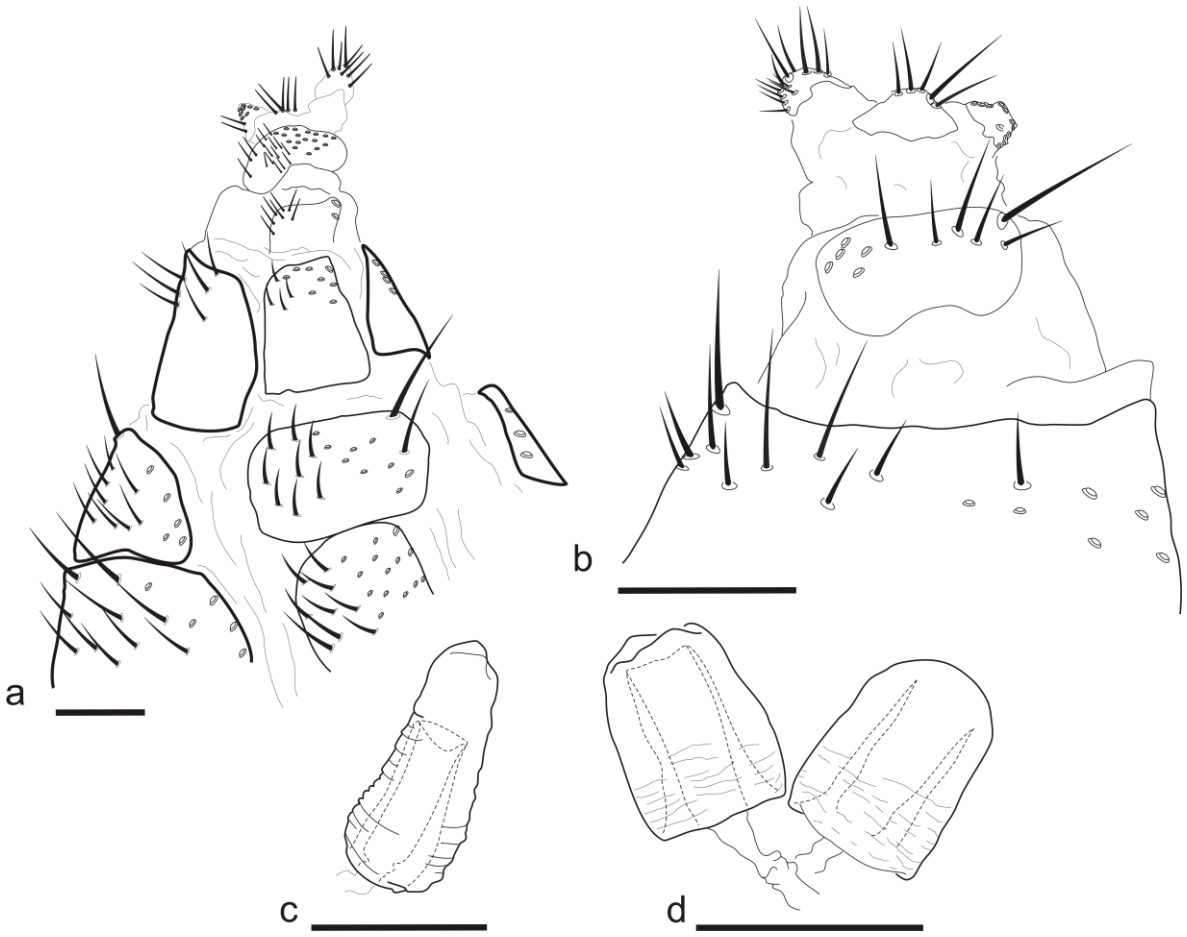


Figure 6: *Rhinoleucophenga fluminensis* (Lima 1950). Puparium of the slide nº 3322, dorsal view (scale bar 1.0 mm).

Figure 7: *Rhinoleucophenga flava* sp. nov. Holotype in alcohol, a: general habitus, dorsal view; b: general habitus, lateral view; c: head, frontal view; d: wing (scale bar 1.0 mm, except in c: 0.5 mm).

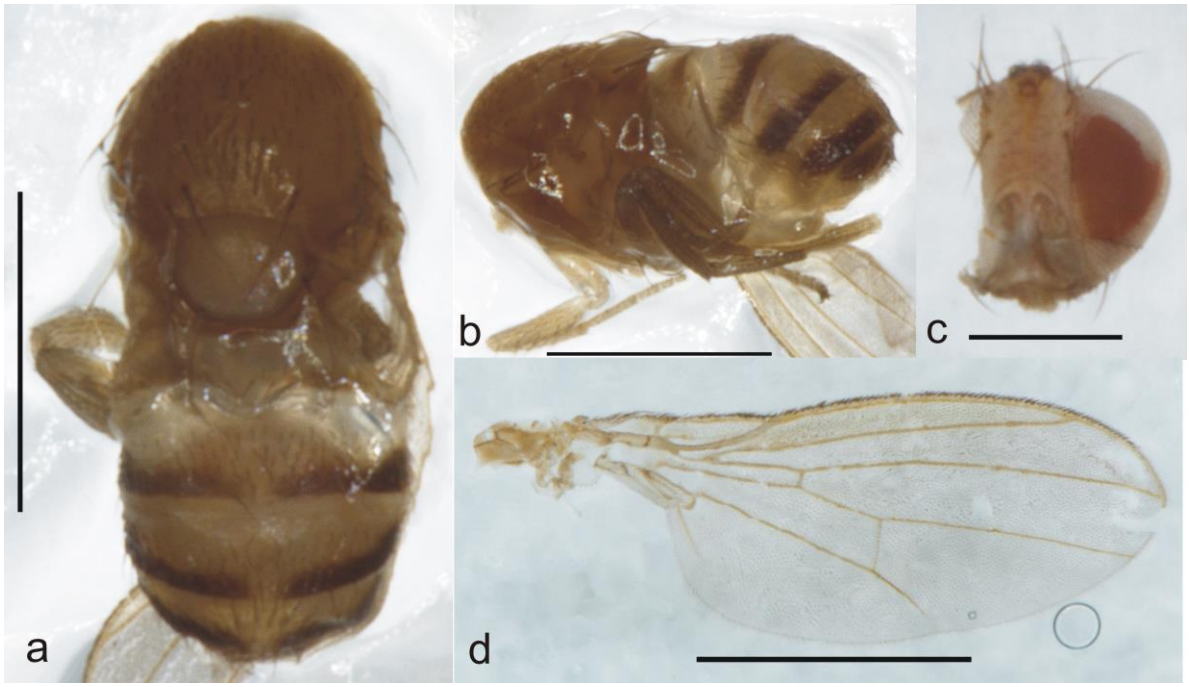


Figure 8: *Rhinoleucophenga flava* sp. nov. Male terminalia, a: aedeagus, dorsal view; b: aedeagus, lateral view; c: aedeagus, ventral view; d: epandrium (scale bar 0.1 mm).

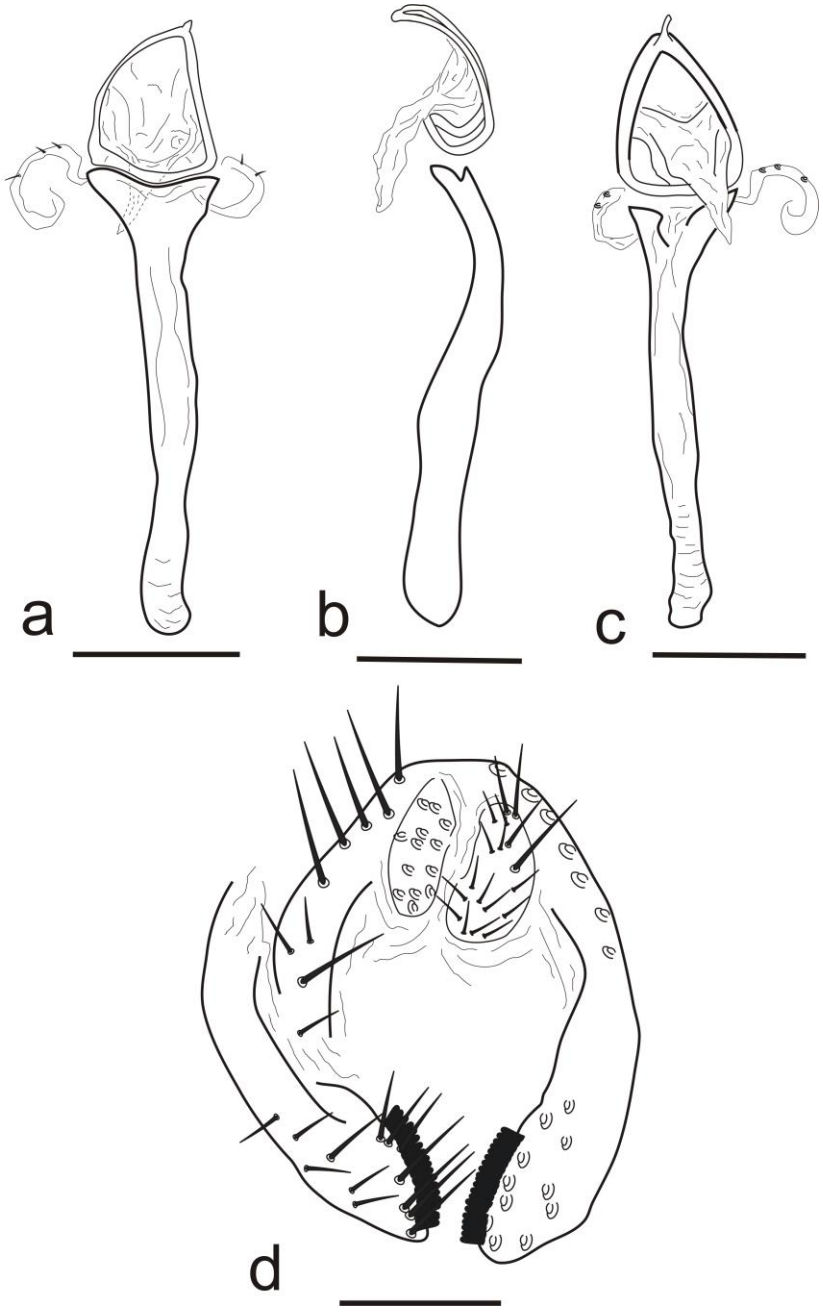


Figure 9: *Rhinoleucophenga grimaldii* sp. nov. Holotype in alcohol, a: general habitus, lateral-dorsal view; b: thorax, dorsal view; c: head, frontal view; d: wing; (scale bar 1.0 mm, except in c: 0.5 mm).

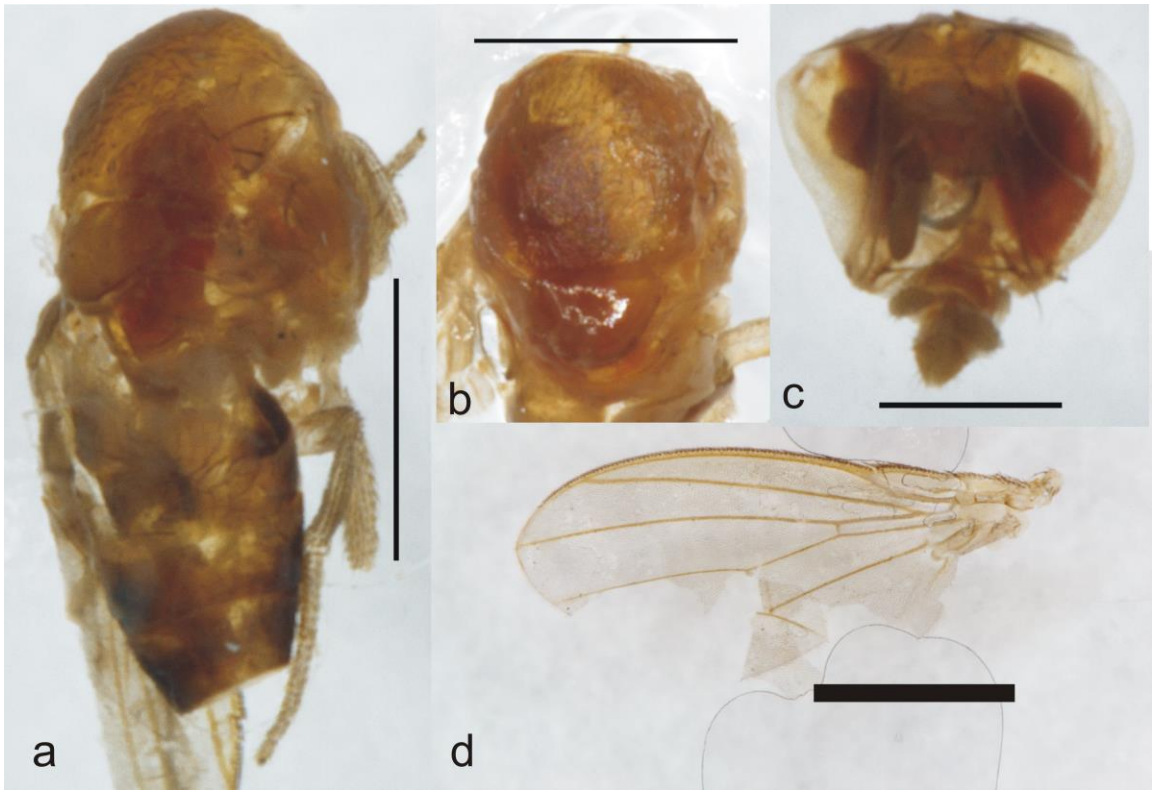
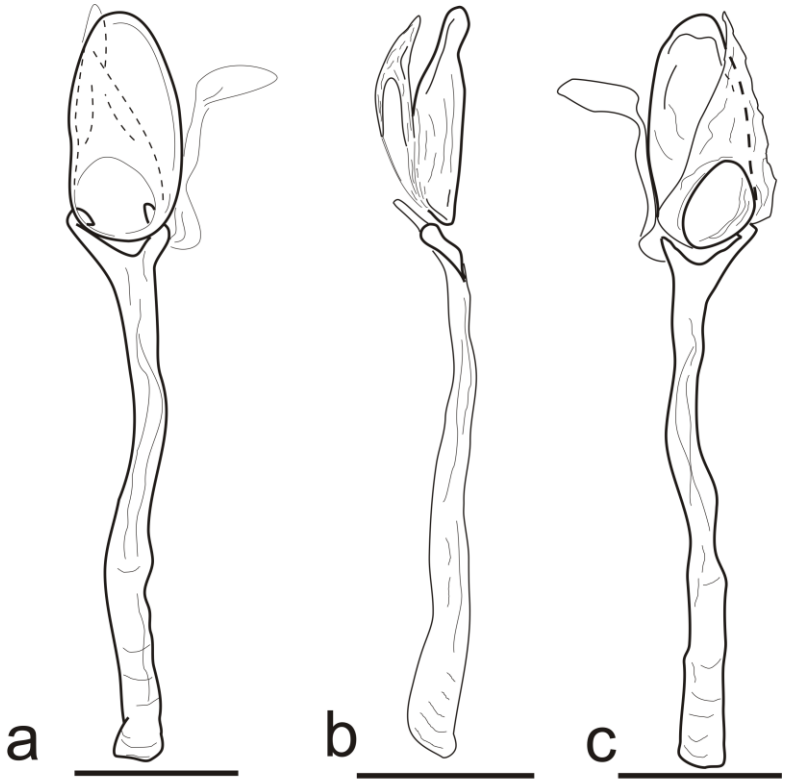


Figure 10 *Rhinoleucophenga grimaldii* sp. nov. Male terminalia, a: aedeagus, ventral view; b: aedeagus, lateral view; c: aedeagus, dorsal view; d: epandrium (scale bar 0.1 mm).



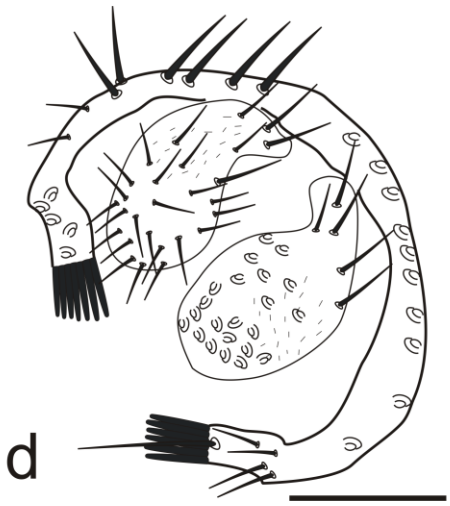


Figure 11: *Rhinoleucophenga exigua* sp. nov. Holotype, a: general habitus, dorsal view; b: head, frontal view; c: general habitus, lateral view; d: wing (scale bar 1.0 mm, except in b: 0.5 mm).

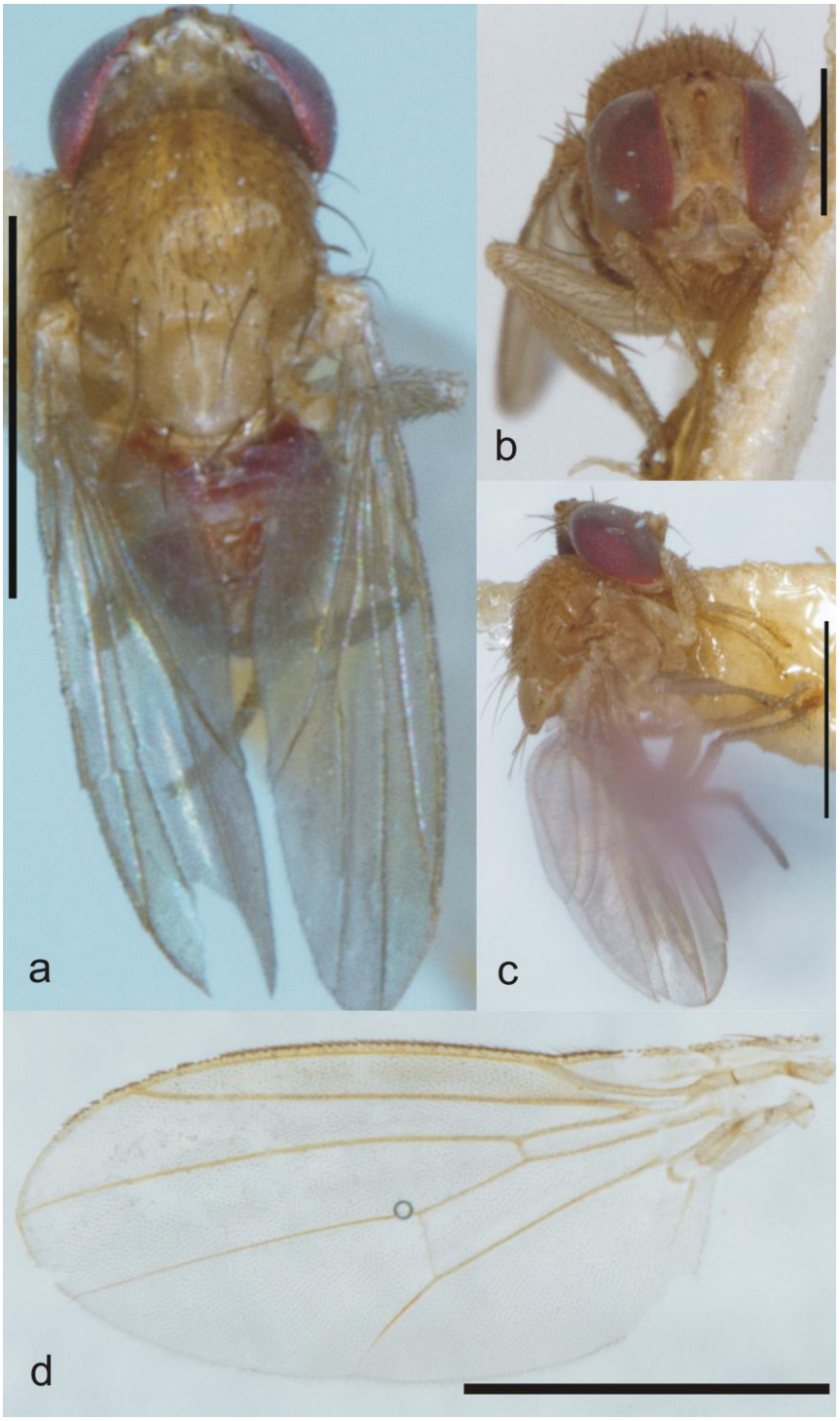
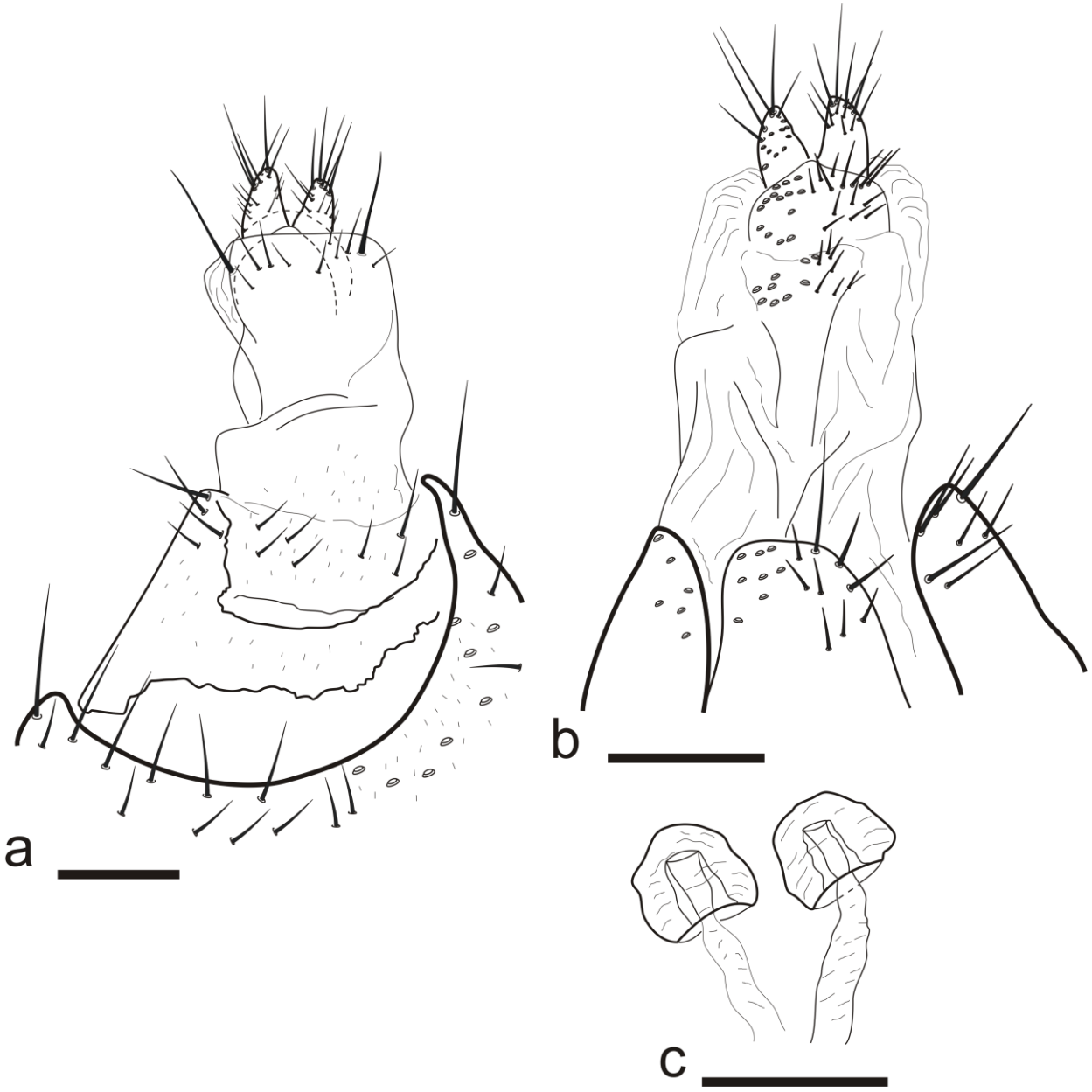


Figure 12: *Rhinoleucophenga exigua* sp nov. Female terminalia, a: dorsal view; b: ventral view; c: spermathecal capsule (scale bar 0.1 mm).



3.1.10. TABLES

Table 1: Complementary measures and indices to the *Rhinoleucophenga brasiliensis* (Lima 1950) and *R. fluminensis* (Lima 1950). Indices according to Bächli et al. (2004). *: measures in millimeters (mm).

	Lote number: 2858							Lote number: 3056					
	R. brasiliensis #01 f#	*R. brasiliensis* #02 f#	*R. brasiliensis* #03 f#	*R. brasiliensis* #04	*R. brasiliensis* #05 m#	*R. brasiliensis* #06 f#	*R. brasiliensis* #07	*R. fluminensis* #01 m#	*R. fluminensis* #02 f#	*R. fluminensis* #03 f#	*R. fluminensis* #04 m# Lectotype	*R. fluminensis* #05 m#	*R. fluminensis* #06 m#
HEAD													
Frontal length *	0.30	0.38	0.30	0.38	-	0.32	0.30	0.40	0.38	0.34	0.38	0.38	0.38
Frontal index	-	-	2.59	2.23	-	2.00	-	2.53	2.47	-	2.69	2.53	2.67
Top-to-bottom frontal width ratio	-	1.21	1.27	1.08	1.08	1.17	1.30	1.27	-	-	1.35	1.20	1.38
Ocellar triangle to front length ratio	-	0.45	0.47	0.39	-	0.38	0.40	0.40	0.45	0.47	0.42	0.45	0.47
Setae or1/or3 ratio	0.93	-	0.88	-	0.70	0.97	1.00	0.77	0.85	0.85	-	0.85	-
Setae or2/or1 ratio	0.71	0.70	0.80	-	0.86	0.71	0.67	1.00	0.91	0.91	-	0.91	0.91
Vibrissal index	0.46	-	0.36	0.33	-	-	-	-	0.44	0.44	0.44	0.47	-
Cheek index	6.29	-	7.67	8.33	-	7.33	-	11.67	11.33	12.80	10.67	11.33	-
Eye index	1.38	1.33	1.35	1.25	1.20	1.29	1.40	1.75	1.36	1.68	1.39	1.42	1.42
THORAX													
Thorax length*	2.45	2.00	2.23	2.36	2.37	2.17	2.75	1.83	2.29	2.31	2.35	2.35	2.31
Strongest prescutellar acrostichal setae, % length related to	60.00	36.00	53.00	-	50.00	-	54.00	54.00	54.00	52.00	54.00	58.00	-

posterior dorsocentral setae													
Dorsocentral setae,transverse distance related to longitudinal distance	2.33X	2.14X	2.40X	2.14X	2.14X	2.40X	2.66X	3.00X	3.00X	-	-	3.50X	-
Sterno index	1.00	-	1.00	1.00	1.00	1.11	1.00	1.00	1.00	-	1.00	1.00	-
WING													
Length*	1.50	-	1.30	-	-	1.50	1.40	2.08	2.10	2.00	2.20	2.08	-
Width*	0.70	-	0.56	-	0.68	-	0.60	0.86	0.94	0.84	-	1.00	-
WING INDICES													
C	2.29	-	2.35	-	-	2.24	2.56	-	2.86	3.00	-	3.10	-
Hb	0.76	-	0.59	-	-	0.59	0.63	-	0.52	0.35	-	0.33	-
Ac	0.17	-	1.79	-	-	-	1.60	-	1.75	1.82	-	1.62	-
4c	-	-	-	-	-	-	-	-	1.31	1.43	-	1.31	-
4v	-	-	-	-	-	-	-	-	3.38	3.57	-	3.25	-
5x	2.71	-	2.86	-	-	-	-	-	2.63	3.00	-	2.30	-
M	-	-	-	-	-	-	-	-	1.31	1.50	-	1.44	-
prox.x	-	-	-	-	-	-	-	-	1.03	1.14	-	1.06	-
Body length*	-	-	1.50	-	1.70	1.50	1.44	2.00	-	-	2.00	2.20	2.00

Table 2: Complementary measures and indices to *Rhinoleucophenga flava* sp nov., *R. grimaldii* sp nov. and *R. exigua* sp nov. Indices according to Bächli et al. (2004). *: measures in millimeters (mm).

			Rhinoleucophenga sp. nov.					
	R. flava Holotype	*R. grimaldii* Holotype	*R. exigua* #01	R. exigua #02	R. exigua #03 f#	R. exigua #04 f# Holotype	R. exigua #05	R. exigua #06
HEAD								
Frontal length *	0.42	0.44	0.44	0.40	0.42	0.42	0.36	0.36
Frontal index	1.40	1.10	1.42	1.48	1.40	1.31	1.44	1.38
Top-to-bottom frontal width ratio	1.13	1.05	1.16	1.19	1.17	1.19	1.20	1.15
Ocellar triangle to front length ratio	0.38	0.41	0.41	0.45	0.43	0.43	0.44	0.44
Setae or1/or3 ratio	1.40	-	-	-	-	-	-	-
Setae or2/or1 ratio	0.57	-	-	-	-	0.57	0.76	-
Vibrissal index	0.57	0.30	0.37	0.32	-	0.32	0.35	0.33
Cheek index	10.67	8.75	11.67	10.67	11.00	9.71	8.29	-
Eye index	1.28	1.35	1.40	1.39	1.32	1.31	1.32	1.28
THORAX								
Thorax length*	1.12	1.30	1.16	1.04	1.15	1.13	0.92	0.95
Strongest prescutellar acrostichal setae, % length related to posterior dorsocentral setae	-	-	50.00	43.48	52.08	-	-	52.38
Dorsocentral setae,transverse distance related to longitudinal distance	2.85X	5.00X	3.80X	2.76X	2.57X	2.66X	3.11X	2.83X
Sterno index	1.00	-	0.94	0.94	0.97	1.00	0.93	1.00
WING								

Length*	2.10	2.40	2.20	2.00	-	2.08	-	1.90
Width*	0.85	1.10	0.90	0.84	0.88	0.84	0.80	0.78
WING INDICES								
C	2.67	3.22	2.59	2.36	-	2.36	-	2.33
Hb	0.57	0.52	0.45	-	-	0.50	-	0.48
Ac	1.75	1.35	1.76	1.91	-	1.83	-	1.91
4c	1.31	0.92	1.47	1.38	-	1.42	-	1.62
4v	2.94	2.00	3.27	2.75	-	3.03	-	3.31
5x	1.65	1.25	1.67	1.67	2.12	2.00	2.86	2.13
M	1.03	0.60	1.00	0.94	1.20	1.10	1.67	1.23
prox.x	1.13	0.80	1.20	1.00	1.07	1.19	1.04	1.15
Body length*	2.30	2.85	2.16	1.92	2.10	2.12	1.70	1.80

4. CAPÍTULO IV

(Manuscrito para submissão ao periódico *Zootaxa*)

4.1. A New Species of the New world Genus *Rhinoleucophenga* (Diptera: Drosophilidae) and Redescription of Five Species Originally Described by Malogolowkin in 1946.

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4.1.1. ABSTRACT

The genus *Rhinoleucophenga* Hendel comprises 29 nominal species with New World distribution. In the present study five species are redescribed: *Rhinoleucophenga personata* Malogolowkin; *R. lopesi* Malogolowkin; *R. angustifrons* Malogolowkin; *R. matogrossensis* Malogolowkin and *R. nigrescens* Malogolowkin. *R. capixabensis* Culik & Ventura is proposed as a new junior synonymy of *R. lopesi*. Other species, *R. jacareacanga* sp. nov., is described from the specimens deposited at CEIOC/Fiocruz. The description of new species and review of some former descriptions of *Rhinoleucophenga* is indispensable since the distribution records of some species are doubtful.

KEY WORDS: *Drosophila*, *Gitona*, Gitonini, Steganinae.

4.1.2. RESUMO

O genero *Rhinoleucophenga* Hendel é composto por 29 espécies com distribuição no Novo Mundo. No presente estudo cinco espécies são redescritas: *Rhinoleucophenga personata* Malogolowkin; *R. lopesi* Malogolowkin; *R. angustifrons* Malogolowkin; *R. matogrossensis* Malogolowkin e *R. nigrescens* Malogolowkin. *R. capixabensis* Culik & Ventura é proposta como um novo sinônimo júnior de *R. lopesi*. Outra espécie, *R. jacareacanga* sp. nov., é descrita a partir de espécimes depositados no CEIOC/Fiocruz. A descrição de novas espécies e a revisão de algumas descrições antigas de *Rhinoleucophenga* é indispensável, uma vez que os registros de distribuição de algumas espécies são duvidosos.

PALAVRAS-CHAVE: *Drosophila*, *Gitona*, Gitonini, Steganinae.

4.1.3. INTRODUCTION

Currently, the new world genus *Rhinoleucophenga* Hendel comprises 29 nominal species (Poppe *et al*. 2015; Vidal & Vilela 2015). As most of these species were described in the first half of the 20th century (Duda 1927, 1929; Lima 1935, 1950; Malogolowkin 1946), many of them lack better morphological characterization in their descriptions. Thus, some problems associated with the species identification and their geographical distribution have been noticed (Vilela 1990; Poppe *et al*. 2015).

Many species from the Neotropical fauna of *Rhinoleucophenga* were described by Malogolowkin (1946), and their type series were deposited in the Entomological Collection of the Instituto Oswaldo Cruz (CEIOC) in Brazil. The most part of the *Rhinoleucophenga* type series were destroyed in an episode known as the "Massacre de Manguinhos" (Massacre of Manguinhos) (Costa *et al*. 2008). As a consequence, it has become even more difficult to identify and establish the geographical distribution of the species proposed by Malogolowkin (1946).

Recently many species of *Rhinoleucophenga* have been described, including sibling sets of species (Poppe *et al*. 2015). In the current conditions, the conference and the complementarity of old descriptions are necessary to avoid synonymy description processes and misidentifications or even uncertainties about the geographical distribution of some species.

Considering the current patterns of species description, the present study aims to redescribe *Rhinoleucophenga personata* Malogolowkin 1946; *R. lopesi* Malogolowkin 1946; *R. angustifrons* Malogolowkin 1946; *R. matogrossensis* Malogolowkin 1946 and

R. nigrescens Malogolowkin 1946 and to describe *R. jacareacanga* sp. nov. We also provide a new junior synonymy and comments about the distribution of some species in the Brazilian territory.

4.1.4. MATERIALS AND METHODS

We consulted specimens from the Coleção Entomológica do Instituto Oswaldo Cruz (CEIOC), Rio de Janeiro, Brazil; Museu Nacional do Rio de Janeiro (MNRJ), Rio de Janeiro, Brazil; and Coleção Entomológica da Universidade Federal do Espírito Santo (UFES). Additionally, ten specimens of *Rhinoleucophenga capixabensis* Culik & Ventura collected in municipality of Tangará da Serra, State of Mato Grosso, Brazil (14º39'05"S; 57º25'25"W), and a specimen from the Ecological Reserve of Raso da Catarina, municipality of Paulo Afonso, Bahia, Brazil (9°30'39"S 38°32'12"W) had their morphology analyzed.

The terminology and the morphology analysis were based on measures and indices given by Bächli *et al*. (2004), which were done with an ocular reticle inserted into a stereomicroscope. Males' terminalia were disarticulated in glycerol after treatment with 10% potassium hydroxide (KOH) and acid fuchsine (Bächli et al. 2004). The genitalia were mounted in a piece of glycerine jelly (ca. 2 x 2 x 2 mm) (Grimaldi 1987), stored in microvials with glycerol and pinned with the respective specimen. Photos of the specimens were taken with a digital camera coupled to an optical stereomicroscope. Drawings of the genitalia were made with a *camera lucida* system attached to a compound microscope with 40× and 10× objective lenses and a 10× ocular lens. We transcribed the type specimens' labels in full and backslash indicates label change.

4.1.5. RESULTS

Rhinoleucophenga Hendel

Rhinoleucophenga Hendel, 1917: 44-45

Pseudophortica Sturtevant, 1918: 37

Gitona (in New world) Brake & Bächli, 2008: 291

Rhinoleucophenga personata Malogolowkin 1946

(Figures 1a-d; 2a-d; 3a-d; Table 1)

Rhinoleucophenga personata Malogolowkin 1946: 417 (key), 422, 423 (figures 11-13);
Rhinoleucophenga personata Mata et al., 2008: 57, 58 (table).

Material examined: HOLOTYPE (dried mounted material, deposited in CEIOC) labeled "*Rhinoleucophenga personata* DET - Ch. Malogolowkin Rio, 5-946 \ Salobra jan. 941 Mato Grosso Com. I.O.C. \ Inst. Osw. Cruz N° 8122 \ Holotypus". PARATYPE (dried mounted material, deposited in CEIOC) labeled "*Rhinoleucophenga personata* DET. Chana Malogolowkin Rio, V.946 \ Salobra jan. 941 Mato Grosso Com. I.O.C.\ Inst. Osw. Cruz N° 8123 \ PARÁTIPO". Both specimens were with their terminalia disarticulated and lost.

Diagnosis. General body color brown; front covered with ca. 28 scattered interfrontal setulae. Top-to-bottom frontal width ratio 1.05 (1.03-1.06); gena brownish with an irregular brown spot. Scutum covered with a longitudinal dark brown stripe; hyaline wings; tergites with large brown stripes widely interrupted medially. Femur proximally brown and distally brownish. Body length ca. 3.87 mm (3.60-4.15).

Description. Holotype and paratype present the same follow characteristics:

Head (Fig 1a, c). Front ventrally brownish and superiorly brown, covered with ca. 28 scattered interfrontal setulae; ocellar triangle and ocelli brown. Carina nose-like and ca. 75% sulcated. Face brownish; gena brownish with an irregular brown spot; antenna with flagellomere proximally brownish and distally brown, pedicel dark brown; arista with 7 long dorsal branches and 5 long ventral branches plus terminal fork. Palpus yellowish with ca. 30 (25-30) setae on lower part.

Thorax (Fig 1b-c). Scutum brown and scutellum dark brown, scutum covered with a longitudinal dark brown stripe. Ten irregular rows of acrostichal setulae. Two pairs of prescutellar acrostichal setae. Pleura dark brown and halteres yellowish. Legs brownish (femur proximally brown and distally brownish).

Wings (Fig 1c). Hyaline, without spots.

Abdomen (Fig 1d). Tergites II-V with large brown stripes widely interrupted medially.

Male terminalia. In the manuscript of Malogolowkin (1946) it is possible to notice an aedeagus ring-like shaped, epandrium with an elongated ventral lobe and ca. 13 prensisetae.

For more measures and indices see Table 1.

Distribution. Known from the type locality (probably Salobra, a district of municipality of Miranda in the State of Mato Grosso do Sul, Brazil) and from the Ecological Reserve of Raso da Catarina, Municipality of Paulo Afonso, Bahia, Brazil (9°30'39"S 38°32'12"W). Mata *et al*. (2008) recorded this species in the Paranã Valley, Central region of Brazil but the exact locality was not mentioned.

Note. A specimen from Ecological Reserve of Raso da Catarina, Municipality of Paulo Afonso, Bahia, Brazil (9°30'39"S 38°32'12"W) collected by G.F. Oliveira, was reviewed and had the morphology described (Fig 2a-d). The specimen (conserved in ethanol 70%) presents the same external morphological characters of *R. personata*, except by the number of scattered interfrontal setulae ca. 50 and the palpus with ca. 40 setae on lower part. The male terminalia (Fig 3a-d) is characterized by aedeagus ring like, wider medially, with a dorsal sclerotized structure shaped like a "duck's bill". Epandrium microtrichose with ca. seven upper and 15 longer lower setae on each side. Ventral lobe elongated. Surstylus with 12 prensisetae. Cerci elongated, with ca. 30 setae each one, eight longer setae in the apical portion. Complementary measures and indices on Table 1.

Rhinoleucophenga lopesi Malogolowkin 1946

(Figures 4a-d; 5a-d; 6a-b; 7; 8a-d; Table 1)

Rhinoleucophenga lopesi Malogolowkin 1946: 417 (key), 424 (figures 14-15);
Rhinoleucophenga sp.1 (Blauth and Gottschalk, 2007);
Rhinoleucophenga lopesi Mata *et al*., 2008: 57, 58 (table);
Rhinoleucophenga capixabensis Culik and Ventura, 2009: 418 (NEW SYNONYMY).

Material examined: HOLOTYPE f# labeled (dried mounted material, deposited in CEIOC) labeled "*Rhinoleucophenga lopesi* DET - Chana Malogolowkin Rio V-946 \ Brasil, Rio de Janeiro, 8-934, H. Souza Lopes \ Inst. Oew. Cruz nº 8.125 \

HOLOTYPUS". The right wing, III-VII tergites and the terminalia were disarticulated and lost. Holotype of *R. capixabensis* (in ethanol 70%, deposited in MNRJ) labeled "Brasil, Espirito Santo, Cachoeiro de Itapemirim, INCAPER FE Pacotuba 16.I.2008, col. M.P. Culik ex. *A. comosus* + *D. brevipes Rhinoleucophenga capixabensis* HOLOTYPE" and 05 paratypes of *R. capixabensis* (in ethanol 70%, 03 deposited in MNRJ and 02 deposited in UFES) labeled "Brasil, Espirito Santo, Cachoeiro de Itapemirim, INCAPER FE Pacotuba 16.I.2008, col. M.P. Culik ex. *A. comosus* + *D. brevipes Rhinoleucophenga capixabensis* PARATYPE".

Diagnosis. General body color brown; front covered with ca. 40 scattered interfrontal setulae. Top-to-bottom frontal width ratio about 1.00; gena brownish with an irregular brown spot. Hyaline wings; tergite II and III with brown stripes interrupted medially.

Description. Based on the holotype. Head (Fig 4a, c). Front homogeneously brownish, covered with ca. 40 scattered interfrontal setulae; ocellar triangle brownish with dark brown ocelli. Carina nose-like and ca. 90% sulcated. Face brownish; gena brownish with an irregular brown spot; antenna with pedicel yellow. Palpus yellowish with ca. 20 (15-20) setae on lower part. Arista lost.

Thorax (Fig 4b-c). Scutum brownish and scutellum brown. Ten irregular rows of acrostichal setulae. Three pairs of prescutellar acrostichal setae. Pleura brown and halteres yellowish. Legs brownish.

Wings (Fig 4d). Hyaline, without spots.

Abdomen. Abdomen brownish; tergite II with brown stripes interrupted medially.

Terminalia unknown.

For more measures and indices see Table 1.

Note. We analyzed the holotype and five paratypes of *R. capixabensis* (the holotypus and two paratypes (Fig. 5a-d and 6) with measurements presented in Table 1) and ten specimens collected in the municipality of Tangará da Serra, State of Mato Grosso, Brasil (14º39'05"S; 57º25'25"W) (measurements presented in Table 1). Both the type specimens of *R. capixabensis* and the specimens collected in State of Mato Grosso present similar morphological characters with the holotypus of *R. lopesi*. Aristae of these specimens with 5-6 dorsal and 3-4 ventral branches. The male terminalia of a

paratype of *R. capixabensis* (Fig. 7a-b) and of the five specimens collected in State of Mato Grosso (Fig. 8a-d) were similar, with the ringed-shape aedeagus typical of *Rhinoleucophenga*. The epandrium is microtricose, with c.a. 60 upper+lower setae. Large cerci, free and ventrally folded. Surstyli fused with epandrium with 16-18 prensisetae. Aedeagus dorsoventrally flattened, with narrowed apex. Two long paraphysis projected anteriorly from the aedeagal apodeme, bearing the hypandrium, with three setulae in apical region. Hypandrium slightly concave, rounding aedeagus, with a cleft in posterior and two lateral projections.

Distribution. Brazil (States of Rio de Janeiro, Mato Grosso, Espírito Santo). Mata *et al*. (2008) recorded this species in Paranã Valley, Central region of Brazil, but the exact locality was not mentioned.

Rhinoleucophenga angustifrons Malogolowkin 1946

(Figures 9a-e; Table 1)

Rhinoleucophenga angustifrons Malogolowkin 1946: 417 (key), 424 (figures 16-17), 425-426;

Rhinoleucophenga angustifrons Roque & Tidon, 2008: 97.

Material examined: HOLOTYPE f# (dried and mounted specimen, deposited in CEIOC) labeled "*Rhinoleucophenga angustifrons* DET - Chana Malogolowkin Rio V-946 \ BRASIL JUSSARAL X-934 L. TRAV. ET LOPES \ Inst. Osw. Cruz nº 8.121 \ HOLOTYPUS. Left wing, posterior right leg and terminalia were disarticulated and lost.

Diagnosis. Body yellow; front covered with ca. 40 scattered interfrontal setulae. Frontal index 2.83; carina nose-like and ca. 50% sulcated. Hyaline wings; tergites with brown stripes interrupted medially. Body length ca. 2.50 mm.

Description. Head (Fig 9c). Front homogeneously yellowish, covered with ca. 40 scattered interfrontal setulae; ocellar triangle yellow with brown ocelli. Carina nose-like and ca. 50% sulcated. Face and gena yellowish; antenna with flagellomore and pedicel homogeneously brownish; arista with 7 long dorsal branches and 6 long ventral branches. Palpus yellowish with ca. 15 setae on lower part.

Thorax (Fig 9a-b). Scutum and scutellum homogeneously yellow. Ten irregular rows of acrostichal setulae. One pair of prescutellar acrostichal setae. Pleura brownish; halteres yellowish. Legs homogeneously yellow.

Wings (Fig 9e). Hyaline, without spots.

Abdomen (Fig 9d). Tergites III-III yellowish and tergites IV-V brownish; with brown stripes interrupted medially.

Terminalia unknown.

For more measures and indices see Table 1.

Distribution. Known only from the type locality (State of Rio de Janeiro, Brazil).

Note. *Rhinoleucophenga angustifrons* recorded by Roque & Tidon (2008) was analyzed and it is not a *Rhinoleucophenga* species, probably a *Leucophenga* Mik specimen.

Rhinoleucophenga matogrossensis Malogolowkin 1946

(Figure 10a-d; Table 1)

Rhinoleucophenga matogrossensis Malogolowkin 1946: 417 (key), 419 (figure 9), 420.

Material examined: HOLOTYPE m# (dried and mounted specimen, deposited in CEIOC) labeled "*Rhinoleucophenga matogrossensis* DET - Chana Malogolowkin Rio, 5-946 \ Salobra 30-8-40 Mato-Grosso Com. I.O.C. \ Inst. Osw. Cruz n° 8.124 \ HOLOTYPUS". Right wing and terminalia were disarticulated and lost.

Diagnosis. General body color brown; front covered with ca. 180 scattered interfrontal setulae. Frontal index 1.53; Carina nose-like and ca. 90% sulcated. Hyaline wings; tergites with dark brown stripes interrupted on tergites II-III and continuous on tergites IV-VI. Legs homogeneously yellow. Body length ca. 6.25 mm.

Description. Head (Fig 10c). Front ventrally brown and superiorly brownish, covered with ca. 180 scattered interfrontal setulae; ocellar triangle brownish with brown ocelli. Carina nose-like and ca. 90% sulcated. Face yellowish; gena brownish; antenna with flagellomore and pedicel homogeneously brownish; arista with 9 long dorsal branches and 6 long ventral branches. Palpus yellowish with more than 50 setae on lower part.

Thorax (Fig 10a-b). Scutum and scutellum homogeneously brownish. 12 irregular rows of acrostichal setulae. Four pairs of prescutellar acrostichal setae, the central one is the shortest. Pleura brown and halteres brownish. Legs homogeneously yellow.

Wings (Fig 10d). Hyaline, without spots.

Abdomen. Abdomen proximally brown and distally dark brown; tergites II-III with dark brown stripes interrupted medially, tergite IV-VI with continuous stripes.

Terminalia unknown.

For more measures and indices see Table 1.

Distribution. Known only from the type locality (probably Salobra, a district of the municipality of Miranda, Mato Grosso do Sul, Brazil).

Rhinoleucophenga nigrescens Malogolowkin 1946

(Figures 11a-e; Table 1)

Rhinoleucophenga nigrescens Malogolowkin 1946: 417 (key), 421 (figure 10), 422.

Material examined: HOLOTYPE f# (dried and mounted specimen, deposited in CEIOC) labeled "*Rhinoleucophenga nigrescens* DET - Chana Malogolowkin Rio, V-946 \ Inst. Osw. Cruz nº 8.120 \ HOLOTYPUS". Right wing, posterior left leg and terminalia were disarticulated and lost.

Diagnosis. Body dark brown; front covered with ca. 200 scattered interfrontal setulae. Frontal index 1.56; Carina nose-like and ca. 80% sulcated. Hyaline wings; abdomen dark brown-black, continuous tergites stripes. Body length ca. 6.50 mm.

Description. Head (Fig 11a). Front ventrally dark brown and superiorly brown, covered with ca. 200 scattered interfrontal setulae; ocellar triangle and ocelli dark brown. Carina nose-like and ca. 80% sulcated. Face brownish; gena brown; antenna with flagellomore and pedicel homogeneously brownish; arista with 11 long dorsal branches and 7 long ventral branches. Palpus brownish with ca. 40 setae on lower part.

Thorax (Fig 11c-d). Scutum and scutellum homogeneously dark brown. 10 irregular rows of acrostichal setulae. Four pairs of prescutellar acrostichal setae, the central one is the longest. Pleura and halteres brown. Legs homogeneously brown.

Wings (Fig 11b). Hyaline, without spots.

Abdomen (Fig 11e). Abdomen proximally dark brown and distally black, tergites with stripes continuous.

Terminalia unknown.

For more measures and indices see Table 1.

Distribution. Known only from the type locality (probably Salobra, a district of the municipality of Miranda, Mato Grosso do Sul, Brazil).

R. jacareacanga sp nov. Poppe, Valente & Gottschalk

(Figures 12a-e; 13a-d; Table 1)

Material examined. Two dried male specimens labeled "Jacareacanga, Pará, Brasil. M. Alvarenga, xii.1968". Deposited in CEIOC.

Type series. Holotype: 1m# (dried and mounted specimen, deposited in CEIOC) labeled "Jacareacanga \ Pará, Brasil \ DET – M. Alvarenga \ X.68 \ Inst. Osw. Cruz nº 8.120 \ HOLOTYPUS". Additionally labeled as "*Rhinoleucophenga jacareacanga*; HOLOTYPE m#; State of Pará, Brazil. Det.: JL Poppe and MS Gottschalk; v.2014". Postabdomen dissected stored in a microvial with glycerin, on the same pin with the respective specimen. Paratype: 1m# (dried and mounted specimen, deposited in CEIOC) labeled "Jacareacanga \ Pará, Brasil \ DET – M. Alvarenga \ X.68 \ Inst. Osw. Cruz nº 8.120 \ HOLOTYPUS". Additionally labeled as "*Rhinoleucophenga jacareacanga*; PARATYPE; State of Pará, Brazil. Det.: JL Poppe and MS Gottschalk; v.2014". All specimens were deposited in Coleção Entomológica do Instituto Oswaldo Cruz (CEIOC), Fiocruz.

Diagnosis. Body brown; front covered with ca. 80 scattered interfrontal setulae. Carina nose-like ca. 90% sulcated. Arista with 15 long dorsal branches and 11 long ventral branches plus terminal fork. Twelve irregular rows of acrostichal setulae. Clouded

wings with M curved to R_{4+5}; tergites with dark brown stripes interrupted medially in the tergite II and continuous from the tergites III. Body length 6.00 mm. Male terminalia as in figure 13a-d.

Description. Head (Fig 12a, d). Front homogeneously brown, covered with ca. 80 scattered interfrontal setulae; ocellar triangle brown with dark brown ocelli. Carina nose-like and ca. 90% sulcated. Face and gena brown; antenna with flagellomere homogeneously brown and pedicel brownish; arista with 15 long dorsal branches and 11 long ventral branches plus terminal fork. Palpus brown with ca. 36 setae on lower part.

Thorax (Fig 12a-b). Scutum brown, scutellum brownish and marginally brown. Twelve irregular rows of acrostichal setulae. Three pairs of prescutellar acrostichal setae, the central one is the longest. Pleura and legs brown; halters brownish.

Wings (Fig 12e). Brownish, with brown clouded around the veins R_{2+3}, C II, C III, R_{4+5} apical distally, M III, M IV and Dm-Cu.

Abdomen (Fig 12c). Abdomen proximally brownish and distally brown; tergites with dark brown stripes interrupted medially in the tergite II and continuous from the tergites III.

Male terminalia (Fig 13a-d) aedeagus shaped like a “water drop”, elongated, wider basally. Slightly curved dorso-ventally with a dorsal apical sharp projection. Epandrium microtrichose with ca. eight upper and 25 long lower setae on each side. Surstylus with 17 prensisetae. Cerci elongated, with ca. 60 setae each one.

Female unknown.

For more measures and indices see Table 1.

Etymology. The species name refers to its original label at CEIOC/Fiocruz: “Jacareacanga \ Pará, Brasil \ M. Alvarenga \ XII.1968”.

Type locality. We believe it is from the municipality of Jacareaganga, State of Pará, north of Brazil (6°13’20”S 57°45’10”W), as it is originally labeled at CEIOC.

Distribution. Known only from the type locality (State of Pará, Brazil).

Biology. Collect method unknown.

4.1.6. DISCUSSION

Rhinoleucophenga is compound by rare or few abundant species, thus, the type series are commonly compound by a single or few individuals (Junges & Gottschalk 2014; Poppe *et al*. 2014, 2015). The species described by C. Malogolowkin in 1946 represent an important part of *Rhinoleucophenga* diversity in the Neotropical region; therefore, redescriptions and a comparative study provided more taxonomic characters to the focus species, which make easier the process of correct species identification.

Rhinoleucophenga angustifrons is a typical yellow species such as *R. brasiliensis* (Lima) and *R. fluminensis* (Lima), but it presents peculiar characteristics of a very narrow front and a rounded head; beyond long arista branches that differs *R. angustifrons* from the other two species. Malogolowkin (1946) mentioned the carina of *R. angustifrons* as not sulcated, the opposite was notice here, the specimen presents a carina ca. 50% sulcated.

Rhinoleucophenga matogrossensis, *R. personata* and *R. lopesi* are both brown species, resembling *R. missionera* Poppe *et al*. and *R. sulina* Poppe *et al*. *Rhinoleucophenga matogrossensis* differs from all species for presenting a high bristled front (ca. 180 interfrontal setae) and dark abdomen with tergites stripes continuous. *Rhinoleucophenga personata* differs from all other species by its “multibrown” antenna color pattern. Furthermore, the specimen collected by G.F. Oliveira in the Ecological Reserve of Raso da Catarina, municipality of Paulo Afonso, is *R. personata* based on the morphological review; thus the male terminalia presented here represents new information to help in the species determination.

Rhinoleucophenga lopesi differs from *R. missionera* and *R. sulina* for the abdominal pigmentation, with the tergites II and III with wide dark bands medially interrupted and not touching the posterior margin, and posterior tergites almost all dark; in addition, the aedeagus morphology is very different among the three species. The available information about the male terminalia of *R. capixabensis*, determined here as junior synonymy of *R. lopesi*, provide important information to the determination of that species.

Rhinoleucophenga nigrescens is a big dark brown \ black species, being it a well noticed characteristic to differentiate it from the other *Rhinoleucophenga* species; although big size is commonly noticed in *Rhinoleucophenga*, such as in *R. obesa*

(Loew), *R. gigantea* (Thomson) and *R. pampeana* Poppe *et al*., the dark general body color is peculiar to *R. nigrescens*.

The new species *R. jacareacanga* sp. nov. is also a typical big brown species, resembling *R. matogrossensis*; but it differs from *R. matogrossensis* for present clouded wings and very plumose arista. Furthermore, *R. jacareacanga* sp. nov. differs from all other *Rhinoleucophenga* species for its aedeagus and epandrium morphology.

4.1.7. ACKNOWLEDGEMENTS

We thank Dr. Jane Costa, Dr. Márcio Felix and Danielle Cerri from the Entomological Collection of the Institute Oswaldo Cruz (IOC), Dr. José Aires Ventura from Instituto Capixaba de Pesquisa, Assitência Técnica e Extensão Rural, and Dr[a] Cátia Antunes de Mello-Partiu from Museu Nacional do Rio de Janeiro (Universidade Federal do Rio de Janeiro) for allowing us to access the studied. Msc. Georgia F. Oliveira for kindly provide a *Rhinoleucophenga* specimen. The National Council of Technological and Scientific Development (CNPq), PRONEX-FAPERGS (10/0028-7) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing grants and fellowships.

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4.1.9. FIGURES

Figure 1: *Rhinoleucophenga personata* Malogolowkin (1946). Holotype, dried and mounted specimen, a: head, frontal view; b: thorax, dorsal view; c: general habitus, lateral view; d: abdomen, dorsal view; (scale bar 1.0 mm).

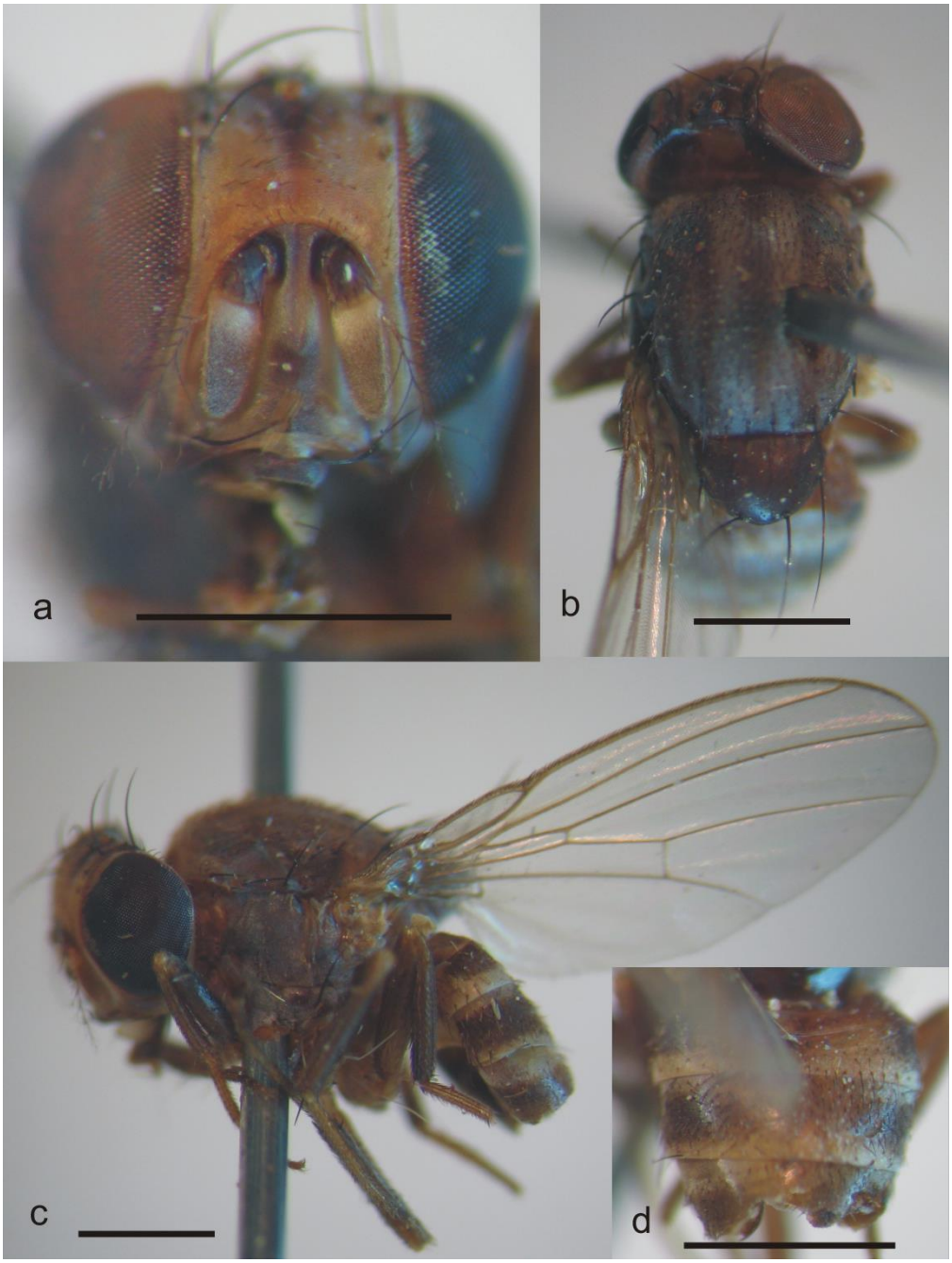


Figure 2: *Rhinoleucophenga personata* Malogolowkin, ordinary specimen collect in the State of Bahia, Brazil, in ethanol 100%, a: general habitus, dorsal view; b: general habitus, lateral view; c: head, frontal view; d: wing (scale bar 1.0 mm, except in c: 0.5 mm).

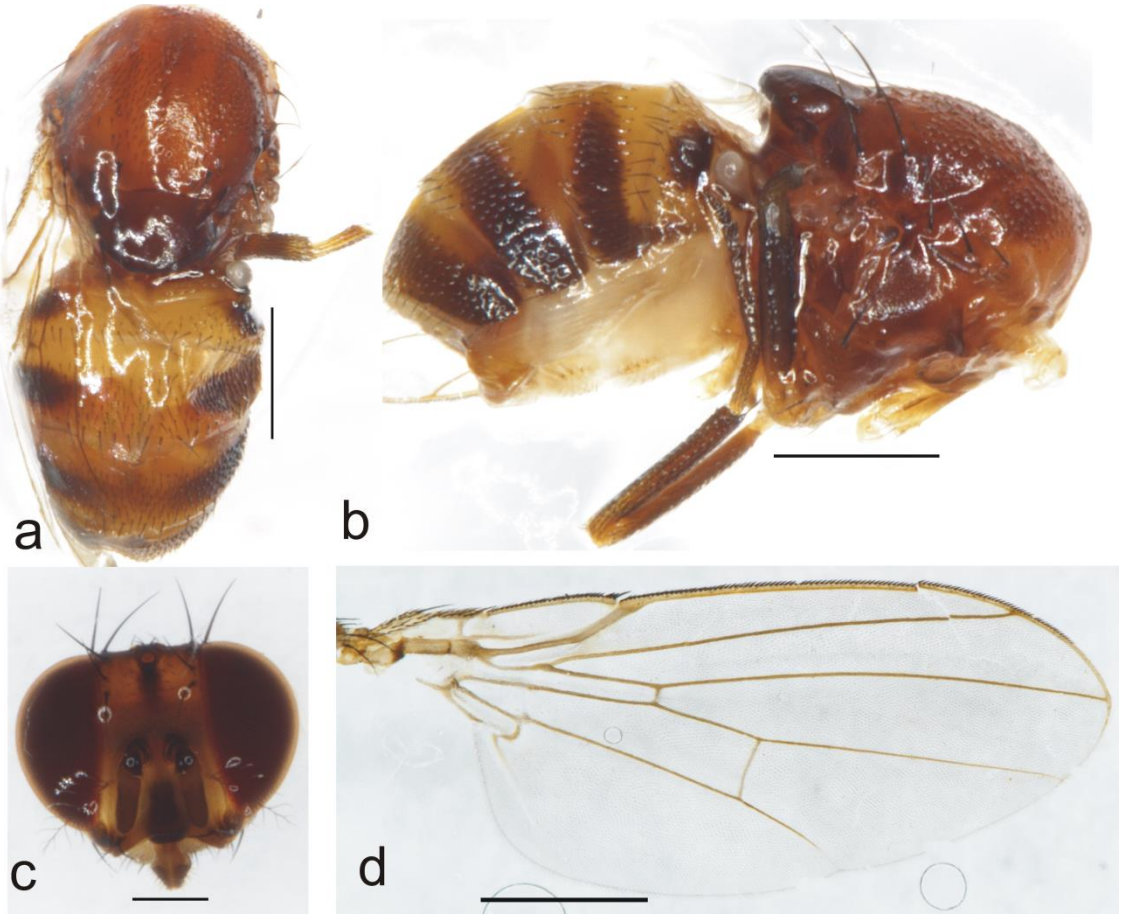


Figure 3: *Rhinoleucophenga personata* Malogolowkin, ordinary specimen collect in the State of Bahia, Brazil. Male terminalia. a: aedeagus, dorsal view; b: aedeagus, lateral view; c: aedeagus, ventral view; d: epandrium (scale bar 0.1 mm).

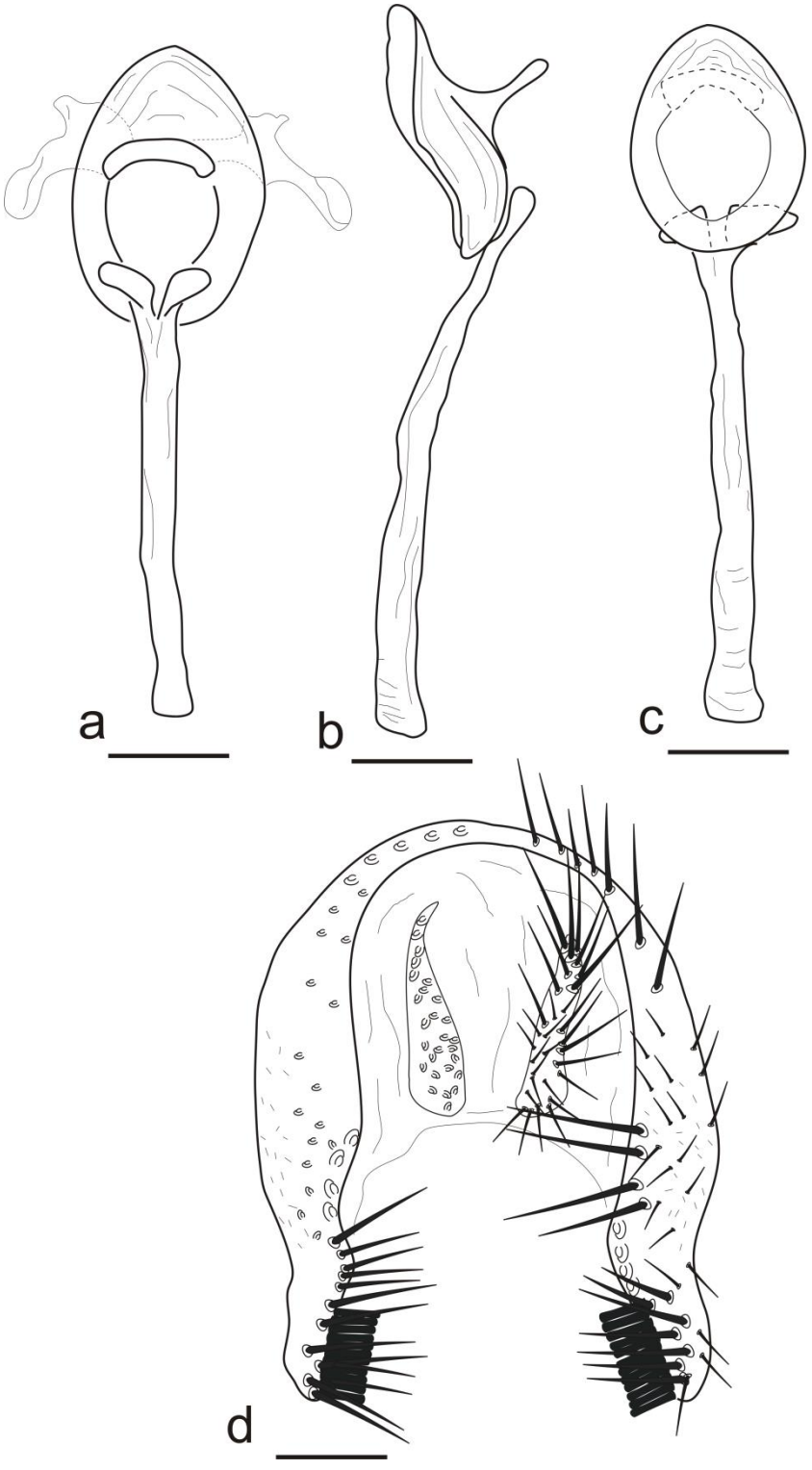


Figure 4: *Rhinoleucophenga lopesi* Malogolowkin (1946). Holotype, dried and mounted specimen, a: head, frontal view; b: thorax, dorsal view; c: general habitus, lateral view; d: wing (scale bar 1.0 mm).

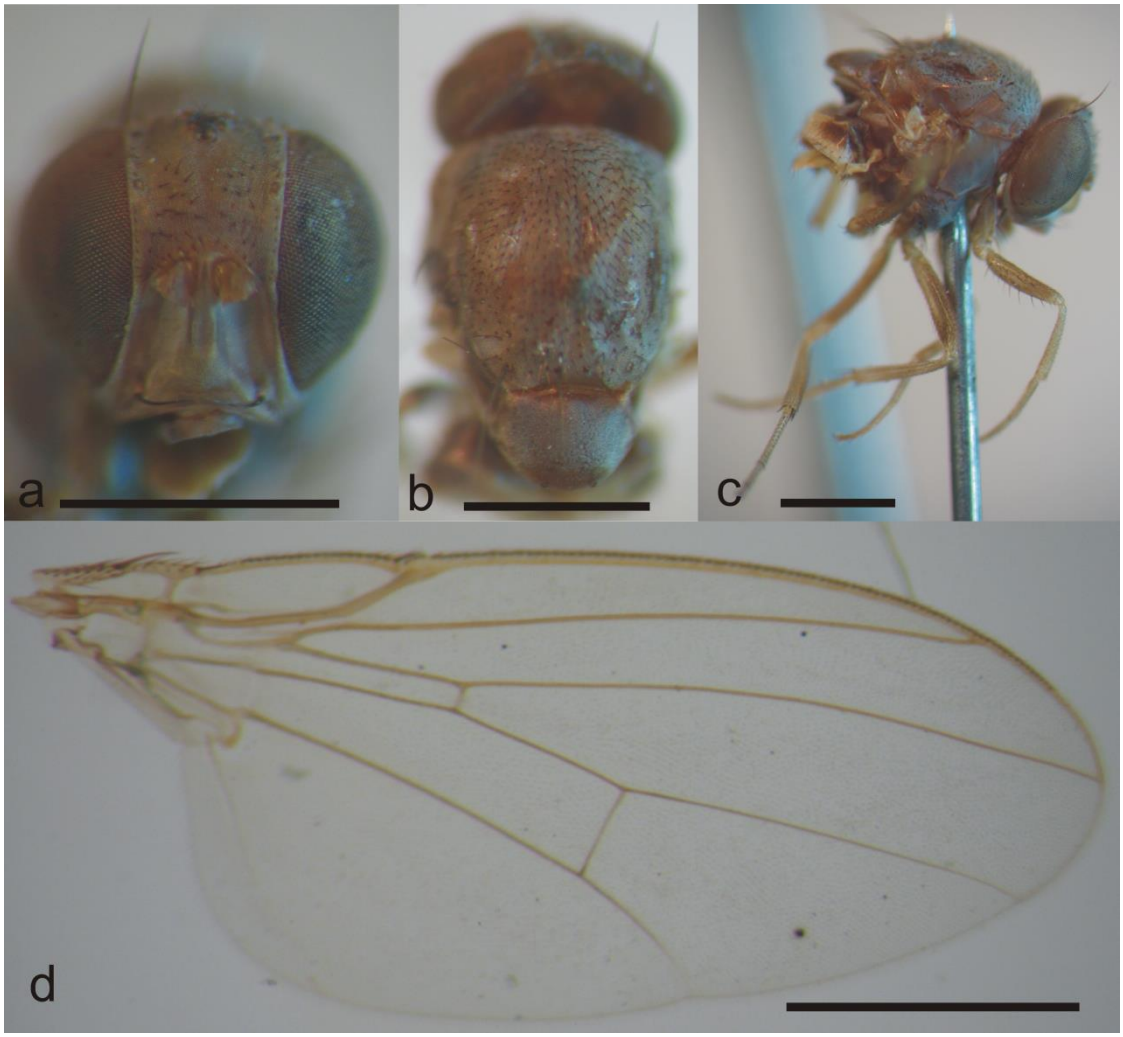


Figure 5: *Rhinoleucophenga lopesi* Malogolowkin. Male paratype of *R. capixabensis*, in ethanol 70%, a: general habitus, lateral view; b: thorax, dorsal view; c: wing; d: head, frontal view (scale bar 1.0 mm, except in d: 0.5 mm).

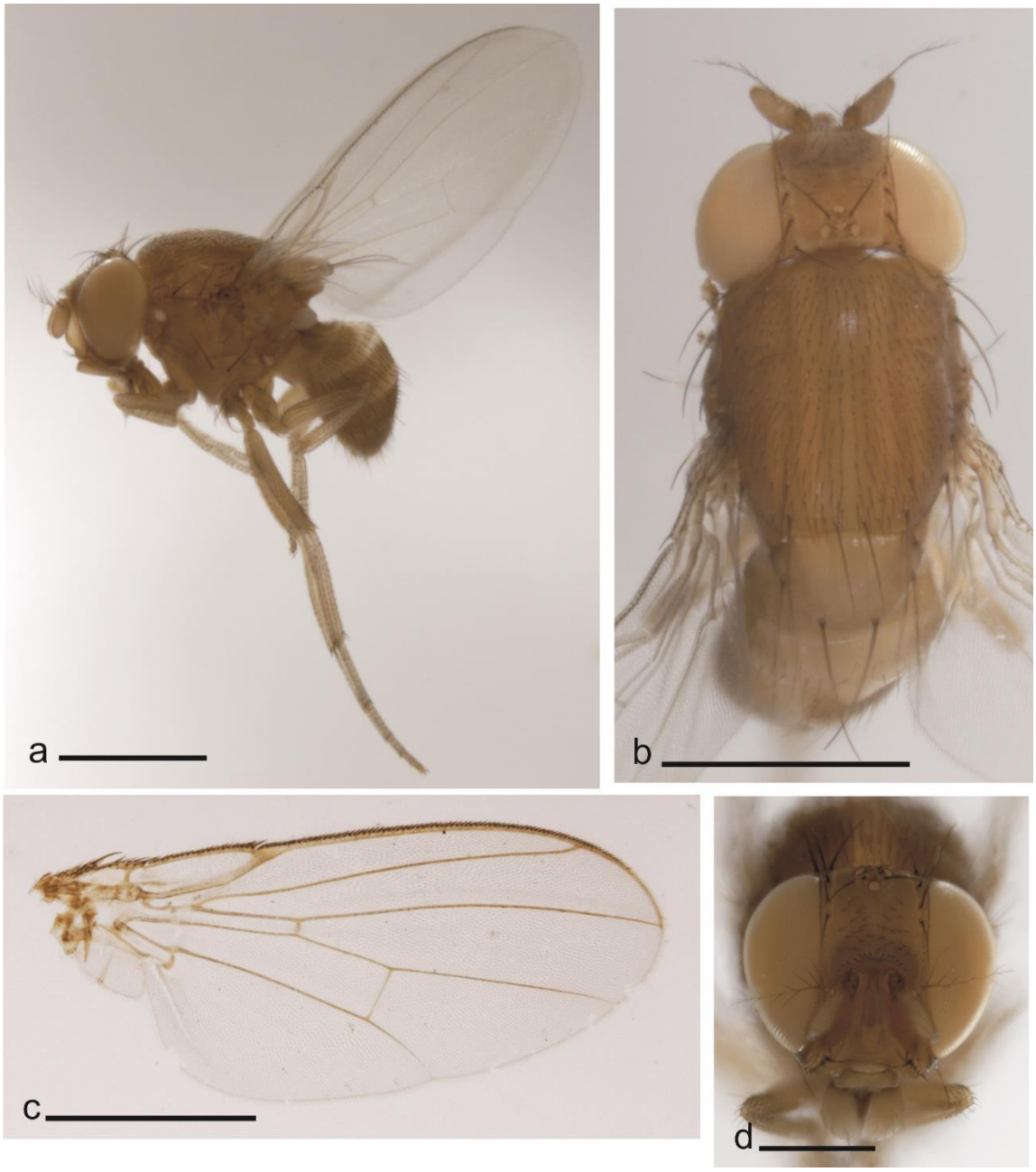


Figure 6: *Rhinoleucophenga lopesi* Malogolowkin. Female paratype of *R. capixabensis*, in ethanol 70%, lateral view (scale bar 0.7 mm).

Figure 7: *Rhinoleucophenga lopesi* Malogolowkin. Paratype of *R. capixabensis*. Male terminalia. a: epandrium + hypandrium, posterior view; b: aedeagus, ventral view (scale bar 0.1 mm).

Figure 8: *Rhinoleucophenga lopesi* Malogolowkin, ordinary specimen collect in State of Mato Grosso, Brazil. Male terminalia. a: aedeagus, lateral view; b: aedeagus, ventral view; c: aedeagus, dorsal view; d: epandrium (scale bar 0.1 mm).

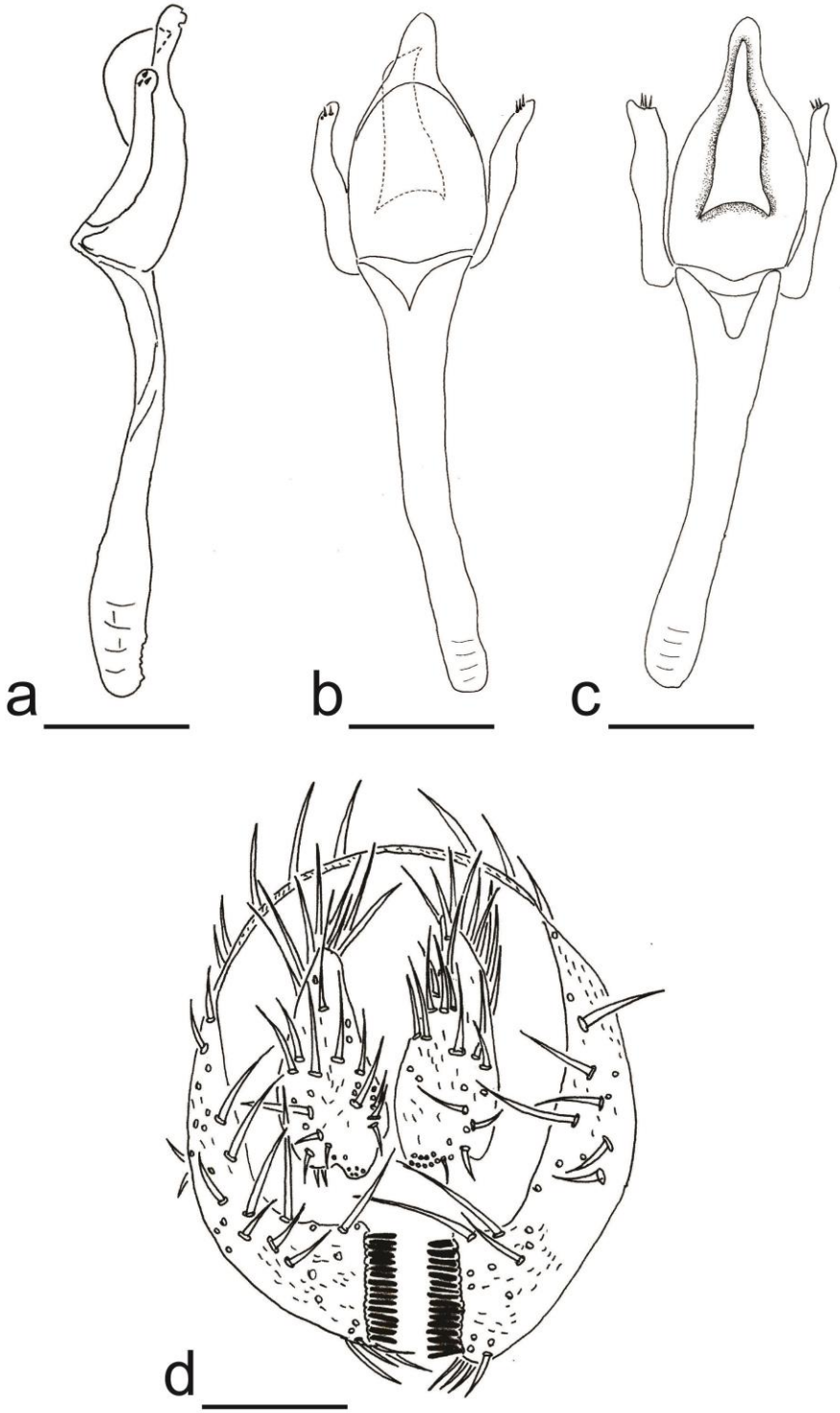


Figure 9: *Rhinoleucophenga angustifrons* Malogolowkin (1946). Holotype, dried and mounted specimen, a: thorax, dorsal view; b: general habitus, lateral view; c: head, frontal view; d: abdomen, dorsal view; e: wing, distal portion (scale bar 1.0 mm).

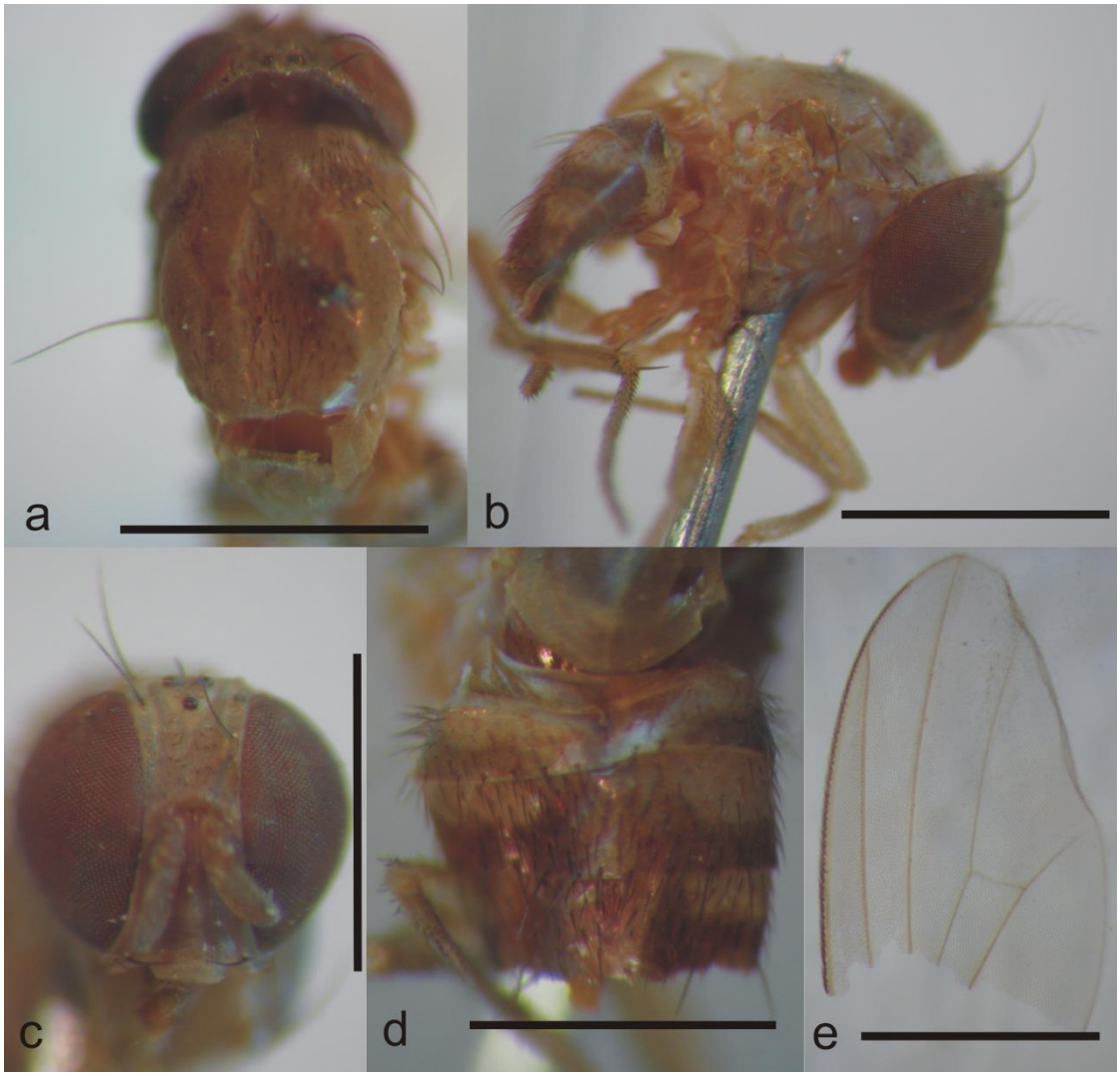


Figure 10: *Rhinoleucophenga matogrossensis* Malogolowkin (1946). Holotype, dried and mounted specimen, a: general habitus, lateral view; b: thorax, dorsal view; c: head, frontal view; d: wing (scale bar 1.0 mm).

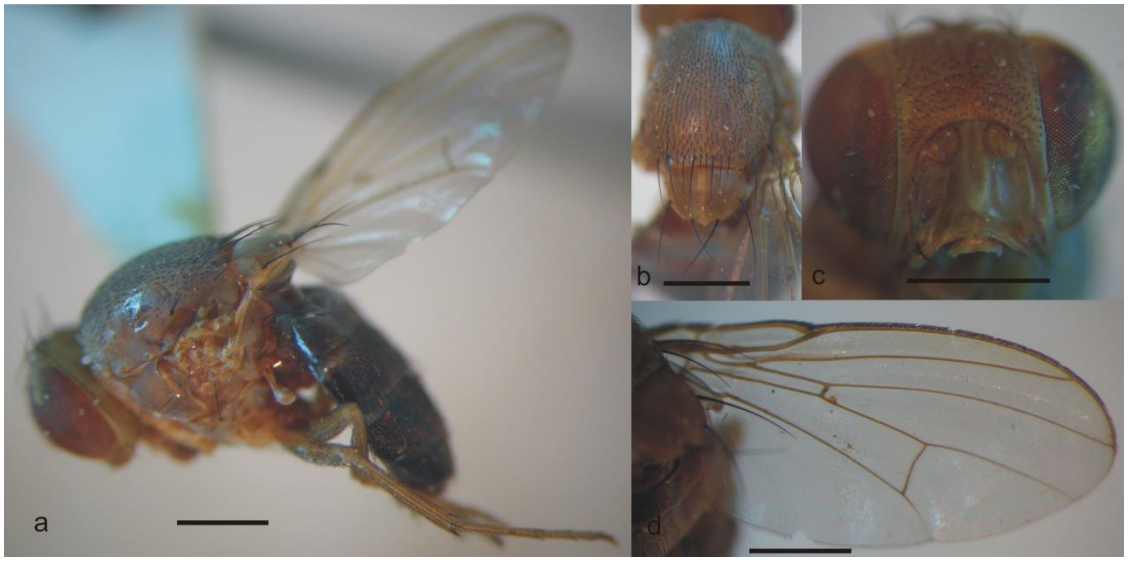


Figure 11: *Rhinoleucophenga nigrescens* Malogolowkin (1946). Holotype, dried and mounted specimen, a: head, frontal view; b: wing; c: general habitus, lateral view; d: thorax, dorsal view; e: abdomen, dorsal view (scale bar 1.0 mm).

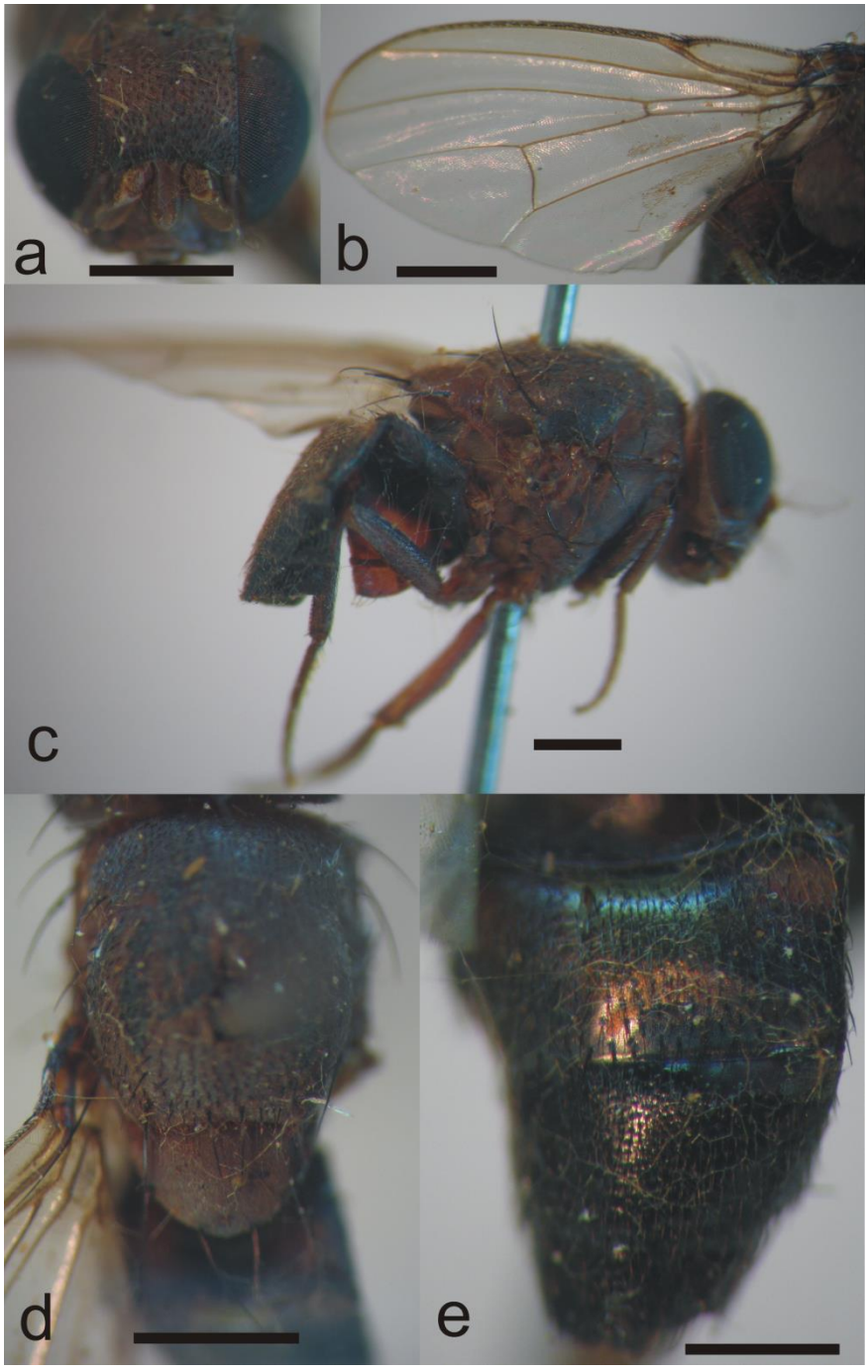


Figure 12: *Rhinoleucophenga jacareacanga* sp. nov. Holotype, dried and mounted specimen, a: general habitus, lateral view; b: thorax, dorsal view; c: abdomen, dorsal view; d: head, frontal view; e: wing (scale bar 1.0 mm, except in d: 0.5 mm).

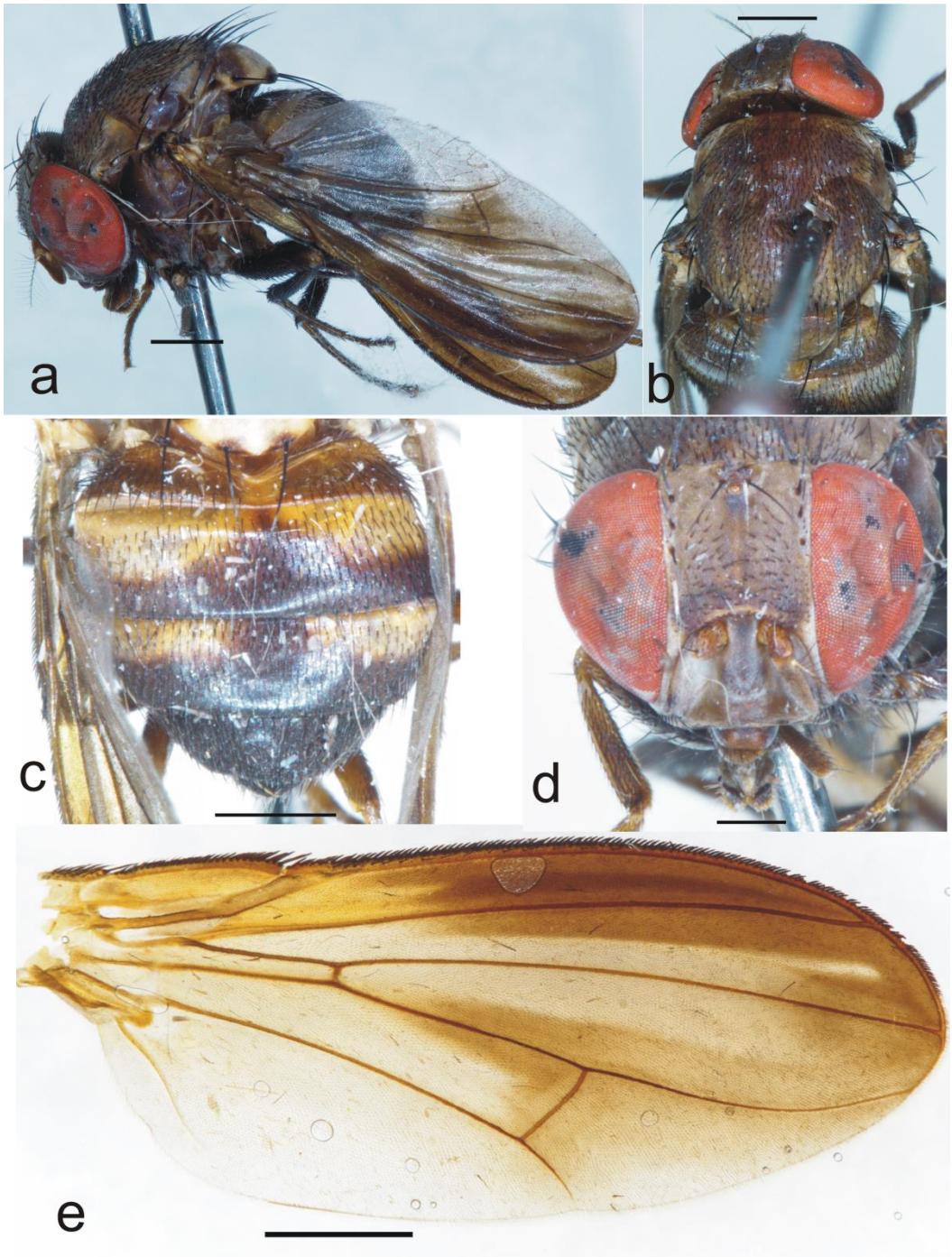
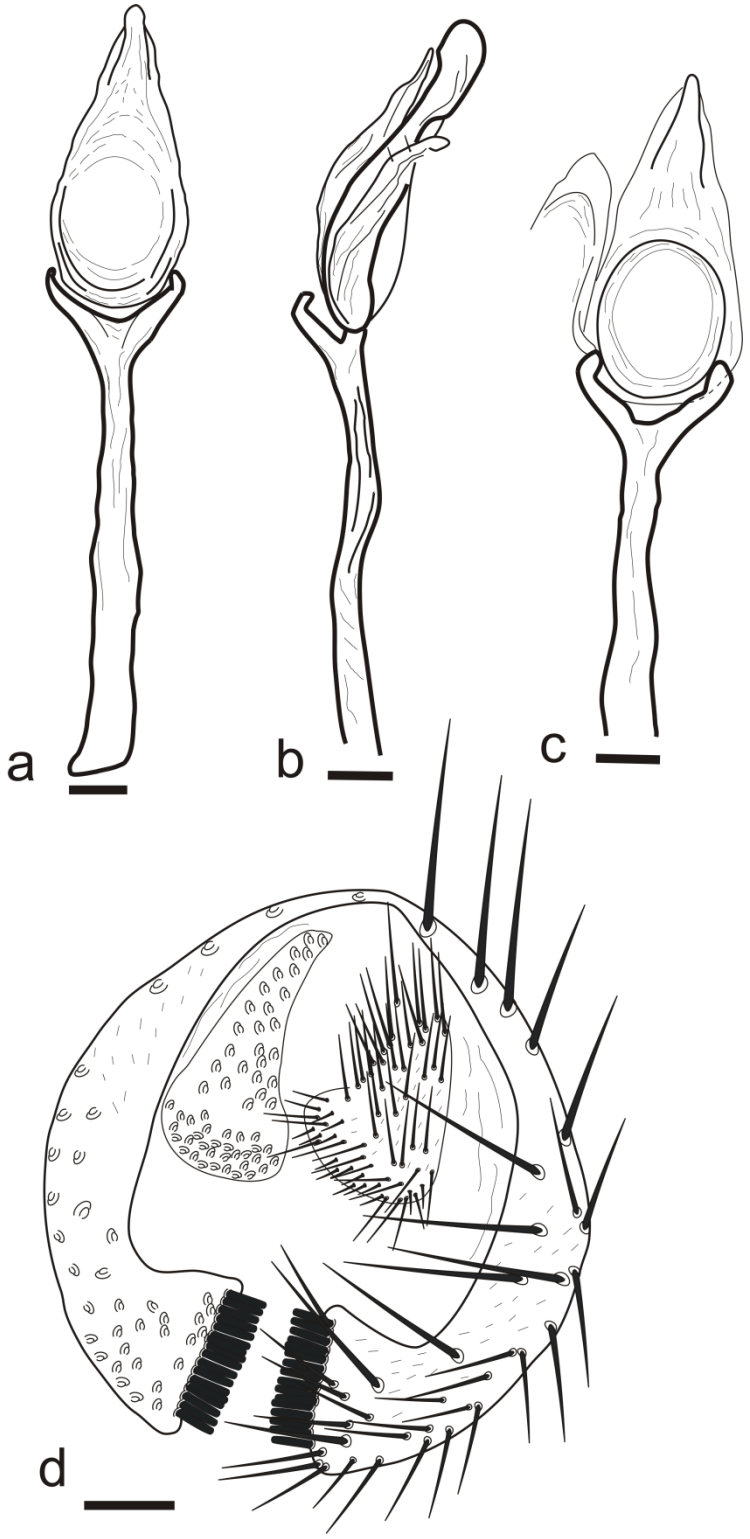


Figure 13: *Rhinoleucophenga jacareacanga* sp. nov. Male terminalia, a: aedeagus, ventral view; b: aedeagus, lateral view; c: aedeagus, dorsal view; d: epandrium (scale bar 0.1 mm).



4.1.10. TABLES

Table 1: Complementary measures and indices to the *Rhinoleucophenga* specimens described by Malogolowkin (1946), ordinary specimens of *R. personata* and *R. lopesi*, the holotype and two paratypes of *R. capixabensis* Culik & Ventura (2009) and *R. jacareacanga* sp nov. Indices according to Bächli *et al*. (2004). *: measures in millimeters (mm).

	R. personata ♂ holotype #8122	*R. personata* ♂ paratype #8123	*R. personata* ♂ from Bahia	*R. lopesi* ♀ holotype	*R. capixabensis* ♀paratype	*R. capixabensis* ♀ holotype	*R. capixabensis* ♀ paratype	*R. lopesi* ♂ from Mato Grosso	*R. lopesi* ♂ from Mato Grosso	*R. lopesi* ♂ from Mato Grosso	*R. lopesi* ♂ from Mato Grosso	*R. lopesi* ♂ from Mato Grosso	*R. lopesi* ♀ from Mato Grosso	*R. lopesi* ♀ from Mato Grosso	*R. lopesi* ♀ from Mato Grosso	*R. lopesi* ♀ from Mato Grosso	*R. lopesi* ♀ from Mato Grosso	*R. angustifrons* ♀ holotype #8121	*R. matogrossensis* ♂ holotype #8124	*R. nigrescens* ♀ holotype #8120	*R. jacareacanga* sp nov. holotype #01♂	*R. jacareacanga* sp nov. paratype #02♂
HEAD																						
Frontal length *	0.70	0.68	0.62	0.58	0.56	0.60	0.58	0.64	0.50	0.56	0.56	0.64	0.61	0.56	0.54	0.67	0.59	0.46	1.04	1.07	1.04	1.10
Frontal index	1.03	1.06	0.91	1.00	1.00	1.03	1.14	1.11	1.03	1.17	1.00	1.03	1.09	1.17	1.03	1.05	1.06	2.83	1.53	1.56	1.04	1.04
Top-to-bottom frontal width ratio	1.03	1.06	0.98	1.12	1.00	1.00	1.03	1.03	0.97	1.00	1.09	1.05	1.00	0.97	1.03	1.05	1.00	1.20	0.92	0.96	1.08	1.06
Ocellar triangle to front length ratio	0.43	0.41	0.48	0.34	0.38	0.35	0.37	0.28	0.32	0.31	0.29	0.25	0.26	0.29	0.29	0.26	0.27	0.39	0.29	0.21	0.35	0.33
Setae or1/or3 ratio	0.82	-	0.85	-	1.15	0.81	1.00	1.05	0.94	0.94	0.94	1.05	0.91	1.00	0.84	1.10	1.05	0.89	-	-	1.08	-
Setae or2/or1	0.85	-	0.82	-	0.72	0.87	0.63	0.60	0.69	0.71	0.73	0.76	0.70	0.60	0.69	0.59	0.55	0.69	-	-	0.74	-

ratio																					
Vibrissal index	0.33	0.36	0.26	0.33	0.33	0.41	0.29	-	-	-	-	-	-	-	-	-	-	0.31	0.41	0.41	0.42	0.38
Cheek index	7.33	6.47	5.70	8.17	8.30	5.47	8.30	5.86	6.00	7.60	6.33	5.22	8.00	8.00	6.17	5.57	5.57	11.43	7.67	13.6	7.82	9.00
Eye index	1.28	1.31	1.32	1.44	1.57	1.41	1.35	1.41	1.67	1.37	1.53	1.28	1.50	1.40	1.35	1.41	1.41	1.38	1.47	1.55	1.46	1.50
THORAX																						
Thorax length*	2.50	2.53	2.16	1.91	1.56	1.55	1.42	1.90	1.54	1.65	1.68	1.92	1.84	1.68	1.66	1.92	1.84	2.55	3.00	2.91	3.20	3.35
Strongest prescutellar acrostichal setae, % length related to posterior dorsocentral setae	65	71	66	59	71	60	59	69	58	57	55	53	52	65	57	60	63	-	73	51	84	85
Dorsocentral setae,transverse distance related to longitudinal distance	2.37	2.37	2.37	3.55	3.42	3.42	3.33	-	-	-	-	-	-	-	-	-	-	4.16	4.80	5.00	28.81	31.66
Sterno index	-	1.06	1.00	0.89	0.82	0.88	1.00	0.83	0.89	0.95	1.08	0.98	1.02	1.03	1.00	-	0.98	0.88	-	0.89	0.84	0.94
WING																						
Length*	3.88	4.25	3.85	3.30	2.66	2.99	2.80	2.86	2.56	2.64	2.88	3.04	2.83	2.72	2.90	3.04	2.82	2.64	4.60	5.00	5.85	6.25
Width*	1.75	1.83	1.75	1.60	1.48	1.55	1.60	1.52	1.38	1.42	1.52	1.60	1.60	1.44	1.52	1.62	1.52	1.18	2.00	2.30	2.50	2.50
WING INDICES																						
C (CII/CIII)	4.00	3.50	3.21	3.16	3.08	2.86	2.94	3.08	3.33	2.89	2.87	3.37	2.85	2.63	2.75	2.88	3.00	-	3.60	3.58	4.11	4.76
Hb (CIIIhb/ CIII)	0.85	0.86	0.57	0.53	0.31	0.39	0.56	0.42	0.33	0.34	0.32	0.34	0.36	0.37	0.50	0.43	0.43	0.62	0.49	0.50	0.62	0.76
Ac (CIII/ CIV)	1.11	1.26	1.34	1.31	1.30	1.44	1.29	1.29	1.15	1.35	1.41	1.35	1.39	1.00	1.33	1.48	1.35	1.53	1.13	1.26	2.14	1.64
4c (CIII/ MIII)	0.85	1.26	1.09	1.00	0.97	1.00	0.90	0.95	1.03	1.13	1.15	0.88	1.03	1.09	1.11	1.11	0.88	0.96	0.66	0.64	0.56	0.48
4v (MIV/ MIII)	2.51	3.43	2.81	2.21	2.11	2.03	1.90	2.13	2.38	2.29	2.45	1.98	2.11	2.14	2.08	2.33	1.75	2.00	1.41	1.32	1.25	1.24
5x (Cua/ dM-cu)	2.29	2.06	1.94	0.95	1.38	1.26	1.63	1.75	1.53	1.67	1.55	1.35	1.25	1.89	1.35	1.67	1.30	1.73	1.00	0.76	0.77	0.87
M (CuA/ MIII)	1.06	1.27	1.09	0.53	0.81	0.67	0.77	0.92	1.00	0.97	0.94	0.78	0.79	0.97	0.75	0.97	0.65	0.70	0.47	0.36	0.30	0.32
prox.x (R4+5	0.81	1.16	1.03	1.03	0.89	0.97	0.80	0.87	1.07	1.00	0.94	0.98	0.82	0.86	0.94	1.11	0.75	-	0.75	0.71	0.59	0.53

basal/ MIII)

Body length*	4.15	3.60	4.75	-	2.89	3.30	2.98	3.18	3.00	2.88	3.15	3.00	2.85	2.85	2.82	3.60	2.79	2.50	6.25	6.50	6.00	6.00

5. CAPÍTULO V

(Manuscrito para submissão ao periódico *Zootaxa*)

5.1. Neotropical fauna of *Rhinoleucophenga* Hendel (Diptera, Drosophilidae): Description of eleven new species

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5.1.1. ABSTRACT

The genus *Rhinoleucophenga* Hendel comprises 29 nominal species with New World distribution. The description of new species of *Rhinoleucophenga* is indispensable to improve the faunistic knowledge of Neotropical region, in which previous studies have shown unidentified or misidentified *Rhinoleucophenga* species. In the present study eleven new species are described: *R. punctata* sp. nov., *R. paraguayensis* sp. nov., *R. ignota* sp. nov., *R. fusca* sp. nov., *R. alata* sp. nov., *R. paulistorum* sp. nov., *R. obscura* sp. nov., *R. fulva* sp. nov., *R. maculosa* sp. nov., *R. nigra* sp. nov. and *R. brasilis* sp.

nov. The new species presented here corroborate the high diversity of *Rhinoleucophenga* that have been recorded in the Neotropical region, and highlight the need of describing species in the current taxonomic patterns to Drosophilidae in order to avoid taxonomic confusion with groups of similar species.

Key words: *Drosophila*, *Gitona*, Steganinae, Taxonomy.

5.1.2. RESUMO

O gênero *Rhinoleucophenga* Hendel é composto por 29 espécies distribuídas nas regiões Neotropical e Neártica. A descrição de novas espécies de *Rhinoleucophenga* é indispensável para melhorar o conhecimento faunístico da região Neotropical, na qual estudos prévios têm mostrado espécies não identificadas ou erroneamente identificadas para *Rhinoleucophenga*. No presente estudo 11 novas espécies são descritas: *R. punctata* sp. nov., *R. paraguayensis* sp. nov., *R. ignota* sp. nov., *R. fusca* sp. nov., *R. alata* sp. nov., *R. paulistorum* sp. nov., *R. obscura* sp. nov., *R. fulva* sp. nov., *R. maculosa* sp. nov., *R. nigra* sp. nov. e *R. brasilis* sp. nov. As novas espécies apresentadas aqui corroboram a alta diversidade de *Rhinoleucophenga* na região Neotropical, e destacam a necessidade de descrição de espécies nos padrões atuais da taxonomia de Drosophilidae na tentativa de evitar problemas taxonômicos com grupos de espécies crípticas.

Palavras-chave: *Drosophila*, *Gitona*, Steganinae, Taxonomia.

5.1.3. INTRODUCTION

Rhinoleucophenga Hendel is a genus of Drosophilidae with Neotropical and Nearctic distribution. In the last years many species of *Rhinoleucophenga* have been described (Junges & Gottschalk 2014; Poppe *et al*. 2014, 2015a; Vidal & Vilela 2015), and new records of *Rhinoleucophenga* species have been done in Neotropical areas (Mata *et al*. 2008; Roque & Tidon 2008, 2013; Vilela & Bächli 2009; Roque *et al*. 2013; Poppe *et al*. 2015a). The increase in the number of records of *Rhinoleucophenga* species highlights the need for reviewing old description studies, as well as the need for performing complementary description of some species (Poppe *et al*. submitted).

Rhinoleucophenga seems to prefer open environments instead of forest areas. Poppe *et al*. (2014, 2015b) stressed the high diversity of *Rhinoleucophenga* in a grassland area of the Pampa biome, and other authors have noticed this genus in open areas of the Brazilian Caatinga (G.F. Oliveira, personal comm.; Poppe *et al*. 2015a), Cerrado (Blauth & Gottschalk 2007; Mata *et al*. 2008; Roque & Tidon 2008, 2013; Roque *et al*. 2013; Vidal & Vilela 2015), and Restinga (Schmitz *et al*. 2009); beyond many other records in open areas between the latitudes 37°N (Texas, United States) (Malloch & McAtee 1924; Vilela 1990) and 34°S (Argentina) (Thomson 1869; Vilela 1990). However, there are some few records of *Rhinoleucophenga* in forest areas (De Toni *et al*. 2007; Hochmüller *et al*. 2010; Poppe *et al*. 2015a) and areas of low urbanization levels (Gottschalk *et al*. 2007).

As general to Drosophilidae, the morphological variability in *Rhinoleucophenga* is very high, that is, this genus presents species of 2.0-7.0 mm with body color patterns varying from yellow to black. Besides, the occurrence of sibling species groups has been commonly noticed (Vilela 1990; Poppe *et al*. 2015a), as well as high levels of intraspecific variation among populations from different environments (Poppe *et al*. submitted). Thus, the description of new specimens collected in previous studies is fundamental to avoid systematic problems of misidentification and uncertainties about the geographical distribution of some species groups; additionally new descriptions are important to improve the distributional and ecological knowledge of *Rhinoleucophenga*.

Considering the current patterns of species description, the present study presents the description of *Rhinoleucophenga punctata* sp. nov., *R. paraguayensis* sp. nov., *R. ignota* sp. nov., *R. fusca* sp. nov., *R. alata* sp. nov., *R. paulistorum* sp. nov., *R. obscura* sp. nov., *R. fulva* sp. nov., *R. maculosa* sp. nov., *R. nigra* sp. nov. and *R. brasilis* sp. nov.

5.1.4. MATERIALS AND METHODS

Descriptions are based on measures and indices given by Bächli *et al*. (2004), which were done with an ocular reticle inserted into a stereomicroscope. Male and female terminalia were disarticulated in glycerol after treatment with 10% potassium hydroxide (KOH) (Bächli *et al*. 2004). The genitalia were mounted in a piece of

glycerine jelly (ca. 2 x 2 x 2 mm) (Grimaldi 1987), stored in microvials with glycerol and pinned with the respective specimen. Photos of the specimens were taken with a digital camera coupled to an optical stereomicroscope. Drawings of the genitalia were made with a *camera lucida* system attached to a compound microscope with 40× and 10× objective lenses and a 10× ocular lens. The terminology follows Bächli *et al*. (2004).

Most of described specimens were deposited in the Coleção Entomológica do Instituto Oswaldo Cruz (CEIOC) at Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil, except *R. paulistorum* sp. nov. and *R. alata* sp. nov., deposited in the Museu de Zoologia at Universidade de São Paulo, Brazil (MZUSP).

5.1.5. RESULTS

Rhinoleucophenga Hendel

Rhinoleucophenga Hendel, 1917: 44-45

Pseudophortica Sturtevant, 1918: 37

Gitona (in New world) Brake & Bächli, 2008: 291

Genus Diagnosis: strong prescutellar acrostichal setae, frons densely covered with scattered interfrontal setulae, surstyli fused to epandrium bearing small peg-like prensisetae, simple aedeagus (Vilela & Bächli 2009), only two katepisternal setae, postpronotum with one setae and a pair of divergent basal scutellar setae (Malogolowkin 1946).

Rhinoleucophenga punctata sp. nov.

(Figures 1a-d, 2a-d; Table 1)

Type series. Holotype: 1m# labelled "*Rhinoleucophenga punctata*; HOLOTYPE m#; Brazil, Rio Grande do Sul, São Luiz Gonzaga (28°24'21"S 54°57'06"W), 12.x.2013.

Col.: JL Poppe; banana bait". Postabdomen disarticulated stored in a microvial with glycerin and attached with the respective exemplar. Holotype preserved in ethanol 100%; deposited at CEIOC/Fiocruz.

Diagnosis. Scutum brown covered with many small dark brown spots at bases of setae and setulae, two central longitudinal dark brown stripes. Scutellum dark brown. Front homogeneously brown, ocellar triangle dark brown. Arista with 4 short dorsal and ventral branches plus terminal fork. Carina nose-like and ca. 90% sulcated. Abdomen yellow with dark brown band which is medially interrupted and laterally broadened. Wings hyaline. Male terminalia as in figure 2a-d.

Description. Head (Fig 1b-c). Front homogeneously brown, covered with ca. 30 scattered interfrontal setulae; ocellar triangle dark brown. Each orbital setae with a brown patch around base. Carina nose-like and ca. 90% sulcated. Face brownish; gena yellow; antenna with flagellomere homogeneously brown and pedicel brownish; arista with 4 short dorsal and ventral branches plus terminal fork. Palpus yellow with ca. 12 setae on lower part.

Thorax (Fig 1a-b). Scutum brown covered with many small dark brown spots at bases of setae and setulae, two central longitudinal dark brown stripes. Scutellum dark brown. Eight irregular rows of acrostichal setulae. One pair of long prescutellar acrostichal setae. Pleura brownish with a longitudinal dark brown stripe; legs yellow with incomplete dark brown annuli in the distal femur and proximal tibia; halteres whitish.

Wings (Fig 1d). Hyaline, without spots.

Abdomen (Fig. 1a-b). Abdomen with yellow ground color, tergite II with a dark brown stripe widely interrupted medially, tergites III to VI each with a broad dark brown stripe which is medially interrupted and laterally broadened.

Male terminalia (Fig 2a-d). Aedeagus membranous with a sclerotized incomplete ring ventrally curved in the apical portion, and a sclerotized "Y" shaped structure on the dorsal face. Epandrium microtrichose with ca. seven upper and 15 lower setae on each side. Surstyli with seven prensisetae. Cerci very elongated, with ca. 60 setae each one, 9-10 longer setae in the apical portion.

To more measures and indices see Table 1.

Female unknown.

Etymology. The species name refers to its spotted thorax.

Type locality. Brazil, Rio Grande do Sul, São Luiz Gonzaga (28°24’21”S 54°57’06”W).

Distribution. Known only from the type locality.

Biology. Collected in fermented-banana traps, in an urban area.

Rhinoleucophenga alata sp. nov.

(Figures 3a-d, 4a-c; Table 1)

Type series. Holotype: 1f# labelled “*Rhinoleucophenga alata*; HOLOTYPE f#; Brazil, São Paulo, Serra do Japi (23°15'27"S 46°58'28"W); 18.iii.2010. Col.: NW Perioto. Malaise”. Postabdomen disarticulated stored in a microvial with glycerin and attached with the respective exemplar. Paratype: 1f# labelled “*Rhinoleucophenga alata*; PARATYPE; Brazil, Santa Catarina, Botuverá (27°11'29"S 49°04'32"W); 16.iv.2013 col.: DC De Toni. Resource: banana bait”. Holotype and paratype preserved in ethanol 100% are deposited at MZUSP.

Diagnosis. Scutum brown. Front homogeneously yellow-brownish, covered with ca. 200 scattered interfrontal setulae. Arista with 9-10 dorsal and 7 ventral long branches plus terminal fork. Carina nose-like and ca. 90% sulcated. Abdomen proximally brownish and distally dark brown-black. Wings non-hyaline Veins Dm-Cu, R-M, costal II-IV, the tip of R_{4+5} apical and M-IV strongly clouded. 3-4 clouded supernumerary veins into Costal-II, and 2 incomplete clouded supernumerary veins into R_{4+5} apical.

Description. Head (Fig 3c). Front homogeneously yellow-brownish, covered with ca. 200 scattered interfrontal setulae; ocellar triangle brownish with brown ocelli. Carina nose-like and ca. 90% sulcated. Face and gena brownish; antenna with flagellomere homogeneously yellow-brownish and pedicel brownish; arista with 9-10 dorsal and 7 ventral long branches plus terminal fork. Palpus brownish with ca. 80 setae on lower part.

Thorax (Fig 3a-b). Scutum brown and scutellum brown, darker apically. 12-14 irregular rows of acrostichal setulae. 2-3 pair of long prescutellar acrostichal setae. Pleura brownish; legs yellow; halteres yellowish.

Wings (Fig 3d). Non-hyaline with costal region brownish. Veins Dm-Cu, R-M, costal II-IV, the tip of R_{4+5} apical and M-IV strongly clouded. 3-4 clouded supernumerary veins into Costal-II, and 2 incomplete clouded supernumerary veins into R_{4+5} apical.

Abdomen (Fig 3a-b). Tergite II brown with posterior edge black; tergite III brown with middle-posterior portion dark brown; tergite IV-VI dark brown-black.

Female terminalia (Fig 4a-c). Epiproct microtrichose with ca. 14 setae. Hypoproct microtrichose with ca. 50 setae, 10 longer ones. Cerci with ca. four longer apical setae on each one. Spermathecal capsule elongated with basal introvert reaching ca. 1/3 of inner capsule, apically thinner than the ½ basal portion, length to width ratio of the spermathecal capsule = 3.70.For more measures and indices see Table 1.

Male unknown.

Etymology. The species name refers to its spotted and exuberant wings.

Type locality. Brazil, São Paulo, Serra do Japi (23°15'27"S 46°58'28"W).

Distribution. Known from São Paulo and Santa Catarina, Brazil.

Biology. Collected in fermented-banana traps in Santa Catarina, and in Malaise traps in São Paulo.

Rhinoleucophenga paulistorum sp. nov.

(Figures 5a-d, 6a-c; Table 1)

Type series. Holotype: 1f# labelled "*Rhinoleucophenga paulistorum*; HOLOTYPE f#; Brazil, São Paulo, Serra do Japi (23°15'27"S 46°58'28"W); 18.iii.2010. Col.: NW Perioto. Malaise". Paratype: 2f# labelled "*Rhinoleucophenga paulistorum*; PARATYPE; Brazil, São Paulo, Serra do Japi (23°15'27"S 46°58'28"W); 18.iii.2010. Col.: NW Perioto. Malaise". Postabdomen disarticulated stored in a microvial with

glycerin and attached with the respective specimens. Holotype and paratypes preserved in ethanol 100%; deposited at MZUSP.

Diagnosis. Front homogeneously yellow-brownish, covered with ca. 80 scattered interfrontal setulae; ocellar triangle yellow with the edge of the ocelli dark brown. Carina nose-like and ca. 80% sulcated. Arista with 6-7 dorsal and 5 ventral long branches plus terminal fork. Scutum and scutellum brownish. 10-12 irregular rows of acrostichal setulae. Abdomen proximally brownish and distally dark brown. Wings non-hyaline with costal region brownish. Female terminalia and the spermathecal capsule as in figure 6a-c.

Description. Head (Fig 5c). Front homogeneously yellow-brownish, covered with ca. 80 scattered interfrontal setulae; ocellar triangle yellow with the edge of the ocelli dark brown. Carina nose-like and ca. 80% sulcated. Face yellow; gena brownish with a diffuse brown spot. Antenna with flagellomere and pedicel homogeneously brownish; arista with 6-7 dorsal and 5 ventral long branches plus terminal fork. Palpus yellow with ca. 30 setae on lower part.

Thorax (Fig 5a-b). Scutum and scutellum brownish. 10-12 irregular rows of acrostichal setulae. 3 pair of long prescutellar acrostichal setae, the central pair is the longest one. Pleura brownish; legs yellow; halteres whitish.

Wings (Fig 5d). Non-hyaline with costal region brownish.

Abdomen (Fig 5a-b). Tergite II brownish-yellow with laterally dark brown stripe; tergite III brownish with ½ posterior covered by narrowly interrupted dark brown stripe; tergite IV-VI brown with 3/4 posterior covered by narrowly interrupted dark brown stripe.

Female terminalia (Fig 6a-c). Epiproct microtrichose with ca. 12 setae. Hypoproct microtrichose with ca. 50 setae, 10 longer ones. Cerci with ca. five longer apical setae on each one. Spermathecal capsule with basal introvert reaching ca. 3/4 of inner capsule, length to width ratio = 1.46.For more measures and indices see Table 1.

Male unknown.

Etymology. The species name refers to its type locality, São Paulo state.

Type locality. Brazil, São Paulo, Serra do Japi (23°15'27"S 46°58'28"W).

Distribution. Known only from the type locality.

Biology. Collected in Malaise traps.

Rhinoleucophenga obscura sp. nov.

(Figures 7a-c, 8a-d; Table 1)

Type series. Holotype: 1m# labelled "*Rhinoleucophenga obscura*; HOLOTYPE m#; Brazil, Bahia, Estação Ecológica Raso da Catarina/ Município de Paulo Afonso (9°30'39"S 38°32'12"W). 22.iv.2012. Col.: GF Oliveira; banana bait". Postabdomen disarticulated stored in a microvial with glycerin and attached with the respective specimen. Holotype preserved in ethanol 100%; deposited at CEIOC/Fiocruz.

Diagnosis. Front homogeneously brown, covered with ca. 40 scattered interfrontal setulae; ocellar triangle brown. Carina nose-like and ca. 40% sulcated. Arista with 5 dorsal and 4 ventral long branches plus terminal fork. Scutum and scutellum brown. 10 irregular rows of acrostichal setulae. Abdomen proximally brown with dark brown stripes widely interrupted medially. Wings hyaline. Male aedeagus and epandrium as in figure 8a-d.

Description. Head (Fig 7a). Front homogeneously brown, covered with ca. 40 scattered interfrontal setulae; ocellar triangle brown. Carina nose-like and ca. 40% sulcated. Face brownish; gena brown. Antenna with flagellomere and pedicel homogeneously brown; arista with 5 dorsal and 4 ventral long branches plus terminal fork. Palpus yellow with ca. 30 setae on lower part.

Thorax (Fig 7b). Scutum and scutellum brown with a diffuse dark brown stripe. 10 irregular rows of acrostichal setulae. 1 pair of long prescutellar acrostichal setae. Pleura brown; legs brownish; halteres yellowish.

Wings (Fig 7c). Hyaline.

Abdomen (Fig 7b). Brown with dark brown stripes widely interrupted medially.

Male terminalia (Fig 8a-d). Aedeagus with a triangular shape, wider in the base and thinner in the apical portion. There is a sharp apical projection dorsally curved, like a spicule. Epandrium microtrichose with ca. seven upper and 35 lower setae on each side. Surstyli with 22 prensisetae. Cerci round shaped, with ca. 20 setae each one, seven longer setae in the apical portion.For more measures and indices see Table 1.

Female unknown.

Etymology. The species name refers to its dark general body color. The word *obscura* means “dark” in Latin.

Type locality. Brazil, Bahia, Estação Ecológica Raso da Catarina (9°30'39"S 38°32'12"W).

Distribution. Known only from the type locality.

Biology. Collected in fermented-banana traps in the Caatinga biome.

Rhinoleucophenga paraguayensis sp. nov.

(Figures 9a-f, 10a-c; Table 1)

Examinated Material. Two dried female specimens labeled “*Rhinoleucophenga* #1f# Paraguay, Asuncion. X.1943. Col.: Unknown. / *R.* paraguayensis #1 (and #02)f# Paraguay, Asuncion. X.1943. Det.: JL Poppe and MS Gottschalk; v.2014”. Deposited at CEIOC/Fiocruz.

Type series. Holotype: 1f# labelled “*Rhinoleucophenga paraguayensis*; HOLOTYPE f#; Paraguay, Asuncion (26°16'56"S 57°38'06"W). Det.: JL Poppe and MS Gottschalk; v.2014”. Postabdomen disarticulated stored in a microvial with glycerin and attached with the respective specimen. Paratype: 1f# labelled “*Rhinoleucophenga paraguayensis*; PARATYPE; Paraguay, Asuncion (26°16'56"S 57°38'06"W). Det.: JL Poppe and MS Gottschalk; v.2014”. Holotype and paratype were deposited at CEIOC/Fiocruz.

Diagnosis. Front ventrally yellow and dorsally brownish, covered with ca. 40 scattered interfrontal setulae; ocellar triangle yellow with the edge of the ocelli dark brown. Arista with 6 dorsal and 5 ventral long branches plus terminal fork. Scutum and scutellum brownish with a central diffuse longitudinal yellow stripe on the scutellum

and posterior portion of the scutum. 12 irregular rows of acrostichal setulae. Abdomen with yellow ground color, tergite II-VI with 1/3 covered by a dark brown stripe widely interrupted medially. Wings hyaline. Female terminalia and spermathecal capsule as in figure 10a-c.

Description. Head (Fig 9a, f). Front ventrally yellow and dorsally brownish, covered with ca. 40 scattered interfrontal setulae; ocellar triangle yellow with the edge of the ocelli dark brown. Carina nose-like and ca. 60-80% sulcated. Face and gena yellow. Antenna with flagellomere and pedicel homogeneously yellow-brownish; arista with 6 dorsal and 5 ventral long branches plus terminal fork. Palpus yellow with ca. 30 setae on lower part.

Thorax (Fig 9a-b). Scutum and scutellum brownish with a central diffuse longitudinal yellow stripe on the scutellum and posterior portion of the scutum. 12 irregular rows of acrostichal setulae. 3 pairs of long prescutellar acrostichal setae, the central pair is the longest one. Pleura brownish; legs yellow; halters yellow.

Wings (Fig 9c). Hyaline.

Abdomen (Fig 9e). Abdomen with yellow ground color, tergite II-VI with 1/3 covered by a dark brown stripe widely interrupted medially. In the paratype the abdomen is black; however, when it is clarified with KOH 10% it reveals a yellow ground color and brown stripes.

Female terminalia (Fig 10a-c). Epiproct microtrichose with ca. six setae. Hypoproct microtrichose with ca. 40 setae. Cerci microtrichose with a longer apical setae on each one. Spermathecal capsule with basal introvert reaching ca. 3/4 of inner capsule, length to width ratio = 1.46.For more measures and indices see Table 1.

Male unknown.

Etymology. The species name refers to its locality type, Paraguay, South America.

Type locality. Paraguay, Asuncion (26°16'56"S 57°38'06"W).

Distribution. Known only from the type locality.

Biology. Unknown.

Rhinoleucophenga fulva sp. nov.

(Figures 11a-d, 12a-c; Table 1)

Type series. Holotype: 1f# labelled "*Rhinoleucophenga fulva*; HOLOTYPE f#; Brazil, Rio Grande do Sul, Bossoroca (28°45'01"S 54°56'55"W). 20.xii.2011. Col.: JL Poppe; banana bait". Postabdomen disarticulated stored in a microvial with glycerin and attached with the respective specimen. Holotype preserved in ethanol 100%; deposited at CEIOC/Fiocruz.

Diagnosis. Front homogeneously brownish, covered with ca. 200 scattered interfrontal setulae. Carina nose-like and ca. 75% sulcated. Arista with 8 dorsal and 5 ventral long branches plus terminal fork. Scutum brown and scutellum dark brown. 14 irregular rows of acrostichal setulae. 4 pair of long prescutellar acrostichal setae. Abdomen proximally brown and distally dark brown. Wings non-hyaline, brownish. Female terminalia and the spermathecal capsule as in figure 12a-c.

Description. Head (Fig 11a). Front homogeneously brownish, covered with ca. 200 scattered interfrontal setulae; ocellar triangle brownish with the edge of the ocelli brown. Carina nose-like and ca. 75% sulcated. Face and gena brownish; antenna with pedicel brownish and flagellomere homogeneously brown; arista with 8 dorsal and 5 ventral long branches plus terminal fork. Palpus brownish with ca. 60 setae on lower part.

Thorax (Fig 11b, d). Scutum brown and scutellum dark brown. 14 irregular rows of acrostichal setulae. 4 pairs of long prescutellar acrostichal setae, the central pair is the longest one. Pleura brown; legs yellow; halters yellow-whitish.

Wings (Fig 11c). Non-hyaline, brownish.

Abdomen (Fig 11b, d). Tergite II brown, laterally dark brown; tergite III brown covered by a dark brown stripe medially narrow; tergite IV-VI brown with 5/4 posterior covered by large dark brown stripe.

Female terminalia (Fig 12a-c). Epiproct microtrichose with ca. six setae. Hypoproct microtrichose with ca. 40 setae, ca. six longer ones. Cerci with five longer apical setae

on each one. Spermathecal capsule with basal introvert reaching ca. 3/4 of inner capsule, length to width ratio = 1.46.For more measures and indices see Table 1.

Male unknown.

Etymology. The species name refers to its dark general body color. The word *fulva* means “brown” in Latin.

Type locality. Brazil, Rio Grande do Sul, Bossoroca (28°45'01"S 54°56'55"W).

Distribution. Known only from the type locality.

Biology. Collected in fermented-banana traps in the Pampa biome.

Rhinoleucophenga maculosa sp. nov.

(Figures 13a-d, 14a-c; Table 1)

Type series. Holotype: 1f# labelled “*Rhinoleucophenga maculosa*; HOLOTYPE f#; Brazil, Rio Grande do Sul, Bossoroca (28°45'01"S 54°56'55"W). 20.xii.2011. Col.: JL Poppe; banana bait”. Postabdomen disarticulated stored in a microvial with glycerin and attached with the respective specimen. Holotype preserved in ethanol 100%; deposited at CEIOC/Fiocruz.

Diagnosis. Front homogeneously brownish, covered with ca. 100 scattered interfrontal setulae. Carina nose-like and ca. 90% sulcated. Arista with 8 dorsal and 6 ventral long branches plus terminal fork. Scutum and scutellum brown. 10 irregular rows of acrostichal setulae. 3 pairs of long prescutellar acrostichal setae. Abdomen proximally brown and distally dark brown. Wings non-hyaline, costal portion brownish. Veins Dm-Cu, R-M, the tip of R_{2+3}, R_{4+5} apical and M-IV strongly clouded, as well as the proximal portion of veins R_{2+3} and R_{4+5}.

Description. Head (Fig 13c). Front homogeneously brown, covered with ca. 100 scattered interfrontal setulae; ocellar triangle brownish with the edge of the ocelli brown. Carina nose-like and ca. 90% sulcated. Face brown and gena dark brown; antenna with pedicel and flagellomere homogeneously brown; arista with 8 dorsal and 6 ventral long branches plus terminal fork. Palpus brownish with ca. 50 setae on lower part.

Thorax (Fig 13a-b). Scutum and scutellum brown with a central longitudinal narrow yellow stripe. 10 irregular rows of acrostichal setulae. 3 pairs of long prescutellar acrostichal setae, the central pair is the longest one. Pleura brown; legs brownish, femur proximally brown; halteres whitish.

Wings (Fig 13d). Non-hyaline, costal portion brownish. Veins Dm-Cu, R-M, the tip of R_{2+3}, R_{4+5} apical and M-IV strongly clouded, as well as the proximal portion of veins R_{2+3}, R_{4+5} and M-III.

Abdomen (Fig 13a-b). Tergite II brown, laterally dark brown; tergite III brown with ¼ posteriorly covered by dark brown stripe medially narrow; tergite IV-VI dark brown.

Female terminalia (Fig 14a-c). Epiproct microtrichose with ca. eight setae. Hypoproct microtrichose with ca. 40 setae, ca. 10 marginal longer ones. Cerci with three longer apical setae on each one. Spermathecal capsule round shaped with basal introvert reaching ca. 2/3 of inner capsule, length to width ratio = 0.9.

For more measures and indices see Table 1.

Male unknown.

Etymology. The species name refers to its spotted wing. The word *macula* means "spotted" in Latin.

Type locality. Brazil, Rio Grande do Sul, Bossoroca (28°45'01"S 54°56'55"W).

Distribution. Known only from the type locality.

Biology. Collected in fermented-banana traps in the Pampa biome.

Rhinoleucophenga nigra sp. nov.

(Figures 15a-d, 16a-c; Table 1)

Type series. Holotype: 1f# labelled "*Rhinoleucophenga nigra*; HOLOTYPE f#; Brazil, Rio Grande do Sul, Bossoroca (28°45'01"S 54°56'55"W). 22.xii.2012. Col.: JL Poppe; banana bait". Postabdomen disarticulated stored in a microvial with glycerin and

attached with the respective specimen. Holotype preserved in ethanol 100%; deposited at CEIOC/Fiocruz.

Diagnosis. Front homogeneously brownish, covered with ca. 50 scattered interfrontal setulae. Carina nose-like and ca. 80% sulcated. Arista with 8 dorsal and 5 ventral long branches plus terminal fork. Scutum and scutellum brown. 12 irregular rows of acrostichal setulae. 2 pairs of long prescutellar acrostichal setae. Abdomen proximally brown and distally dark brown. Wings hyaline.

Description. Head (Fig 15c). Front homogeneously brownish, covered with ca. 50 scattered interfrontal setulae; ocellar triangle brownish with the edge of the ocelli dark brown. Carina nose-like and ca. 80% sulcated. Face brownish and gena brown; antenna with pedicel and flagellomere homogeneously brownish; arista with 8 dorsal and 5 ventral long branches plus terminal fork. Palpus yellow with ca. 25 setae on lower part.

Thorax (Fig 15a-b). Scutum and scutellum brown. 12 irregular rows of acrostichal setulae. 2 pairs of long prescutellar acrostichal setae, the central pair is the longest one. Pleura brown; legs yellow; halteres whitish.

Wings (Fig 15d). Hyaline.

Abdomen (Fig 15a-b). Tergite II brownish with a laterally dark brown stripe; tergite III brownish covered by a large dark brown stripe medially broadly interrupted; tergite IV-VI with 6/5 posteriorly covered by a dark brown stripe.

Female terminalia (Fig 16a-c). Epiproct microtrichose with ca. eight setae. Hypoproct microtrichose with ca. 40 setae. Cerci with three longer apical setae on each one. Spermathecal capsule round shaped with basal introvert reaching ca. 2/3 of inner capsule, length to width ratio = 0.8.

For more measures and indices see Table 1.

Male unknown.

Etymology. The species name refers to its dark color. The word *nigra* means “dark” in Latin.

Type locality. Brazil, Rio Grande do Sul, Bossoroca (28°45'01"S 54°56'55"W).

Distribution. Known only from the type locality.

Biology. Collected in fermented-banana traps in the Pampa biome.

Rhinoleucophenga brasilis sp. nov.

(Figures 17a-e, 18a-c; Table 1)

Examinated Material. Two dried female specimens labeled "*Rhinoleucophenga* #1 (and #02)f# Brazil, Mato Grosso, ii.1937. col.: unknown. Det.: JL Poppe and MS Gottschalk. v.2014". Deposited at CEIOC/Fiocruz.

Type series. Holotype: 1f# labeled "*Rhinoleucophenga brasilis*; HOLOTYPE f#; Brazil, Mato Grosso. ii.1937. col.: unknown. Det.: JL Poppe and MS Gottschalk; v. 2014". Postabdomen disarticulated stored in a microvial with glycerin and attached with the respective specimen. Paratype: 1f# labeled "*Rhinoleucophenga brasilis*; PARATYPE; Brazil, Mato Grosso. ii.1937. col.: unknown. Det.: JL Poppe and MS Gottschalk; v.2014". Holotype and paratype are deposited at CEIOC/Fiocruz.

Diagnosis. Front homogeneously brownish, covered with ca. 50 scattered interfrontal setulae. Carina nose-like and ca. 90% sulcated. Arista with 7 dorsal and 5 ventral long branches plus terminal fork. Scutum and scutellum brown. 10 irregular rows of acrostichal setulae. 1 pair of long prescutellar acrostichal setae. Wings hyaline. Female terminalia and the spermathecal capsule as in figure 18a-c.

Description. Head (Fig 17a-b). Front homogeneously brownish, covered with ca. 50 scattered interfrontal setulae; ocellar triangle brownish with dark brown ocelli. Carina nose-like and ca. 90% sulcated. Face and gena brownish; antenna with pedicel yellow, flagellomere homogeneously brownish; arista with 7 dorsal and 5 ventral long branches plus terminal fork. Palpus yellow.

Thorax (Fig 17a, d). Scutum and scutellum brown. 10 irregular rows of acrostichal setulae. 1 pair of long prescutellar acrostichal setae. Pleura brown; legs yellow; halteres yellowish.

Wings (Fig 17e). Hyaline.

Abdomen (Fig 17c). Abdomen with brownish ground color, distally dark brown-black. However, as noticed in *R. paraguayensis* sp. nov., we tend to believe that after clarified the abdomen can reveals a different color pattern.

Female terminalia (Fig 18a-c). Epiproct microtrichose with ca. six setae. Hypoproct microtrichose with ca. 30 setae, ca. six longer marginal ones. Cerci with three longer apical setae on each one. Spermathecal capsule round shaped with basal introvert reaching ca. ½ of inner capsule, length to width ratio = 0.9.

To more measures and indices see Table 1.

Male unknown.

Etymology. The species name refers to Brazil, country of its type locality.

Type locality. Brazil, Mato Grosso state.

Distribution. Known only from the type locality.

Biology. Unknown.

Rhinoleucophenga ignota sp. nov.

(Figures 19a-d; Table 1)

Examinated Material. A dried male specimen labeled "*Rhinoleucophenga* #1m IOC/ Fiocruz/ RJ. N°: 32552. Loc.: unknown. col.: unknown. Det.: JL Poppe". Deposited at CEIOC/Fiocruz.

Type series. Holotype: 1m# labelled "*Rhinoleucophenga ignota*; HOLOTYPE m#; Locality unknown. Det.: JL Poppe and MS Gottschalk; v.2014". Holotype deposited at CEIOC/Fiocruz.

Diagnosis. Front homogeneously brownish, covered with ca. 40 scattered interfrontal setulae. Carina nose-like. Arista with long branches. Scutum and scutellum brownish. 10 irregular rows of acrostichal setulae. Wings hyaline. Abdomen with brownish-yellow ground color, tergite II-VI with dark brown continuous stripes.

Description. Head (Fig 19b). Front homogeneously brownish, covered with ca. 40 scattered interfrontal setulae; ocellar triangle brownish. Carina nose-like. Face and gena yellow; antenna with pedicel yellow, flagellomere homogeneously brownish; arista with long branches. Palpus yellow.

Thorax (Fig 19a). Scutum and scutellum brownish. 10 irregular rows of acrostichal setulae. At least 1 pair of long prescutellar acrostichal setae. Pleura brownish; legs yellow; halteres yellowish.

Wings (Fig 19d). Hyaline.

Abdomen (Fig 21a). Abdomen with brownish-yellow ground color, tergite II-VI with dark brown continuous stripes.

Female and male terminalia unknown. It was not possible to disarticulate the male terminalia due its very dried condition.

To more measures and indices see Table 1.

Etymology. The species name refers to its unknown type locality, distribution and biology. The word *ignota* means "unknown" in Latin.

Type locality. Unknown.

Distribution. Unknown.

Biology. Unknown.

Rhinoleucophenga fusca sp. nov.

(Figures 20a-e; Table 1)

Examinated Material. A dried specimen labeled as "*Rhinoleucophenga* #2 Brazil, São Paulo, C. do Jordão. xi.1936. Col.: J Lane. Det.: JL Poppe and MS Gottschalk". Deposited at CEIOC/Fiocruz.

Type series. Holotype: labelled "*Rhinoleucophenga fusca*; HOLOTYPE; sex undetermined. Locality São Paulo, C. do Jordão. xi.1936. Col.: J Lane. Det: JL Poppe and MS Gottschalk; v.2014". Holotype deposited at CEIOC/Fiocruz.

Diagnosis. Front homogeneously brownish, covered with ca. 40 scattered interfrontal setulae. Carina nose-like with ca. 50% sulcated. Arista with 8 dorsal and 7 ventral long branches. Scutum and scutellum brownish. 8 irregular rows of acrostichal setulae. Wings non-hyaline with costal region brownish.

Description. Head (Fig 20a, c). Front homogeneously brownish, covered with ca. 40 scattered interfrontal setulae; ocellar triangle brownish with ocelli brown. Carina nose-like with ca. 50% sulcated. Face yellow, gena brownish; antenna with pedicel and flagellomere homogeneously brownish; arista with 8 dorsal and 7 ventral long branches. Palpus yellow.

Thorax (Fig 20a, d). Scutum and scutellum brownish. 8 irregular rows of acrostichal setulae. At least 2 pairs of prescutellar acrostichal setae, the central pair is the longest one. Pleura brownish; legs yellow; halters yellowish.

Wings (Fig 20e). Non-hyaline, costal region brownish.

Abdomen (Fig 20b). Abdomen with brownish-yellow ground color, with dark brown irregular spots. Probably the abdominal color is not preserved, as noticed to *R. paraguayensis* sp. nov.

Female and male terminalia unknown.

To more measures and indices see Table 1.

Etymology. The species name refers to its brownish general body color. The word *fusca* means “obscure” in Latin.

Type locality. Brazil, São Paulo state, Campos do Jordão.

Distribution. Known only from type locality.

Biology. Unknown.

5.1.6. DISCUSSION

The species described here belong to *Rhinoleucophenga* based on the following features: strong prescutellar acrostichal setae, frons densely covered with scattered

interfrontal setulae, surstyli fused to epandrium bearing small peg-like prensisetae, simple aedeagus (Vilela & Bächli 2009), only two katepisternal setae, postpronotum with one setae and a pair of divergent basal scutellar setae (Malogolowkin 1946).

Commonly the male's terminalia morphology has been used to identify Drosophilidae species (Vilela & Bächli 1990), but some authors have also shown the females' terminalia as a useful structure to differentiate species (Sturtevant 1921; Throckmorton 1962, 1975; Poppe *et al*. 2015a). Here, nine of the eleven new species proposed do not present information about the male's reproductive structures. However, a comprehensive *Rhinoleucophenga* species revision was performed, as well as phylogenetic analyses (data not shown); thus, the set of morphological traits of each new species proposed, associated to the other analysis not presented in this manuscript, provide enough information to ensure the described species as new ones.

Rhinoleucophenga punctata sp. nov. resembles *R. punctulata* Duda and *R. punctuloides* Poppe, Schmitz & Valente by its body general morphology, mainly by the spotted brownish thorax. Beyond differences in the aedeagus structure, *R. punctata* sp. nov. differs from the other species by presenting ocellar triangle dark brown, arista with four dorsal and four ventral short branches and scutellum dark brown without spots.

Rhinoleucophenga paraguayensis sp. nov. resembles *R. sulina* Poppe *et al*. by its general brownish body color and size, abdominal pattern of stripes, number of prescutellar acrostichal setae and by the length of arista branches. But it differs from *R. sulina* by presenting less interfrontal setulae (ca. 40), six dorsal and five ventral long arista branches, 12 rows of acrostichal setulae and differences in the female terminalia.

Rhinoleucophenga ignota sp. nov., resembles *R. missionera* Poppe *et al*. by its general brownish body color and abdominal pattern of stripes, ca. 40 interfrontal setulae, arista plumose and hyaline wings. But it differs of *R. missionera* mainly by its smaller body size and for presenting only one pair of prescutellar acrostichal setae.

Rhinoleucophenga fusca sp. nov., resembles *R. joaquina* Schmitz, Gottschalk & Valente and *R. tangaraensis* Junges & Gottschalk by the body general color and size, and by the wing indices. But it differs from both species by the abdominal color pattern and, from *R. joaquina* by the length of arista branches.

Rhinoleucophenga alata sp. nov., *R. paulistorum* sp. nov., *R. fulva* sp. nov., and *R. maculosa* sp. nov. are all big brown species. Thus, these species resemble *R. obesa* (Loew), *R. pampeana* Poppe *et al*. and *R. gigantea* (Thomson) by the body size, and *R. matogrossensis* Malogolowkin and *R. nigrescens* Malogolowkin by the body size and color. Furthermore, *Rhinoleucophenga alata* sp. nov., resembles *R. pampeana* by the wings with supernumerary veins in the vein R_{2+3}, *R. matogrossensis* and *R. nigrescens* by the front covered with ca. 200 interfrontal setulae. But *R. alata* sp. nov. differs from *R. pampeana* by its body color and from all species by its pattern of spots in the wings and by the morphology of its spermathecal capsule. *Rhinoleucophenga paulistorum* sp. nov., also resembles the previously mentioned species by presenting 10-12 rows of acrostichal setulae, but it differs from them by presenting three pairs of prescutellar acrostichal setae, wings with costal region clouded and a brownish gena with a diffuse brown spot. *Rhinoleucophenga fulva* sp. nov., beyond the big size, also presents ca. 200 interfrontal setulae such as *R. gigantea*, *R. obesa*, *R. pampeana*, *R. matogrossensis* and *R. nigrescens*; but it differs from the three first species by its brown body color, and from the last two species by its abdominal color pattern and brownish wing. *Rhinoleucophenga maculosa* sp. nov. differs from all big brown species mentioned above by its peculiar spotted wing.

Rhinoleucophenga obscura sp. nov., *R. nigra* sp. nov., and *R. brasilis* sp. nov. are also brown species, but smaller than the species mentioned in the previous paragraph (ca. 3.5 mm). All three species present ca. 40-50 interfrontal setulae and hyaline wings, such as *R. missionera*, *R. punctuloides* and *R. angustifrons* Malogolowkin, but they differ from *R. punctuloides* by the long length of arista branches, thorax and front without spots and only 1-2 prescutellar acrostichal setae. Moreover, they differ from *R. angustifrons* by the body color and head not round shaped, and from *R. missionera* mainly by the abdominal color pattern and number of prescutellar acrostichal setae. Furthermore, they differ among each other by the abdominal color pattern and by their terminalia traits.

The new species presented here corroborate the high diversity of *Rhinoleucophenga* that has been recorded in the Neotropical region, and highlight the need of describing the species in the current taxonomic patterns of Drosophilidae in order to avoid taxonomic confusion with similar groups of species, such as the small

yellow species originally described by Lima (1950) and reviewed by Poppe *et al*. (submitted), as well as among the brown species presented here.

5.1.7. ACKNOWLEDGEMENTS

We thank Dr. Jane Costa, Dr. Márcio Felix and Danielle Cerri from the Entomological Collection of the Institute Oswaldo Cruz (CEIOC) for allowing us to access the many specimens deposited there; Msc. Georgia F. de Oliveira, Msc Gabriela Piani, Dr. Dalton Amorim and Dr. Daniela De Toni for the specimens kindly provided; the National Council of Technological and Scientific Development (CNPq), PRONEX-FAPERGS (10/0028-7) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing grants and fellowships.

5.1.8. REFERENCES

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5.1.9. FIGURES

Figure 1: Holotype of *R. punctata* sp. nov., male, in ethanol. a: general habitus, dorsal view; b: general habitus, lateral-dorsal view; c: head, frontal view; d: wing (scale bar 1.0 mm, except in c: 0.5 mm).

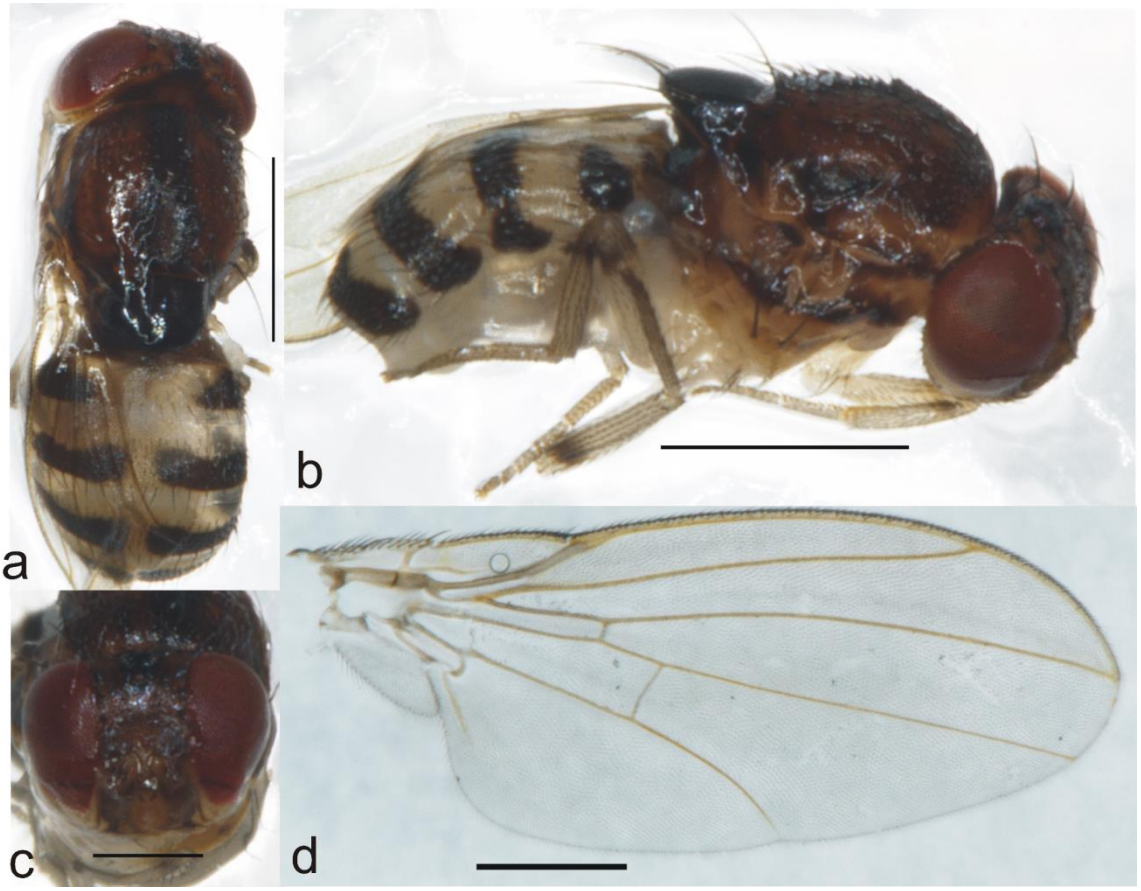


Figure 2: Holotype of *R. punctata* sp. nov. Male terminalia, a: aedeagus, ventral view; b: aedeagus, lateral view; c: aedeagus, doral view; d: epandrium, posterior view (scale bar 0.1 mm).

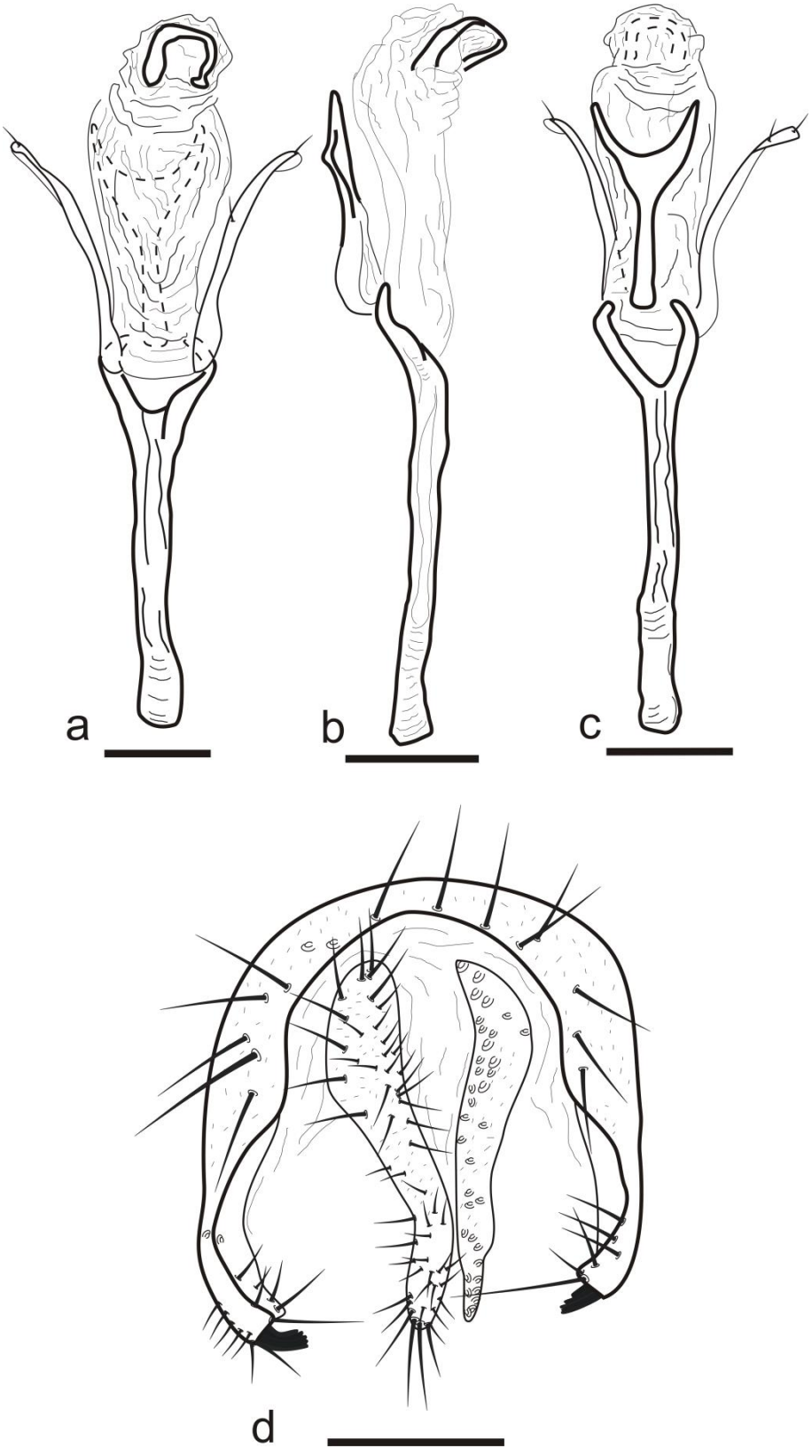


Figure 3: Holotype of *R. alata* sp. nov., female, in ethanol. a: general habitus, dorsal view; b: general habitus, lateral view; c: head, frontal view; d: wing (scale bar 1.0 mm, except in c: 0.5 mm).

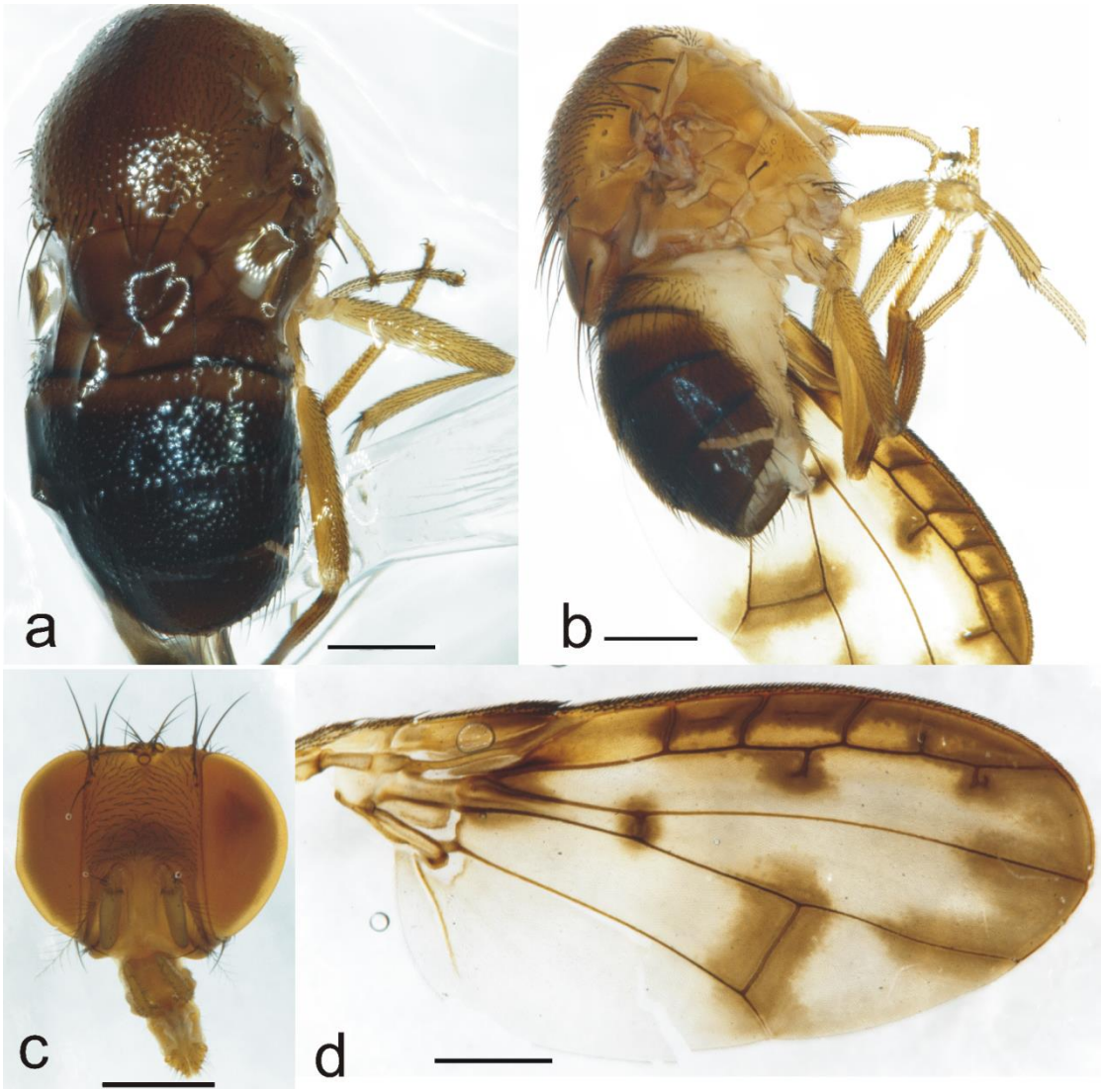


Figure 4: Holotype of *R. alata* sp. nov. Female terminalia, a: dorsal view; b: spermathecal capsule; c: ventral view (scale bar 0.1 mm).

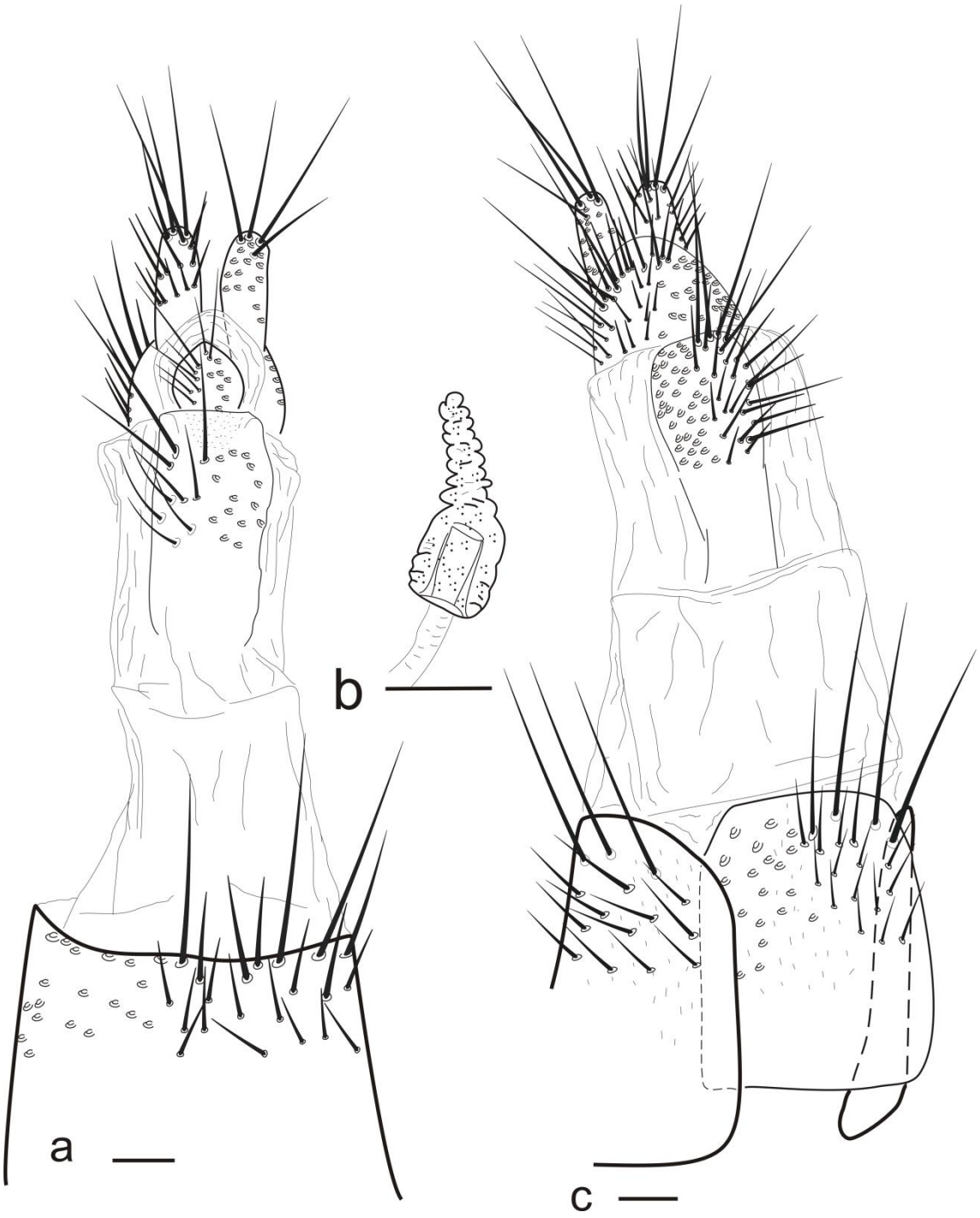


Figure 5: Holotype of *R. paulistorum* sp. nov., female, in ethanol. a: general habitus, dorsal view; b: general habitus, lateral view; c: head, frontal view; d: wing (scale bar 1.0 mm).

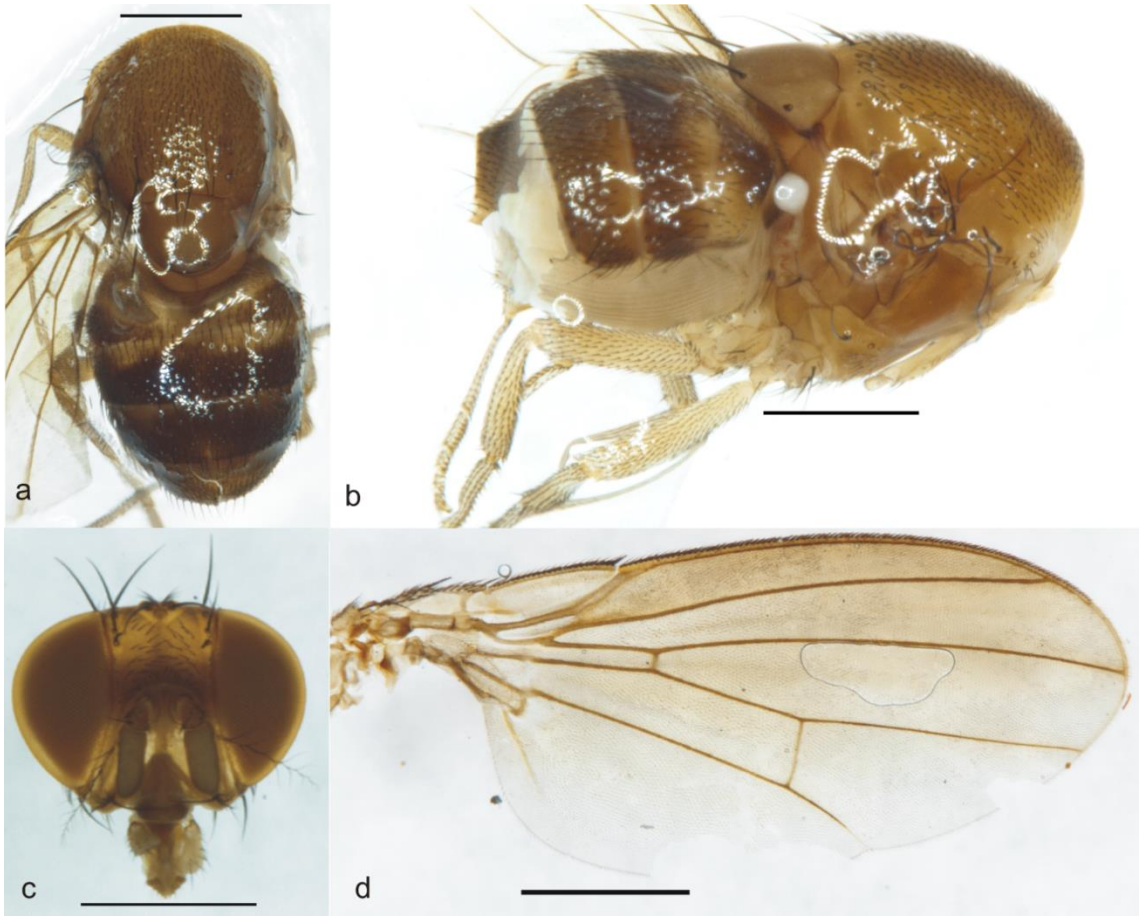


Figure 6: Holotype of *R. paulistorum* sp. nov. Female terminalia, a: dorsal view; b: spermathecal capsule; c: ventral view (scale bar 0.1 mm).

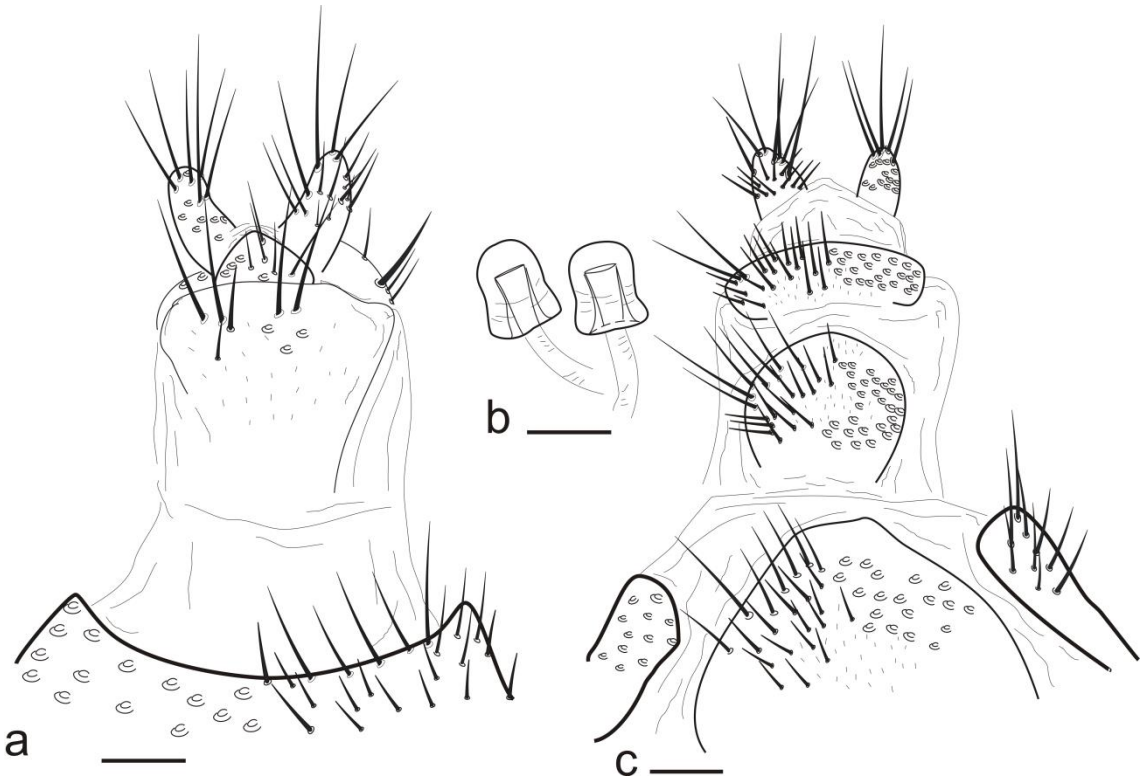


Figure 7: Holotype of *R. obscura* sp. nov., male, in ethanol. a: head, frontal view; b: general habitus, lateral-dorsal view; c: wing (scale bar 1.0 mm, except in a: 0.5 mm).

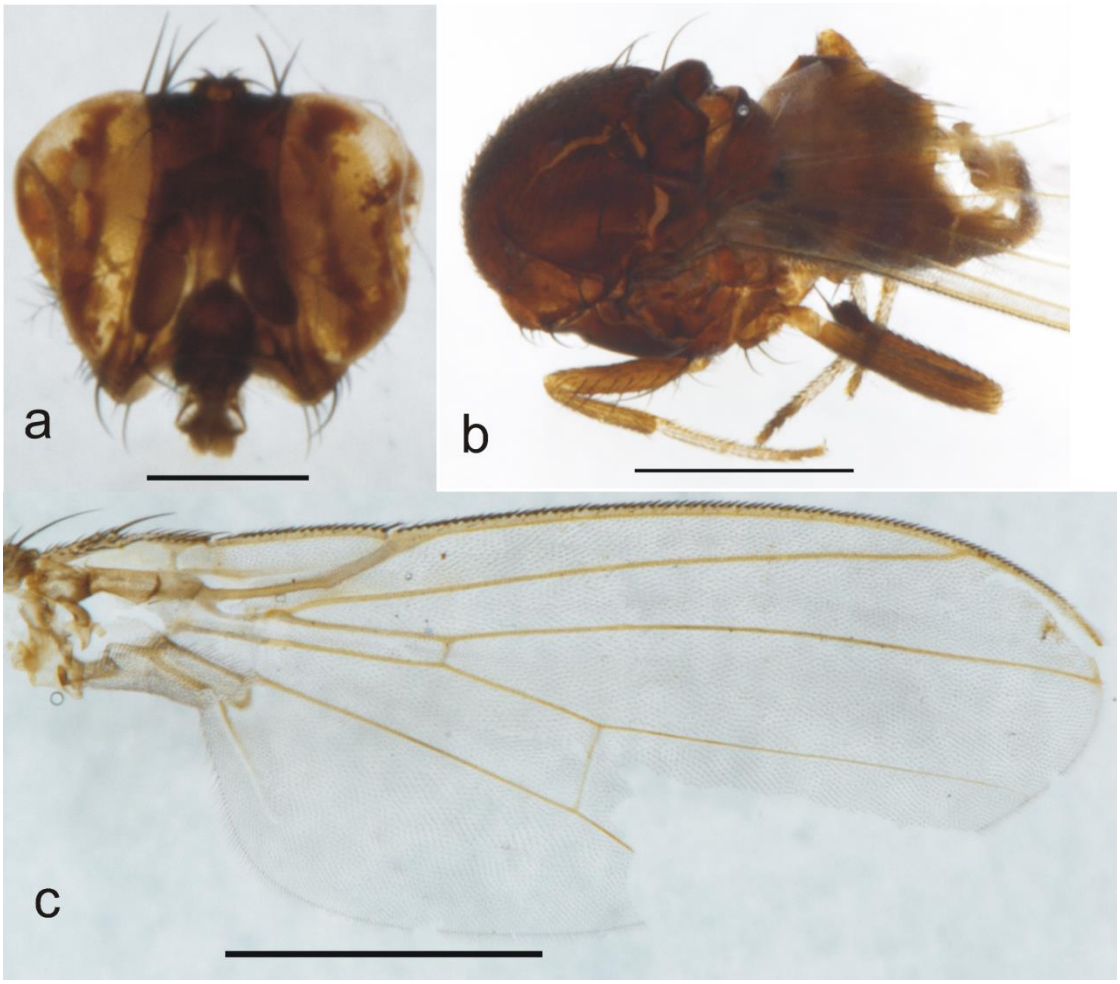


Figure 8: Holotype of *R. obscura* sp. nov. Male terminalia, a: aedeagus, dorsal view; b: aedeagus, lateral view; c: aedeagus, ventral view; d: epandrium, posterior-lateral view (scale bar 0.1 mm).

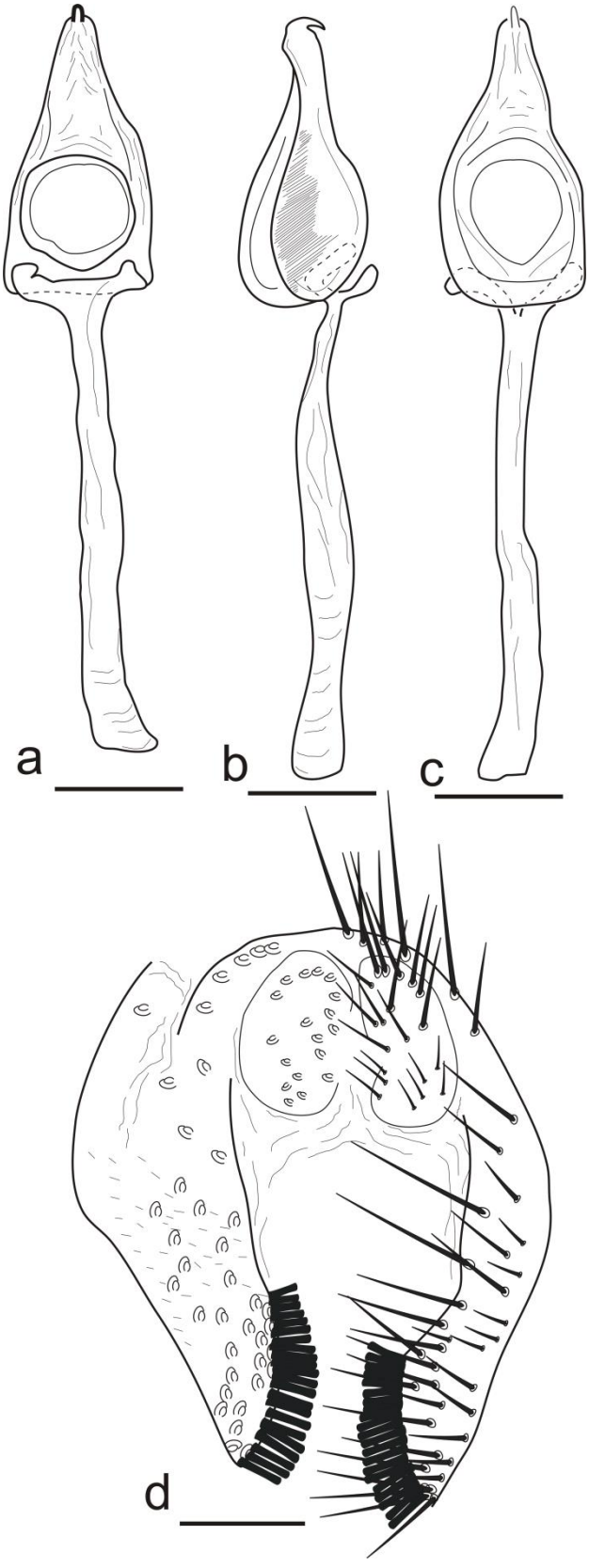


Figure 9: Holotype of *R. paraguayensis* sp. nov., female, dried. a: general habitus, lateral view; b: thorax, dorsal view; c: wing; d: abdomen, dorsal view ; e: clarified abdomen, dorsal view; f: head, frontal view; (scale bar 1.0 mm, except in f: 0.5 mm).

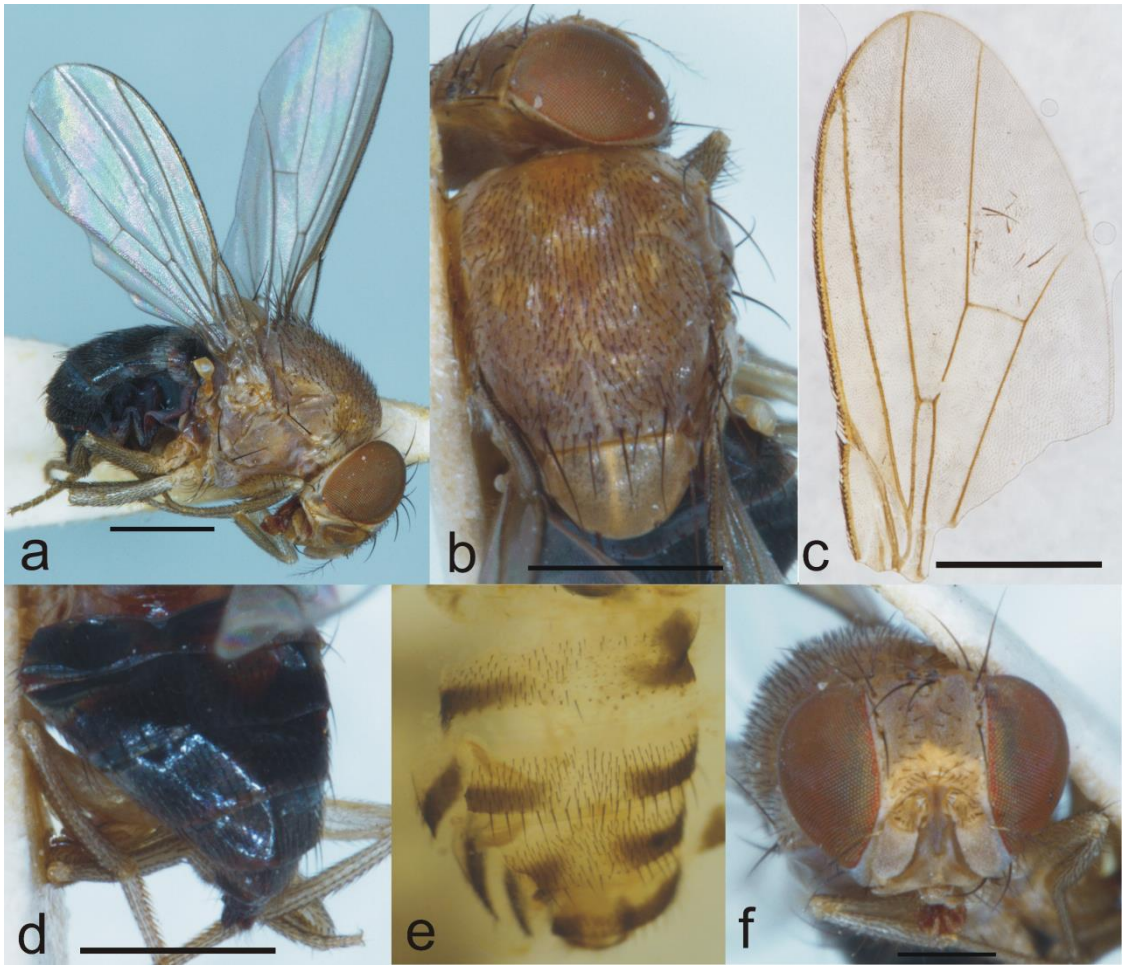


Figure 10: Holotype of *R. paraguayensis* sp. nov. Female terminalia, a: dorsal view; b: spermathecal capsule; c: ventral view (scale bar 0.1 mm).

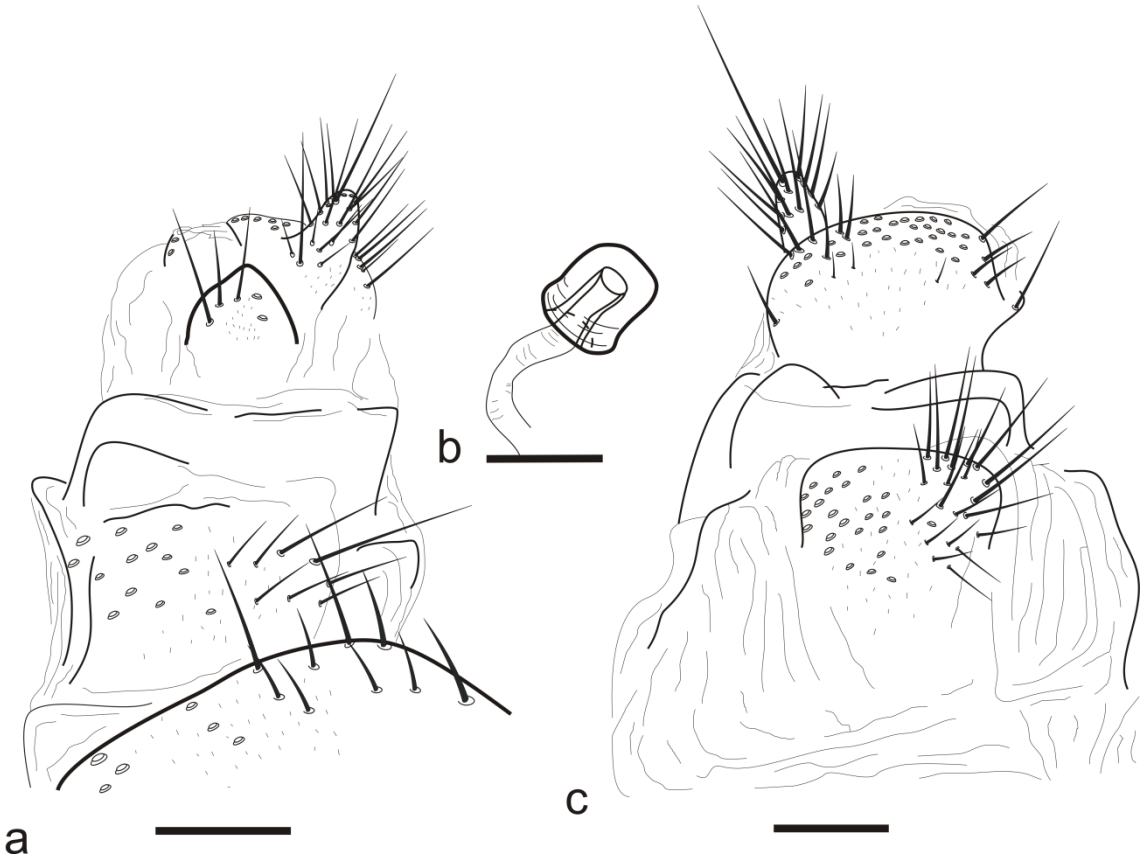


Figure 11: Holotype of *R. fulva* sp. nov., female, in ethanol. a: head, frontal view; b: general habitus, lateral view; c: wing; d: general habitus, dorsal view (scale bar 1.0 mm, except in a: 0.5 mm).

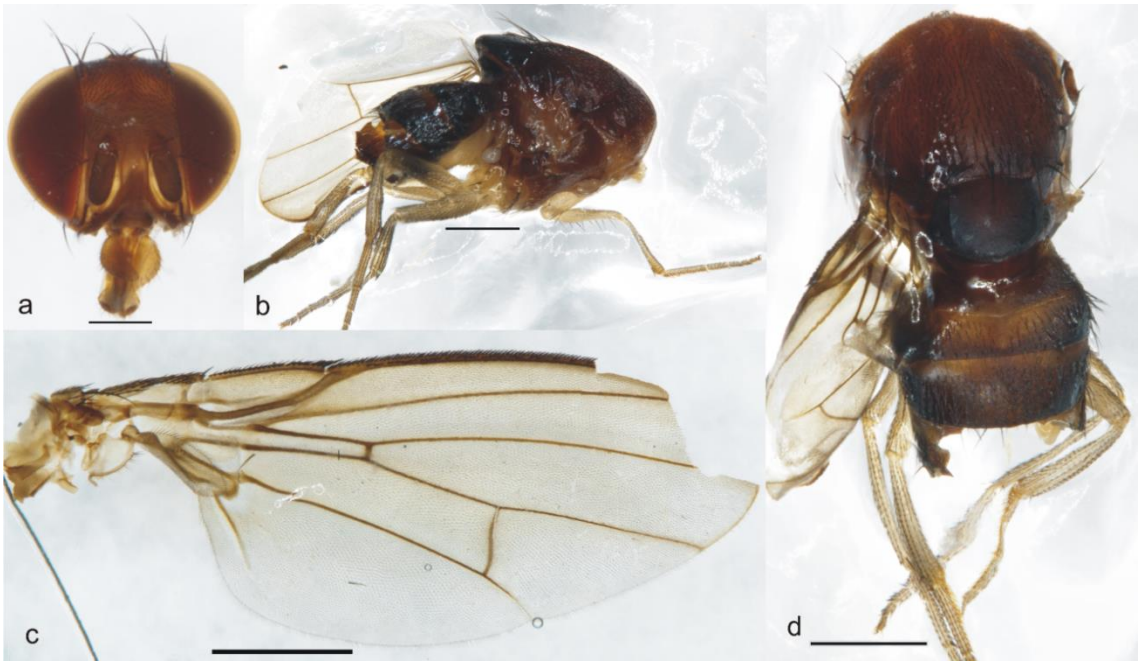


Figure 12: Holotype of *R. fulva* sp. nov. Female terminalia, a: ventral view; b: spermathecal capsule; c: dorsal view (scale bar 0.1 mm).

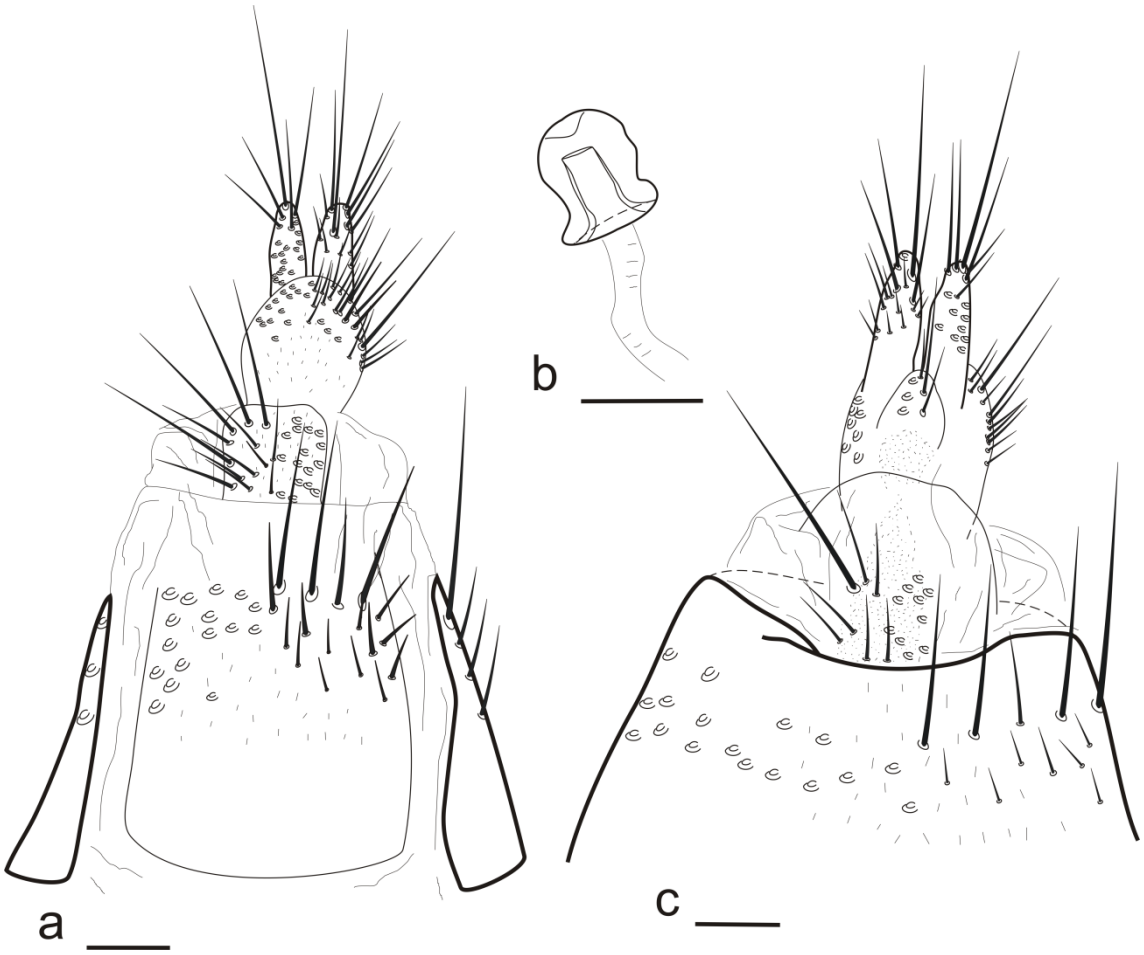


Figure 13: Holotype of *R. maculosa* sp. nov., female, in ethanol. a: general habitus, dorsal view; b: general habitus, lateral view; c: head, frontal view; d: wing (scale bar 1.0 mm, except in c: 0.5 mm).

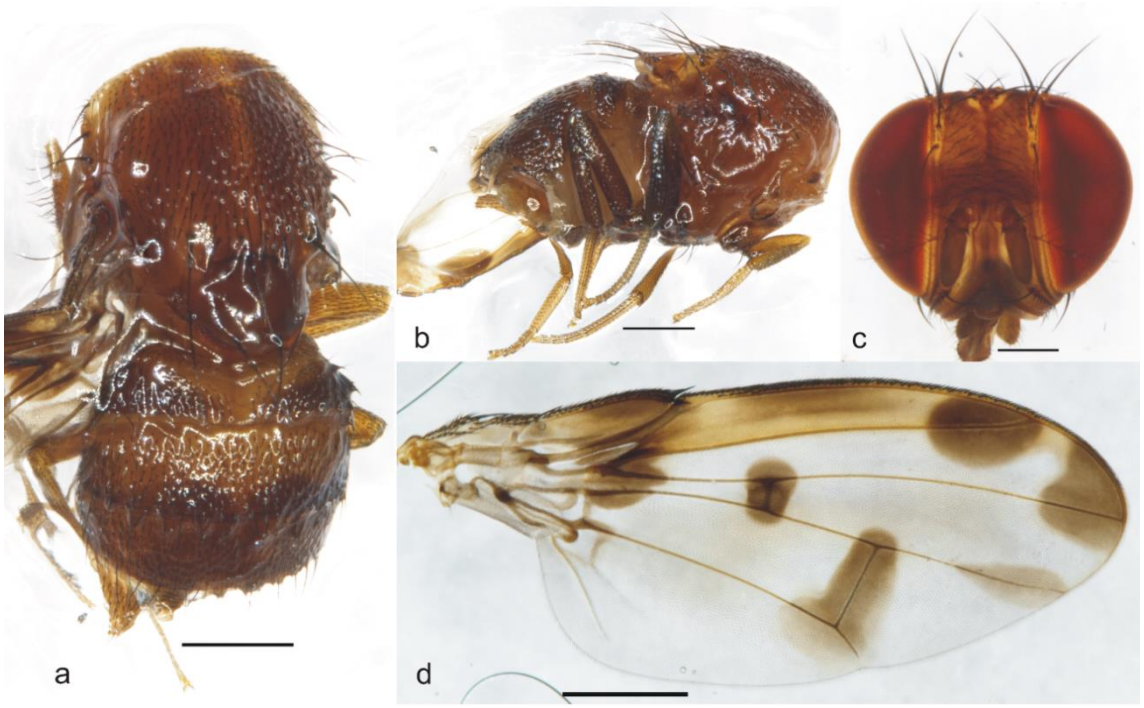


Figure 14: Holotype of *R. maculosa* sp. nov. Female terminalia, a: dorsal view; b: spermathecal capsule; c: ventral view (scale bar 0.1 mm).

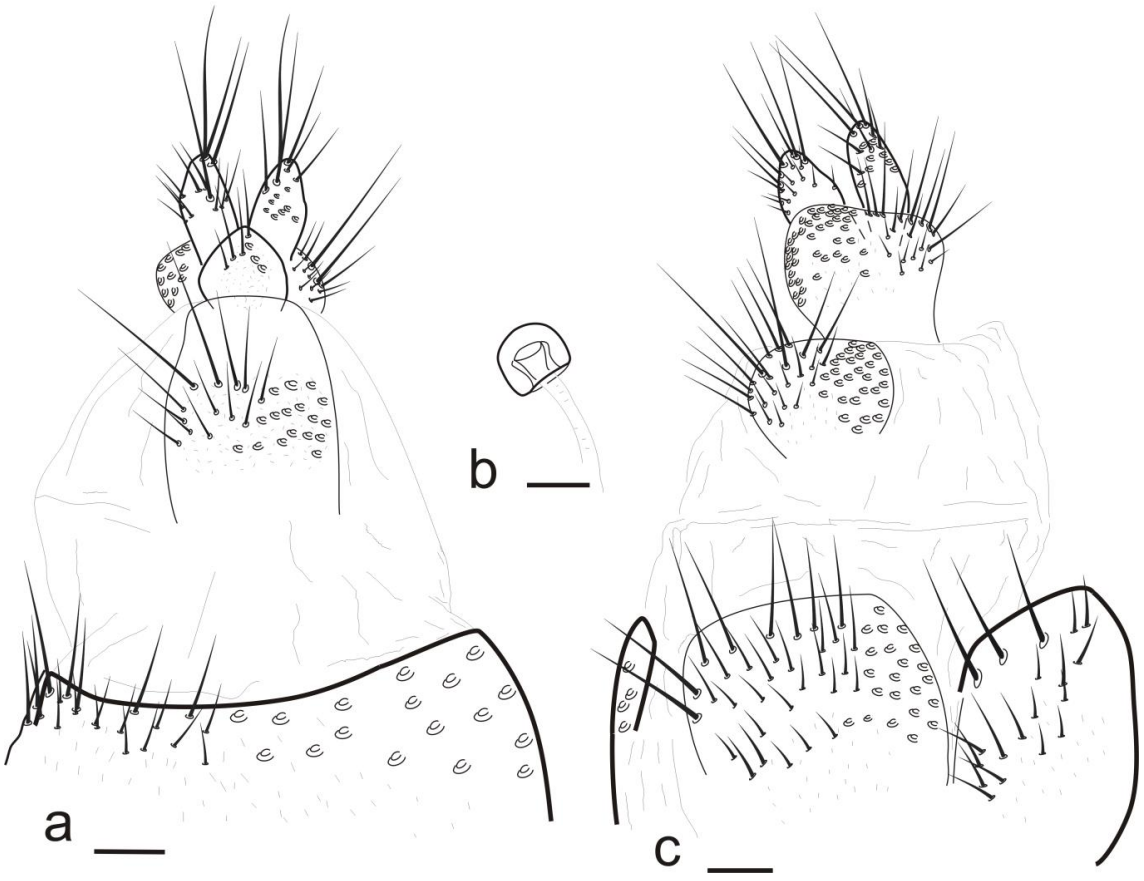


Figure 15: Holotype of *R. nigra* sp. nov., female, in ethanol. a: general habitus, dorsal view; b: general habitus, lateral view; c: head, frontal view; d: wing (scale bar 1.0 mm, except in c: 0.5 mm).

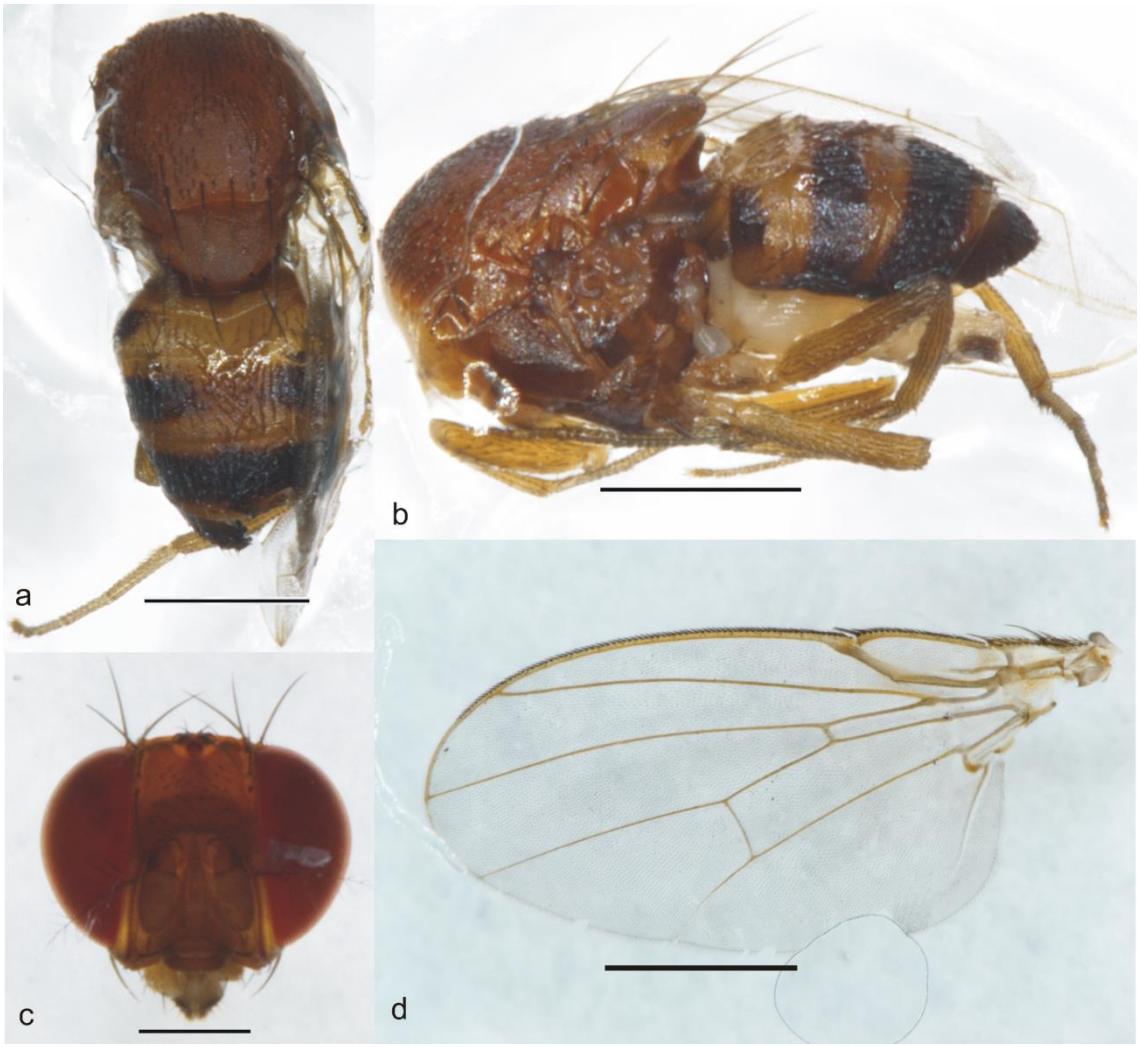


Figure 16: Holotype of *R. nigra* sp. nov. Female terminalia, a: dorsal view; b: spermathecal capsule; c: ventral view (scale bar 0.1 mm).

Figure 17: Holotype of *R. brasilis* sp. nov., female, dried. a: general habitus, lateral-dorsal view; b: head, frontal view; c: abdomen, dorsal view; d: thorax, dorsal view; e: wing (scale bar 1.0 mm, except in b: 0.5 mm).

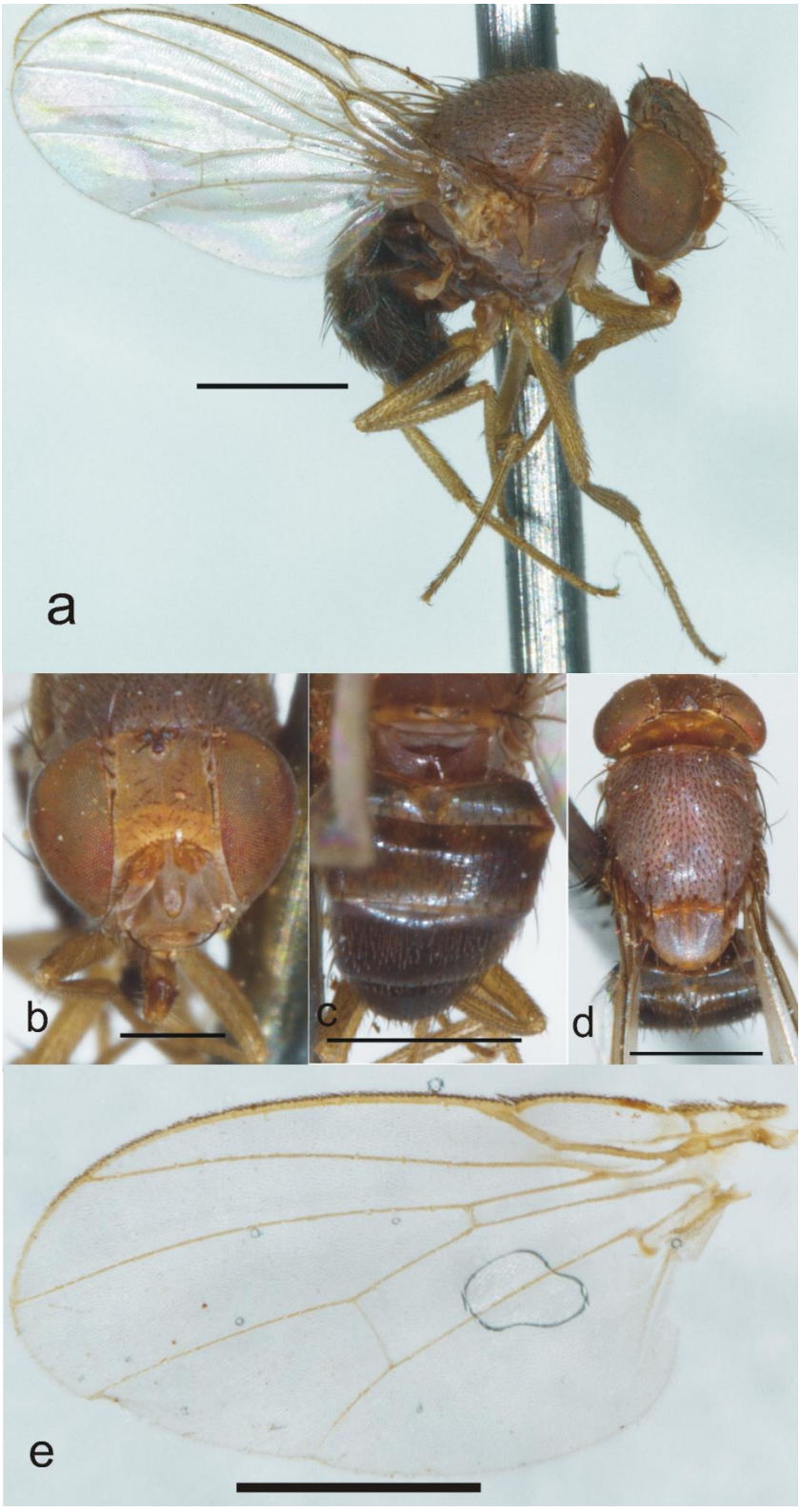


Figure 18: Holotype of *R. brasilis* sp. nov. Female terminalia, a: dorsal view; b: ventral view; c: spermathecal capsule (scale bar 0.1 mm).

Figure 19: Holotype of *R. ignota* sp. nov., dried. a: general habitus, dorsal view; b: head, frontal view; c: general habitus, lateral view; d: wing (scale bar 1.0 mm, except in b: 0.5 mm).

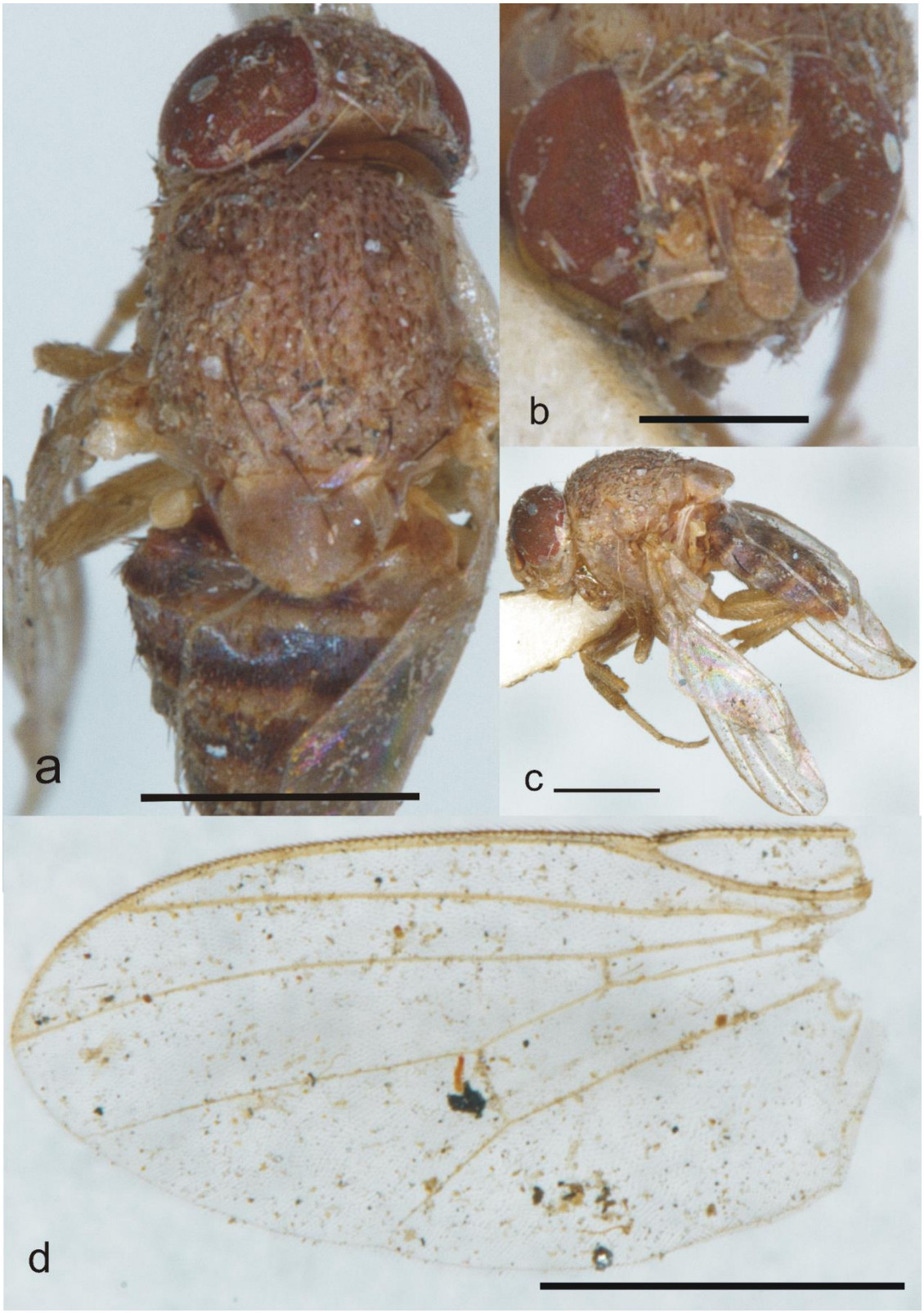
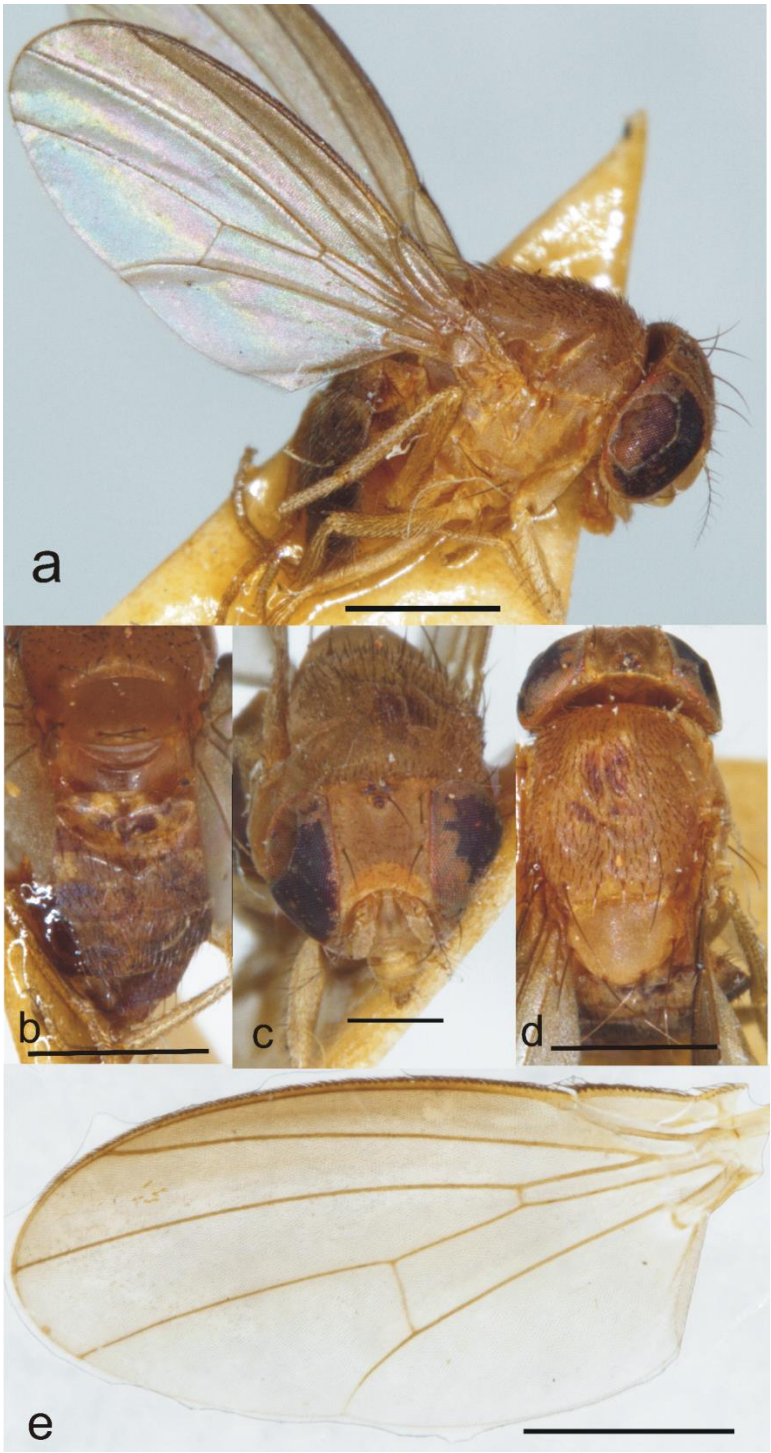


Figure 20: Holotype of *R. fusca* sp. nov., dried. a: general habitus, lateral view; b: abdomen, dorsal view; c: head, frontal view; d: thorax, dorsal view; e: wing (scale bar 1.0 mm, except in c: 0.5 mm).



5.1.10. TABLE

Table 1: Complementary measures and indices to the new *Rhinoleucophenga* specimens. Measures and indices according to Bächli et al. (2004). *: measures in millimeters (mm); -: measures not available.

	R. punctata Holotype	*R. alata* Holotype	*R. alata* Paratype	*R. paulistorum* Holotype	*R. paulistorum* Paratype	*R. paulistorum* Paratype	*R. obscura* Holotype	*R. paraguayensis* Holotype	*R. paraguayensis* Paratype
HEAD									
Frontal length *	0.46	1.10	1.00	0.68	0.62	0.70	0.54	0.62	0.60
Frontal index	1.15	1.03	1.02	1.03	1.03	1.16	1.35	1.10	1.00
Top-to-bottom frontal width ratio	1.20	0.98	1.04	1.13	1.20	1.13	1.15	1.07	1.06
Ocellar triangle to front length ratio	0.60	0.25	0.26	0.44	0.38	0.41	0.48	0.38	0.43
Setae OR1/OR3 ratio	1.03	0.88	0.92	-	-	-	1.15	1.06	1.05
Setae OR2/OR1 ratio	0.74	0.66	0.65	-	-	-	0.60	0.58	0.61
Vibrissal index	0.42	0.41	0.57	0.25	0.34	0.28	0.33	0.38	0.31
Cheek index	2.21	4.30	9.37	6.00	6.37	6.62	8.80	6.12	5.44
Eye index	1.18	1.58	1.44	1.26	1.37	1.29	1.22	1.34	1.32

THORAX

Thorax length*	1.52	3.23	2.96	2.38	2.12	2.38	1.68	1.92	1.94
Strongest prescutellar acrostichal setae, % length related to posterior dorsocentral setae	66.00	0.95	0.90	59.00	-	59.00	-	60.00	57.00
Dorsocentral setae, transverse distance related to longitudinal distance	4.66X	6.66X	4.46X	3.65X	3.80	4.21X	4.28X	3.75X	4.37X
Sterno index	1.00	1.07	0.82	0.94	1.00	0.92	0.91	0.96	1.00

WING

Length*	2.50	6.06	5.93	4.20	3.80	4.20	3.25	3.25	3.30
Width*	1.14	2.93	2.75	1.90	1.70	1.90	1.30	1.40	1.60

WING INDICES

C	2.10	4.00	4.22	3.31	2.82	3.42	3.10	3.50	3.44
Hb	0.75	0.26	0.22	0.45	0.39	0.40	0.60	0.53	0.55
Ac	2.06	1.19	1.15	1.23	1.46	1.40	1.42	1.33	1.31
4c	3.00	0.70	0.69	0.92	1.00	0.77	1.11	1.07	0.96
4v	6.59	1.26	1.38	2.02	2.05	1.82	2.59	2.96	2.50

5x	3.00	0.46	0.60	1.42	1.44	1.57	1.69	-	1.76
M	3.00	0.29	0.38	0.75	0.68	0.66	0.81	1.23	1.00
Prox. X	1.95	0.91	1.04	1.00	0.84	0.71	1.03	1.15	1.13
Body length*	3.38	7.20	6.80	4.75	4.50	4.60	3.50	3.50	3.80

Table 1: continued.

	R. fulva Holotype	*R. maculosa* Holotype	*R. nigra* Holotype	*R. brasilis* Holotype	*R. brasilis* Paratype	*R. ignota* Holotype	*R. fusca* Holotype
HEAD							
Frontal length *	0.92	1.02	0.62	0.56	0.54	0.50	0.58
Frontal index	1.15	1.15	1.03	1.03	1.12	1.08	1.16
Top-to-bottom frontal width ratio	1.00	1.06	0.93	1.01	1.04	1.17	1.12
Ocellar triangle to front length ratio	0.28	0.29	0.35	0.32	0.33	0.44	0.37
Setae OR1/OR3 ratio	1.38	0.89	0.90	1.00	0.85	-	1.12
Setae OR2/OR1 ratio	0.52	1.04	0.72	0.66	0.79	-	0.72
Vibrissal index	0.45	0.33	0.23	0.26	0.36	-	0.28
Cheek index	11.81	8.10	8.16	6.92	8.20	3.85	9.00
Eye index	1.38	1.44	1.44	1.45	1.46	1.26	1.40
THORAX							
Thorax length*	2.58	2.86	1.80	1.66	1.51	1.60	1.72

Strongest prescutellar acrostichal setae, % length related to posterior dorsocentral setae	68.00	83.00	62.00	58.00	52.00	-	56.00
Dorsocentral setae, transverse distance related to longitudinal distance	4.60X	3.12X	3.36X	3.52X	2.94X	-	4.83X
Sterno index	0.97	0.92	0.96	-	-	-	1.00
WING							
Length*	4.50	5.25	3.40	3.00	3.00	2.90	3.50
Width*	1.95	2.35	1.60	1.40	1.40	-	1.50
WING INDICES							
C	4.16	2.50	1.13	7.58	7.69	-	3.18
Hb	0.53	0.85	0.18	0.35	0.34	0.44	0.57
Ac	1.00	1.44	3.58	1.19	1.30	1.66	1.32
4c	0.63	1.14	2.50	0.96	0.86	-	1.00
4v	1.53	1.60	2.00	2.00	1.83	-	2.30
5x	0.95	0.54	1.93	1.71	1.85	-	1.47
M	0.48	0.41	0.93	0.82	0.86	-	0.75

Prox. X	0.80	1.29	0.77	0.93	0.71	-	0.90
Body length*	5.40	5.75	3.50	3.45	3.20	3.25	3.40

6. CAPÍTULO VI

(Manuscrito para submissão ao periódico *Entomological Science*)

6.1. Review of *Rhinoleucophenga obesa* (Loew) (Diptera: Drosophilidae) recorded in the Neotropical region based on molecular, morphological and distributional data

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6.1.1. ABSTRACT

Rhinoleucophenga is a genus endemic to the New World and *R. obesa* seems to be the most widespread of its species, being recorded from United States of America up to the South of Brazil. Nevertheless, there are some uncertainties about the real identity and distribution of this interesting species. In this paper, we looked for morphological characters able to discriminate *R. obesa* from its sibling species, *R. gigantea*. Redescription of *R. obesa* Lima (1935) and *R. obesa* Malogolowkin (1946) are presented and comparisons of *R. obesa* specimens from different localities in Brazil are

performed. Plates of females' terminalia of *R. obesa* and *R. gigantea* are presented for the first time. The epandrium and the spermathecal capsules revealed useful characteristics to differentiate *R. obesa* from its sibling species, and these morphological differences were corroborated by a sequence fragment of *COI*. The specimens described by Malogolowkin (1946) and Lima (1935) seem to comprise the same species, probably *R. obesa*. Specimens from the South of Brazil, previously identified as *R. obesa*, were determined as *R. gigantean*, which restricts the distribution limits of *R. obesa* in South America. *Rhinoleucophenga obesa* sensu Malogolowkin (1946) and *R. gigantea* were recorded for the first time in a coast environment and in the Brazilian savannah, respectively. Therefore, the present study corresponds to an advance to the taxonomic definition of *R. obesa*.

Keywords: *COI*; *Drosophila*; *Rhinoleucophenga gigantea*; Steganinae; Systematics.

6.1.2. INTRODUCTION

The genus *Rhinoleucophenga* encompasses at least 29 species (Poppe et al. 2015, Vidal & Vilela 2015), being recorded in the New World from 37°N to 34°S (Vilela 1990). In this context, *R. obesa* (Loew) seems to be the most widespread of the *Rhinoleucophenga* species. This species was described from specimens collected in Texas (Loew 1872) and, currently, it presents records ranging from the United States of America up to the south of Brazil.

Lima (1935) firstly described important ecological aspects about the behavior of *R. obesa,* identifying it as a predator of *Aclerda campinensis* (Hemiptera: Coccidae). Malogolowkin (1946) described many species to *Rhinoleucophenga*, and identified nine specimens collected in Mato Grosso State, Brazil, as *R. obesa*. After that, other specimens identified as *R. obesa* were recorded in different Brazilian biomes (De Toni *et al*. 2007, Blauth & Gottschalk 2007, Hochmüller *et al*. 2010, Poppe *et al*. 2014). However, many uncertainties about the identity of *R. obesa* are mentioned by some authors (Wheeler 1952, Wheeler & Takada 1971, Vilela 1990, Poppe *et al*. 2015a). McAtee (1924) mentioned *R. obesa* as a widespread species and also as a synonym of *R. pallida* Hendel; that species are highly similar morphologically but they differ by the aedeagus traits (Grimaldi 1990) and by vein M-IV strongly clouded in *R. pallida*

(personal observation). Consequently, the real distribution of *R. obesa* remains uncertain; furthermore, some authors also stress that the name *R. obesa* may indeed comprise a group of sibling species, including *R. gigantea* (Thomson) (Vilela 1990).

The possibility of occurrence of other species among this "*obesa – gigantea*" sibling group is acceptable due to three main reasons. First, as a consequence of the high diversity of this genus that has been recently noticed (Poppe *et al*. 2015a). Second, due to the wide latitudinal range of the records of *R. obesa* (37°N-34ºS) (Vilela 1990, Bächli 2015), once this wide distribution is associated with great climatic variation, which directly influence the distribution patterns of Drosophilidae species (Poppe *at al*. 2015b), being able to conduct to evolutionary bottlenecks or even to the raising of geographic races (Parsons 1989, Hasson *et al*. 1993). And finally, due to the lack of a review of old descriptions of *Rhinoleucophenga* species collected along Nearctic and Neotropical regions.

Reviewing the taxonomic status of *R. obesa* is especially important due to the high representativeness of this species in many phylogenetic studies (Throckmorton 1975, Grimaldi 1990, Remsen & O'Grady 2002, Sidorenko 2002, Otranto *et al*. 2008, Van der Linde *et al*. 2010, Yassin 2013). So, besides elucidating some taxonomic problems in *Rhinoleucophenga*, especially related to long time processes of misidentification of sibling species, and uncertainties on the geographic distribution of the related species, the review of specimens identified as *R. obesa* in the Neotropical region will add to the knowledge related to the evolution of the genus as a whole. So, in this paper, we look for morphological characters able to discriminate *R. obesa* from its sibling species, *R. gigantea*, as supported by molecular data. Since we could not analyze the type-series of *R. obesa* and there are no terminalia illustrations available for such specimen, we provided redescriptions of *R. obesa* sensu Lima (1935) and *R. obesa* sensu Malogolowkin (1946), once the definition of *R. obesa* is generally based on these specimens. After that, some specimens mentioned in literature as *R. obesa* were reviewed and determined as *R. gigantea* (Poppe *et al*. 2014); which restricted the distribution range of the target species.

6.1.3. MATERIALS AND METHODS

Specimens Morphology Analysis

Descriptions are based on the terminology, measures and indices given by Bächli *et al*. (2004), which were done with an ocular reticle inserted into a Zeiss stereomicroscope. Measurements in the text represent averages followed by the ranges in parentheses. Male and female terminalia were disarticulated in glycerol after treatment with 10% potassium hydroxide (KOH) and acid fuchsine (Bächli *et al*. 2004). The terminalia were mounted in a piece of glycerine jelly (ca. 2 x 2 x 2 mm) (Grimaldi 1987), stored in microvials with glycerol and pinned with the respective specimen. Photos of the specimens were taken with a digital camera coupled to an optical stereomicroscope. Drawings of the terminalia were made with a *camera lucida* system attached to a compound microscope with 40× and 10× objective lenses and a 10× ocular lens, with terminalia in the glycerine jelly, avoiding any compression of the structures that could alter the morphology.

Specimens identified as *R. obesa* collected in Brazil from the municipality of Bossoroca, Rio Grande do Sul (28°45'01"S 54°56'55"W – Poppe *et al*. 2014); from São Domingos, Goiás (13°39'S 46°45'W – Mata et al. 2008); from Tangará da Serra, Mato Grosso State (14°39'05"S 57°25'25"W) and from Porto de Galinhas, Pernambuco (8°30'30"S 35°0'20"W) (Fig. 1) were morphologically compared among each other and also with *R. obesa* described by Lima (1935) and Malogolowkin (1946).

All the analyzed specimens are deposited in the Entomological Collection of the Institute Oswaldo Cruz (CEIOC), at Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil.

DNA Extraction and PCR Amplification

Total DNA of individuals flies preserved in 70% ethanol was extracted according to the DNeasy Blood & Tissue Kit (Qiagen) instructions. A fragment of the mitochondrial *Cytochrome oxidase* subunit *I* (*COI)* gene was amplified by PCR, using a TY-J-1460 and C1-N-2329 primer set (Simon *et al*. 1994).

The *COI* gene amplification was performed in reactions of 10μL using 20 ng of DNA, 1U Taq Platinum, 1 × reaction buffer, 1.5 mM $MgCl_2$, 20 pM of each primer and 200 μM of each nucleotide. The PCR conditions were 35 cycles (45 s at 95°C, 45 s at

53°C, and 1 min at 72°C), with initial denaturation at 95°C for 5 min and final extension at 72°C for 5 min.

The effectiveness of each amplification was verified by electrophoresis. PCR amplicons were purified by Exonuclease I (10 U/µl) and Shrimp Alkaline Phosphatase (1 U/µl). DNA sequencing was performed directly from the purified amplicons at Macrogen (Seoul, South Korea), employing the same forward and reverse *COI* primers described by Simon *et al*. (1994).

The list of specimens employed in this study as well as the accession numbers of all generated *COI* gene sequences are shown in Table 1. DNA extractions were performed only with specimens from Bossoroca (four specimens) and Porto de Galinhas (one specimen). Specimens from the other regions were not included in the molecular analysis due to sampling or conditioning shortages.

Sequence Analysis

The obtained sequences were assembled and edited using the Staden Package (Staden 1996). Consensus sequences were aligned using the Clustal W algorithm, implemented with Mega 6 (Tamura *et al*. 2013). The final alignment of the *COI* data set was verified against published *COI* sequences of other Drosophilidae species available in GenBank. The authenticity of the produced mtDNA sequences was verified by using an on-line protein translator system available at http://web.expasy.org/translate/. The nucleotide substitution saturation of the sequences was accessed using the Xia's method in DAMBE 5 (Xia 2013) software.

Pairwise genetic divergences of *COI* sequences were calculated using the Kimura two-parameter (K2P) model in Mega 6 (Tamura *et al*. 2013), with 10,000 bootstrap replicates, as suggested by Hebert *et al*. (2003) for DNA barcoding.

The automatic barcode gap discovery (ABGD) species delineation tool (Puillandre *et al*. 2012) was used as a supplementary method to verify species delimitation between the specimens from Bossoroca and Porto de Galinhas. ABGD is an automated iterative process that sorts sequences into putative species based on pairwise distances, without an *a priori* species hypothesis. This algorithm automatically detects significant differences between intra and interspecific variations (barcoding gaps). The aligned sequences of all haplotypes were uploaded to the web interface at

http://wwwabi.snv.jussieu.fr/public/abgd/ and the run was performed with the default settings.

Phylogenetic Analyses

To the phylogenetic analyses, additional *Rhinoleucophenga* species were included (*R. pampeana* Poppe *et al*. and *R. trivisualis* Poppe, Schmitz & Valente (GenBank accession number: KU756239 and KU728936, respectively)). As outgroup, *COI* sequences from *Leucophenga angusta* and *L. quadripunctata* were included in the analyses (GenBank accession number HQ842780.1 and HQ842781.1, respectively).

Phylogenetic analyses were conducted using neighbor-joining (NJ) in Mega 6 (Tamura *et al*. 2013) and Bayesian inference (BI) in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). For NJ analyses, we used the model of nucleotide substitution suggested by Hebert *et al*. (2003) for DNA barcoding with 10,000 bootstrap replicates. Bayesian phylogenetic analysis was performed using the GTR+I model according to the Akaike information criterion (Akaike 1974) obtained in MrModeltest 2.2.1 (Nylander 2004). Posterior distributions of parameters, including tree topology and branch lengths, were estimated using Markov chain Monte Carlo sampling. Samples from the posterior distribution were drawn every 1,000 generations over a total of 1,000,000 generations. The first 25% of samples were discarded as burn-in.

The analysis involved nine nucleotide sequences. Codon positions included were 1st+2nd+3rd. There were a total of 847 positions in the final dataset.

6.1.4. RESULTS

Redescription of *R. obesa* specimens determined by Lima (1935) and Malogolowkin (1946)

Rhinoleucophenga Hendel

Rhinoleucophenga Hendel (1917): 44-45

Pseudophortica Sturtevant (1918): 37

Gitona (in New world) Brake & Bächli (2008): 291

Rhinoleucophenga obesa

(Figures 2a-c, 3a-d, 4a-d, 5a-c, 7a-e, 8a-b, 9a-b, 13a-c; Table 2)

Drosophila obesa Loew (1872): 102-103; *Phortica hirtifrons* Johnson (1913): 88; *Pseudophortica obesa* Sturtevant (1918): 37; *Rhinoleucophenga obesa* Malloch & McAtee (1924): 33.

Rhinoleucophenga obesa sensu Lima 1935

(Figure 2a-c; Table 2)

Examined Material: A female labeled "*R. obesa*. Tube N° 1085/2116, specimen N° 2419". A male specimen with the terminalia disarticulated labeled "*R. obesa*. Tube N° 1085/2116, specimen N° 2419". A wing in a slide numbered as 2365. Specimens from Rio de Janeiro, Brazil. All specimens are deposited in the Coleção Entomológica do Instituto Oswaldo Cruz (CEIOC)/ Fiocruz.

Diagnosis. General body color brownish; front covered with ca. 200 scattered interfrontal setulae. Frontal index 1.87 (1.75-2.00); Carina nose-like and ca. 90% sulcated; arista with 9 long dorsal branches and 7 long ventral branches. Spotted wing. Body length ca. 5.00 mm.

Description. Head (Fig. 2c). Front ventrally brownish and superiorly yellowish, covered with ca. 200 scattered interfrontal setulae; ocellar triangle yellowish (or brown in some specimens) with dark brown ocelli. Carina nose-like and ca. 90% sulcated. Face yellowish; gena brownish; antenna with flagellomore and pedicel homogeneously brownish; arista with 9 long dorsal branches and 7 long ventral branches. Palpus brownish with ca. 50 (40-60) setae on lower part.

Thorax (Fig. 2a-b). Scutum brownish (with diffuse brown stripes in some specimens) and scutellum homogeneously yellow. 12 irregular rows of acrostichal setulae. Three pairs of prescutellar acrostichal setae. Pleura yellowish; halteres yellow-brownish. Legs homogeneously yellow.

Wings (Fig. 2a). Non-hyaline, with distal and proximal spots. Vein Dm-Cu and R-M clouded.

Abdomen. Abdomen all black (color probably not preserved).

Terminalia. Probably the aedeagus and epandrium depicted in Lima (1950) belonged to the dissected specimen. The terminalia was not localized.
To more measures and indices see Table 2.

Rhinoleucophenga obesa sensu Malogolowkin 1946
(Figures 3a-d, 4a-d, 5a-c; Table 2)

Examined Material: Nine specimens labeled "*R. obesa* #1 Brazil, Mato Grosso, Salobra; 2. ix. 1940 Det.: C. Malogolowkin, in 1946. N° 8127". "*R. obesa* #2♀ Brazil, Mato Grosso, Salobra; 2. ix. 1940 Det.: C. Malogolowkin, in 1946". "*R. obesa* #3♂ Brazil, Mato Grosso, Salobra; 2. ix. 1940 Det.: C. Malogolowkin, in 1946". "*R. obesa* #4 Brazil, Mato Grosso, Salobra; 2. ix. 1940 Det.: C. Malogolowkin, in 1946. N° 8129". "*R. obesa* #5 Brazil, Mato Grosso, Salobra; 2. ix. 1940 Det.: C. Malogolowkin, in 1946. N° 8130". "*R. obesa* #6♀ Brazil, Mato Grosso, Salobra; 2. ix. 1940 Det.: C. Malogolowkin, in 1946". "*R. obesa* #7♂ Brazil, Mato Grosso, Salobra; 2. ix. 1940 Det.: C. Malogolowkin, in 1946. N° 8126". "*R. obesa* #8 Brazil, Mato Grosso, Salobra; 2. ix. 1940 Det.: C. Malogolowkin, in 1946. N° 8128". "*R. obesa* #9♀ Brazil, Mato Grosso, Salobra; 2. ix. 1940 Det.: C. Malogolowkin, in 1946". Specimens #03♂, #07♂ and #09♀ had the postabdomen disarticulated stored in a microvial with glycerin and attached with the respective specimen. All specimens are deposited in the Coleção Entomológica do Instituto Oswaldo Cruz (CEIOC)/ Fiocruz.

Diagnosis. General body color yellow-brownish; front covered with ca. 200 scattered interfrontal setulae. Frontal index 1.79 (1.65-1.91); Carina nose-like and ca. 70% sulcated; arista with 10 (8-11) long dorsal branches and 7 (6-8) long ventral branches. Spotted wing. Body length ca. 5.02 (4.80-5.50) mm. Male and female terminalias as in figures 4a-d and 5a-c, respectively.

Description. Head (Fig. 3a-c). Front homogeneously brownish, covered with ca. 200 scattered interfrontal setulae. Ocellar triangle brownish with brown ocelli. Carina nose-like and ca. 70% (60-75) sulcated. Face and gena yellowish; antenna with flagellomore homogeneously brownish, pedicel yellow; arista with 10 (8-11) long dorsal branches and 7 (6-8) long ventral branches. Palpus yellowish with ca. 50 setae on lower part.

Thorax (Fig 3a-b). Scutum and scutellum brownish (with diffuse brown stripes in some specimens). 12 irregular rows of acrostichal setulae. Three pairs of prescutellar acrostichal setae, the central one is the longest. Pleura brownish; halteres yellowish. Legs homogeneously yellow.

Wings (Fig. 3a). Non-hyaline, with distal and proximal spots. Vein Dm-Cu, R-M and costal cell clouded.

Abdomen (Fig. 3d). Proximally brownish and distally dark brown; tergites with dark brown stripes continuous.

Male terminalia (Fig. 4a-d). Aedeagus round shaped, the base slightly wider than the apical portion, curved dorsal-ventrally, with a dorsal structure projected medially into the top. Epandrium microtrichose, fused with surstyli with ca. 26 prensisetae, curvedly inserted. Ventral lobe with ca. 30 setae each one. Cerci microtrichose elongated, basely wider, with ca. 10 apical longer setae each one.

Female terminalia (Fig. 5a-c). Cerci long and well sclerotized with 2 longer apical setae on each one. Epiproct with ca. 10 setae. Hypoproct with ca. 50 setae. Spermathecal capsule elongated with basal introvert reaching ca. 2/3 of inner capsule, length to width ratio = 1.56.

To more measures and indices see Table 2.

Other specimens sampled in the Neotropical region

The specimens from the municipality of Bossoroca (Poppe *et al*. 2014) (Fig. 6a-d), São Domingos (Mata *et al*. 2008), Porto de Galinhas (Fig. 7a-e) and Tangará da Serra, all presented the following diagnosis: General body color yellow-brownish; front covered with ca. 200 scattered interfrontal setulae. Carina nose-like and ca. 90% sulcated; arista with 7-9 long dorsal branches and 7 long ventral branches. 2-3 pairs of long prescutellar acrostichal setae. Wing non-hyaline, with tip of veins R_{2+3} and R_{4+5} apical, vein C-III, Dm-Cu and R-M clouded. Abdomen dorsal-proximally yellow-brownish, laterally brown and distally dark brown. Body length ca. 5.00 mm.

Nervertheless, despite these similarities in the body external morphology, differences in the male terminalia were noticed among the specimens, mainly in the epandrium (Fig. 8a-f). The specimens collected in Porto de Galinhas (Fig. 9a-b) and Tangará da Serra presented aedeagus and epandrium similar to the species described by Lima (1935) and Malogolowkin (1946) (Fig. 4a-d); the main similarity in the epandrium

is the ventral lobe with ca. 26 prensisetae in a concave curved row, and a larger ventral prominence without prensisetae. Differently, specimens from Bossoroca present the ventral lobe of epandrium with ca. 20 prensisetae in a straight row inserted in the total ventral extension (Fig. 10). However, among the specimens from Bossoroca, some variation in the shape of epandria ventral lobe was also noticed (Figs. 8c-d and 8e-f).

Concerning the females reproductive structures, females from São Domingos and Bossoroca present spermathecal capsules with "spicules" (Figs. 11 and 12, respectively). This is the most evident difference with the specimens from Porto de Galinhas and from the specimens identified by Malogolowkin (1946) (Figs. 13 and 5, respectively).

Molecular and phylogenetic diagnostic

The final alignment consisted of nine sequences of 847bp of the *COI* gene. These sequences contained 158 variable sites (18.65%), of which 101 (11.92%) were parsimony informative. The sequences were not saturated, based on an Iss that was significantly lower than the critical Iss (Iss = 0.1093, $Iss._c$ = 0.7792, $P < 0.0001$). All ordinary specimens from Bossoroca compound a strongly supported clade through the NJ and BI analyses (Figs. 14 and 15, respectively). Interesting, in both analyses that clade was closer to *R. pampeana* than to the branch of *R. obesa* from Porto de Galinhas, although support for this relationship was low (0.82 and 64 in the BI and NJ analyses, respectively). The only difference between the phylogenetic analyses was in the clades support values, which were higher in the BI analysis.

Based on *COI* sequences, genetic divergence values higher than 4% were noticed among the specimens from Porto de Galinhas and Bossoroca (Table 3). The divergence between individuals from Bossoroca ranged from 0.00% to 0.49%, while the mean divergence presented by this population was 0.24%. The divergence between Bossoroca males with different epandrium (Figure 8c-d and 8e-f) ranged from 0.00% to 0.12%; whereas males with equal epandrium morphology (Boss_spp_2 and Boss_spp_3; Figs. 8c and 8d, respectively) presented a divergence of 0.12%. Differently, the divergence level among specimens from Bossoroca and from Porto de Galinhas was more pronounced, ranging from 4.52% to 4.78%, with a mean divergence of 4.58%. Complementarily, the result obtained applying the ABGD algorithm to the

COI data set showed a barcoding gap between populations from Porto de Galinhas and Bossoroca.

6.1.5. DISCUSSION

Previously the number of branches in the arista was considered as a diagnose character (Malogolowkin 1946, Poppe *et al*. 2014) to distinguish *R. obesa* from *R. gigantea*, but after analyzing many specimens, we noticed that this is a highly variable character. Thus, through the body external general morphology patterns, it was impossible to distinguish the specimens of *R. obesa* sensu Lima (1935), sensu Malogolowkin (1946), from Tangará da Serra and Porto de Galinhas from their sibling species, *R. gigantea*, sampled in Bossoroca and São Domingos. Likewise, the aedeagus general form is really similar between those species; if comparisons are done with each aedeagus in different angles, they can erroneously present the same form. On the other hand, the epandrium presents useful characteristics to differentiate the specimens: there are 20 prensisetae in a straight row in the specimens from Bossoroca, as well as in *R. gigantea* redescribed by Vilela (1990) (Figs. 8c-f and 10c; Figs. 1-2 in Vilela 1990), and 26 prensisetae in a concave curved row in the *R. obesa* specimens of C. Malogolowkin (Fig. 4d) and in the specimen from Porto de Galinhas (Fig. 9a) and Tangará da Serra. Additionally, the epandrium ventral lobe in the three last specimens present a larger ventral portion without prensisetae (Figs. 4d and 9a), while in *R. gigantea* the edge of ventral lobe is almost totally inserted by prensisetae, again as noticed to the specimens from Bossoroca (Figs. 8c-f and 10c; Figs. 1-2 in Vilela 1990). Thus, the specimens from Bossoroca, South of Brazil, were misidentified as *R. obesa* by Poppe *et al*. (2014), and they actually belong to *R. gigantea*. So, the specimens described by Malogolowkin (1946), Lima (1935) and those sampled in Porto de Galinhas and Tangará da Serra are the same species, defined as *R. obesa*. Furthermore, the specimen of *R. obesa* with the disarticulated terminalia in the examined material of A.C. Lima probably corresponds to the specimen whose aedeagus and epandrium was depicted by Lima (1935, Fig. 3). Although that terminalia could not be directly analyzed, in his depiction it is possible to observe clearly the same disposition of the prensisetae in a concave row as in Malogolowkin's specimens. Vilela (1990) suggested that the concave shape of the prensisetae row in the epandrium depicted by Lima (1935) could be an artifact of

compression by a slide or the specimen could belong to another species; however, we observed this same concave shape of the prensisetae row in the other *R. obesa* specimens analyzed, without compression by any slide. So, we suggest that the Lima's specimen is conspecific with Malogolowkin specimens, and that the concave shape is the original shape of that structure.

There are many available literatures that mention *R. obesa* in the results or discussions (Duda 1927: 41-43, Brimley 1938: 388, Patterson 1943: 15 (table 1), 19 (table 4), 21 (table 5), 36, Parish & Cushing 1938: 754 (table 3), Wheeler 1952: 193-194, Grimaldi 1988: 185, Grimaldi 1990: 100 (figure 542), 134, Remsen & O'Grady 2002: 261 (appendix B), Van der Linde *et al*. 2010: 29 (figure 3) and Yassin 2013: suplementary table 1, suplementary file S2), and others recording the species in the states of Rio Grande do Sul and Santa Catarina, South of Brazil (De Toni *et al*. 2007: 366 (table 1), 367 (table 2), 368 (table 3), 370 (table 4), 371 (table 5), 372 (table 6), 375 (table 8), Gottschalk *et al*. 2007: 854 (table 1) and Hochmüller *et al*. 2010: 290 (table 2), 294). However, we are not sure if all of them refer to the same species, as well as if that is the same *R. obesa* sensu Malogolowkin (1946). Even that some of them provided illustrations of terminalia (such as Wheeler & Takada (1971: 227, figures 4a-e), specimen collected in Texas), but the epandrium was illustrated in an angle that does not allow to safely confirm it as a conspecific specimen to *R. obesa* sensu Malogolowkin. On the other hand, the female spermathecal capsule presented by Throckmorton (1962: 272, figure 33.2), also for a specimen collected in Texas, differs from the spermathecal capsule of the female of Malogolowkin's specimens (Fig. 5c). In the same way, the spermathecal capsules of the specimens from São Domingos and Bossoroca (Figs. 11b and 12b, respectively) differ from the morphology of Malogolowkin and Porto de Galinhas specimens (Figs. 5c and 13b, respectively) by the presence of "spicules" in the former ones. Thus, the female specimen from Bossoroca must be a female of *R. gigantea*, once all males were defined as *R. gigantea* specimens in that region. It is the first female terminalia representation of *R. gigantea*. Furthermore, it is the first record of *R. gigantea* in the Brazilian savannah (São Domingos), Cerrado biome.

Additionally, fragments of *COI* revealed to provide complementary evidence to morphology, helping to distinguish *R. gigantea* specimens from *R. obesa*, as well as to

indicate intraspecific variation among the *R. gigantea* specimens with different epandrium shape. DNA barcoding is based on the premise that a short standardized sequence of DNA can distinguish between individuals of a species, because genetic variation between species is likely to exceed that found within a single species (Hebert *et al*. 2003). Thus, it is a valuable genetic tool to reveal cryptic species previously unrecognized through the analysis of standard morphological variation (Hebert *et al*. 2004). The distinction between *R. obesa* and *R. gigantea* specimens is further corroborated by the clades obtained in the phylogenetic analysis. The phylogenetic position of *R. pampeana* as sister to *R. gigantea*, to the exclusion of *R. obesa* also reinforces this distinction. *Rhinoleucophenga pampeana* presents a high morphological similarity with both species, except by the supernumerary veins present in the wings (Poppe *et al*. 2014, 2015a).

According to some authors (Hajibabaei *et al*. 2006, 2007, Waugh 2007, Yassin *et al*. 2010, 2013) no single approach can provide a definitive conclusion on species boundaries. So, molecular, distributional and morphological data must provide complementary evidences, and this defines the approach commonly known as Integrative Taxonomy. The presented morphological evidences to differentiate *R. obesa* and *R. gigantea* were corroborated by the molecular data. Considering this, the geographical distribution of both species must be revised. Beyond the type series of *R. gigantea* from Buenos Aires, Argentina (Vilela 1990), its occurrence is confirmed for southern Brazil (Bossoroca, Rio Grande do Sul) and firstly recorded in Central Brazil (São Domingos; Cerrado biome), its new northernmost locality. On the other hand, *R. obesa*, as defined here, is confirmed only for Rio de Janeiro (Lima 1935), Salobra, Mato Grosso do Sul (Malogolowkin 1946), Tangará da Serra, Mato Grosso and Porto de Galinhas, Pernambuco. Its occurrence in other localities previously mentioned in the literature needs further confirmation, and several of them may be, actually, its sibling *R. gigantea*, or even other sister cryptic species.

So, the problem related to the identity of *R. obesa* was not totally solved, since the holotype, from Texas, USA, has yet to be checked and compared to the Brazilian specimens; according to Vilela (1990) it is probably deposited in the Museum of Comparative Zoology at Harvard. However, the present study represents an important

advance in discriminating the two species occurring in Brazil, one probably *R. obesa* and the other certainly *R. gigantea*.

6.1.6. ACKNOWLEDGEMENTS

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6.1.7. REFERENCES

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6.1.8. FIGURES

Figure 1: Geopolitical map of South America with the points of recorded *R. gigantea* specimens: (1) Bossoroca, Rio Grande do Sul, (2) São Domingos, Goiás; and *R. obesa* specimens: (3) Tangará da Serra, Mato Grosso, (4) Porto de Galinhas, Pernambuco. (L) Rio de Janeiro, Lima's specimens; (M) Salobra, Mato Grosso do Sul, Malogolowkin's specimens.

Figure 2: Ordinary specimen of *R. obesa* determined by A. C. Lima (1935). a: general habitus, lateral view; b: general habitus, dorsal view; c: head, frontal view (scale bar 1.0 mm).

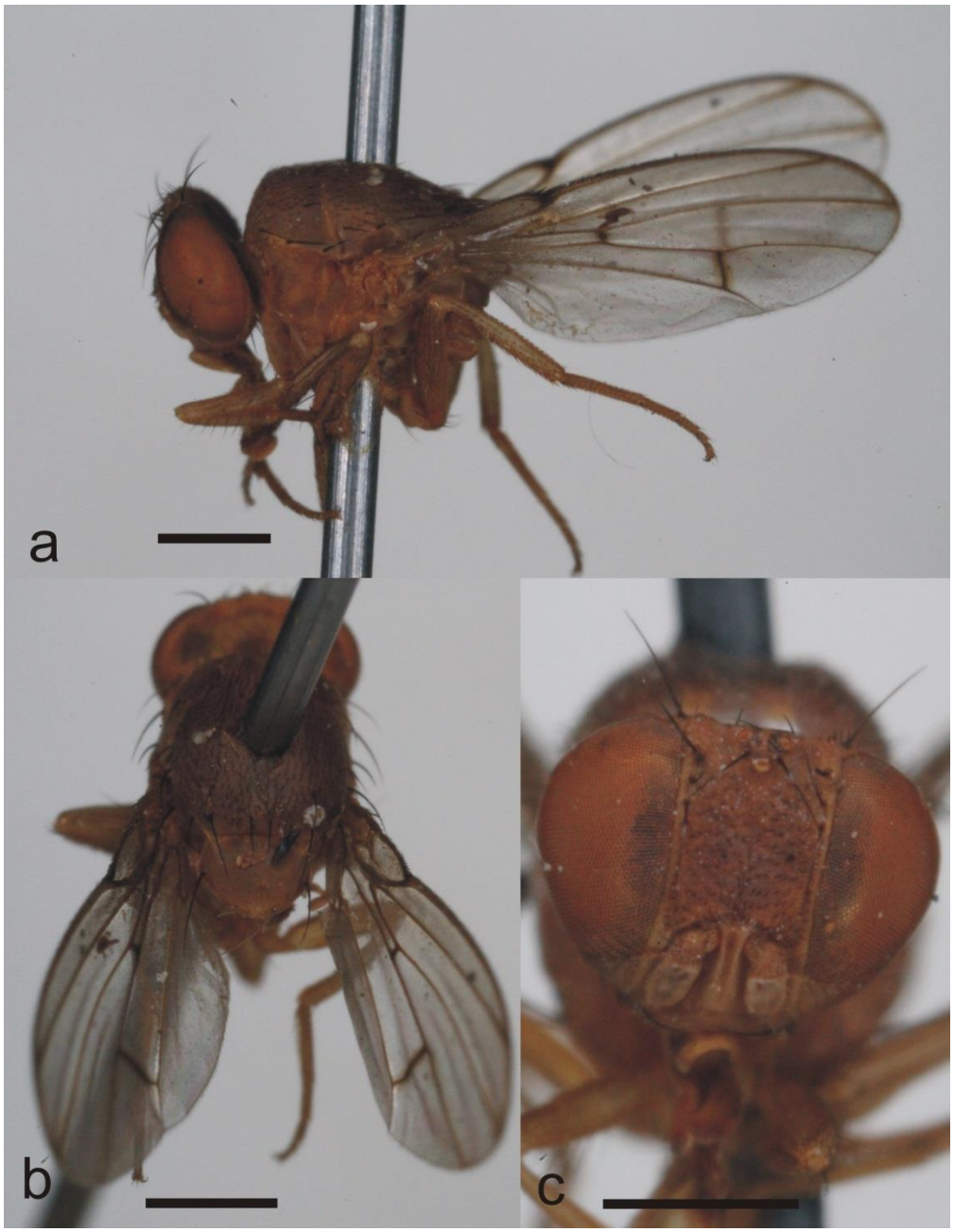


Figure 3: Ordinary specimen of *R. obesa* determined by C. Malogolowkin (1946). a: general habitus, lateral view; b: thorax, dorsal view; c: head, frontal view; d: abdomen, dorsal view (scale bar 1.0 mm, except in c: 0.5 mm).

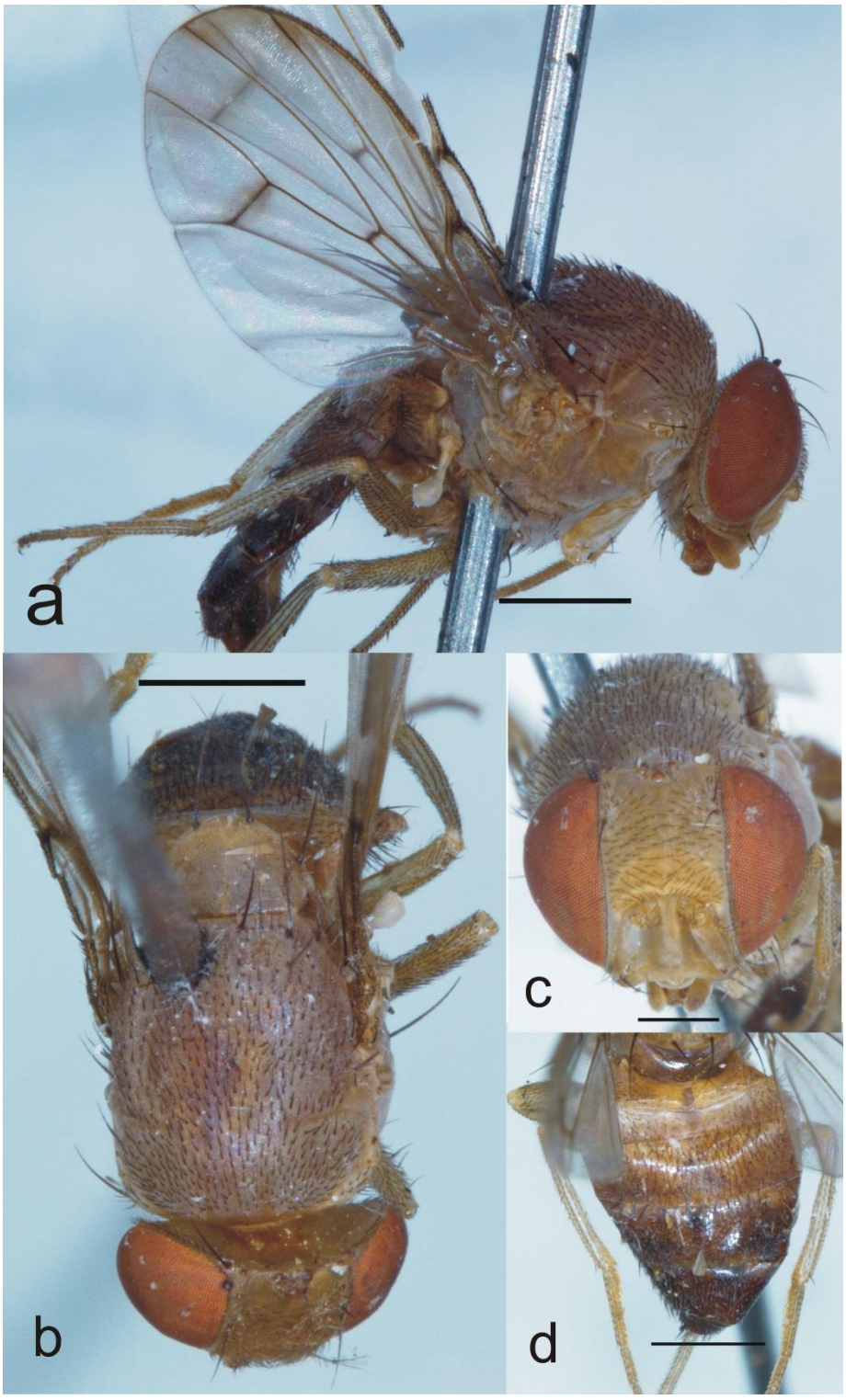


Figure 4: Male terminalia of an ordinary specimen of *R. obesa* (#03♂) determined by C. Malogolowkin (1946). a: aedeagus, dorsal view; b: aedeagus, lateral view; c: aedeagus, ventral view; d: epandrium (scale bar 0.1 mm).

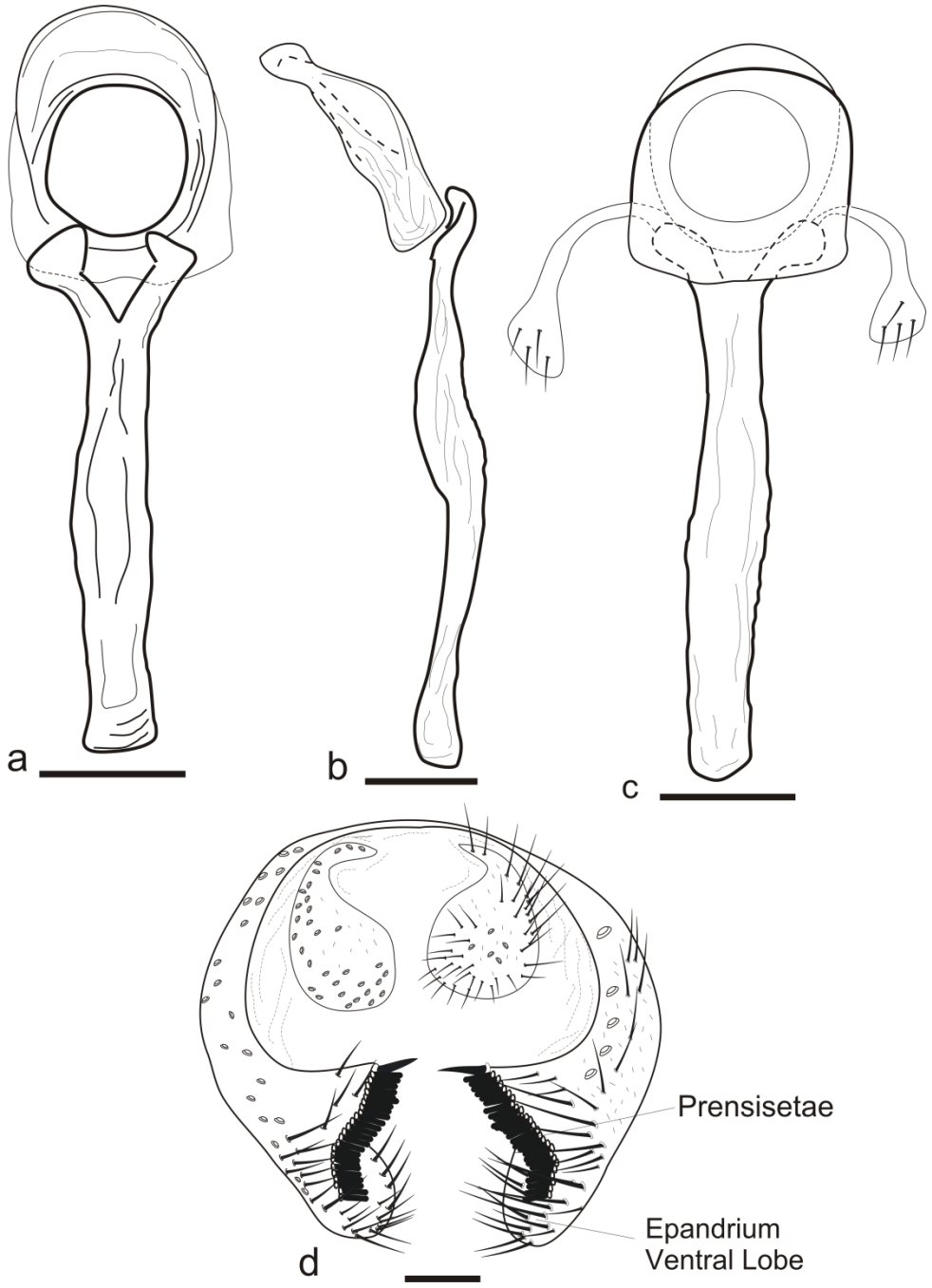


Figure 5: Female terminalia of an ordinary specimen of *R. obesa* (#09♀) determined by C. Malogolowkin (1946). a: dorsal view; b: ventral view; c: spermathecal capsule (scale bar 0.1 mm).

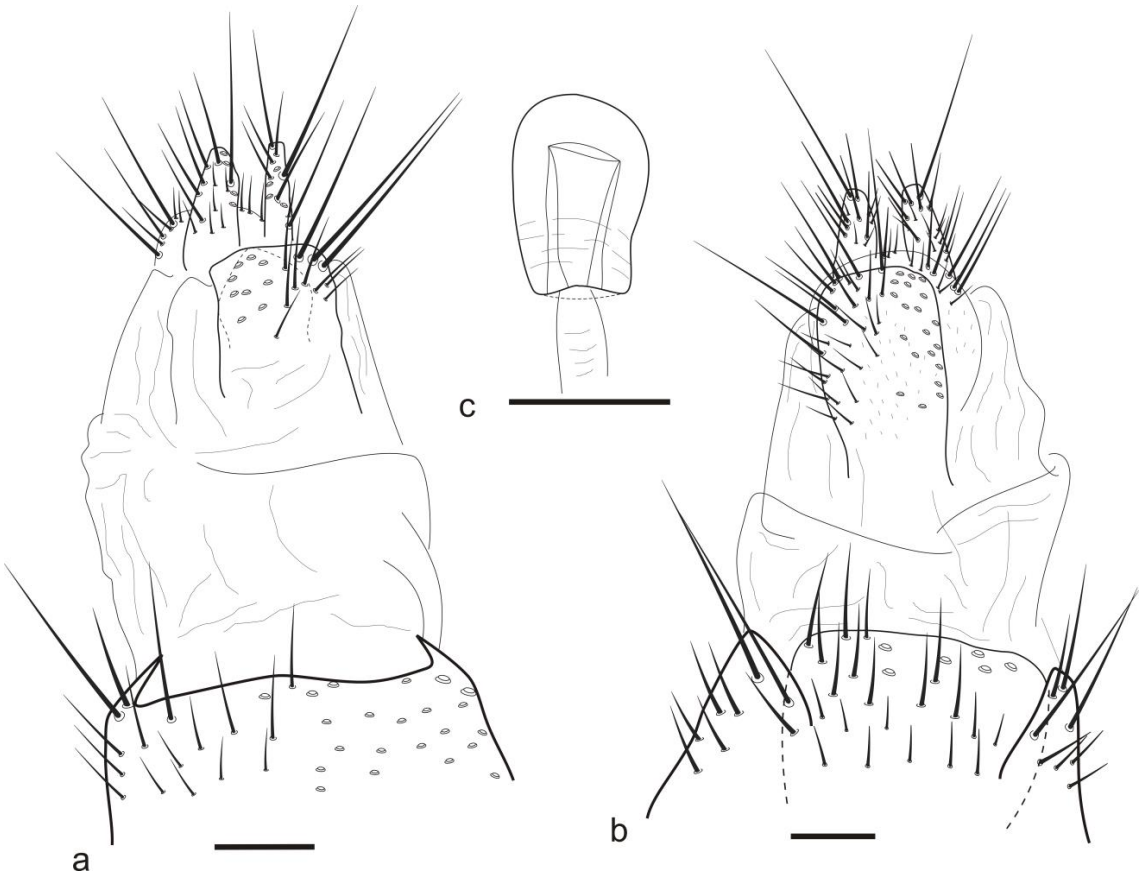


Figure 6: Ordinary specimen of *Rhinoleucophenga gigantea* from Bossoroca, Rio Grande do Sul, previously identified as *R. obesa* (Poppe et al. 2014). a: head, frontal view; b: thorax, dorsal view; c: abdomen, dorsal view; d: wing (scale bar 0.5 mm).

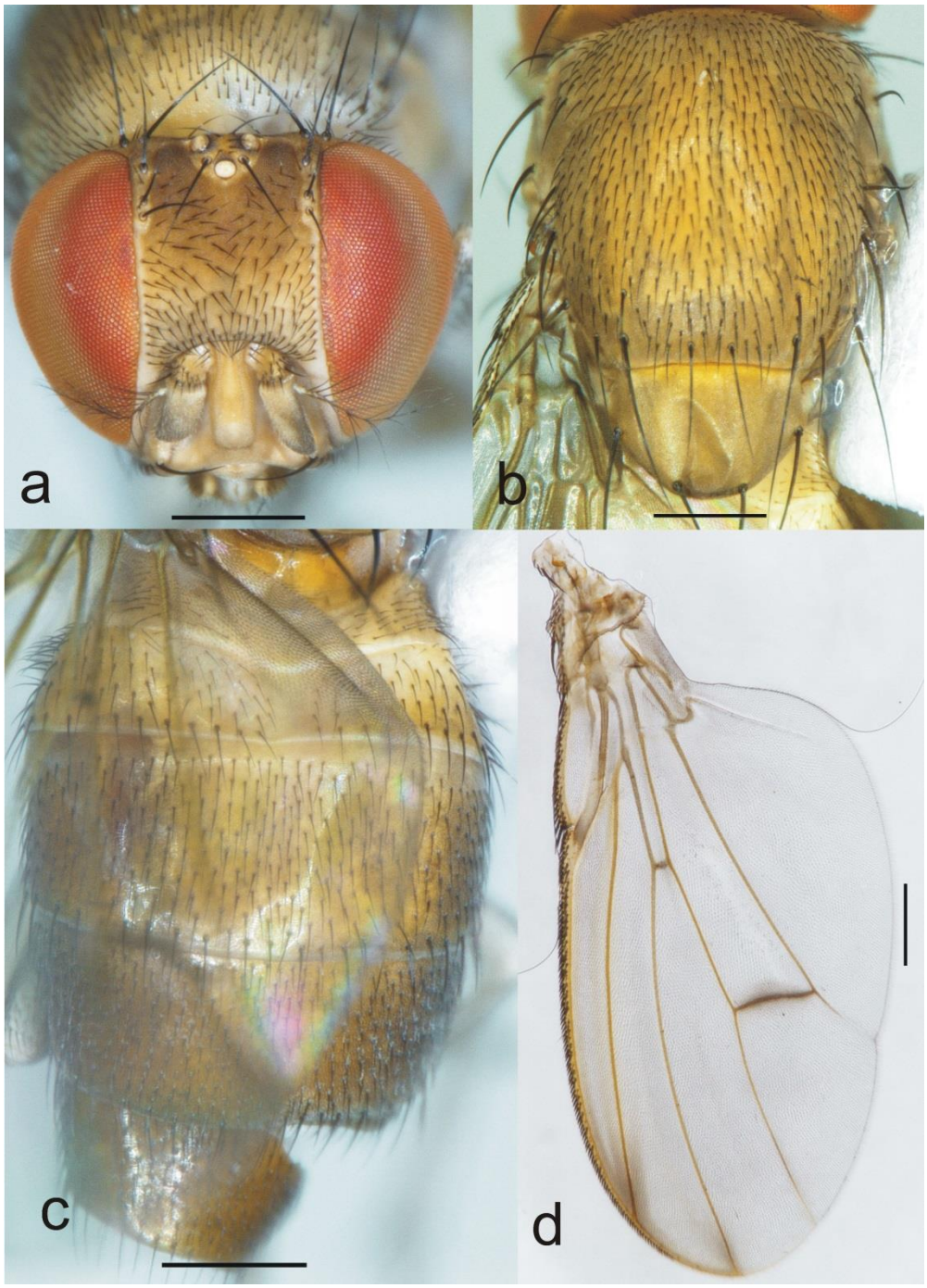


Figure 7: Ordinary specimen of *R. obesa* from Porto de Galinhas, Pernambuco. a: general habitus, dorsal view; b: general habitus, lateral view; c: wing; d: head, frontal view; e: abdomen, dorsal view (scale bar 1.0 mm).

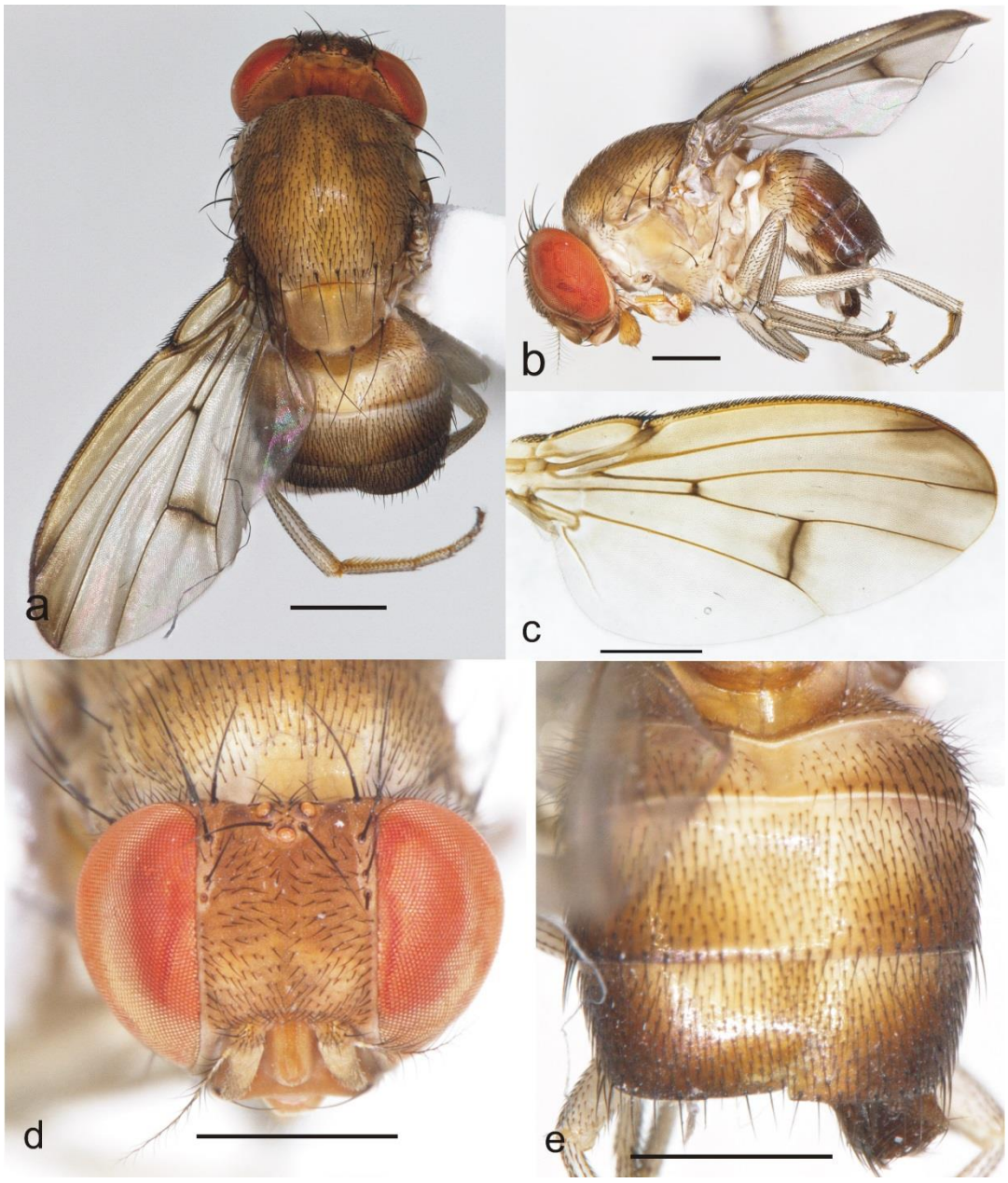


Figure 8: Epandrium of ordinary specimens of *Rhinoleucophenga*. a: *R. obesa* specimen from Porto de Galinhas, Pernambuco; b: *R. obesa* specimen determined by C. Malogolowkin (1946); c: Boss_spp_3; d: Boss_spp_2; e: Boss_spp_4; f: specimen from Bossoroca, Rio Grande do Sul. Arrows point to the different epandrium ventral lobe morphology among the specimens from Bossoroca. Note: here the specimens c-f were determined as *R. gigantea*.

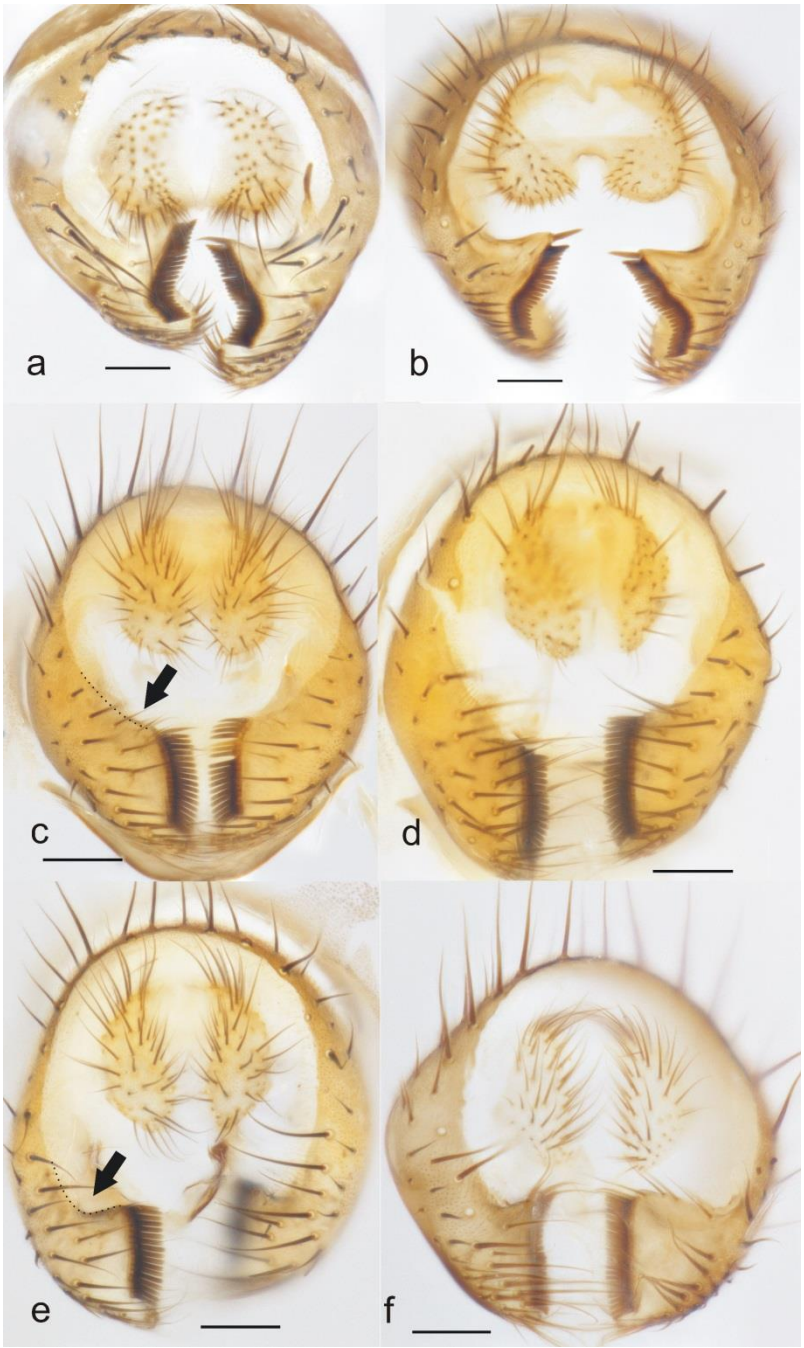


Figure 9: Male terminalia of an ordinary specimen of *R. obesa* from the Porto de Galinhas, Pernambuco. a: epandrium; b: aedeagus, ventral view (scale bar 0.1 mm).

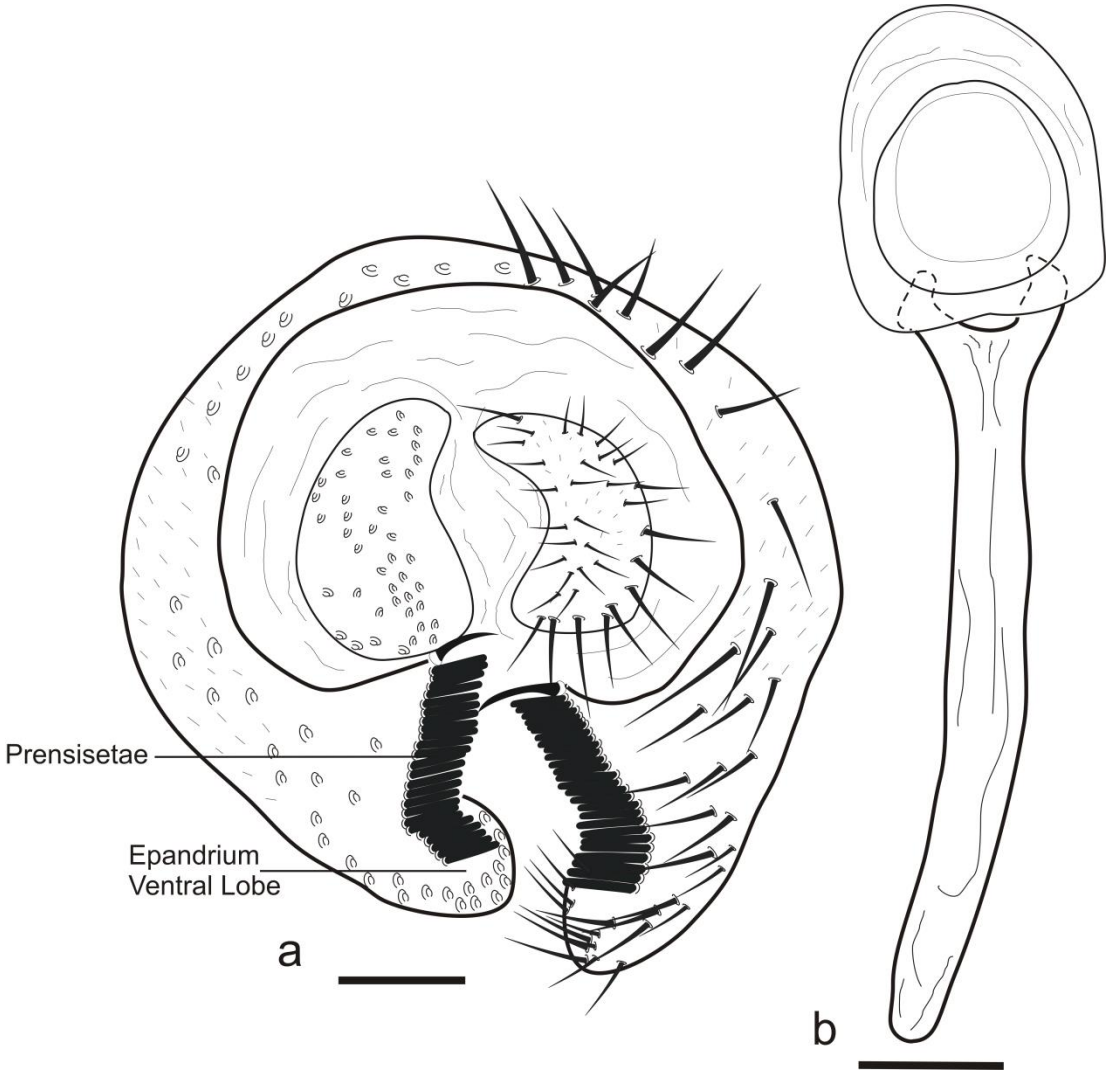


Figure 10: Male terminalia of an ordinary specimen of *Rhinoleucophenga gigantea* from Bossoroca, Rio Grande do Sul, previously identified as *R. obesa* (Poppe et al. 2014). a: aedeagus, dorsal view; b: aedeagus, ventral view; c: epandrium (scale bar 0.1 mm).

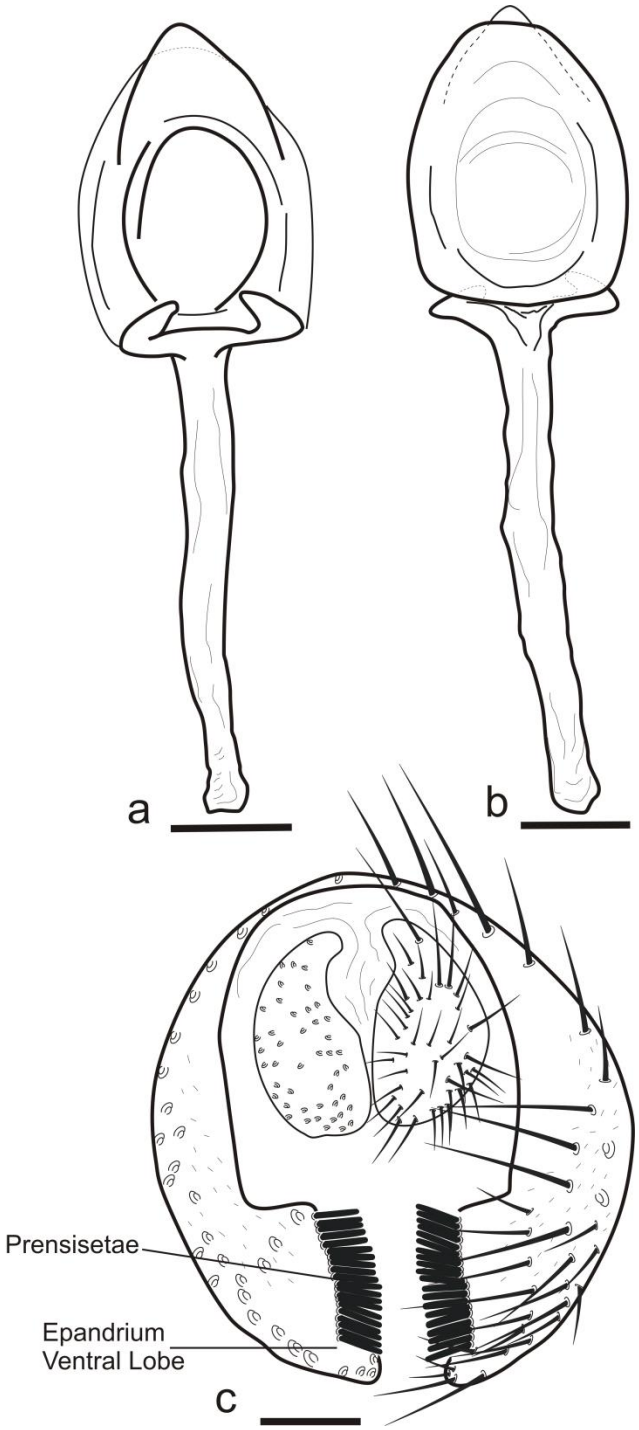


Figure 11: Female terminalia of an ordinary specimen of *R. gigantea* from São Domingos, Goiás. a: ventral view; b: spermathecal capsule; c: dorsal view (scale bar 0.1 mm).

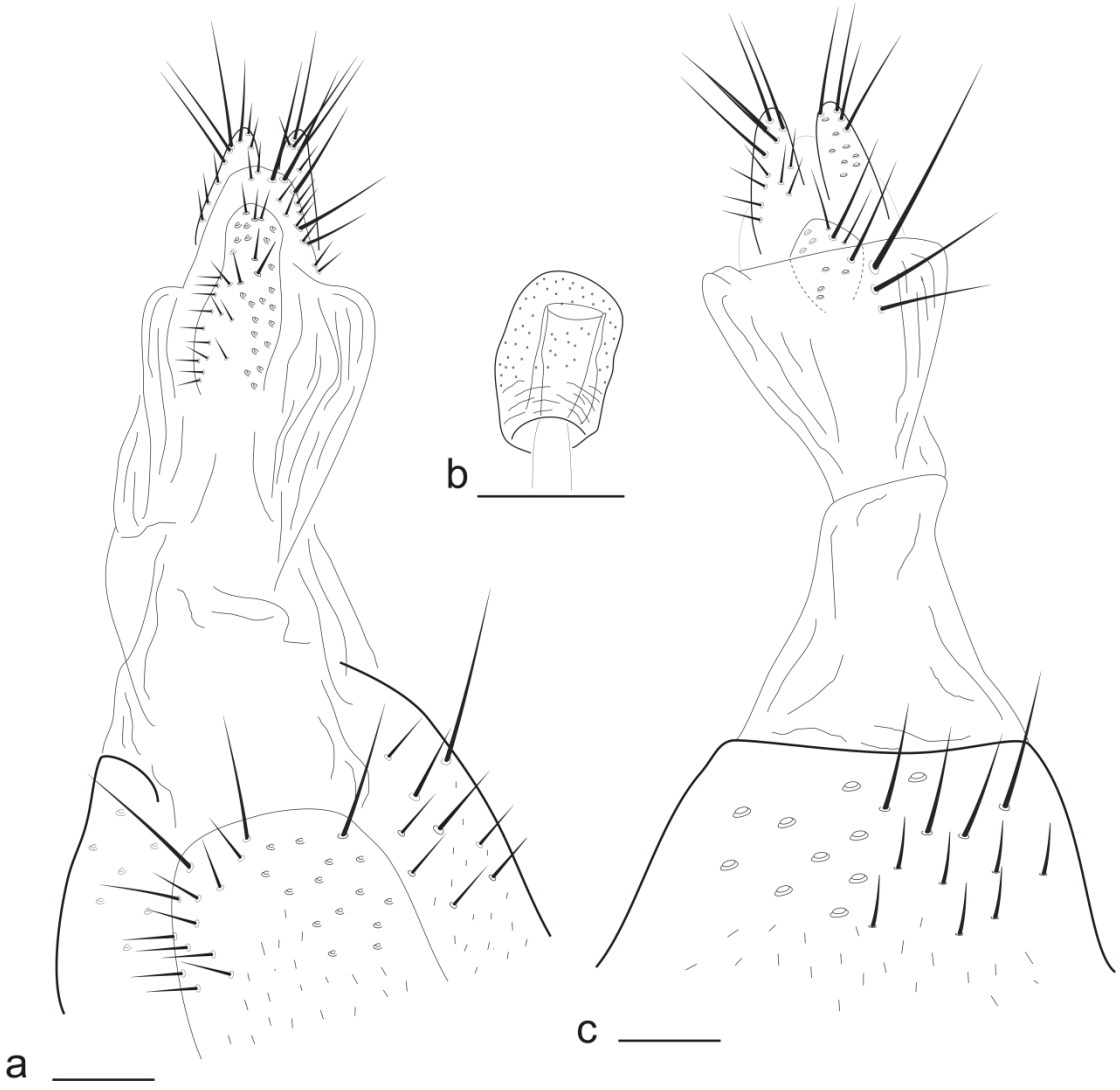


Figure 12: Female terminalia of an ordinary specimen of *Rhinoleucophenga gigantea* from Bossoroca, Rio Grande do Sul, previously determined as *R. obesa* (Poppe et al. 2014). a: dorsal view; b: spermathecal capsule; c: ventral view (scale bar 0.1 mm).

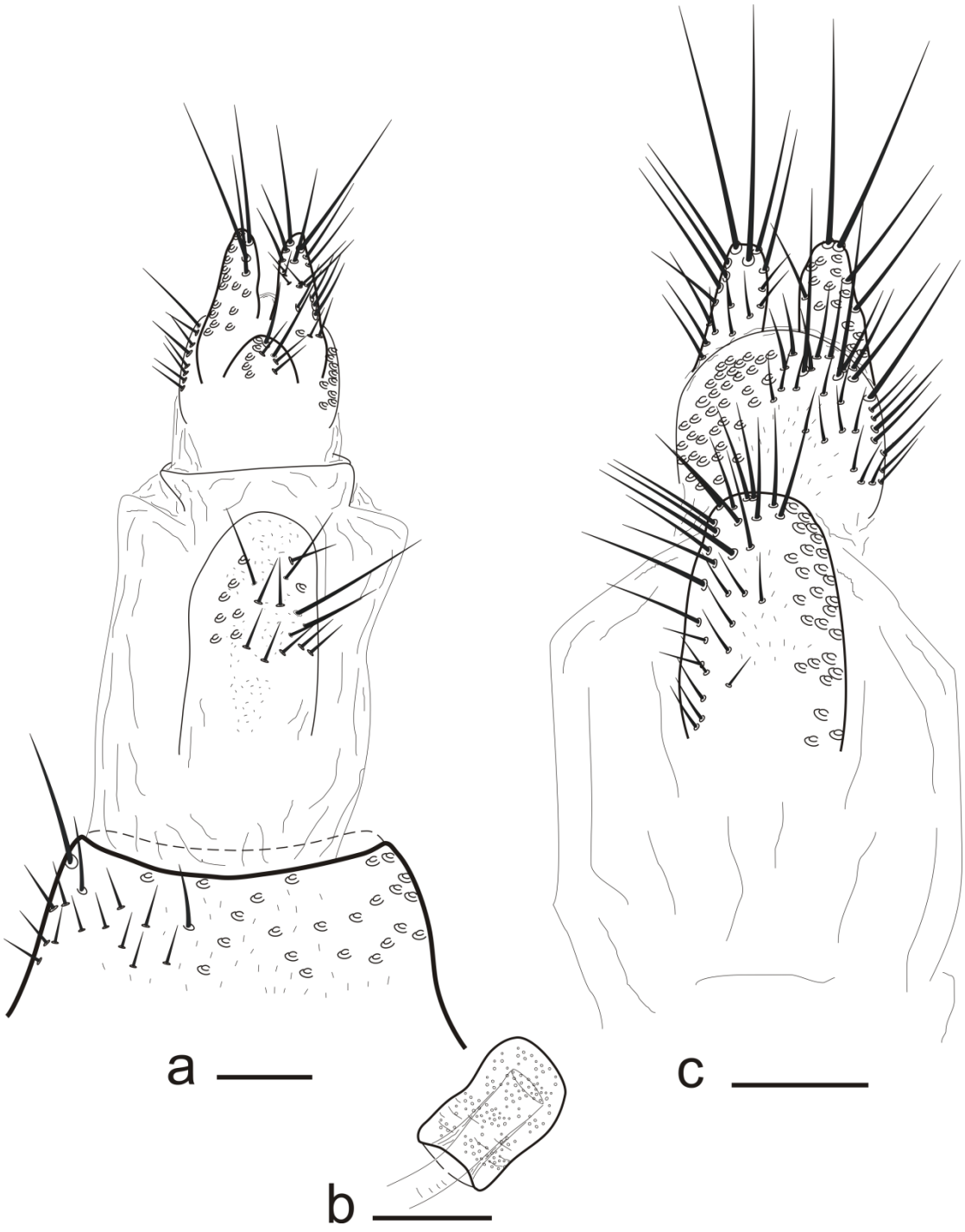


Figure 13: Female terminalia of an ordinary specimen of *R. obesa* from Porto de Galinhas, Pernambuco. a: ventral view; b: spermathecal capsule; c: dorsal view (scale bar 0.1 mm).

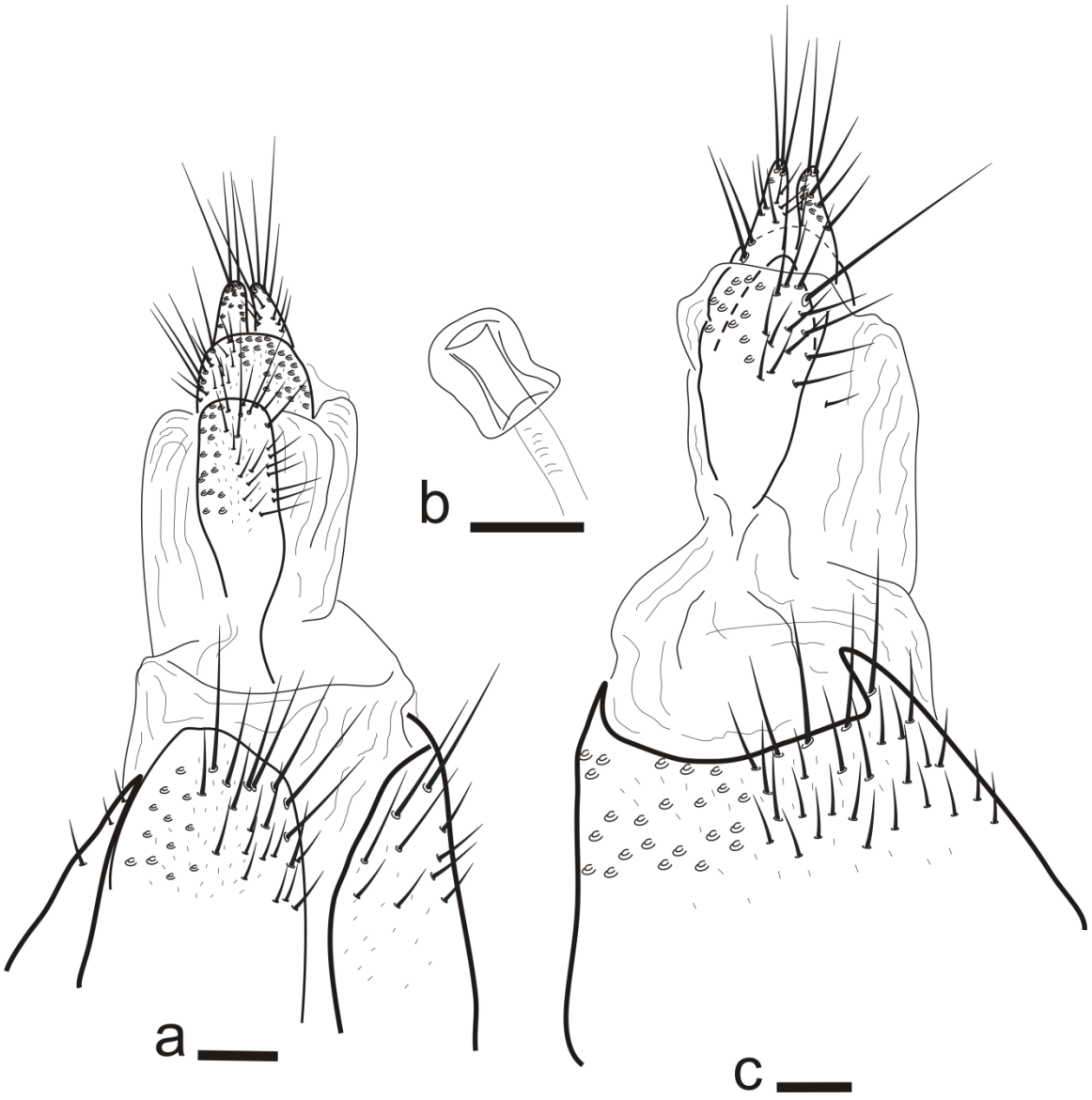


Figure 14: Neighbour-joining consensus tree obtained from a 847-bp alignment of *cytochrome c oxidase subunit I* (COI) gene sequences of *Rhinoleucophenga* specimens. Numbers at nodes represent support values (10,000 bootstrap replications).

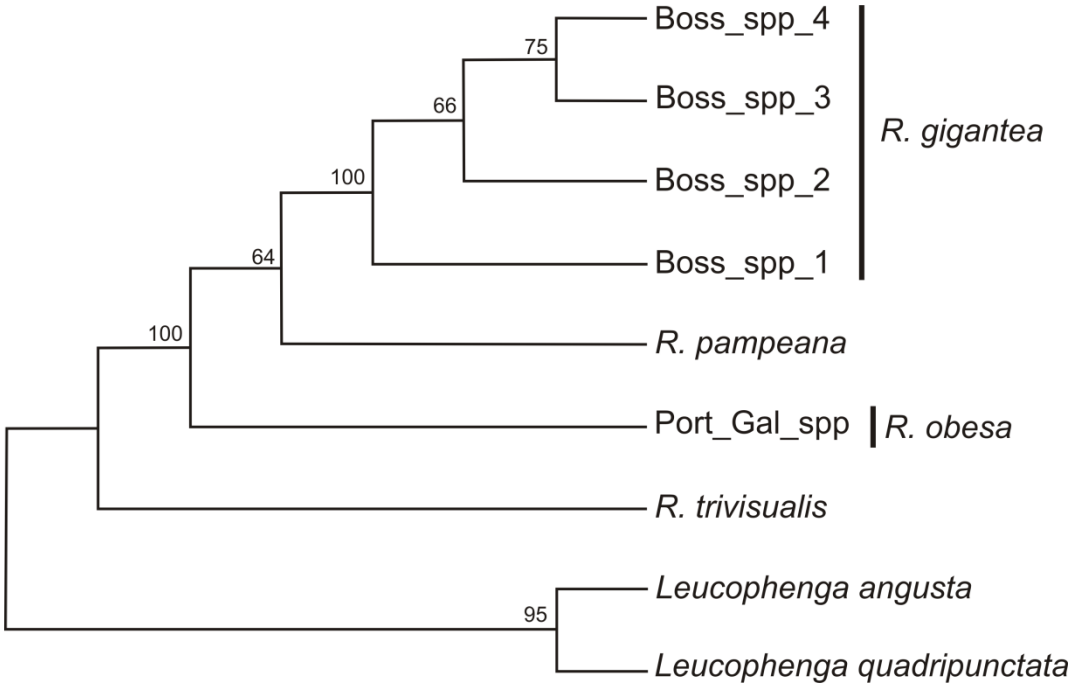
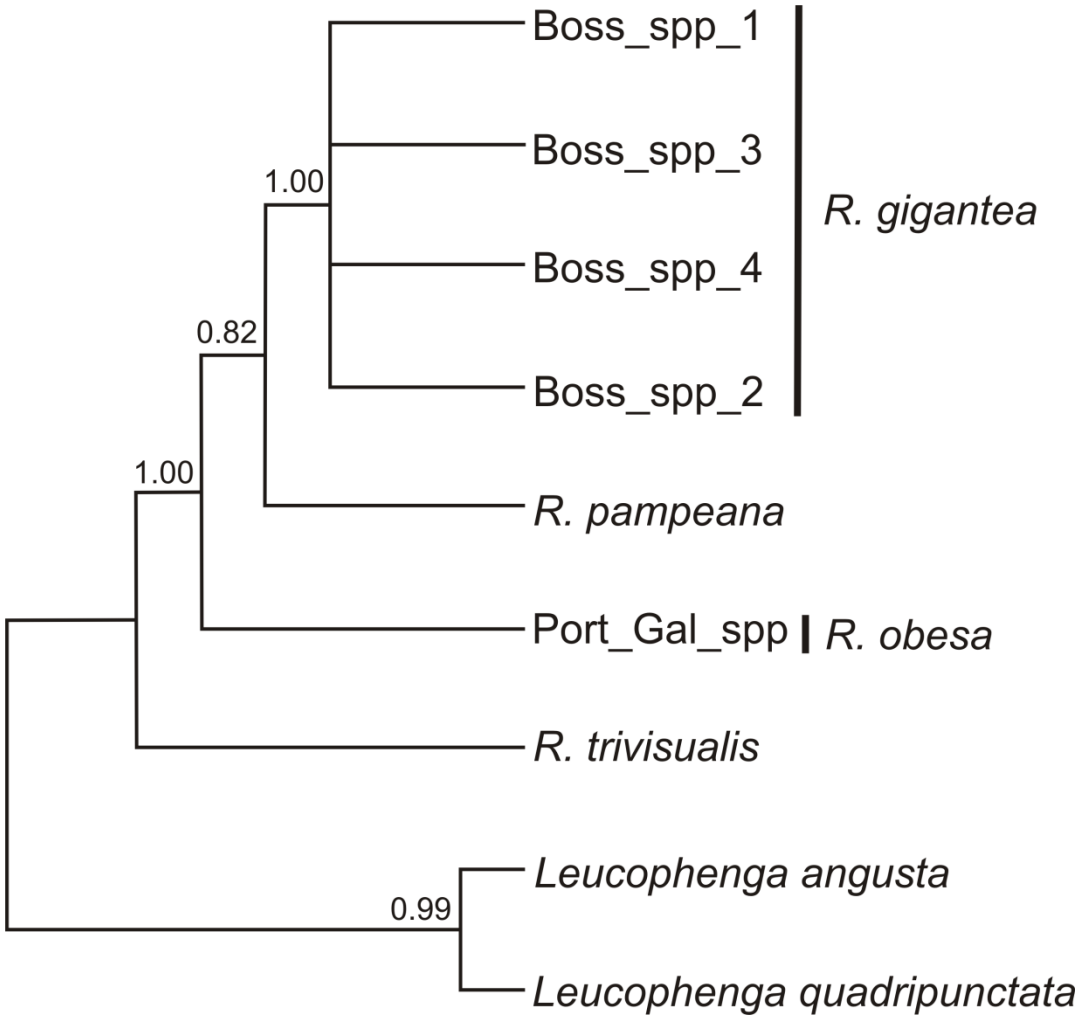


Figure 15: Consensus tree obtained from a 847-bp alignment of *cytochrome c oxidase subunit I* (COI) gene sequences of *Rhinoleucophenga* specimens by using Bayesian inference. Numbers at nodes represent posterior probabilities values (1,000,000 generations).



6.1.9. TABLES

Table 1: Collecting sites data and GenBank accession numbers to the gene sequences of *cytochrome c oxidase* subunit *I* (COI) of *Rhinoleucophenga* sibling specimens used in the phylogenetic analysis. Boss_spp: specimen of *R. gigantea* from Bossoroca; Port_Gal_spp: specimen of *R. obesa* from Porto de Galinhas.

Specimens	Collection site	Latitude (S)	Longitude (W)	GenBank accession numbers
Boss_spp_1	Bossoroca, Rio Grande do Sul, Brazil	28°45'01"	54°56'55"	KU728955
Boss_spp_2	Bossoroca, Rio Grande do Sul, Brazil	28°45'01"	54°56'55"	KU728956
Boss_spp_3	Bossoroca, Rio Grande do Sul, Brazil	28°45'01"	54°56'55"	KU728957
Boss_spp_4	Bossoroca, Rio Grande do Sul, Brazil	28°45'01"	54°56'55"	KU728958
Port_Gal_spp	Porto de Galinhas, Pernambuco, Brazil	8°30'30"	35°0'20"	KU728954

Table 2: Measures and indices of the *Rhinoleucophenga obesa* specimens described by A. C. Lima (1935) and C. Malogolowkin (1946). Indices according to Bächli *et al*. (2004). *: measures in millimeters (mm); -: measures not available.

	Species originally described by A. Costa Lima in 1935			Species originally described by S. Malogolowkin in 1946								
HEAD	*R. obesa* ♂ Tube #1085/2116-2419	*R. obesa* ♀ Tube #1086/2116-2419	*R. obesa* wing #2365	*R. obesa* #01 ♂	*R. obesa* #02 ♀	*R. obesa* #03 ♂	*R. obesa* #04	*R. obesa* #05	*R. obesa* #06	*R. obesa* #07 ♂	*R. obesa* #08	*R. obesa* #09 ♀
Frontal length *	0.93	0.84	-	1.00	0.88	0.86	0.70	0.96	0.92	-	0.94	0.86
Frontal index	2.00	1.75	-	1.82	1.82	1.87	1.91	1.74	1.73	-	1.65	1.75
Top-to-bottom frontal width ratio	1.08	1.00	-	0.95	1.00	1.00	1.00	0.97	1.03	-	0.97	0.99
Ocellar triangle to front length ratio	0.32	0.26	-	0.26	0.30	0.33	0.31	0.29	0.24	-	0.26	0.26
Setae or1/or3 ratio	1.17	1.09	-	-	0.95	-	-	-	-	-	-	-
Setae or2/or1 ratio	0.57	0.50	-	-	0.53	-	-	-	0.54	-	-	-
Vibrissal index	0.39	0.45	-	0.24	0.34	-	0.35	0.30	0.34	-	0.29	0.41
Cheek index	11.33	10.50	-	10.00	10.00	10.67	10.60	11.17	11.09	-	9.71	10.91
Eye index	1.51	1.34	-	1.43	1.36	1.45	1.36	1.46	1.53	-	1.51	1.50
THORAX												
Thorax length*	3.06	2.91	-	2.63	3.00	2.72	2.41	3.03	2.50	2.66	2.67	2.73

Strongest prescutellar acrostichal setae. % length related to posterior dorsocentral setae	61	67	-	62	60	63	-	71	66	67	67	65
Transverse distance between dorsocentral setae, related to longitudinal distance	4.36X	4.50X	-	3.92X	4.44X	4.40X	-	4.60X	-	4.00X	4.57X	3.80X
Sterno index	0.92	0.93	-	-	1.00	0.97	1.00	-	-	0.95	-	0.94
WING												
Length*	4.65	4.50	4.10	4.65	4.00	4.25	-	4.50	4.00	4.30	4.40	4.00
Width*	2.00	2.10	2.10	2.15	1.90	1.90	1.80	2.10	1.85	1.90	2.20	1.90
WING INDICES												
C	3.38	3.33	3.36	3.44	2.96	3.05	3.12	3.15	3.46	3.42	3.46	3.26
Hb	0.38	0.37	0.43	0.43	0.45	0.39	0.40	0.44	0.38	0.45	0.41	0.36
Ac	1.21	1.14	1.21	1.25	1.27	1.32	1.36	1.24	1.17	1.19	1.18	1.33
4c	0.73	0.78	0.71	0.75	0.83	0.82	0.79	0.79	0.72	0.68	0.68	0.73
4v	1.31	1.48	1.53	1.43	1.46	1.36	1.49	1.42	1.37	1.23	1.33	1.31
5x	0.81	1.00	1.08	0.90	0.92	0.88	0.91	0.86	0.81	0.88	0.94	0.91
M	0.38	0.52	0.53	0.49	0.48	0.44	0.49	0.47	0.4	0.39	0.44	0.43
Prox.x	0.73	0.90	0.71	0.85	0.87	0.84	0.70	0.92	0.76	0.71	0.75	0.84
Body length*	-	5.00	-	5.00	4.80	5.50	-	5.00	5.00	5.00	-	4.90

Table 3: Pairwise genetic divergence (Kimura two-parameter) among specimens of *Rhinoleucophenga* from Bossoroca (Boss_spp_1-4) and Porto de Galinhas (Port_Gal_spp) using *cytochrome c oxidase* subunit *I* (COI) gene sequences.

	Specimens	1	2	3	4
1	Boss_spp_1				
2	Boss_spp_2	0.49%			
3	Boss_spp_3	0.37%	0.12%		
4	Boss_spp_4	0.37%	0.12%	0.00%	
5	Port_Gal_spp	4.78%	4.52%	4.52%	4.52%

7. CAPÍTULO VII

(Manuscrito para submissão ao periódico *Entomological Science*)

7.1. Latitudinal variation in *Rhinoleucophenga punctulata* populations (Diptera, Drosophilidae) from South America: combined analyses of morphological and molecular data

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7.1.1. ABSTRACT

Despite the fact that Drosophilidae is a very diverse and well-studied taxon, the New World genus *Rhinoleucophenga* is yet poorly understood even in regard to species distribution and morphological variability pattern. In this sense, *R. punctulata* is a dot-spotted thorax species widely distributed in the Neotropical region. Specimens of *R. punctulata* were collected in different biomes in Brazil: Pampa, Cerrado and Caatinga *sensu strictu*, and in a southern Amazonian savannah enclave area. Geographical variations in the body external morphology and in the morphology of spermathecal capsules were noticed among the different populations. The hypothesis that each population could be a different species was tested through molecular data. A fragment of the mitochondrial *Cytochrome oxidase* subunit *I* (*COI*) gene was sequenced to perform phylogenetic analyses through Neighbor-joining and Bayesian inferences.

Pairwise genetic divergences of *COI* sequences were calculated using DNA barcode premises. The analyzed populations presented different variation levels, considering both, morphology and molecular traits, which could be linked to environmental characteristics of each biome. However, new species were not proposed because the intrapopulations nucleotide variations exceed the interpopulations ones. Based on the phylogenetic analyses, it is suggested that *R. punctulata* originated in Caatinga and after spread into the Cerrado and Amazon areas, and subsequently to the Pampa. So, morphological and molecular data are complementary and indispensable to understand the biological diversity and the distribution of *R. punctulata* through the Neotropical environments. Furthermore, this is the first study to raise the hypothesis that environmental characteristics, such as climate heterogeneity, can affect the reproductive traits of *Rhinoleucophenga* species.

Keywords: *Drosophila*; Neotropical; spermathecal capsules; Steganinae.

7.1.2. INTRODUCTION

Drosophilidae is a highly diverse taxon that has been investigated by morphologists since long time (Sturtevant 1921; Bächli *et al*. 2004). Reproductive organs of males and females are used by drosophilists as the main diagnostic characters to differentiate sibling species (Bächli *et al*. 2004). Nevertheless, in the last years, the advances in molecular biology results have represented a complementary data set to morphological data, improving the species boundaries and definition (Hebert *et al*. 2003a, 2003b; Yassin *et al*. 2010).

Most of the studies performed with Drosophilidae are related to species of *Drosophila*, and most of other genera lack general knowledge. This is the casefor the New World genus *Rhinoleucophenga*, whose species distribution and morphological variability is yet poorly studied (Poppe *et al*. 2015a). Species distributional data is fundamental to understand how new species come into existence, once different environmental conditions can represent interbreeding barriers between populations and promote genetic modifications into new phenotype records (Pitnick *et al*. 1999; Carreira *et al*. 2013).

One of the most widespread species of *Rhinoleucophenga* is *R. punctulata* Duda which is broadly found in open environments of South America (Vilela & Bächli 2009;

Roque & Tidon 2013; Poppe *et al*. 2014), ranging from subtropical-temperate to tropical climates, but apparently absent or rare in forest biomes. However, recently Poppe *et al*. (2015a) described *R. punctuloides* Poppe, Schmitz and Valente, a sibling species to *R. punctulata* recorded in the Pampa and Cerrado biomes, triggering the need of reviewing the geographical records of the last species.

Considering the recent discovery of a sibling species, we analyzed specimens of *R. punctulata* from different geographic localities and found striking morphological differences in the female spermathecal capsules among populations. So, the hypothesis that each population could be a different species was tested through molecular data. However, we show here that the differences are better explained by intraspecific variation and the hypothesis that environmental characteristics, such as climate heterogeneity, may be affecting the reproductive traits of *Rhinoleucophenga* species was proposed.

7.1.3. MATERIAL AND METHODS

Sampling areas

Specimens of *Rhinoleucophenga punctulata* were collected in banana-baited traps (Tidon & Sene 1988) in different latitudes through the Brazilian *sensu strictu* biomes: Pampa, municipality of Bossoroca, Rio Grande do Sul (28°45’01”S 54°56’55”W, 200 m); Caatinga, Raso da Catarina Ecological Station, Bahia (9°33'39"S 38°44'12"W, 500 m); Cerrado, Parque Nacional das Emas, Goiás (18º15’S 52º53’W, 600 m – Roque & Tidon 2008) and municipality of Tangará da Serra, Mato Grosso (14°04’38”S 57°03’45”W, 500 m); and in a southern Amazonian savanna enclave (6°13’36”S 48°27’55”W, 300 m), in the Parque Estadual Serra das Andorinhas, municipality of São Geraldo do Araguaia, Pará (Fig. 1). All areas consist predominantly of open vegetation with a mosaic of forest patches compounded mainly by typical arboreal species of each region.

Climatically, the Caatinga area is characterized as an arid environment, with annual temperatures higher than 18°C and low rainfall levels (ca. 500 mm) (Paes & Dias 2008). Cerrado presents wet summers (ca. 1,500 mm) and dry winter periods, with annual temperatures higher than 18°C (Tidon 2006; Przybylska *et al*. 2014). Pampa biome presents huge oscillations of temperatures, which range from negative values

during the winter up to 40°C during the summer, with rainfall well distributed along the year (ca. 1,300 mm) (Poppe *et al*. 2014; Pillar & Lange 2015). The Amazon region presents constant high humidity levels regulated by rainfall levels higher than 1,500 mm, distributed along the year, and average annual temperatures higher than 18°C (Köppen 1931).

Specimens Morphology Analysis

Female descriptions are based on measures and indices given by Bächli *et al*. (2004), at least five specimens from each locality were measured. Female terminalia and spermathecal capsules were disarticulated in glycerol after treatment with 10% potassium hydroxide (KOH) and acid fuchsine (Bächli *et al*. 2004). The structures were mounted in a piece of glycerine jelly (ca. 2 x 2 x 2 mm) on a clean slide (Grimaldi 1987). Photos of the specimens were taken with a digital camera coupled to an optical stereomicroscope after the specimens were dried with hexamethyldisilazane (HMDS) (Brown 1993) and pinned. Drawings of the terminalia and spermathecal capsule were made with a camera lucida system attached to a compound microscope with 40× and 10× objective lenses and a 10× ocular lens. The terminology follows Vilela and Bächli (1990) and Bächli *et al*. (2004). All examined specimens are deposited in the Entomological Collection of the Instituto Oswaldo Cruz (CEIOC), at Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil. The disarticulated terminalias are stored in microvials with glycerol and pinned with the respective specimens.

A total of 45 female specimens had their spermathecal capsules analyzed to confirm the morphological pattern of each population: 15 from Caatinga, 15 from Amazon, 10 from Cerrado and 5 from Pampa. A comparative study of the males of the same populations is not shown; since the number of specimens available was small and no consistent pattern of variation was observed.

DNA Extraction and PCR Amplification

Total DNA of individual fly preserved in 70% ethanol was extracted according to the DNeasy Blood & Tissue Kit (Qiagen) instructions. PCR amplifications were performed using the TY-J-1460 and C1-N-2329 primer pair (Simon *et al*. 1994), which amplifies a fragment of approximately 850-base pair (bp) of the mitochondrial

Cytochrome oxidase subunit *I* (*COI)* gene. The PCR reactions were carried out in volumes of 10μL, using 20 ng of DNA, 2.5 mM $MgCl_2$, 1X PCR buffer reaction, 200 mM of each dNTPs, 20 pM of each primer and 1 U of Taq DNA polymerase. The reactions conditions were 95°C for 5 minutes, followed by 35 cycles of 95°C for 45 seconds, 53°C for 45 seconds and 72°C for 1 minute, finishing with a final extension at 72°C for 5 minutes.

The amplicons were purified with Exonuclease I (10U/μl) and Shrimp Alkaline Phosphatase (1U/μl) and sent to a sequencing service (www.macrogen.com). Each sample was sequenced in both, the forward and reverse directions using the same primers as those used in amplification. The obtained accession numbers of the sequences, as well as the specimens used, are shown in Table 1.

Sequence Analysis

The obtained sequences were assembled and edited using the Staden Package (Staden 1996). Consensus sequences were aligned using the Clustal W algorithm, implemented with Mega 6 (Tamura *et al*. 2013). The final alignment of the *COI* data set was verified against published *COI* sequences of other Drosophilidae species available in GenBank. The authenticity of the produced mtDNA sequences was verified by using an on-line protein translator system available at http://web.expasy.org/translate/. The nucleotide substitution saturation of the sequences was accessed using the Xia's method in DAMBE 5 (Xia 2013) software.

Pairwise genetic divergences of *COI* sequences were calculated using the Kimura two-parameter (K2P) model in Mega 6 (Tamura *et al*. 2013), with 10,000 bootstrap replicates, as suggested by Hebert *et al*. (2003a) for DNA barcoding. This approach was employed to test our previous hypothesis that populations of the *R. punctulata* from each environment could represent different species.

Phylogenetic Analyses

To the phylogenetic analyses, the final alignment consisted of 23 sequences. Beyond those from *R. punctulata* populations, which encompassed 15 sequences, we generated and included three sequences from *R. trivisualis* Poppe, Schmitz and Valente, two from *R. punctuloides* and one from *R. obesa* Loew. The first two species were

included since they are also dot-spotted thorax species, and *R. punctuloides* is sibling to *R. punctulata*. As outgroup, *COI* sequences from *Leucophenga angusta* Okada and *L. quadripunctata* (de Meijere) were included in the analyses (GenBank accession number HQ842780.1 and HQ842781.1).

Phylogenetic analyses were conducted using neighbor-joining (NJ) in Mega 6 (Tamura *et al*. 2013) and Bayesian inference (BI) in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). For NJ analyses, we used the model of nucleotide substitution suggested by Hebert *et al*. (2003a) for DNA barcoding, with 10,000 bootstrap replicates. For Bayesian phylogenetic analysis, the GTR+I+G model was used as suggest by jModelTest2 (Darriba *et al*. 2012) in the analysis of the best fitting substitution model for the *COI* alignment. Posterior distributions of parameters, including tree topology and branch lengths, were estimated using Markov chain Monte Carlo sampling. Samples from the posterior distribution were drawn every 1,000 generations over a total of 1,000,000 generations. The first 25% of samples were discarded as burn-in.

7.1.4. RESULTS

Morphological comparison among females of *R. punctulata* populations

Based on the external morphology, *R. punctulata* from Cerrado detaches from the others mainly by its darker thorax color and smaller body size, whereas populations from Pampa and Amazon are more similar, followed by the Caatinga one (Fig 2; Table 2). In general, the females specimens present the following morphological characters:
The female specimens present the following morphological characters:

Head (Fig 2 a-d). Front homogeneously brownish, covered with ca. 28 scattered interfrontal setulae, except to the Cerrado specimens (ca. 22 setulae); ocellar triangle brownish with brown ocelli. Each orbital setae with a brown patch around the base. Carina nose-like and ca. 75% sulcated. Face and gena brownish; antenna with flagellomere homogeneously brown and pedicel brownish; arista with 6 long dorsal branches and 4 long ventral branches plus terminal fork. Palpus yellow with ca. 20 setae on lower part, except to the Cerrado specimens (ca. 15 setae).

Thorax (Fig 2e-h). Scutum brown, except to the Cerrado specimens (brown-grayish), covered with many small dark brown spots at bases of setae and setulae, two

diffuse longitudinal dark brown stripes. Scutellum brown with four lateral dark brown spots and also an elongated central-posterior spot. Six irregular rows of acrostichal setulae. Two pairs of prescutellar acrostichal setae, the central one is the longest. Pleura brownish with longitudinal dark brown stripe; legs yellow; halters whitish. Hyaline wings (Fig 2m-p).

Abdomen (Fig 2i-l) with yellow ground color, tergite II with a dark brown stripe widely interrupted medially, tergites III to VI each with a broad, dark brown stripe which is medially interrupted and laterally broadened; the stripes are gradually enlarged towards tip of abdomen.

In addition to external morphology variation, we observed that the main difference among the populations is related to the females' spermathecal capsules (Fig 3a-d). Female spermathecal capsules are most elongated in the Amazonian population (Fig 3a). The Caatinga population presents the most reduced, rounded spermathecal capsules (Fig. 3b). Females from Pampa and Cerrado biomes presented similar spermathecal capsules (Fig. 3c and d, respectively) with intermediary dimensions between Amazon and Caatinga ones (Table 2).

The sampled specimens from Caatinga, Amazon and Pampa did not presented differences in the body size (2.7-3.0 mm). For more measures and indices see Table 2.

Molecular comparison among *R. punctulata* populations

To the genetic divergence analysis, we generated 15 sequences from different *R. punctulata* populations (seven specimens of *R. punctulata* from Caatinga, three from Cerrado, three from Pampa and two from Amazon). In 847-bp of the *COI* gene, no indel or premature stop codon was detected. These sequences contained 63 variable sites (7.43%), from which 29 (3.42%) were parsimony informative; the sequences were not saturated.

The K2P mean divergence value observed among all *R. punctulata* specimens was 1.6%. Comparing the sequence divergence between the four populations, the average value was 0.7-1.0% among the Amazon, Cerrado and Pampa population, whereas the variation raised to 2.0-2.4% among the Caatinga and the other populations (Table 3). Though, when we analyze the intrapopulationals nucleotide variation, we observed that the divergence ranged from none (0% in Amazon) to a maximum of 2.6%

within the Caatinga population (Table 3). In the pairwise comparisons (Appendix S1), the Caatinga population showed to be the most divergent one, mainly comparing to the specimens from the Pampa. Populations from Cerrado and Amazon are less divergent between each other, while population from Pampa is more similar to the Amazon one, followed by the Cerrado population (Appendix S1).

As concerns the phylogenetic analyses, trees produced by the BI and NJ methods presented similar topologies (Fig. 4 and 5, respectively). The main difference was in the clades support values, which were higher in the BI analysis (Fig. 4). Equally, a clade exclusively with *R. punctulata* specimens from Caatinga was highly supported in both phylogenetic searches; however, one specimen from Caatinga grouped in a separated clade with a specimen from Cerrado. The individuals from Cerrado, Pampa and Amazon intermingled in the other supported clades. As well as, *R. punctulata* was a sister clade closest to *R. punctuloides*, a sibling species recorded in the Pampa and Cerrado. So, these results corroborate those of the spermathecal capsule morphology, but it indicates an intraspecific variation among *R. punctulata* populations.

7.1.5. DISCUSSION

Rhinoleucophenga punctulata was described by Duda (1927) and redescribed by Vilela and Bächli (2009); however, in both cases, only males were described. Although male reproductive structures are commonly used to identify Drosophilidae species (Vilela & Bächli 1990), the female spermathecal capsules also present morphological traits that can be taxonomically informative to distinguish the species (Sturtevant 1921; Throckmorton 1962, 1975; Poppe *et al*. 2015a). Poppe *et al*. (2015a) presented a plate of the female's terminalia of *R. punctulata* from the Pampa biome, while pointing differences in the morphology of the spermathecal capsules of *R. punctulata* and its sibling *R. punctuloides*. Here, variations in the spermathecal capsules among populations of *R. punctulata* were noticed, and a complementary description of females is presented. Even so, the described variation in regard to *R. punctulata* spermathecal capsules does not impair the diagnosis of *R. punctuloides* (Poppe *et al*. 2015a).

Rhinoleucophenga punctulata specimens from Cerrado differ slightly from the other populations by the body length and external color. In addition, the spermathecal capsule morphology presents an outstanding differentiation among most populations,

mainly from Amazon and Caatinga. The high differentiation presented by the Caatinga population was further confirmed by the *COI* sequences. So, we hypothesize here that the observed variation among *R. punctulata* populations possibly reflects differences among their environments of origin.

Molecularly, the populations from Pampa and Cerrado are more similar with the Amazon specimens. Probably the higher similarity between Cerrado and Amazon populations is due to the higher geographic proximity between them (Nekola & White 1999; Hebert *et al*. 2003a) and also by the fact that the Amazon locality sampled here is a savannah enclave. Based on the divergence analyses of *COI*, Hebert *et al*. (2003b) suggested that low divergence values point to recent origin events. The divergence of the specimens from Caatinga to the other populations (0.02-0.024) was twice higher than the divergence noticed among the Pampa, Cerrado and Amazon specimens (0.007-0.01). So, it is plausible to suppose that *R. punctulata* may have originally migrated from Caatinga into the Cerrado and, subsequently from Cerrado to the Amazon and Pampa areas. Furthermore, the resulted clade exclusively with specimens from Caatinga; the paraphyletic clades of Pampa and Amazon related to Cerrado specimens; as well as, the strongly supported clade compound by a specimen from Caatinga and other from Cerrado, all reinforce the dispersal route hypothesis of that species.

Despite the high divergence of the population from Caatinga, the maximum genetic divergence within this population is higher than its mean divergence with the other populations; so, one of the main requirements to species delimitation through DNA barcoding was broken (Hebert *et al*. 2003a). Therefore, the hypothesis that each population would represent a different species was not corroborated, but the molecular data reinforces the morphological evidence of geographical differentiation within *R. punctulata*.

According to some authors (Parsons 1989; Poppe *et al*. 2013), temperature in stressing conditions is an important climatic factor determining species distribution, being able to lead to evolutionary bottlenecks and even to the raising of geographic races (Hasson *et al*. 1993). Temperature is a similar environmental factor among Cerrado, Amazon and Caatinga biomes, presenting higher annual oscillations in the Pampa. However, there are striking differences in the humidity levels among these environments, with Caatinga highlighted as the most arid one. The higher humidity

values found in the other three evaluated Biomes could also attenuate the high temperature effects, especially in regard to Amazon and Cerrado. Thus, these contrasting climatic factors could have promoted the differentiation between Caatinga and the other environments as suggested by the phylogenetic results.

On the other hand, since the ecology of *Rhinoleucophenga* is poorly known, we cannot discard that there are many other biotic and abiotic factors able to influence the species dispersion, such as the breeding and feeding behavior. Some species of *Rhinoleucophenga* are known by their predatory feeding behavior of coccids (*R. obesa*, *R. brasiliensis* (Lima), *R. fluminensis* (Lima), *R. capixabensis* Culik & Ventura) and ants (*R. mymercophaga* Vidal & Vilela) (Lima 1935, 1950; Culik & Ventura 2009; Vidal & Vilela 2015), thus the presence or absence of other organisms in the environment might influence *Rhinoleucophenga* distribution. As the breeding and feeding natural resources used by *R. punctulata* are unknown we cannot discard such an effect. Furthermore, intrinsic factors also can determine Drosophilidae species as "good" or "bad" dispersers (Janzen 1967; Poppe *et al*. 2015b), or in a more unpredicted way the dispersion of *R. punctulata* could be resulted from random or stochastic events (Hubbell 2001).

Concerning the body size, the specimens from Caatinga, Amazon and Pampa presented the same length and are bigger than Cerrado specimens. Many studies have concluded that flies emerging from crowded resources are smaller, presenting low capability of dispersion and higher mortality level by desiccation (Roff 1977, Roper *et al*. 1996, Soto *et al*. 2011; Willi & Hoffmann 2012). Mata *et al*. (2015) pointed that during wet the period in Cerrado the species populations are abundantly increased by the higher resources availability. So, few and crowded resources during the dry seasons could justify the smaller body size noticed to the specimens from Cerrado. While the reduced spermathecal capsules size of females from Caatinga could represent an adaptive strategy to the population maintenance; that is, the flies would store fewer sperm and would oviposit fewer in each available resource, avoiding larval intraspecific competition and the decrease in the adult body length. According to Nunney and Cheung (1997), the phenotype induced by a particular set of environmental conditions may represent a fitness gain to the species, and this adaptive response may lead to differentiation among populations from different environments. So, the high

sclerotization level and shape variation noticed in the spermathecal capsules of *R. punctulata* from different environments point to possible differences in the organ utilization level by the females. However, the influence of the resources availability on the population fitness and on the spermathecal capsules morphology was not tested here; thus, it is a hypothesis to be analyzed in future researches. Markow (2015) highlighted the lack of studies investigating the relation between environmental variability and the species reproductive morphological traits.

Therefore, the analyzed populations of *R. punctulata* present different levels of intraspecific variation, considering either morphology or molecular traits, and this is probably linked to the environmental characteristics of each biome. So, morphological, distributional and molecular data revealed here complementary to explain the biological diversity of *R. punctulata* through the Neotropical environments.

7.1.6. ACKNOWLEDGEMENTS

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7.1.7. REFERENCES

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7.1.8. FIGURES

Figure 1: Geopolitical map of South America with the limits of the sampled biomes. Localities, 1: Pampa, Bossoroca, Rio Grande do Sul (28°45’01”S 54°56’55”W, 200 m); 2: Cerrado, Tangará da Serra, Mato Grosso (14°04’38”S 57°03’45”W, 500 m); 3: Cerrado, Parque Nacional das Emas, Goiás (18º15’S 52º53’W, 600 m – Roque & Tidon 2008); 4: Amazon, Parque Estadual Serra das Andorinhas, São Geraldo do Araguaia (6°13’36”S 48°27’55”W, 300 m); 5: Caatinga, Raso da Catarina Ecological Station, Bahia (9°33'39"S 38°44'12"W, 500 m).

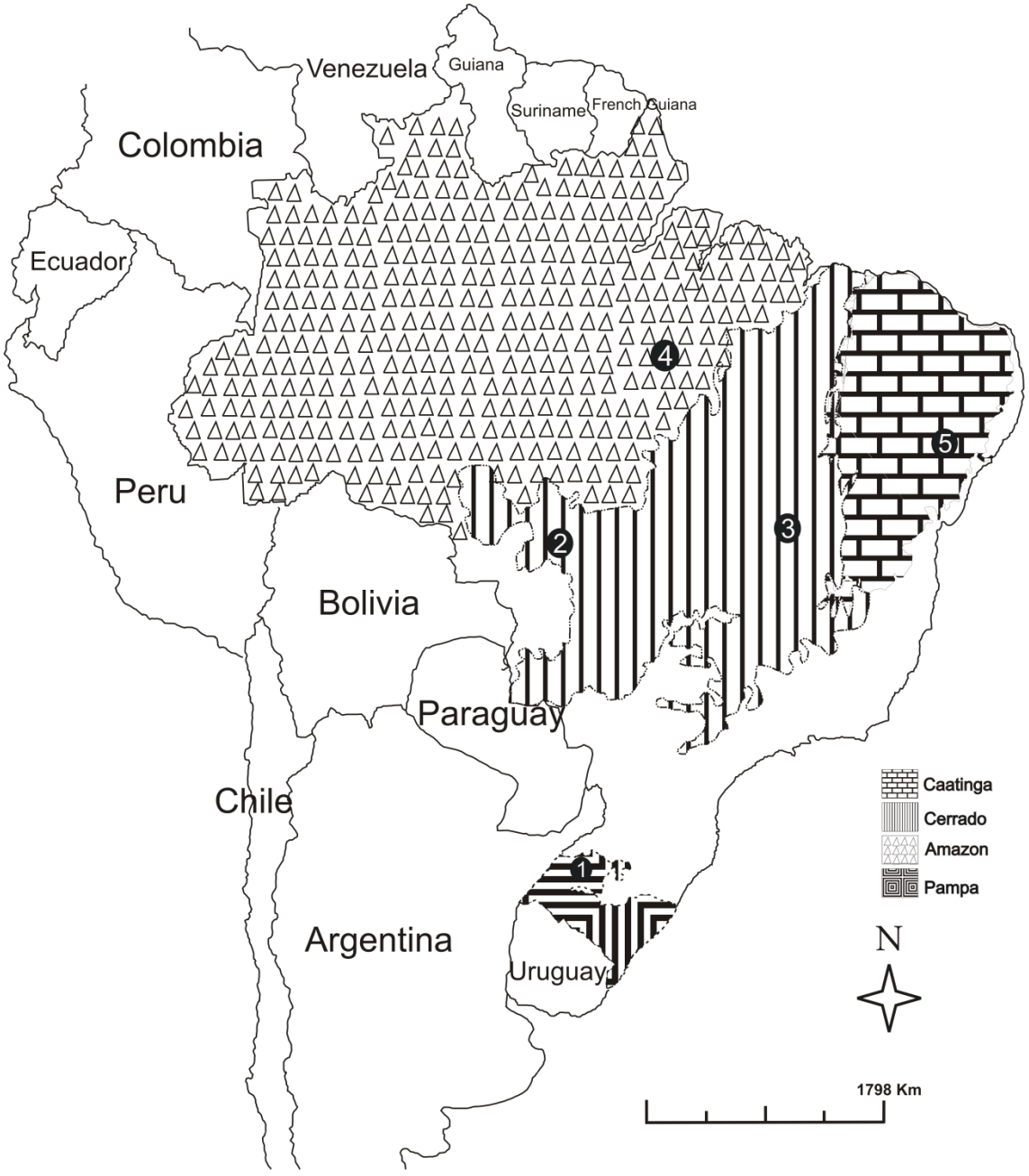


Figure 2: Ordinary specimens of *Rhinoleucophenga punctulata* from Amazon (a, e, i, m), Caatinga (b, f, j, n), Pampa (c, g, k, o) and Cerrado (d, h, l, p). Scale bar 1.0 mm; except in a, b, c and d: 0.5 mm.

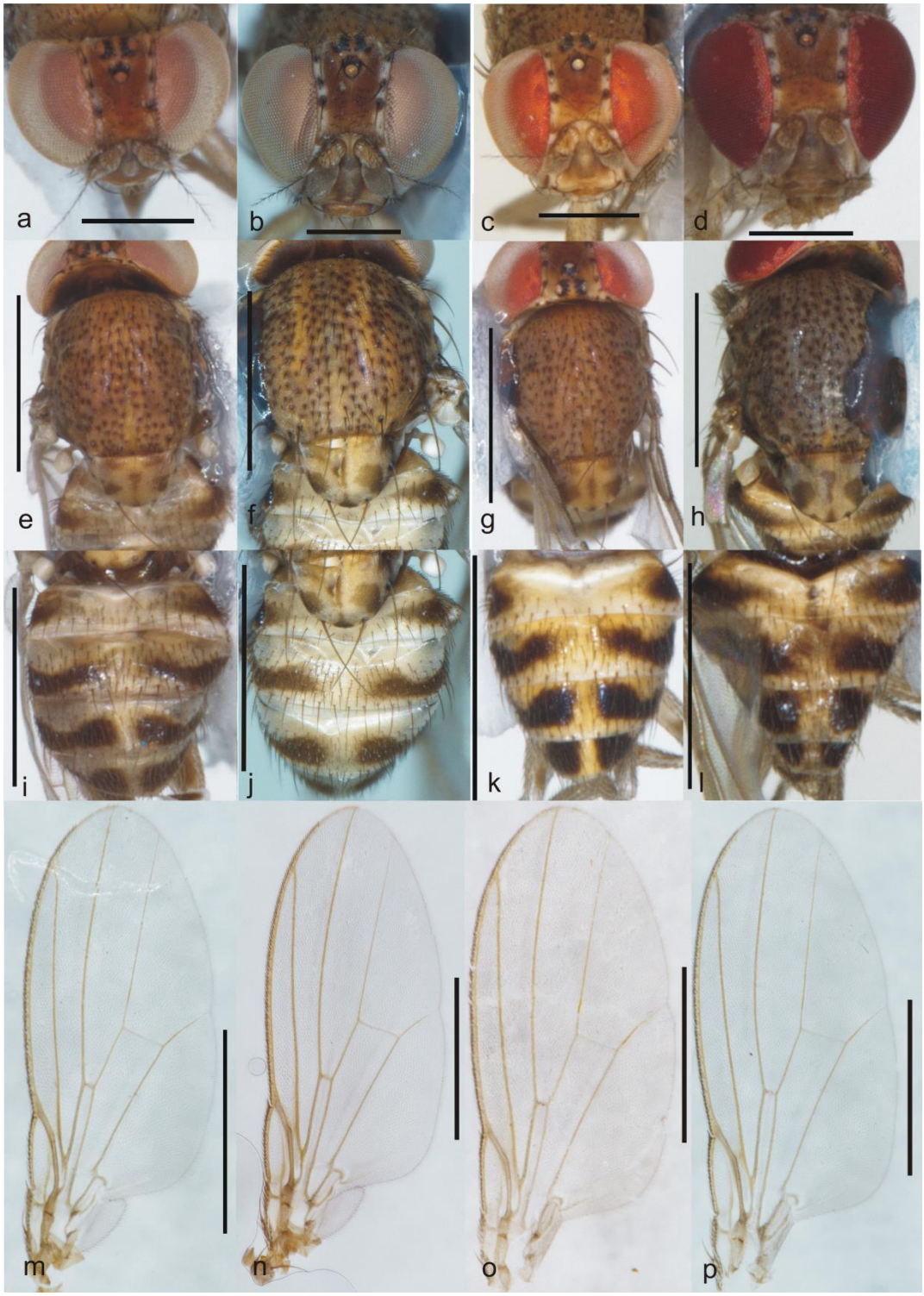


Figure 3: Spermathecal capsules of ordinary specimens of *Rhinoleucophenga punctulata* from Amazon, a. Caatinga, b. Pampa, c. Cerrado, d. scale bar 0.1 mm.

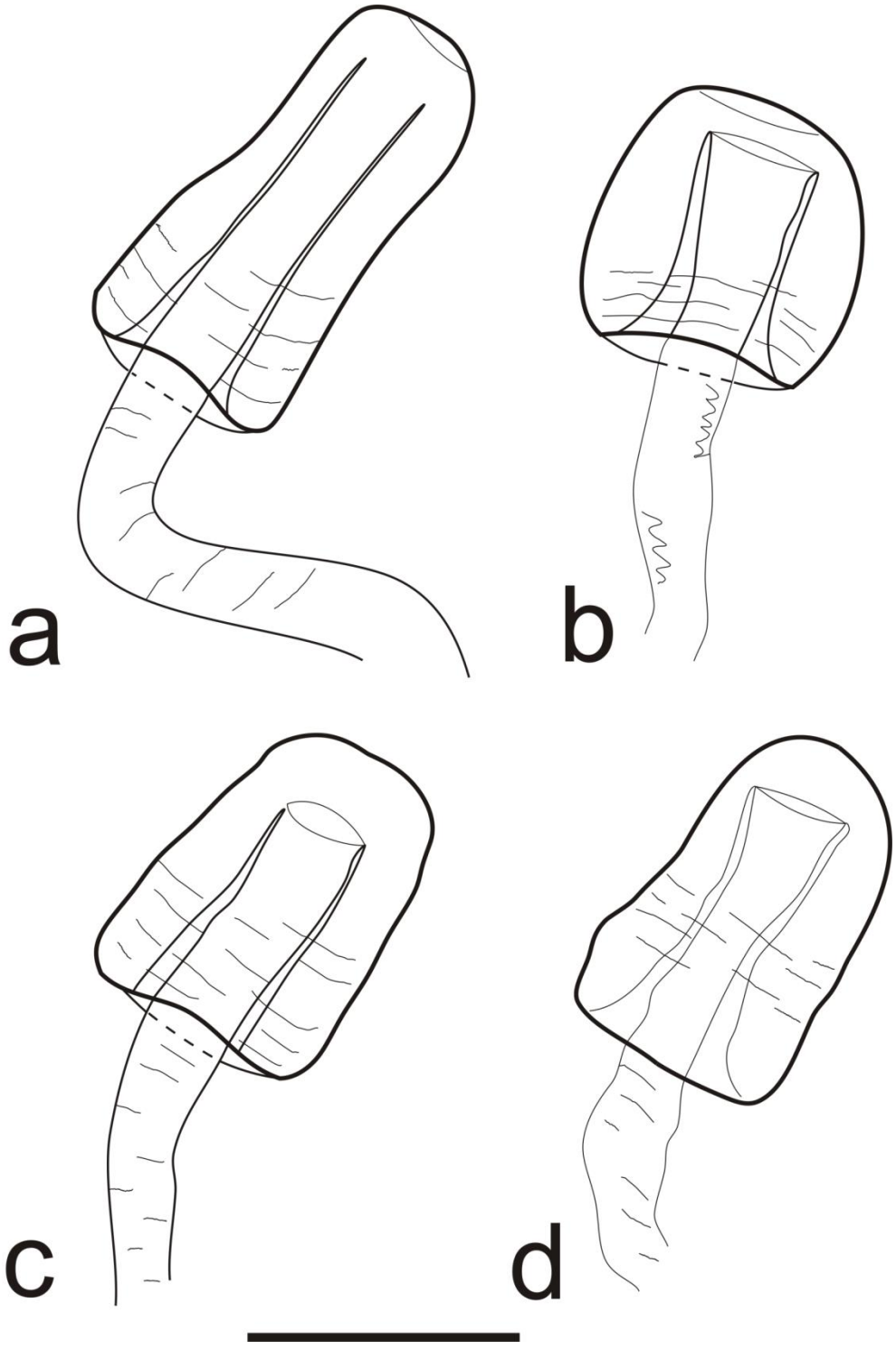


Figure 4: Consensus tree obtained from a 847-bp alignment of *cytochrome c oxidase* subunit *I* (*COI*) gene sequences of the *Rhinoleucophenga* specimens by using Bayesian inference. Numbers at nodes represent posterior probabilities values (1,000,000 generations).

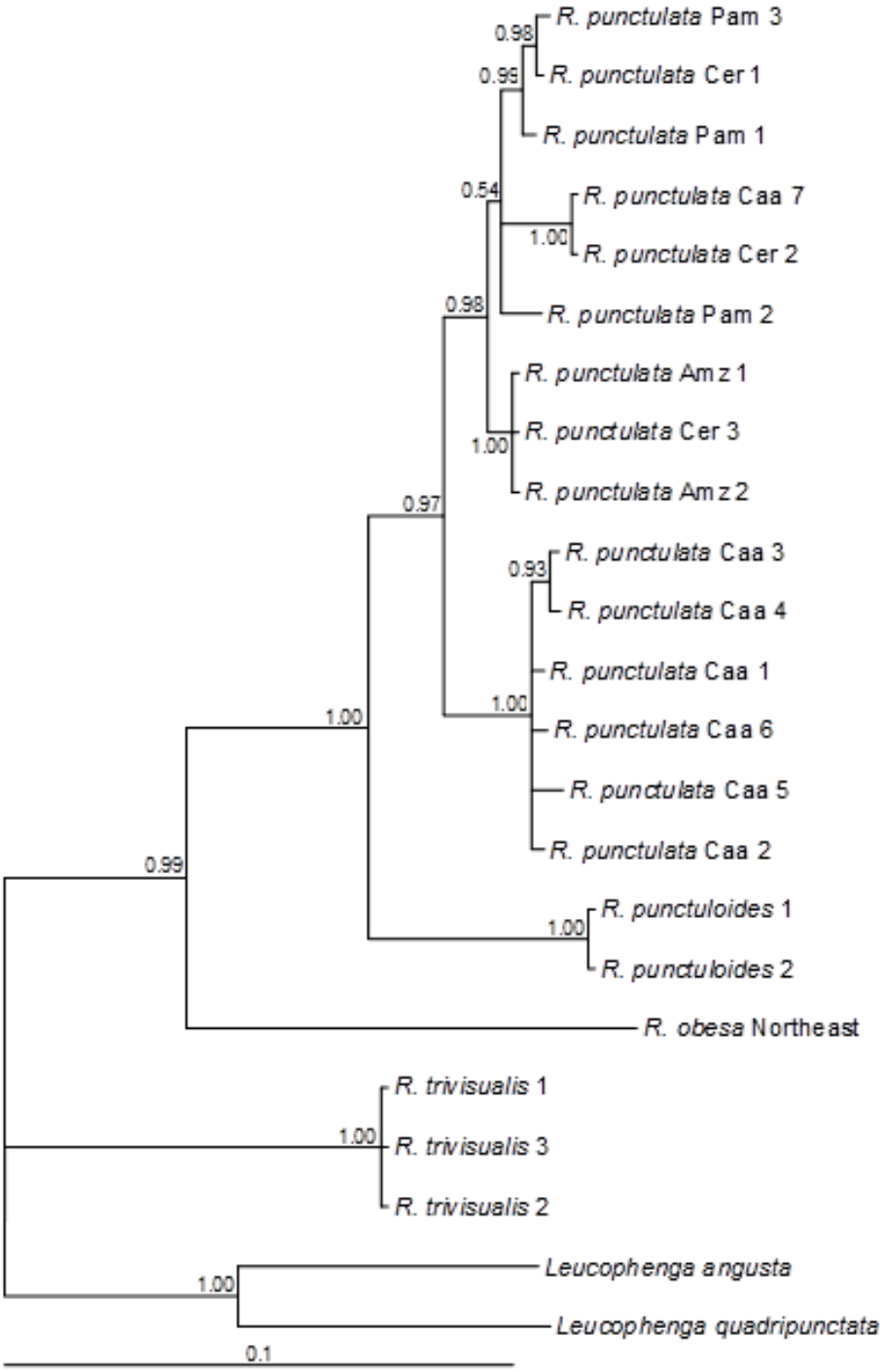
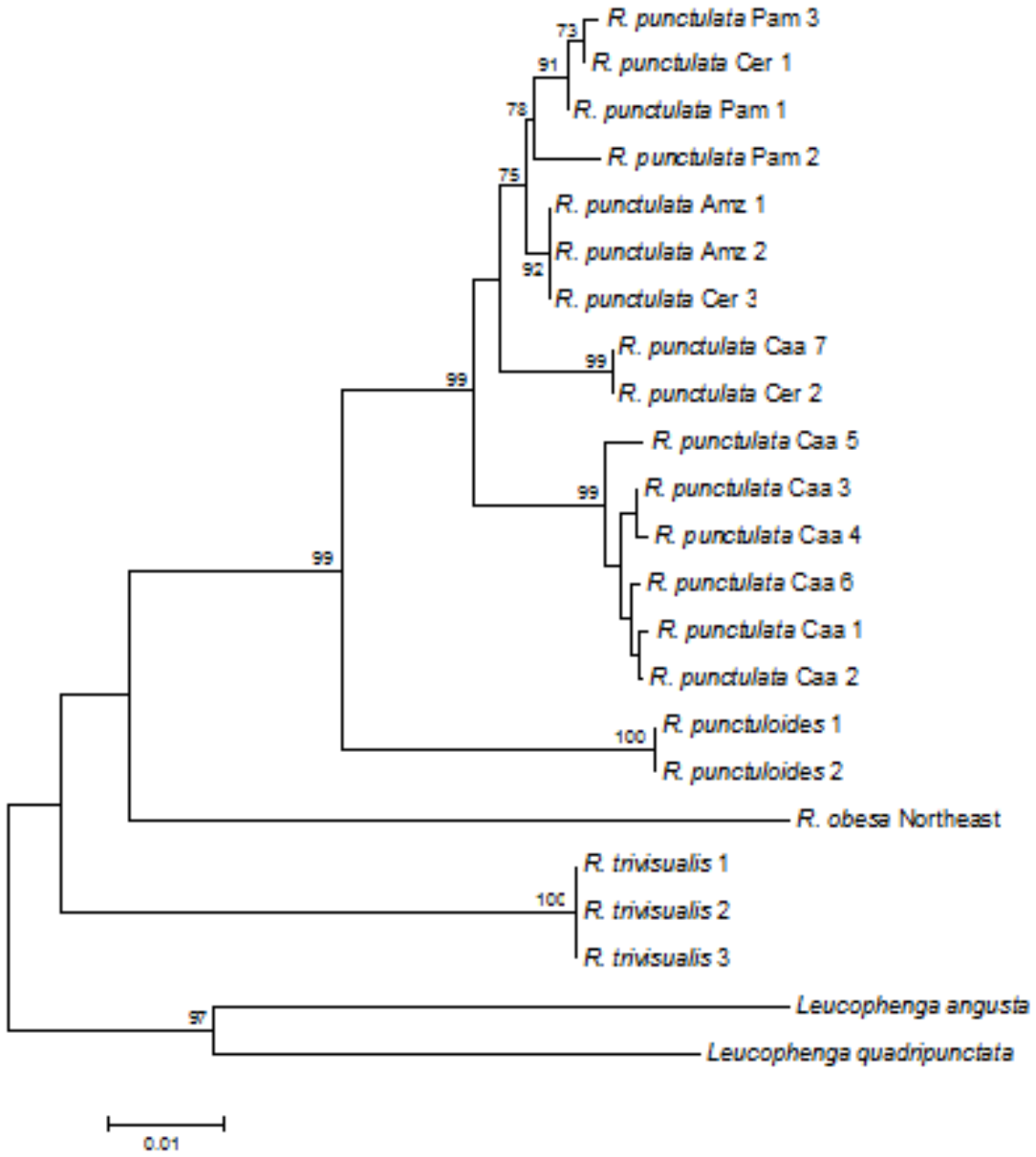


Figure 5: Neighbor-joining consensus tree obtained from an 847-bp alignment of *cytochrome c oxidase* subunit *I* (*COI*) gene sequences of the *Rhinoleucophenga* specimens. Numbers at nodes represent support values (10,000 bootstrap replications).



7.1.9. TABLES

Table 1: Geographical coordinates and the GenBank accession number of the specimens used for this study. Amz: specimen from Amazon region; Pam: specimen from Pampa; Caa: specimen from Caatinga; Cer: specimen from Cerrado.

Specimens	Collection site	Biome	Latitude (S)	Longitude (W)	GenBank accession numbers
R. punctulata Amz1	Parque Estadual Serra das Andorinhas, São Geraldo do Araguaia	Amazonian Savannah enclave	6°13’36”	48°27’55”	KU728939
R. punctulata Amz 2	Parque Estadual Serra das Andorinhas, São Geraldo do Araguaia	Amazonian Savannah enclave	6°13’36”	48°27’55”	KU728940
R. punctulata Pam 1	Bossoroca, Rio Grande do Sul	Pampa	28°45’01”	54°56’55”	KU728941
R. punctulata Pam 2	Bossoroca, Rio Grande do Sul	Pampa	28°45’01”	54°56’55”	KU728942
R. punctulata Pam 3	Bossoroca, Rio Grande do Sul	Pampa	28°45’01”	54°56’55”	KU728943
R. punctulata Caa 1	Raso da Catarina Ecological Station, Bahia	Caatinga	9°33'39"	38°44'12"	KU728944
R. punctulata Caa 2	Raso da Catarina Ecological Station, Bahia	Caatinga	9°33'39"	38°44'12"	KU728945
R. punctulata Caa 3	Raso da Catarina Ecological Station, Bahia	Caatinga	9°33'39"	38°44'12"	KU728946
R. punctulata Caa 4	Raso da Catarina Ecological Station, Bahia	Caatinga	9°33'39"	38°44'12"	KU728947
R. punctulata Caa 5	Raso da Catarina Ecological Station, Bahia	Caatinga	9°33'39"	38°44'12"	KU728948
R. punctulata Caa 6	Raso da Catarina Ecological Station, Bahia	Caatinga	9°33'39"	38°44'12"	KU728949

R. punctulata Caa 7	Raso da Catarina Ecological Station, Bahia	Caatinga	9°33'39"	38°44'12"	KU728950
R. punctulata Cer 1	Parque Naciona das Emas, Goiás	Cerrado	18°15’	52°53’	KU728951
R. punctulata Cer 2	Parque Naciona das Emas, Goiás	Cerrado	18°15’	52°53’	KU728952
R. punctulata Cer 3	Parque Naciona das Emas, Goiás	Cerrado	18°15’	52°53’	KU728953
R. punctuloides 1	Bossoroca, Rio Grande do Sul	Pampa	28°45’01”	54°56’55”	KU728934
R. punctuloides 2	Bossoroca, Rio Grande do Sul	Pampa	28°45’01”	54°56’55”	KU728935
R. obesa Northeast	Porto de Galinhas, Pernambuco	Coast environment	8°30’30”	35°0’20”	KU728954
R. trivisualis 1	Raso da Catarina Ecological Station, Bahia	Caatinga	9°33'39"	38°44'12"	KU728936
R. trivisualis 2	Raso da Catarina Ecological Station, Bahia	Caatinga	9°33'39"	38°44'12"	KU728937
R. trivisualis 3	Raso da Catarina Ecological Station, Bahia	Caatinga	9°33'39"	38°44'12"	KU728938

Table 2: Complementary measures and indices to the *R. punctulata* specimens. Measures and indices according to Bächli et al. (2004); measurements represent averages followed by the ranges in parentheses. *: measures in millimeters (mm); - : not available measure.

	Females of *Rhinoleucophenga punctulata* Duda			
	Amazon	Caatinga	Pampa	Cerrado
HEAD				
Frontal length *	0.51 (0.50-0.52)	0.47 (0.46-0.48)	0.45	0.48 (0.45-0.53)
Frontal index	1.21 (1.19-1.23)	1.38 (1.33-1.43)	1.14 (1.10-1.18)	1.33 (1.27-1.43)
Top-to-bottom frontal width ratio	1.09 (1.04-1.14)	1.22	1.15	1.15 (1.08-1.24)
Ocellar triangle to front length ratio	0.51 (0.50-0.52)	0.53 (0.52-0.54)	0.56 (0.53-0.59)	0.50 (0.45-0.54)
Setae OR1/OR3 ratio	1.10 (1.00-1.20)	-	1.04 (0.83-1.25)	1.46
Setae OR2/OR1 ratio	0.81 (0.79-0.83)	0.83	0.90 (0.84-0.95)	-
Vibrissal index	0.39 (0.37-0.40)	0.54	0.57 (0.54-0.60)	0.44 (0.40-0.50)
Cheek index	6.45 (6.00-6.90)	7.21 (7.20-7.22)	5.50 (5.00-6.00)	6.34 (4.64-7.60)
Eye index	1.33 (1.30-1.35)	1.21 (1.18-1.24)	1.25	1.27 (1.25-1.28)
THORAX				
Thorax length*	1.41 (1.40-1.42)	1.31 (1.26-1.36)	1.43 (1.42-1.44)	1.35 (1.26-1.53)
Strongest prescutellar acrostichal setae, % length related to posterior dorsocentral setae (pre-esc/ dorso posterior)	58 (55-61)	74 (62-86)	0.72 (0.64-0.80)	64 (61-68)
Transverse distance between dorsocentral setae, related to longitudinal distance	3.09X (2.85-3.33)	4.03X (3.46-4.60)	4.06X (3.46-4.66)	4.34X (3.29-5.75)
Sterno index	1.00	1.00	1.00	1.00
WING				
Length*	2.55 (2.35-2.75)	2.23 (2.15-2.30)	2.48 (2.45-2.50)	2.28 (2.20-2.30)

Width*	1.08 (1.05-1.10)	1.04 (1.00-1.08)	1.17 (1.16-1.18)	1.07 (1.02-1.12)
WING INDICES				
C	3.33	2.81 (2.80-2.82)	2.85 (2.68-3.01)	2.95 (2.53-3.27)
Hb	0.66	0.71 (0.65-0.76)	0.56 (0.50-0.62)	0.46 (0.41-0.53)
Ac	1.50	1.71 (1.66-1.76)	1.69 (1.68-1.70)	1.71 (1.57-2.00)
4c	1.12	1.30 (1.21-1.38)	1.16 (1.10-1.22)	1.19 (1.04-1.30)
4v	2.62	2.92 (2.73-3.11)	2.62 (2.56-2.68)	2.65 (2.47-2.87)
5x	1.38 (1.36-1.40)	1.41 (1.37-1.45)	1.39 (1.25-1.52)	1.50 (1.18-1.72)
M	0.86 (0.85-0.87)	0.88 (0.84-0.91)	0.80 (0.77-0.82)	0.84 (0.80-0.95)
prox.x	1.14 (1.12-1.15)	1.22 (1.05-1.38)	1.05 (1.00-1.09)	1.09(0.92-1.35)
SPERMATHECAL CAPSULE				
Length to width ratio	2.27	0.95	1.47	1.54
Body length*	2.80 (2.70-3.20)	2.78 (2.70-3.00)	2.98 (2.7-3.2)	2.56 (2.55-2.60)

Table 3: Pairwise genetic divergence (Kimura two-parameter) among and within species of the *Rhinoleucophenga* genus using *cytochrome c oxidase* subunit *I* (*COI*) gene sequences. SD: Standard Deviation.

		Interpopulation genetic distance				Intrapopulation genetic distance		
	Specimens	**1**	**2**	**3**	**4**	**Minimum**	**Mean ± SD**	**Maximum**
1	*R. punctulata* Amazon					0	0 ± 0	0
2	*R. punctulata* Pampa	0.008				0.003	0.0077 ± 0.0025	0.012
3	*R. punctulata* Caatinga	0.020	0.024			0.001	0.0104 ± 0.0024	0.026
4	*R. punctulata* Cerrado	0.007	0.010	0.022		0.007	0.0126 ± 0.0034	0.016

7.1.10. SUPPORTING INFORMATION

Appendix S1: Pairwise genetic divergence (Kimura two-parameter) among specimens of the *Rhinoleucophenga punctulata* using *cytochrome c oxidase* subunit *I* (*COI*) gene sequences. Amz: specimen from Amazon region; Pam: specimen from Pampa; Caa: specimen from Caatinga; Cer: specimen from Cerrado.

	Specimen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	R_punctulata_Amz_1		0.000	0.003	0.003	0.003	0.005	0.005	0.005	0.006	0.005	0.005	0.004	0.003	0.004	0.000
2	R_punctulata_Amz_2	0.000		0.003	0.003	0.003	0.005	0.005	0.005	0.006	0.005	0.005	0.004	0.003	0.004	0.000
3	R_punctulata_Pam_1	0.006	0.006		0.003	0.002	0.006	0.006	0.006	0.006	0.006	0.006	0.005	0.001	0.005	0.003
4	R_punctulata_Pam_2	0.009	0.009	0.009		0.004	0.006	0.006	0.006	0.007	0.006	0.006	0.005	0.004	0.005	0.003
5	R_punctulata_Pam_3	0.009	0.009	0.003	0.012		0.006	0.006	0.006	0.006	0.006	0.006	0.005	0.001	0.005	0.003
6	R_punctulata_Caa_1	0.022	0.022	0.025	0.028	0.028		0.001	0.002	0.002	0.002	0.001	0.006	0.006	0.006	0.005
7	R_punctulata_Caa_2	0.020	0.020	0.023	0.026	0.026	0.001		0.003	0.002	0.003	0.002	0.006	0.006	0.006	0.005
8	R_punctulata_Caa_3	0.020	0.020	0.023	0.026	0.023	0.004	0.006		0.001	0.004	0.002	0.006	0.006	0.006	0.005
9	R_punctulata_Caa_4	0.022	0.022	0.025	0.028	0.025	0.003	0.004	0.001		0.003	0.002	0.006	0.006	0.006	0.006
10	R_punctulata_Caa_5	0.020	0.020	0.023	0.026	0.026	0.004	0.006	0.009	0.007		0.003	0.006	0.006	0.006	0.005
11	R_punctulata_Caa_6	0.020	0.020	0.023	0.026	0.026	0.001	0.003	0.003	0.004	0.006		0.006	0.006	0.006	0.005
12	R_punctulata_Caa_7	0.015	0.015	0.015	0.017	0.017	0.026	0.025	0.025	0.026	0.028	0.025		0.005	0.000	0.004
13	R_punctulata_Cer_1	0.007	0.007	0.001	0.010	0.001	0.026	0.025	0.022	0.023	0.025	0.025	0.016		0.005	0.003
14	R_punctulata_Cer_2	0.015	0.015	0.015	0.017	0.017	0.026	0.025	0.025	0.026	0.028	0.025	0.000	0.016		0.004
15	R_punctulata_Cer_3	0.000	0.000	0.006	0.009	0.009	0.022	0.020	0.020	0.022	0.020	0.020	0.015	0.007	0.015	

8. CAPÍTULO VIII

(Manuscrito para submissão ao periódico *Zoological Journal of the Linnean Society*)

8.1. Morphological phylogeny of *Rhinoleucophenga* Hendel (Drosophilidae, Steganinae) under different treatments of continuous data

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Running Title: Morphological phylogeny of *Rhinoleucophenga*

8.1.1. Abstract

There are many rare species of Drosophilidae available only to morphological phylogenetic studies, such as most species of the New World genus *Rhinoleucophenga*. The objective of the present study was to test the monophyletic condition of *Rhinoleucophenga*. Moreover, we also investigated the influence of continuous morphological characters under different methodological treatments in the proposition of a first phylogenetic hypothesis to the genus. It is the first time that a large set of continuous characters are not discretized in a morphological phylogenetic research with Drosophilidae. Thirty-eight species compound the ingroup; seventeen species of Steganinae were included as outgroup. One hundred and four measures were taken; 93 of them are measures of body parts and 11 are setae meristic counting. From these 104 continuous characters 58 ratios were obtained, also treated as continuous characters. Additionally, 62 discrete characters were evaluated. Five strategies of combination and treatment of morphological data were explored: (*A*) 58 continuous ratio characters and 62 discrete characters; (*B*) 104 absolute measures and 62 discrete characters; (*C*) 58 continuous ratio characters *log-transformed* and 62 discrete characters; (*D*) 104 absolute measures *log-transformed* and 62 discrete characters and (*E*) only the 62 discrete characters. All strategies were performed with equal weighting (treatments *A*-*E*) and after performed through implied weighting (treatments *A'*-*E'*). The phylogenetic searches were performed by TNT Software. Continuous characters were fundamental to increase the support of the results, and treating them as ratios or absolute measures highly influenced the trees topology. Trees generated by the *log-transformed* continuous data presented improvement on the average of clades support; but implied weighting represents higher influence than log transformation of continuous characters on the obtained topologies. *Rhinoleucophenga* was presented as a paraphyletic genus in most analyses due its position concerning the genus *Pararhinoleucophenga*. Some *Rhinoleucophenga* clades were repeatedly obtained in most analyzed treatments. The results highlighted the influence of different treatments of data set on the phylogenies found and provided preliminary hypotheses to be further tested into a larger-scale phylogeny of the subfamily Steganinae, with multiple data partitions, including continuous morphological characters.

Keywords: *Drosophila* – Evolution – *Gitona* – Monophyletic – *Pararhinoleucophenga* – Systematics.

8.1.2. Introduction

The information contained in the morphological data is fundamental to perform phylogenetic studies with Drosophilidae (Remsen & O'Grady, 2002; Yassin, 2013), since many species are only rarely collected in the nature (Markow & O'Grady, 2006), and there are a huge amount of taxa deposited in museums which are available to be included mainly in morphological studies. However, few comprehensive studies on the systematics of Drosophilidae were developed based only on morphological data: Throckmorton (1975), Grimaldi (1988, 1990), Okada (1989) and Sidorenko (2002). Moreover, studies with other genera than *Drosophila* are scarce, and the monophyletic status of several Drosophilidae genera is yet unknown. In this sense, no phylogenetic information based on molecular data is available for *Rhinoleucophenga* and the only phylogenetic studies of the genus included few taxa and have dealt only with its relationships with other genera (Grimaldi, 1988, 1990; Okada, 1989). No study on the species relationships inside the genus was performed yet.

Rhinoleucophenga is a New World genus of the subfamily Steganinae, compounded mainly by species recorded preferentially in open environments between the latitudes 37°N (Texas, United States) (Malloch & McAtee, 1924; Vilela, 1990) and 34°S (Argentina) (Thomson, 1869; Vilela, 1990). According to some authors Steganinae was one of the first lineages to derive in Drosophilidae (Grimaldi, 1988; Bächli *et al.*, 2004; Markow & O'Grady, 2006). The two most comprehensive phylogenetic studies of Steganinae were performed by Sidorenko (2002) and Otranto *et al.* (2008); but none of them included *Rhinoleucophenga* species. The first considered morphological data (78 discretized characters evaluated for 34 species of nine genera) to establish phylogenetic relationships to the taxa from the subtribe Steganina (Grimaldi 1990); the second was based on sequences of the mitochondrial *Cytochrome oxidase I* gene to perform phylogenetic analysis of some common Steganinae species.

Other studies based on morphological data, but including *Rhinoleucophenga* among the sampled taxa were performed by Throckmorton (1975), Grimaldi (1988, 1990) and Okada (1989). However, Throckmorton (1975) and Okada (1989) did not present methodological details enough to allow repeatability. Furthermore, Okada (1989) proposed subtribes to Drosophilidae based on 14 binary discretized characters, raising some criticism and doubts about the presented results (Grimaldi, 1990).

Through the use of 25 discretized morphological characters (18 of adults and seven of larve), and 22 terminal species, of which three belonged to *Rhinoleucophenga*,

Grimaldi (1988) proposed a phylogeny of the family Drosophilidae. In this study, the genus *Rhinoleucophenga* was supported by the presence of more than two long pairs of prescutellar acrostichal setae and by the high number of scattered interfrontal setulae (ca. 200 setulae). Additionally, Grimaldi (1990) analyzed the largest morphological data set yet evaluated in regard to systematic studies of Drosophilidae: 217 discrete characters and 120 terminal species, of which two belonged to *Rhinoleucophenga*. According to Grimaldi (1990) the genus is part of the tribe Gitonini, subtribe Acletoxenina, *Pseudiastata* genus group, and is supported by the presence of more than 35 supracervical setae, 50 or more interfrontal setulae and acrostichal setulae in 12 rows.

Continuous characters are usually discretized in intervals of setulae number and length proportions in morphological phylogenetic studies with Drosophilidae (Grimaldi, 1988, 1990; Okada, 1989; Sidorenko, 2002). So, historically, morphological data have been criticized in phylogenetic studies mainly by the arbitrariness performed by most systematists during the discretizing data process, since it is determinant to the phylogeny proposed (Farris, 1983; de Pinna, 1991; Wiens, 2000; Sereno, 2007; Brazeau, 2011; Koch, Soto & Ramírez, 2014, 2015). Conversely, some authors have looked for alternatives able to minimize the arbitrariness noticed on the continuous characters definitions, in order to promote the use of morphological data in phylogenetic studies. Goloboff (1993) argued the use of implied weighting against the homoplasies. Goloboff, Mattoni & Quinteros (2006) implemented the use of continuous characters as they are, without discretization to phylogenetic analyses in TNT software. Koch *et al*. (2015) pointed that differences to perform ratio characters influence the tree topology, and proposed log-transformations as a good solution to avoid these "phylogenetic noise". These methodological advances in morphological phylogenetics remain to be applied in Drosophilidae, especially as concerns a better definition on characters coding, potentially bringing improvements to the available trees from previous studies (Grimaldi, 1990; Sidorenko, 2002) and making the interpretation of some results easier.

Recently Poppe *et al*. (2014, 2015) found a high richness of *Rhinoleucophenga* species in Brazil, especially in the South of the country (Pampa biome), recording ten species to that region and describing five species to the genus. In addition, other 15 species are under description process by our research group. In total, this represents an increase of ca. 70% in the number of *Rhinoleucophenga* species. However, as most *Rhinoleucophenga* species are rarely collected, and many of them are represented only

by museum specimens, morphological data is fundamental to infer the evolutionary hypothesis of this genus. Therefore, the objective of the present study was to test the monophyletic condition of *Rhinoleucophenga*, while infering the phylogenetic relationships among the species included in the analysis, and investigating the influence of continuous morphological characters under different methodological treatments in the proposition of a first phylogenetic hypothesis to the genus. Additionally, the role of continuous characters as a complementary data set to discrete morphological characters in the phylogeny resolution was also analyzed. So, this study represents the first phylogenetic proposal in Drosophilidade including a large number of continuous morphological characters not discretized, and contributes to the knowledge of the influence of different treatments on morphological data set to generate phylogenetic hypotheses.

8.1.3. Material and Methods

Selection of taxa

The phylogenetic analyses were performed including 65 terminal taxa. Thirty-eight species encompassing 23 of the 29 described *Rhinoleucophenga* species and fifteen new species that have not yet been formally described were used as ingroup, and this represents ca. 85% of the known *Rhinoleucophenga* species. All the new species are under description process (manuscripts submitted), and their epithet is not typed in italic in the text. *Rhinoleucophenga* species with widespread distributions were represented by specimens from different localities whenever as possible (Table 1). Furthermore, most species had their type series analyzed.

Since more than one outgroup is recommended in order to avoid spurious interpretations of the characters in the ingroup (Miyaki, Russo & Pereira, 2001), seventeen taxa of Steganinae were included as outgroup (Table 1), and these represented species from all major biogeographic regions inhabited by the subfamily. *Drosophila melanogaster* Meigen, that belongs to the subfamily Drosophilinae, was used to root the analysis.

Selection of characters

Only characters of adult individuals were considered. Characters are described following Sereno (2007), where the character statements are composed of four

fundamental functional components identified as locator, variable, variable qualifier, and character states. The terminology follows Bächli *et al*. (2004).

One hundred and four measures were taken; 93 of them are measures of body parts and 11 are setae meristic counting. From these 104 continuous characters, 58 ratios were performed and also treated as continuous characters. The objective of using ratios is to reduce the effects of different body sizes among the species, as well as represent shapes numerically (Koch *et al*., 2014; for more detailed discussion). Additionally, 62 discrete characters were performed, related to color pattern, shape and presence/absence of body structures.

The character describing the number of interfrontal setulae (character 2, method *B* and *D*) had to be scaled according to the Tree New Technology (TNT) software limitations, that is, the greatest observed value of 200 interfrontal setulae was converted to 65.000, and the minimum present number of 4 interfrontal setulae was scaled to 1.300.

In order to minimize the possibility of error occurrence during the characters observations, as well as to increase the sample of terminal taxa and notice the occurrence of intraspecific variation among the specimens, the highest possible number of specimens of each species was checked (Table 1). All the specimens were directly checked in order to avoid different interpretations about color pattern or measure definitions, which sometimes are not clearly defined in the taxonomic literature.

Statistical tests to verify the correlation and variation between and within continuous characters, respectively, were not performed because many species are represented by only one specimen, hampering a satisfactory statistical performance. Thus, the influence of covarying characters on the analyses will be an issue for further studies. On the other hand, the discrete characters were assumed as independent ones.

Phylogenetic Analysis

The analyses were performed with the TNT Software (Goloboff, Farris & Nixon, 2008) through heuristic Traditional Search Method. The parameters of the analyses were set as follow: 500 replications with 30 trees retained per replication, Tree Bisection Reconnection algorithm (TBR), first performing equal weighting (EW) and after performing implied weighting of $K = 6$ (IW). The implied weighting is proposed as a data set refinement, that is, higher weight is assigned to those characters with less homoplasy, and the sum of weights over all characters is maximized during tree

searches (Goloboff, 1993; Koch *et al*., 2014). The stipulated $K = 6$ to the implied weighted analyzes is in accordance with the proposed by some authors as a good *K* value (Ramírez, 2003; Koch *et al*., 2014), also by the higher stability of trees' topologies generated with values next to six in previous analyses with different *K* values (data not shown).

All 62 discretized characters were treated as non-additive (Fitch, 1971). The characters polarization was performed by outgroup comparison (Nixon & Carpenter, 1993). Continuous characters were analyzed without discretization as proposed by Goloboff *et al*. (2006).

In order to avoid an excessive weighing of character transformation for those characters with higher amplitudes of variation among taxa (e.g. number of interfrontal setulae from 0 (absent) up to 200), all continuous characters were transformed into ranges from 0 (= smallest value, e.g. 0 interfrontal setulae) to 1 (= greatest observed value, e.g. 200 interfrontal setulae), by running the TNT script "*stand.run*". Thus, the range of discrete and continuous characters becomes the same; it may reduce the effect of the scale magnitude of different continuous characters on phylogenetic hypothesis proposed (Koch *et al*., 2014). Missing and undetermined data were assigned in the matrix by '?'.

Five strategies of combination and treatment of morphological data were explored (Figure 1): (*A*) 58 continuous ratio characters and 62 discrete characters (Supp. File 1, 2 and 3); (*B*) 104 absolute measures and 62 discrete characters (Supp. File 4, 5 and 6); (*C*) 58 continuous ratio characters *log-transformed* as proposed by Koch *et al*. (2015) and 62 discrete characters (Supp. File 7); (*D*) 104 absolute measures *log-transformed* and 62 discrete characters (Supp. File 8), and (*E*) only the 62 discrete characters (Supp. File 9, 10 and 11). All strategies were performed with equal weighting (treatments *A*-*E*) and after performed through implied weighting (treatments *A'*-*E'*).

Branch supports were calculated through Bootstrap by absolute group frequencies (*Standard*) and frequency differences (*GC*). Jackknife was also performed with 33% of removal probability. With implied weighting the resampling was performed through symmetric resampling in order to avoid distortions in the analyses due to weight costs. Both analyses were calculated with 1,000 pseudoreplicates. Retention (RI) and Consistency (CI) Indices were calculated using the w*stats.run* script, which is part of the TNT package. The preferred phylogenetic hypothesis obtained to

Rhinoleucophenga was selected based on the cladogram with highest values of support and topological stability to most of the species groups found.

In order to compare the phylogenetic trees obtained by the different treatments performed on the continuous data, topological comparisons of the most parsimonious trees (MPTs) obtained in each case were performed through the Robinson-Foulds distances (*RF*) (Robinson & Foulds, 1981) and Distortion-coefficients (*DistCoef*) (Farris, 1973). In both cases, values range from 0 to 1. Nevertheless, whereas to the *RF*, values closer to zero mean higher congruence among tree topologies, to the *DistCoef* the relationship is opposite. Additionaly, SPR distances analyses was alternatively performed by TNT default settings as implemented by Goloboff (2007).

8.1.4. Results

Comparison of data set treatments

One most parsimonious tree was obtained for each data treatment (Figures 2-5), except when only discrete data were considered, so, a strict consensus tree is presented (Figure 6). The individual support of species groups oscillated according to the treatment applied on the data set, and some differences related to species grouping and also to the number of synapomorphies supporting each group were noticed (Supp. Files 12-16). Average values of clades support by Jackknife and Bootstrap was increased with continuous characters; mainly continuous characters *log-transformed* (Table 2). Likewise, average values of clades support were improved with implied weighting. Thus, based on the highest individual support values to most of *Rhinoleucophenga* species groups, the phylogenic tree obtained with ratio continuous characters *log-transformed* under implied weighting (treatment *C'*) was selected as the preferred phylogenetic hypothesis to the focus genus (Figure 4B). Some comparisons among the results obtained with different treatments are briefly discussed to characterize the phylogenetic relationships of *Rhinoleucophenga*.

Through the *RF* and *DistCoef* tree topology comparisons analyses (Table 3), topologies obtained with ratio characters (treatments *A* and *C*) presented high congruence (100% by *DistCoef*). However, performing implied weighting, the tree topologies obtained by those treatments (*A'* and *C'*) were not totally congruent, with some species fluctuating in their position and a species group losing its monophyletic status in *A'* (species group *a*) (Figure 2B). Similarly, the trees obtained with characters treated as absolute measures (treatments *B*) presented highest congruency with the same

data set *log-transformed* (treatment *D*). Finally, the topology obtained only with discrete characters under equal weighting was highly incongruent with other topologies, but under implied weighting (treatment *E'*) it was more congruent with the topologies obtained with continuous characters treated as ratios (treatments *A* and *C*); and less congruent with absolute measures *log-transformed* under implied weighting (treatment *D'*).

Rhinoleucophenga *and other genera of Steganinae*

Rhinoleucophenga was presented as a paraphyletic genus in most analyses due its position concerning the genus *Pararhinoleucophenga* (Figure 2-4A-B, 5A, 6B). Thus, *Rhinoleucophenga* and *Pararhinoleucophenga* represented a sister clade to the other Steganinae genera. The resemblance of *Pararhinoleucophenga* and some species of *Rhinoleucophenga* was noticed mainly by the convergence of the vein R_{4+5} with M-IV (character 76, treatments *A* and *C*; character 122, treatments *B* and *D;* character 18, treatment *E*), wings with costal cell clouded (character 82, treatments *A* and *C*; character 128, treatments *B* and *D*; character 24, treatment *E*), aedeagus not ring-like shaped (character 91, treatments *A* and *C*; character 137, treatments *B* and *D*; character 33, treatment *E*), scutellum bicolored (character 113, treatments *A* and *C*; character 159, treatments *B* and *D*; character 55, treatment *E*), wings not hyaline (character 115, treatments *A* and *C*; character 161, treatments *B* and *D*; character 57, treatment *E*) and by legs with unicolor pattern (character 117, treatments *A* and *C*; character 163, treatments *B* and *D*; character 58, treatment *E*).

Only through the treatment *D' Pararhinoleucophenga* was phylogenetically closer to the genera *Phortica*, *Stegana* and *Leucophenga* species. Nevertheless, in this case, *Rhinoleucophenga* was recovered as paraphyletic in regard to these four genera (Figure 5B). While by treatment *E*, the obtained phylogeny was poorly solved (Figure 6A).

In each case, most characters noticed to diagnose *Rhinoleucophenga* were continuous ones, and most of these measures were repeatedly found supporting the genus through the treatments analyzed. However, as previously mentioned, the treatments applied on the data set influence tree topologies obtained, thus, diagnose characters are potentially subject to be altered through different treatments performed. Apart from that, characters related to general body color were the most homoplastic in all analyses.

Based on the preferred tree (treatment *C'*), *Rhinoleucophenga* was diagnosed by the following characters, whose values not *log-transformed* are presented in parenthesis: Higher number of interfrontal setulae relative to body length (≥9.496-10.769) (character 1); high proportionality between the front superior and inferior width (≤1.173) (character 2); higher proportionality between the palpus length and width (≤2.600) (character 12); higher proportionality between the genal length and width (≥0.333) (character 14); high length proportionality between the posterior and anterior katepisternal setae (≤0.92-1.21) (character 32); higher proportionality between the length of vein C-IV and wing width (≥0.16-0.33) (character 39); higher number of setae on the III sternite relative to the abdomen length (≥11-38) (character 49); lower number of prensisetae relative to the abdomen length (≤4.11-18.18) (character 54); the pair of dorsolateral tentorial apodeme parallel oriented (character 66); wings with vein R_{2+3} weakly curved into the costal margin (character 75); tergite stripes interrupted (character 88 and 89); surstyli fused to the epandrium (character 98) and surstyli with rod-shaped prensisetae (character 100).

Internal group: relationship of Rhinoleucophenga *species*

Six monophyletic groups of *Rhinoleucophenga* species were identified on trees through most of the treatments analyzed (Figures 2-6). But most of them were individually best supported by the treatment *C'* (Figure 4B), as previously mentioned; thus, they are presented and discussed based on the tree obtained through that data set treatment.

Species group *a*

The group is compounded by *R.* grimaldii, *R.* flava, *R. agustifrons* Malogolowkin, *R.* exigua, *R. brasiliensis* (Lima) and *R. fluminensis* (Lima); all yellow flies (ca. 2.0-2.6 mm) with ca. 30-40 interfrontal setulae and abdomen with brown tergite stripes. The species that compound this group were presented as early *Rhinoleucophenga* offshoots through most treatments (Figures 2-6). In the treatment *C'* it was obtained as a sister group of *R. subradiata* Duda (Figure 4B).

Although this group was recovered through the use of continuous characters as absolute measures (treatments *B* and *D*; Figure 3A and 5A, respectively), it was not supported by these when implied weighting was performed (Figure 3B and 5B). Likewise, it was also not recovered in the treatment *A'* (Figure 2B). Considering only

the discrete data set (treatment *E*) the monophyletic group was found (Figure 6A), through implied weighting it was obtained as sister of *R.* paraguayensis (Figure 6B).

Species group *b*

The clade is compounded by *R. punctulata* Duda from different regions of Brazil (Central, North, Northeast and South), *R. punctuloides* Poppe, Schmitz & Valente, *R.* punctata, *R. americana* (Patterson), *R. bivisualis* (Patterson) and *R. trivisualis* Poppe, Schmitz & Valente*;* all dot-spotted thorax species with brown stripes on the pleura, front with ca. 35 interfrontal setulae presenting spots on the base of OR setae. As noticed to the species from group *a*, the species that compound group *b* were also positioned at the base of *Rhinoleucophenga* through the treatment *C'* (Figure 4B). By the treatment *C,* the species group *b* was a sister clade of *R.* paraguayensis and *R. joaquina* Schmitz, Gottschalk & Valente.

The monophyletic group was also obtained through treatments *A* and *A'* (Figure 2A-B). By the use of continuous characters as absolute measures, the monophyletic species group was not recovered (Figure 3A-B and 5A-B). However, a group compounded only by *R. punctulata* specimens and *R. punctuloides* was always recovered as monophyletic and well supported, mainly in the treatments *B* and *D* (Figure 3 and 5, respectively). Similarly, considering only the discrete characters (treatment *E*), the clade was not obtained; but, after applying implied weighting (treatment *E'*), a monophyletic clade was obtained only without *R. americana* (Figure 6B).

Species group *c*

The group was compounded by *R. obesa* (Loew), *R. gigantea* (Thomson) and *R. pallida* Hendel, all yellow flies (ca. 5.0 mm) with ca. 200 interfrontal setulae, prescutellar acrostichal setae well-developed, front bicolored, abdomen proximally yellow, vein dM-Cu curved, wings with distal spots and ring-like aedeagus with dorsal projection. Through equal weighting, it was obtained as a sister clade of *R. pampeana* Poppe *et al.* (Figure 4A); by implied weighting (Figure 4B), as well as by treatments *A* and *A'* (Figure 2A-B), it was found as sister to *R. pampeana* and *R.* alata (Figure 4B).

By only discrete characters (treatments *E* and *E'*) the clade was also obtained (Figure 6A-B). However, in the analyses of continuous characters as absolute measures, the monophyletic group was obtained only through *log-transformed* data (treatment *D*; Figure 5A).

Species group *d*

The group is compound by *R*. alata specimens and *R. pampeana*; yellow or brownish specimens (ca. 6.0-7.0 mm) with 200 interfrontal setulae and supernumerary veins (Figure 4B). That clade was also obtained and well supported through treatment *A'* (Figures 2B).

Species group *e*

The group is compound by *R.* brasilis specimens and *R. lopesi* Malogolowkin (Figure 4A); brown specimens (ca. 3.5 mm) with ca. 40 interfrontal setulae and hyaline wings. Equally, the clade was found by treatment *A* (Figure 2A) and also through the analyses of discrete data (treatments *E* and *E'*) (Figure 6A-B). Through continuous characters treated as absolute measures under equal weighting (treatment *B*), it was obtained as a sister clade of *R. capixabensis* Culik & Ventura and *R.* obscura (Figure 3A), while through *log-transformed* measures under implied weighting (treatment *D'*), *R.* brasilis grouped with *R. capixabensis* instead of *R. lopesi* (Figure 5B).

*S*pecies group *f*

The clade is compounded by *R. matogrossensis* Malogolowkin, *R. nigrescens* Malogolowkin and *R.* fulva, being characterized by dark brown specimens (ca. 6.0 mm), with ca. 200 scattered interfrontal setulae (Figure 4A-B). A monophyletic group was also obtained through treatment *A* (Figure 2A-B). However, through continuous characters treated as absolute measures (treatments *B* and *D*), the monophyletic group was obtained only in regard to *R. matogrossensis* and *R. nigrescens* (Figure 3A-B and 5A-B, respectively).

8.1.5. Discussion

Comparison among different treatments of continuous data set

Through each method of analyses, only one most parsimonious tree was obtained, except by the analysis of discrete data only (treatments *E* and *E*'). According to Koch *et al*. (2014), it is commonly noticed through analyses with continuous characters and should not be taken as evidence of a strong phylogenetic signal. Nonetheless, continuous characters provide fundamental information to the phylogenies, as well as best branches support values (Goloboff *et al*., 2006). The problem of using continuous characters to propose phylogenetic hypothesis is linked to the arbitrariness to perform them (Rae, 2002; Goloboff *et al*., 2006; Koch *et al*., 2015), but all measures were performed by only one of us (JL Poppe), thus, that problem was partially avoided. Therefore, the addition of continuous characters resulted in

phylogenetic hypotheses with species groups best defined and supported to *Rhinoleucophenga*, mainly with continuous characters treated as ratios.

The most widely used strategies proposed to treat continuous data to phylogenetic analyses are range rescaling and implied weighting (Koch *et al*., 2014), and both approaches were applied to at least some of our treatments. Although many authors have stressed that no single search strategy or treatment methods to characters is equally efficient to all data sets (Goloboff *et al*., 2006; Wheeler, 2012). Rescaling continuous characters is an alternative manner to decrease the scales differences caused by body size from different taxa (Rae, 2002, Goloboff *et al*., 2006; Koch *et al*., 2014). Similarly, implied weighting is employed in the data sets to reduce the scale differences among characters, as well as the homoplasies effects on the resulting phylogeny (Goloboff, 1993; Goloboff *et al*., 2006; Koch *et al*., 2014), where lower implied weights are performed to larger scaled characters, and higher implied weights to smaller scales, thus balancing the overall influence of the different characters, providing stronger phylogenetic signal. Complementary, continuous characters treated as ratios are also commonly used in phylogenetic studies in order to avoid spurious clades compounded by species of similar body size (Magalhães & Santos, 2012). According to Koch *et al*. (2015), some phylogenetic noises can be included in the results even through characters treated as ratios, which could be avoided by log transformation of continuous characters. The same authors also propose that rescaled and *log-transformed* ratio characters improve the branches support in the proposed phylogeny. Likewise, through analyses with continuous ratio characters, the species groups were individually more stable and best supported, mainly with *log-transformed* data set (treatment *C*). Conversely, the phylogenetic tree obtained by the continuous characters treated as absolute measures with implied weighting, *log-transformed* (treatment *D'*) or not (treatment *B'*) presented higher average Jackknife and Bootstrap support, but most *Rhinoleucophenga* species groups were weakly supported and less stable in these analysis. So, the phylogenetic hypothesis obtained by continuous ratio characters l*og-transformed* under implied weighting (treatment *C'*) was selected as theoretically the best phylogenetic hypothesis available to *Rhinoleucophenga*, in accordance to the methodological approach proposed by Koch *et al*. (2015).

On the trees topologies, log transformation was less influent than the implied weighting, since the trees obtained by the treatments *A* and *C* presented the same topology, and the tree found in *B* was topologically more similar with *D* than with any

other tree. Additionally, the composition of each data set also influenced on tree topology, that is, the higher the similarity in the proportion between discretized and continuous characters in the matrixes, the more similar were the topologies obtained from different data sets; it can be also noticed by the examples presented by Goloboff *et al*. (2006). Furthermore, the same authors mentioned SPR distances as a good analysis to verify the similarity among trees topologies; here it was alternatively performed, and our previous results were equally supported (Supp. File 17).

Relations within Rhinoleucophenga *and with other genera of Steganinae*

The paraphyletic condition of *Rhinoleucophenga*, based on its proximity with *Pararhinoleucophenga,* was recovered through most analyzing methods and it is in accordance with the few phylogenetic studies including *Rhinoleucophenga* species. Okada (1989) included *Pararhinoleucophenga* and *Rhinoleucophenga* into the tribe Leucophengini. Grimaldi (1990) also included *Pararhinoleucophenga* and *Rhinoleucophenga* into the same tribe, Gitonini, but pointed it as a doubtful position to *Pararhinoleucophenga*. Grimaldi (1990) and Cao & Chen (2009) highlighted that *Pararhinoleucophenga* presents characters highly similar with *Leucophenga*, mainly the presence of pegs on the costal vein (also noticed here: character 74, methods *A* and *C*; character 120, methods *B* and *D*; character 16, method *E*). Out of that, we also observed other characters resembling *Pararhinoleucophenga* and *Leucophenga* such as the costal vein going up to the insertion point of vein R_{4+5} (character 73, methods *A* and *C*; character 119, methods *B* and *D*; character 15, method *E*), wings not hyaline (character 115, methods *A* and *C*; character 161, methods *B* and *D*; character 57, method *E*), aedeagus not ring-like shaped (character 91, methods *A* and *C*; character 137, methods *B* and *D*; character 33, method *E*) and susrtyli not fused in the epandrium (character 98, methods *A* and *C*; character 144, methods *B* and *D;* character 40, method *E*). Many of those characters are applied to the taxonomy of those genera (to more details see Bächli *et al*., 2004 and Cao & Chen, 2009) and probably represent convergence events between them. Moreover, Cao & Chen (2009) pointed that beyond the higher similarity with *Leucophenga, Pararhinoleucophenga* differs of *Rhinoleucophenga* by its recorded geographical distribution; it is endemic from Oriental region, while the last one is endemic from Neartic and Neotropical regions. Nevertheless, this argument on the basis of biogeography is losely justified, since the crucial aspect to include a taxon in a taxonomic entity is by sharing a unique and exclusive ancestor (Schuh & Brower,

2009). Additionally, the fused condition of surstyli in the epandrium, noticed to *Rhinoleucophenga*, is a pronounced morphological difference from *Pararhinoleucophenga*. Here, the characters resembling *Rhinoleucophenga* and *Pararhinoleucophenga* were highly homoplastic ones, with *CI* values equal or lower than 0.2, except by the convergence of the vein R_{4+5} with M-IV (character 76, methods *A* and *C* – *CI*: 0.5; character 122, methods *B* and *D* – *CI*: 0.25). Differently, Malogolowkin (1946) and Okada (1989) highlighted the convergence between the veins R_{4+5} and M-IV as a useful character to segregate those genera, but here it was a synapomorphy among *Pararhinoleucophenga* species and *R.* jacareacanga (species under description). The position of the last species in *Rhinoleucophenga* is ensured mainly by the aedeagus with ring-like shape, surstyli fused to the epandrium, absence of pegs on the costal vein and front with high number of interfrontal setulae. Recently many new *Rhinoleucophenga* species have been described (Junges & Gottschalk, 2014; Poppe *et al*., 2014, 2015; Vidal & Vilela, 2015), and other similar situations of shared morphological traits arise, such as cercus with ventral processes in *R. punctuloides* and in *Pararhinoleucophenga* species (Cao & Chen, 2009). Therefore, the grouping of *Pararhinoleucophenga* with *Rhinoleucophenga* remains doubtful, as well as the position of the former in Steganinae is dependent of more comprehensive studies.

Likewise, according to Bächli *et al*. (2004) and Vilela & Bächli (2009), the limit between *Gitona* and *Rhinoleucophenga* is ambiguous. Those genera share some characters, such as broad gena, front with scattered setulae, long pairs of prescutellar acrostichal setae, crossvein bM-Cu absent and surstyli fused in the epandrium. In fact, the main difference is concerning their geographical distribution: *Rhinoleucophenga* is exclusively recorded in the Neotropical and Nearctic regions, as already mentioned, while *Gitona* is from the Old Word region (Vilela & Bächli, 2009). Our results are in accordance with Brake & Bächli (2008) who allocated five species of *Gitona* from New World to *Rhinoleucophenga*, among them *R. brasiliensis*, *R. fluminensis*, *R. bivisualis* and *R. americana* that were within *Rhinoleucophenga* through all performed analyses. Here, any species currently classified as *Gitona* was included, which may be considered to further studies. However, an important perception in the phylogenetic tree presented by Grimaldi (1988, 1990) is that *R. brasiliensis* and *R. bivisualis* were grouped with *Gitona* instead of *Rhinoleucophenga*. Here, those species and all other species from their clades (groups *a* and *b*, respectively) were recovered as early offshoots in

Rhinoleucophenga. Possibly, this represents additional evidence to the non-monophyletic condition of *Rhinoleucophenga*.

Remsen & O'Grady (2002) highlighted that some characters sets only present strong phylogenetic signal through combined analyses. The reinforcement of the results obtained by different treatments of continuous data can be taken as indication of strong internal consistency and congruence of our data overall. Therefore, the proposition of species groups seems to be applicable, since most monophyletic species groups were repeatedly obtained through the tested methods; on other words, most methods are congruent with the same hypothesis of relationship among taxa. This can be seen as a robust evidence of the reliability of the proposed *Rhinoleucophenga* phylogeny, even though the support values of some species clades are apparently low. According to Egan (2006), the best phylogenetic hypothesis is not the highest supported one by a specific resampling test, but the hypothesis supported through different analyses. The same author also mentioned that when only one tree is generated in a phylogenetic search, it may be considered as the best hypothesis of relationship among taxa based in a specified data set, independently of the support values, however, low support values can represent ambiguity on the data (Grant & Kluge, 2003), and this cannot be ignored. More important than support values, the repeatability of our results over different treatments can be taken as an indication of their reliability. Even though, the results need to be interpreted with caution, being theoretically most interesting to further studies, since they represent the first phylogenetic propositions to *Rhinoleucophenga*.

The presented results highlight the influence of different treatment methods on the phylogenetic analyses, in agreement with previous studies (Goloboff, 1993; Goloboff *et al*., 2006; Koch *et al*., 2014). However, the consideration of ecological traits in future studies can provide new information to *Rhinoleucophenga* phylogeny or stronger support to the results presented here. Behavior patterns of the target genus should be a useful data set to phylogenetic analyses, once it presents peculiar larval predatory habits (Lima, 1935; Jiménez, 1993; Vidal & Vilela, 2015). Therefore, the present study represents a "starting point", providing preliminary hypotheses which should be tested in a larger-scale Steganinae phylogeny with multiple data partitions, including continuous morphological characters.

8.1.6. Acknowledgments

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8.1.7. References

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8.1.8. Figures

Figure 1. Methodological approach. Arrangement of matrixes of morphological data set to perform the phylogenetic analyses; EW: equal weighting, IW: implied weighting. All strategies were performed with equal weighting (*A*-*E*) and after performed through implied weighting (*A'*-*E'*). The figure number below each tree diagram indicates the correspondent tree in the article.

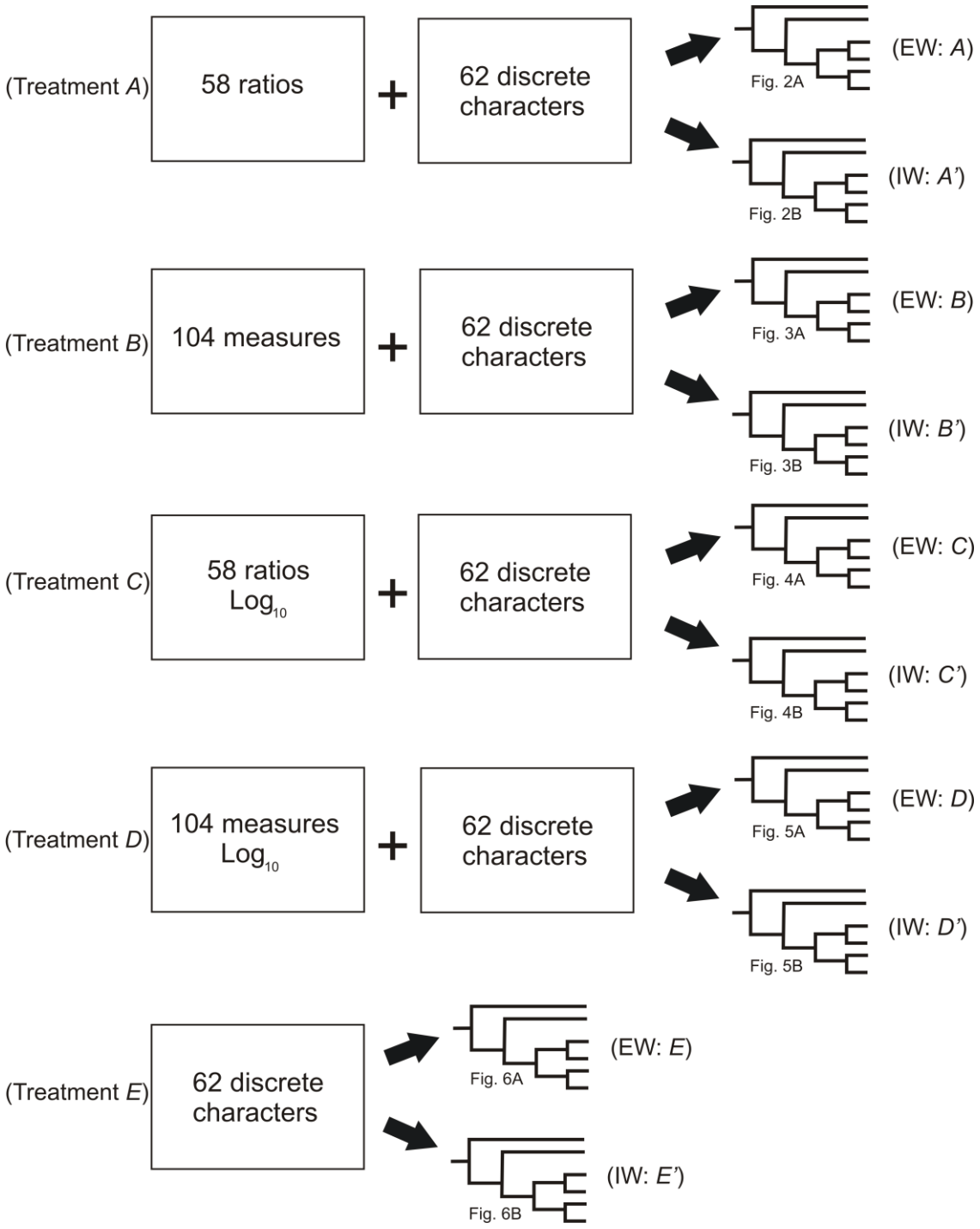


Figure 2. Most parsimonious tree obtained from the analysis using 58 rationed continuous characters without discretization, and 62 discrete characters: 20 neomorphic and 42 transformational (treatment *A*). Numbers below nodes are Bootstrap *standard*, *GC* and Jackknife support values, respectively; - : collapsed nodes or very weakly supported. A: tree obtained through equal weighting analyses (tree length 571.191); B: tree obtained through implied weighting analyses (tree length 39.194).

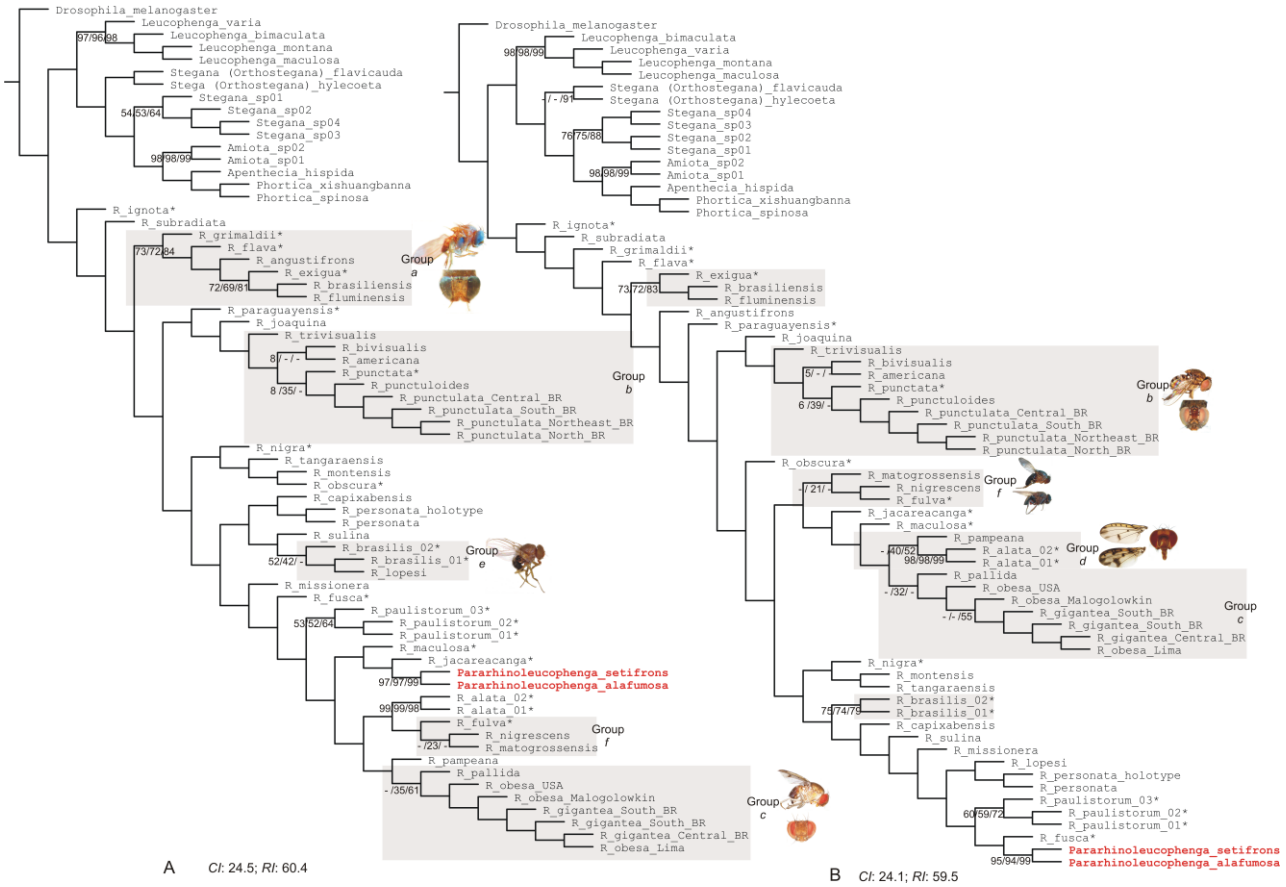


Figure 3. Most parsimonious tree obtained from the analysis using 104 continuous characters: 93 measures of body parts and 11 setae meristic counting without discretization, and 62 discrete characters: 20 neomorphic and 42 transformational (treatment *B*). Numbers below nodes are Bootstrap *standard*, *GC* and Jackknife support values, respectively; - : collapsed nodes or very weakly supported. A: tree obtained through equal weighting analyses (tree length 725.666); B: tree obtained through implied weighting analyses (tree length 50.121).

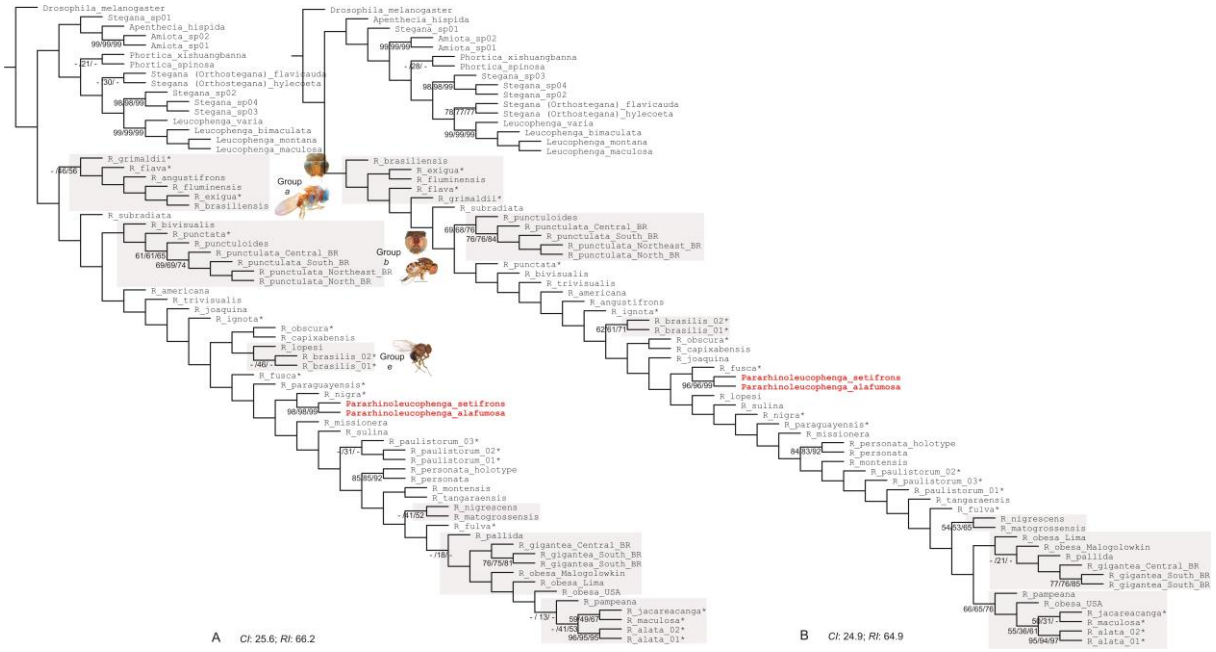


Figure 4. Most parsimonious tree obtained from the analysis using 58 rationed continuous characters *log-transformed* without discretization, and 62 discrete characters: 20 neomorphic and 42 transformational (treatment *C*). Numbers below nodes are Bootstrap *standard*, *GC* and Jackknife support values, respectively; - : collapsed nodes or very weakly supported. A: tree obtained through equal weighting analyses (tree length 580.999); B: tree obtained through implied weighting analyses (tree length 40.016).

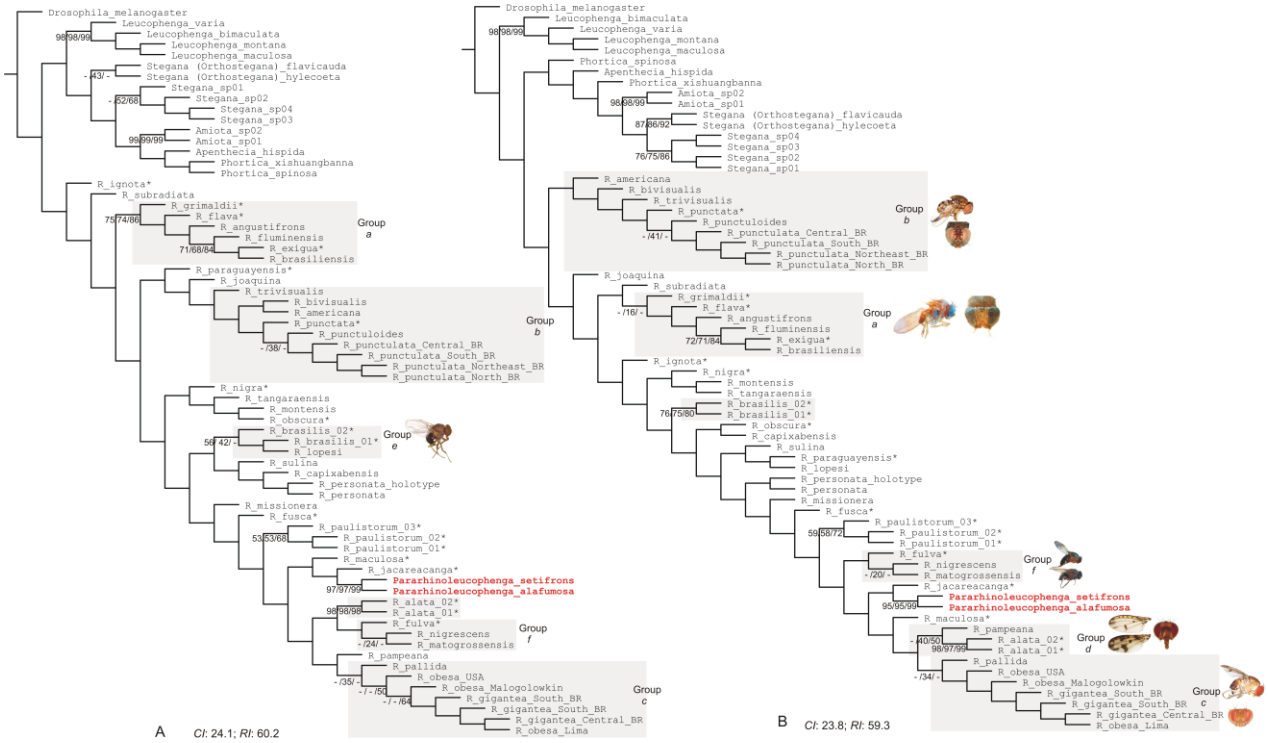


Figure 5. Most parsimonious tree obtained from the analysis using 104 continuous characters: 93 measures of body parts and 11 setae meristic counting *log-transformed* without discretization, and 62 discrete characters: 20 neomorphic and 42 transformational (treatment *D*). Numbers below nodes are Bootstrap *standard*, *GC* and Jackknife support values, respectively; - : collapsed nodes or very weakly supported. A: tree obtained through equal weighting analyses (tree length 739.529); B: tree obtained through implied weighting analyses (tree length 51.156).

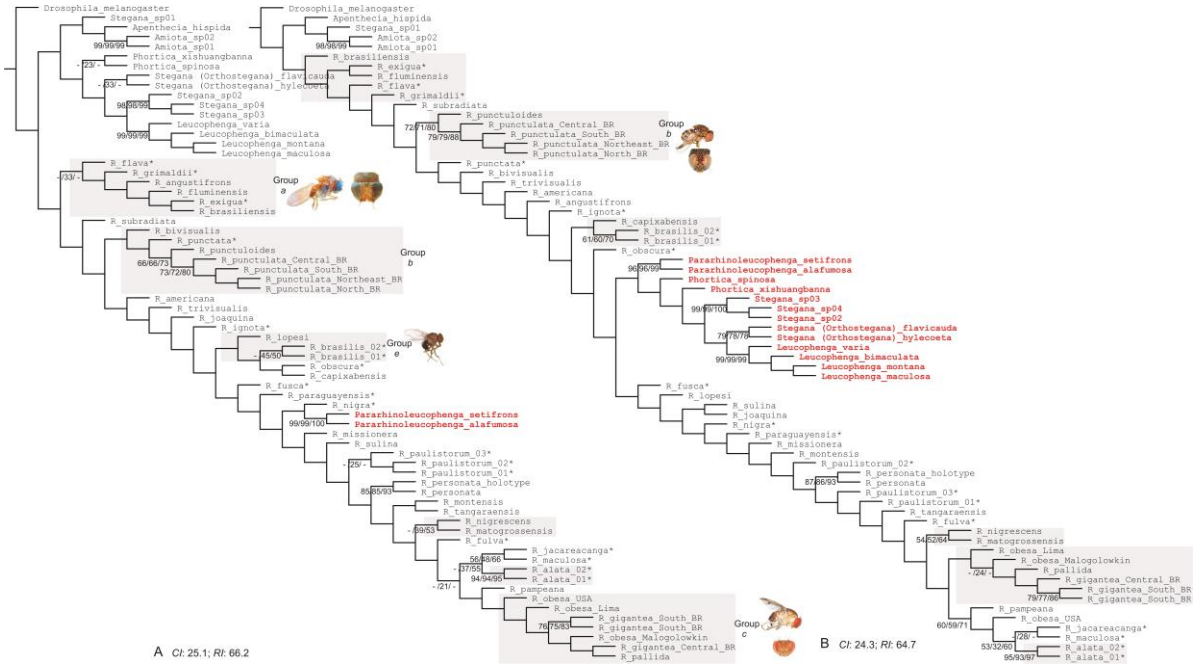
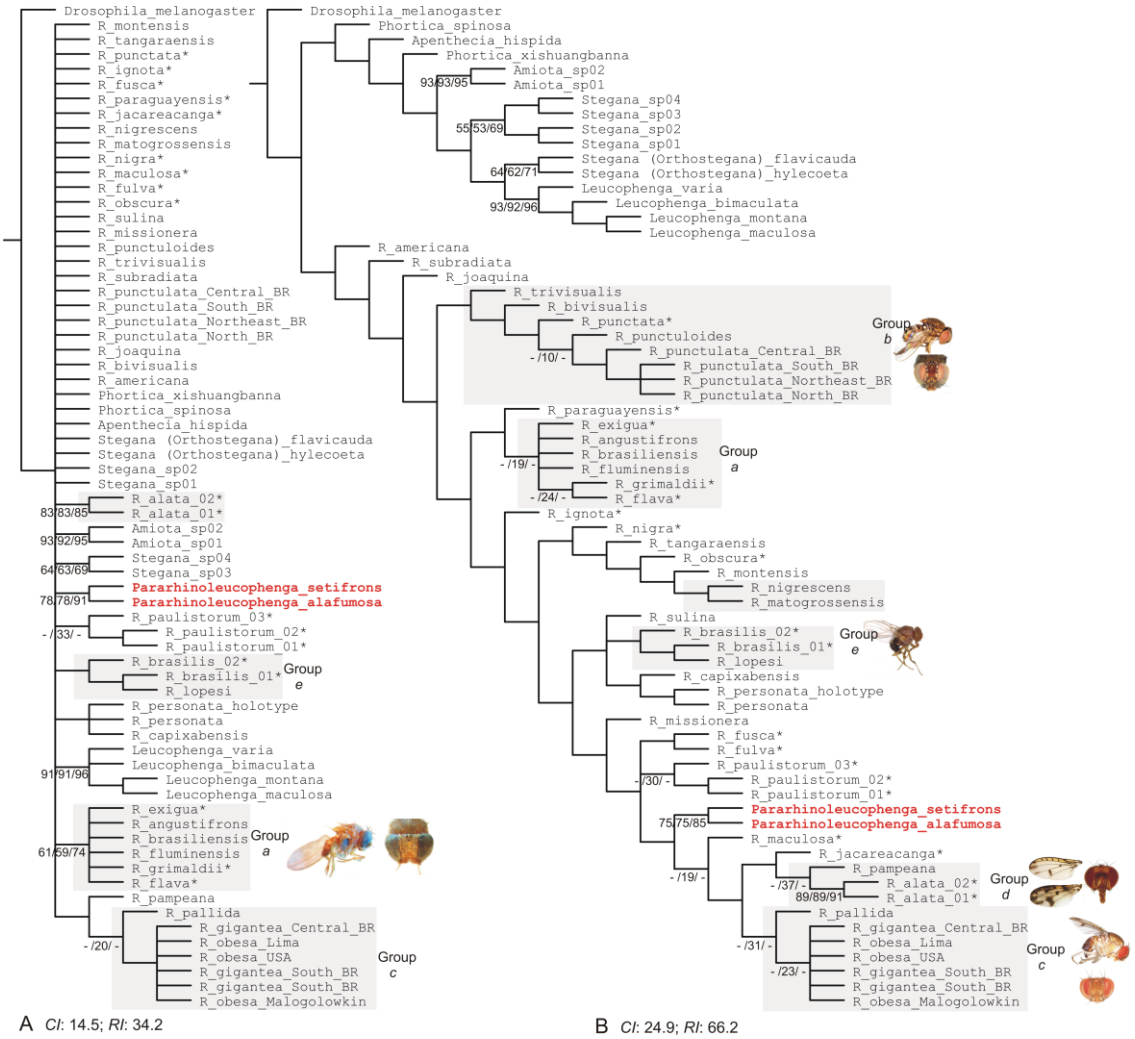


Figure 6. Most parsimonious tree obtained by strict consensus from the analysis using 62 discrete characters: 20 neomorphic and 42 transformational (treatment *E*). Numbers below nodes are Bootstrap *standard*, *GC* and Jackknife support values, respectively; - : collapsed nodes or very weakly supported. A: tree obtained through equal weighting analyses (tree length 599); B: tree obtained through implied weighting analyses (tree length 20.643).



A *CI*: 14.5; *RI*: 34.2

B *CI*: 24.9; *RI*: 66.2

8.1.9. Tables

Table 1. List of species checked and included in the phylogenetic analysis. The number of specimens checked and the institution where they are deposited are also provided.

	Species	Number of specimens analyzed	Deposited at:
Root	*Drosophila melanogaster* Meigen, 1830	5	Fiocruz
Outgroup			
Subtribe Steganini	*Leucophenga bimaculata* (Loew, 1866)	3	Fiocruz
	Leucophenga maculosa (Coquillett in Johnson, 1895)	6	Fiocruz
	Leucophenga montana Wheeler, 1952	1	Fiocruz
	Leucophenga varia (Walker, 1849)	2	Fiocruz
	Pararhinoleucophenga alafumosa Cao & Chen, 2009	1	SCAU
	Pararhinoleucophenga setifrons Cao & Chen, 2009	1	SCAU
	Stegana (Orthostegana) flavicauda Zhang & Chen, 2012 (in Zhang et al., 2012)	1	SCAU
	Stegana (Orthostegana) hylecoeta Zhang & Chen, 2012 (in Zhang et al., 2012)	1	SCAU
	Stegana sp01	1	MZUSP
	Stegana sp02	1	MZUSP
	Stegana sp03	1	MZUSP
	Stegana sp04	1	MZUSP
Subtribe Gitonini	*Amiota* sp01	5	Fiocruz
	Amiota sp02	5	Fiocruz
	Apenthecia hispida Chen & Toda, 2008 (in Cao et al., 2008)	1	SCAU
	Phortica spinosa Chen & Gao, 2005 (in Chen et al., 2005)	1	SCAU
	Phortica xishuangbanna Chen & Chen, 2008 (in Chen et al., 2008)	1	SCAU
Ingroup	**Rhinoleucophenga* alata (From São Paulo, Brazil)	1	MZUSP

**Rhinoleucophenga* alata (From Botuverá, Santa Catarina, Brazil)	1	MZUSP
Rhinoleucophenga americana (Patterson, 1943)	1	AMNH
Rhinoleucophenga angustifrons Malogolowkin, 1946	1	Fiocruz
Rhinoleucophenga brasiliensis (Lima, 1935)	6	Fiocruz
**Rhinoleucophenga* brasilis	2	Fiocruz
Rhinoleucophenga bivisualis (Patterson, 1943)	1	AMNH
Rhinoleucophenga capixabensis Culik & Ventura, 2009	5	Fiocruz
**Rhinoleucophenga* exigua	6	Fiocruz
**Rhinoleucophenga* flava	1	Fiocruz
Rhinoleucophenga fluminensis (Lima, 1935)	7	Fiocruz
**Rhinoleucophenga* fulva	1	Fiocruz
Rhinoleucophenga gigantea (Thomson, 1869) (from South of Brazil)	5	Fiocruz
Rhinoleucophenga gigantea (Thomson, 1869) (from South of Brazil; previously identified as *R. obesa* by Poppe et al. 2014)	5	Fiocruz
Rhinoleucophenga gigantea (Thomson, 1869) (from Central Brazil)	1	Fiocruz
**Rhinoleucophenga* grimaldii	1	Fiocruz
**Rhinoleucophenga* ignota	1	Fiocruz
**Rhinoleucophenga* fusca	1	Fiocruz
**Rhinoleucophenga* jacareacanga	2	Fiocruz
Rhinoleucophenga joaquina Schimitz, Gottschalk & Valente, 2009	9	Fiocruz
Rhinoleucophenga lopesi Malogolowkin, 1946	1	Fiocruz
**Rhinoleucophenga* maculosa	1	Fiocruz
Rhinoleucophenga matogrossensis Malogolowkin, 1946	1	Fiocruz
Rhinoleucophenga missionera Poppe et al., 2014	5	Fiocruz
Rhinoleucophenga montensis Junges & Gottschalk, 2014	2	Fiocruz

**Rhinoleucophenga* nigra	1	Fiocruz
Rhinoleucophenga nigrescens Malogolowkin, 1946	1	Fiocruz
Rhinoleucophenga obesa (Loew, 1872) (described by Malogolowkin, 1946)	9	Fiocruz
Rhinoleucophenga obesa (Loew, 1872) (described by Lima, 1935)	7	Fiocruz
Rhinoleucophenga obesa (Loew, 1872) (from Texas, USA)	1	AMNH
**Rhinoleucophenga* obscura	1	Fiocruz
Rhinoleucophenga pallida Hendel, 1917	1	AMNH
Rhinoleucophenga pampeana Poppe et al., 2014	4	Fiocruz
**Rhinoleucophenga* paraguayensis	2	Fiocruz
**Rhinoleucophenga* paulistorum	3	MZUSP
Rhinoleucophenga personata Malogolowkin, 1946 (Holotype)	1	Fiocruz
**Rhinoleucophenga personata* Malogolowkin, 1946 (from Northeast of Brazil)	1	Fiocruz
**Rhinoleucophenga* punctata	1	Fiocruz
Rhinoleucophenga punctulata Duda, 1929 (from Northeast of Brazil)	5	Fiocruz
Rhinoleucophenga punctulata Duda, 1929 (from North of Brazil)	5	Fiocruz
Rhinoleucophenga punctulata Duda, 1929 (from Central Brazil)	5	Fiocruz
Rhinoleucophenga punctulata Duda, 1929 (from South of Brazil)	5	Fiocruz
Rhinoleucophenga punctuloides Poppe, Schmitz & Valente, 2015	5	Fiocruz
Rhinoleucophenga subradiata Duda, 1929	5	Fiocruz
Rhinoleucophenga sulina Poppe et al., 2014	5	Fiocruz
Rhinoleucophenga tangaraensis Junges & Gottschalk, 2014	2	Fiocruz

Rhinoleucophenga trivisualis Poppe, Schmitz & Valente, 2015	5	Fiocruz	

*: Species in description process, manuscript submitted. AMNH: American Museum of Natural History, USA; MZUSP: Zoology Museum at University of São Paulo, Brazil; Fiocruz: Entomological collection of Fundação Oswaldo Cruz, Rio de Janeiro, Brazil; SCAU: Department of Entomology of South China Agricultural University.

Table 2. Indices and measures of phylogenetic trees obtained through each treatment applied in the data set. -: not calculated.

	Treatment											
	A	***A'***	***B***	***B'***	***C***	***C'***	***D***	***D'***	***E***	***E'***	**Consensus *E***	**Consensus *E'***
Tree length	571.191	-	725.666	-	580.999	-	739.529	-	343	-	599	-
Tree Fit	-	39.19416	-	50.12124	-	40.0169	-	51.156	-	20.5033	-	20.6434
Number of generate trees	1	1	1	1	1	1	1	1	3510	27	-	-
Consistence Index (*CI*)	24.5	24.1	25.6	24.9	24.1	23.8	25.1	24.3	-	-	14.5	24.9
retention Index (*RI*)	60.4	59.5	66.2	64.9	60.2	59.3	66.2	64.7	-	-	34.2	66.2
Average of Bootstrap group frequency	13.9	13.9	15.2	22.5	14	14	15.3	21.2	8.7	10.4	-	-
Average of Bootstrap group frequency difference	21.1	21.4	23.9	26.6	21.2	21.2	23.7	27.5	13	15.2	-	-
Jackknife Clade Average	14.8	18.0	18.2	24.0	16.0	17.3	19.5	25.8	9.3	12.0	-	-

Table 3. Topological comparison tests among the most parsimonious trees obtained by each treatment performed to the data set. Bellow the diagonal: Distortion-Coefficients (*DistCoef*), higher values mean higher topology congruency. Above the diagonal: Robinson-Foulds distances (*RF*), lower values mean higher topology congruency.

Treatment	***A***	***B***	***C***	***D***	***E***	***A'***	***B'***	***C'***	***D'***	***E'***
A	-	0.59	0.03	0.59	0.38	0.38	0.73	0.44	0.77	0.5
B	0.86	-	0.58	0.12	0.45	0.64	0.53	0.58	0.59	0.58
C	1	0.84	-	0.58	0.38	0.39	0.73	0.42	0.77	0.5
D	0.86	0.98	0.86	-	0.44	0.62	0.53	0.58	0.59	0.58
E	0.44	0.43	0.44	0.45	-	0.45	0.53	0.44	0.53	0.32
A'	0.93	0.86	0.92	0.87	0.88	-	0.71	0.45	0.77	0.56
B'	0.81	0.93	0.81	0.93	0.7	0.82	-	0.76	0.39	0.67
C'	0.91	0.87	0.91	0.88	0.9	0.88	0.87	-	0.79	0.55
D'	0.77	0.9	0.77	0.9	0.68	0.76	0.97	0.8	-	0.7
E'	0.87	0.84	0.87	0.85	0.97	0.84	0.83	0.88	0.68	-

8.1.10. Supporting files

Supp. File 1: List of characters from the matrixes *A* and *C* used in the phylogenetic analyses. Characters 1 to 57: continuous ratio characters. Characters 58 to 119: discretized characters; numbers in parentheses indicate the characters states in the matrix. Name of body structures according to Bächli et al. (2004).

Character number	Character
0	Body, length:
1	Front, interfrontal setulae, number relative to body length:
2	Head, front, shape, superior width versus inferior width, proportion:
3	Head, front, orbital setae 01 versus orbital setae 03, length proportion:
4	Head, front, orbital setae 02 versus orbital setae 03, length proportion:
5	Head, front, orbital setae 01 versus orbital setae 02, length proportion:
6	Head, front, vtl setae versus vtm setae, length proportion:
7	Front, ocellar setae versus ocellar triangle, length proportion:
8	Antenna, first flagellomere, shape, length versus width, proportion:
9	Front, carina, percentage sulcated relative to body length:
10	Front, carina, shape, length versus basal width, proportion:
11	Arista, ventral branches, number relative to body length:
12	Face, palpus, shape, length versus width, proportion:
13	Face, palpus, ventral setulae, number relative to body length:
14	Head, gena, shape, length versus width, proportion:
15	Gena, longest genal setae, length versus genal width, proportion:
16	Gena, first genal setae, length versus genal length, proportion:
17	Gena, longest genal setae versus first genal setae, length proportion:
18	Head, posterior region, dorsolateral tentorial apodeme versus dorsocentral tentorial apodeme, length proportion:
19	Head, posterior region, dorsolateral tentorial apodeme, length relative to body length:
20	Head, posterior region, dorsolateral tentorial apodeme, basal distance versus apical distance, proportion:
21	Front, orbital setae 01, distance to orbital setae 03, relative to body length:
22	Front, orbital setae 01, distance to orbital setae 02, relative to body length:
23	Front, orbital setae 03, distance to vtm setae, relative to body length:
24	Front, orbital setae 02, distance to orbital setae 03, relative to body length:
25	Scutum, acrostichal prescutelar setae, number of pairs relative to body length:
26	Scutum, longest acrostichal prescutelar setae, length relative to body length:
27	Scutum, anterior dorsocentral setae, distance to posterior scutum edge, relative to body length:
28	Scutellum, apical setae, distance to anterior scutellum edge, relative to body length:
29	Scutum, posterior dorsocentral setae versus anterior dorsocentral setae, length proportion:
30	Scutellum, basal setae versus apical setae, length proportion:
31	Thorax, katepisternum plate, small setulae, number relative to body length:

32 Thorax, Katepisternum plate, posterior setae versus anterior setae, length proportion:
33 Thorax, postpronotal setae, length relative to scutum length, proportion:
34 Wing, shape, length versus width, proportion:
35 Wing, vein A_1, length versus wing length, proportion:
36 Wing, vein R_{2+3}, length versus wing length, proportion:
37 Wing, vein C-II, length versus wing width, proportion:
38 Wing, vein C-III with heavy bristles versus vein C-III as all, length proportion:
39 Wing, vein C-IV, length versus wing width, proportion:
40 Wing, vein M-IV versus vein M-III, length proportion:
41 Wing, vein CuA, length versus wing width, proportion:
42 Legs, profemur, length relative to scutum length, proportion:
43 Legs, profemur, basal long setae, number relative to profemur length, proportion:
44 Legs, procoxa, length relative to scutum length, proportion:
45 Legs, procoxa setulae, number relative to procoxa length:
46 Legs, long procoxa setulae, number relative to procoxa length:
47 Legs, protibia, length relative to scutum length, proportion:
48 Abdomen, III sternite, shape, length versus width, proportion:
49 Abdomen, III sternite setae, number relative to abdomen length:
50 Ring-like aedeagus, internal circumference, vertical diameter versus horizontal diameter, proportion:
51 Male terminalia, aedeagus, length relative to abdomen length:
52 Male terminalia, aedeagus, shape, length versus largest width proportion:
53 Epandrium, cerci, length relative to abdomen length:
54 Male terminalia, surtylus, prensisetae, number relative to abdomen length:
55 Female terminalia, spermathecal capsule, shape, length versus width, proportion:
56 Female terminalia, dorsal region, epiproct, shape, length versus width, proportion:
57 Female terminalia, ventral region, hypoproct, shape, length versus width, proportion:
58 Head, front, spots on the base of orbital setae: (0) absent; (1) present
59 Head, front, orbital setae, insertion position of one to each other: (0) aleatory; (1) in line
60 Head, front, orbital setae 01, vertical position in relation to the front mean point: (0) ventral; (1) dorsal
61 Head, posterior region, post-ocellar setae: (0) present; (1) absent
62 Head, front, carina, shape: (0) noselike; (1) flat
63 Head, face, carina, groove: (0) absent; (1) present
64 Antennae, arista, branches, lenght: (0) long; (1) micropubescent; (2) short/ pubescent
65 Head, ventral region, gena, spots: (0) absent; (1) present
66 Head, posterior region, dorsolateral tentorial apodeme, orientation of the pair: (0) divergent; (1) parallel; (2) convergent
67 Thorax, scutum, acrostichal prescutellar setae: (0) absent; (1) present
68 Thorax, scutum, spots: (0) present; (1) absent
69 Thorax, scutum, spots type: (0) diffuse stripes; (1) dots and stripes; (2) dots; (3) stripes
70 Thorax, scutellum, basal setae, orientation of the pair: (0) convergent; (1) divergent
71 Thorax, lateral region, pleura, spots: (0) absent; (1) present
72 Thorax, katepisternum plate, longer setae, number: (0) 3; (1) 2

73 Thorax, wing, costal vein, position: (0) crossing the insertion point of vein R_{4+5}; (1) up to the insertion point of vein R_{4+5}.
74 Wing, costal vein, costal spinules: (0) absent; (1) present
75 Thorax, wing, vein R_{2+3}, intensity of convergence into the costal vein: (0) weak; (1) very weak; (2) strong
76 Thorax, wing, vein M-IV, position to the vein R_{4+5} apical: (0) parallel; (1) convergent
77 Thorax, wing, vein dM-Cu, shape: (0) straight; (1) curved
78 Thorax, wing, spots: (0) absent; (1) present
79 Thorax, wing, spots, position: (0) proximal-distal; (1) distal
80 Thorax, wing, vein dM-Cu, clouded: (0) absent; (1) present
81 Thorax, wing, transversal vein R-M, clouded: (0) absent; (1) present
82 Thorax, wing, costal cell, clouded: (0) absent; (1) present
83 Thorax, wing, supernumerary veins: (0) absent; (1) present
84 Thorax, wing, supernumerary veins, number: (0) 4; (1) 6
85 Thorax, wing, supernumerary veins with free ends, number: (0) 4; (1) 3
86 Thorax, wing, supernumerary veins without free ends, number: (0) 0; (1) 3
87 Thorax, wing, vein bm-cu: (0) absent; (1) present
88 Abdomen, proximal tergites, stripes pattern: (0) continuousus; (1) interrupted
89 Abdomen, distal tergites, stripes pattern: (0) continuousus; (1) interrupted
90 Abdomen, distal tergites, interupted stripes, interruption position: (0) laterally; (1) medially
91 Male terminalia, aedeagus, general form: (0) non ring-like; (1) ring-like
92 Male terminalia, aedeagus, apical portion, setae: (0) absent; (1) present
93 Male terminalia, aedeagus, dorsal projection: (0) absent; (1) present
94 Male terminalia, aedeagus, ventral projection: (0) absent; (1) present
95 Aedeagus, dorsal view, basal width related to the apice: (0) equal; (1) wider
96 Male terminalia, aedeagus apodeme, general shape: (0) stick; (1) other
97 Male terminalia, adeagus apodeme, apical fork: (0) present; (1) absent
98 Male terminalia, surstyli, fused to epandrium: (0) no; (1) yes
99 Male terminalia, surstilus, preensisetae: (0) present; (1) absent
100 Male terminalia, surstyli, prensisetae, form: (0) pointed; (1) rod-shaped
101 Female terminalia, spermathecal capsule, basal width related to the apice: (0) wider; (1) narrower; (2) equal
102 Female terminalia, spermathecal capsule, spinules: (0) absent; (1) present
103 Head, front, color pattern: (0) unicolor; (1) bicolor
104 Head, front, ventral portion, color: (0) brownish; (1) yellow; (2) brown; (3) dark brown
105 Head, front, dorsal portion, color: (0) brownish; (1) brown; (2) dark brown; (3) yellow
106 Head, front, ocellar traingle, color: (0) brownish; (1) brown; (2) dark brown; (3) yellow; (4) black
107 Front, ocellar triangle, ocelli, highlighted color: (0) present; (1) absent
108 Head, face, color: (0) yellow; (1) brownish; (2) brown
109 Head, antena, first flagellomere, color pattern: (0) unicolor; (1) bicolor
110 Antena, pedicel, color: (0) brownish; (1) yellow; (2) brown; (3) dark brown
111 Thorax, scutum, color: (0) yellow; (1) brownish; (2) brown; (3) dark brown; (4) black
112 Thorax, scutum, spots color: (0) brownish; (1) dark brown; (2) brown; (3) yellow
113 Thorax, scutellum, color pattern: (0) unicolor; (1) bicolor

114	Thorax, lateral region, pleura, spots color: (0) dark brown; (1) white
115	Thorax, wing, color: (0) hyaline; (1) not-hyaline
116	First pair of legs, femur, color pattern: (0) unicolor; (1) bicolor
117	Thorax, second and third pair of legs, color pattern: (0) unicolor; (1) bicolor
118	Abdomen, dorsal region, proximal portion, color: (0) yellow; (1) brownish; (2) dark brown; (3) brown
119	Abdomen, tergites, stripes, color: (0) black; (1) brown; (2) dark brown; (3) brownish

Supp. File 2: Fifty-eight continuous ratio characters used to perform the matrixes of treatments *A* and *C*. .xlsx file.

Supp. File 3: Matrix used to perform the analysis with the treatment *A*; .tnt file.

Supp. File 4: List of characters from the matrixes *B* and *D* used in the phylogenetic analyses. Characters 1 to 103: continuous characters. Characters 104 to 165: discretized characters; numbers in parentheses indicate the characters states in the matrix. Name of body structures according to Bächli et al. (2004).

Character number	Character
0	Head, vertical length:
1	Head, horizontal length:
2	Head, front, interfrontal setulae, number:
3	Head, front, superior portion, width:
4	Head, front, inferior portion, width:
5	Head, front, vertical length:
6	Head, front, orbital setae 01, length:
7	Head, front, orbital setae 02, length:
8	Head, front, orbital setae 03, length:
9	Head, dorsal region, vtl setae, length:
10	Head, dorsal region, vtm setae, length:
11	Head, dorsal region, post-ocellar setae, length:
12	Head, front, ocellar triangle, length:
13	Head, front, ocellar triangle, basal width:
14	Front, ocellar triangle, ocellar setae, length:
15	Head, face, vibrissa, length:
16	Head, antenna, first flagellomere, length:
17	Head, antenna, first flagellomere, width:
18	Head, front, carina, percentage sulcated:
19	Head, front, noselike carina, length:
20	Head, front, carina, basal length:
21	Head, arista, dorsal branches, number:
22	Head, arista, ventral branches, number:
23	Face, arista, most basal dorsal branch, length:
24	Face, arista, most basal ventral branch, length:
25	Head, face, arista, length:
26	Head, face, palpus, length:
27	Head, face, palpus, width:
28	Face, palpus, ventral setae, number:
29	Head, eye, length:
30	Head, eye, width:
31	Head, gena, length:
32	Head, gena, width:
33	Head, gena, longest genal setae, length:
34	Head, gena, first genal setae, length:
35	Head, posterior region, dorsolateral tentorial apodeme, length:
36	Head, posterior region, dorsocentral tentorial apodeme, length:
37	Head, posterior region, dorsolateral tentorial apodeme, basal distance:
38	Head, posterior region, dorsolateral tentorial apodeme, apical distance:
39	Head, front, orbital setae 01, distance to orbital setae 03:

40 Head, front, orbital setae 01, distance to orbital setae 02:
41 Head, front, orbital setae 03, distance to orbital vtm setae:
42 Head, front, orbital setae 02, distance to orbital setae 03:
43 Thorax, scutum, length:
44 Thorax, scutum, width:
45 Thorax, scutum, acrostichal prescutelar setae, number of pairs:
46 Thorax, scutum, longest acrostichal prescutelar setae, length:
47 Thorax, scutum, anterior dorsocentral setae, distance to posterior scutum edge:
48 Thorax, scutum, posterior dorsocentral setae, distance to posterior scutum edge:
49 Thorax, scutum, anterior dorsocentral setae, transversal distance to the center of scutum:
50 Thorax, scutum, acrostichal setulae, number of lines:
51 Thorax, scutellum, basal setae, distance to anterior scutellar edge:
52 Thorax, scutellum, apical setae, distance to anterior scutellar edge:
53 Thorax, scutum, anterior dorsocentral setae, length:
54 Thorax, scutum, posterior dorsocentral setae, length:
55 Thorax, scutellum, basal setae, length:
56 Thorax, scutellum, apical setae, length:
57 Thorax, scutellum, length:
58 Thorax, scutellum, width:
59 Thorax, katepisternum plate, small setulae, number:
60 Thorax, katepisternum plate, anterior setae, length:
61 Thorax, katepisternum plate, posterior setae, length:
62 Thorax, postpronotum, setae, length:
63 Thorax, wing, length:
64 Thorax, wing, width:
65 Thorax, wing, vein A_1, length:
66 Thorax, wing, vein R_{2+3}, length:
67 Thorax, wing, apical vein R_{4+5}, length:
68 Thorax, wing, basal vein R4+5, length:
69 Thorax, wing, vein C-II, length:
70 Thorax, wing, vein C-III, length:
71 Thorax, wing, vein C-III with heavy bristles, length:
72 Thorax, wing, vein C-IV, length:
73 Thorax, wing, vein M-III, length:
74 Thorax, wing, vein M-IV, length:
75 Thorax, wing, vein Dm-Cu, length:
76 Thorax, wing, vein CuA, length:
77 Legs, profemur, length:
78 Legs, profemur, basal longest setae, lehgth:
79 Legs, procoxa, length:
80 Legs, procoxa, setae, number:
81 Legs, procoxa, long apical setae, number:
82 Legs, procoxa, longest apical setae, length:
83 Legs, protibia, length
84 Legs, protarsus, length:
85 Legs, protarsus, first tarsomere, length:
86 Abdomen, anterior tergites stripes, interruption interval, length:

87 Abdomen, posterior tergites stripes, interruption interval, length:
88 Abdomen, III sternite, setae, number:
89 Abdomen, length:
90 Body, length:
91 Ring-like aedeagus, internal circumference, horizontal radius:
92 Ring-like aedeagus, internal circumference, vertical radius:
93 Male terminalia, aedeagus, length:
94 Male terminalia, aedeagus, middle region, width:
95 Male terminalia, aedeagus, highest width:
96 Male terminalia, aedeagus apodeme, length:
97 Aedeagus apodeme, apical arms, longitudinal distance:
98 Male terminalia, epandrium, cerci, length:
99 Male terminalia, epandrium, cerci, width:
100 Male terminalia, epandrium, basal portion, length:
101 Male terminalia, surtylus, prensisetae, number:
102 Female terminalia, spermathecal capsule, length:
103 Female terminalia, spermathecal capsule, width:
104 Head, front, spots on the base of orbital setae: (0) absent; (1) present
105 Head, front, orbital setae, insertion position of one to each other: (0) aleatory; (1) in line
106 Head, front, orbital setae 01, vertical position in relation to the front mean point: (0) ventral; (1) dorsal
107 Head, posterior region, post-ocellar setae: (0) present; (1) absent
108 Head, front, carina, shape: (0) noselike; (1) flat
109 Head, face, carina, groove: (0) absent; (1) present
110 Antennae, arista, branches, lenght: (0) long; (1) micropubescent; (2) short/ pubescent
111 Head, ventral region, gena, spots: (0) absent; (1) present
112 Head, posterior region, dorsolateral tentorial apodeme, orientation of the pair: (0) divergent; (1) parallel; (2) convergent
113 Thorax, scutum, acrostichal prescutellar setae: (0) absent; (1) present
114 Thorax, scutum, spots: (0) present; (1) absent
115 Thorax, scutum, spots type: (0) diffuse stripes; (1) dots and stripes; (2) dots; (3) stripes
116 Thorax, scutellum, basal setae, orientation of the pair: (0) convergent; (1) divergent
117 Thorax, lateral region, pleura, spots: (0) absent; (1) present
118 Thorax, katepisternum plate, longer setae, number: (0) 3; (1) 2
119 Thorax, wing, costal vein, position: (0) crossing the insertion point of vein R_{4+5}; (1) up to the insertion point of vein R_{4+5}.
120 Wing, costal vein, costal spinules: (0) absent; (1) present
121 Thorax, wing, vein R_{2+3}, intensity of convergence into the costal vein: (0) weak; (1) very weak; (2) strong
122 Thorax, wing, vein M-IV, position to the vein R_{4+5} apical: (0) parallel; (1) convergent
123 Thorax, wing, vein dM-Cu, shape: (0) straight; (1) curved
124 Thorax, wing, spots: (0) absent; (1) present
125 Thorax, wing, spots, position: (0) proximal-distal; (1) distal
126 Thorax, wing, vein dM-Cu, clouded: (0) absent; (1) present
127 Thorax, wing, transversal vein R-M, clouded: (0) absent; (1) present
128 Thorax, wing, costal cell, clouded: (0) absent; (1) present

129 Thorax, wing, supernumerary veins: (0) absent; (1) present
130 Thorax, wing, supernumerary veins, number: (0) 4; (1) 6
131 Thorax, wing, supernumerary veins with free ends, number: (0) 4; (1) 3
132 Thorax, wing, supernumerary veins without free ends, number: (0) 0; (1) 3
133 Thorax, wing, vein bm-cu: (0) absent; (1) present
134 Abdomen, proximal tergites, stripes pattern: (0) continuousus; (1) interrupted
135 Abdomen, distal tergites, stripes pattern: (0) continuousus; (1) interrupted
136 Abdomen, distal tergites, interupted stripes, interruption position: (0) laterally; (1) medially
137 Male terminalia, aedeagus, general form: (0) non ring-like; (1) ring-like
138 Male terminalia, aedeagus, apical portion, setae: (0) absent; (1) present
139 Male terminalia, aedeagus, dorsal projection: (0) absent; (1) present
140 Male terminalia, aedeagus, ventral projection: (0) absent; (1) present
141 Aedeagus, dorsal view, basal width related to the apice: (0) equal; (1) wider
142 Male terminalia, aedeagus apodeme, general shape: (0) stick; (1) other
143 Male terminalia, adeagus apodeme, apical fork: (0) present; (1) absent
144 Male terminalia, surstyli, fused to epandrium: (0) no; (1) yes
145 Male terminalia, surstilus, preensisetae: (0) present; (1) absent
146 Male terminalia, surstyli, prensisetae, form: (0) pointed; (1) rod-shaped
147 Female terminalia, spermathecal capsule, basal width related to the apice: (0) wider; (1) narrower; (2) equal
148 Female terminalia, spermathecal capsule, spinules: (0) absent; (1) present
149 Head, front, color pattern: (0) unicolor; (1) bicolor
150 Head, front, ventral portion, color: (0) brownish; (1) yellow; (2) brown; (3) dark brown
151 Head, front, dorsal portion, color: (0) brownish; (1) brown; (2) dark brown; (3) yellow
152 Head, front, ocellar traingle, color: (0) brownish; (1) brown; (2) dark brown; (3) yellow; (4) black
153 Front, ocellar triangle, ocelli, highlighted color: (0) present; (1) absent
154 Head, face, color: (0) yellow; (1) brownish; (2) brown
155 Head, antena, first flagellomere, color pattern: (0) unicolor; (1) bicolor
156 Antena, pedicel, color: (0) brownish; (1) yellow; (2) brown; (3) dark brown
157 Thorax, scutum, color: (0) yellow; (1) brownish; (2) brown; (3) dark brown; (4) black
158 Thorax, scutum, spots color: (0) brownish; (1) dark brown; (2) brown; (3) yellow
159 Thorax, scutellum, color pattern: (0) unicolor; (1) bicolor
160 Thorax, lateral region, pleura, spots color: (0) dark brown; (1) white
161 Thorax, wing, color: (0) hyaline; (1) not-hyaline
162 First pair of legs, femur, color pattern: (0) unicolor; (1) bicolor
163 Thorax, second and third pair of legs, color pattern: (0) unicolor; (1) bicolor
164 Abdomen, dorsal region, proximal portion, color: (0) yellow; (1) brownish; (2) dark brown; (3) brown
165 Abdomen, tergites, stripes, color: (0) black; (1) brown; (2) dark brown; (3) brownish

Supp. File 5: One hundred and four absolute measures used to perform the matrixes of treatments *B* and *D*. .xlsx file.

Supp. File 6: Matrix used to perform the analysis with the treatment *B*; .tnt file.

Supp. File 7: Matrix used to perform the analysis with the treatment *C*; .tnt file.

Supp. File 8: Matrix used to perform the analysis with the treatment *D*; .tnt file.

Supp. File 9: List of 62 discretized characters from the matrix *E* used in the phylogenetic analyses. Numbers in parentheses indicate the characters states in the matrix. Name of body structures according to Bächli et al. (2004).

Character number	Character
0	Head, front, spots on the base of orbital setae: (0) absent; (1) present
1	Head, front, orbital setae, insertion position of one to each other: (0) aleatory; (1) in line
2	Head, front, orbital setae 01, vertical position in relation to the front mean point: (0) ventral; (1) dorsal
3	Head, posterior region, post-ocellar setae: (0) present; (1) absent
4	Head, front, carina, shape: (0) noselike; (1) flat
5	Head, face, carina, groove: (0) absent; (1) present
6	Antennae, arista, branches, lenght: (0) long; (1) micropubescent; (2) short/ pubescent
7	Head, ventral region, gena, spots: (0) absent; (1) present
8	Head, posterior region, dorsolateral tentorial apodeme, orientation of the pair: (0) divergent; (1) parallel; (2) convergent
9	Thorax, scutum, acrostichal prescutellar setae: (0) absent; (1) present
10	Thorax, scutum, spots: (0) present; (1) absent
11	Thorax, scutum, spots type: (0) diffuse stripes; (1) dots and stripes; (2) dots; (3) stripes
12	Thorax, scutellum, basal setae, orientation of the pair: (0) convergent; (1) divergent
13	Thorax, lateral region, pleura, spots: (0) absent; (1) present
14	Thorax, katepisternum plate, longer setae, number: (0) 3; (1) 2
15	Thorax, wing, costal vein, position: (0) crossing the insertion point of vein R_{4+5}; (1) up to the insertion point of vein R_{4+5}.
16	Wing, costal vein, costal spinules: (0) absent; (1) present
17	Thorax, wing, vein R_{2+3}, intensity of convergence into the costal vein: (0) weak; (1) very weak; (2) strong
18	Thorax, wing, vein M-IV, position to the vein R_{4+5} apical: (0) parallel; (1) convergent
19	Thorax, wing, vein dM-Cu, shape: (0) straight; (1) curved
20	Thorax, wing, spots: (0) absent; (1) present
21	Thorax, wing, spots, position: (0) proximal-distal; (1) distal
22	Thorax, wing, vein dM-Cu, clouded: (0) absent; (1) present
23	Thorax, wing, transversal vein R-M, clouded: (0) absent; (1) present
24	Thorax, wing, costal cell, clouded: (0) absent; (1) present
25	Thorax, wing, supernumerary veins: (0) absent; (1) present
26	Thorax, wing, supernumerary veins, number: (0) 4; (1) 6
27	Thorax, wing, supernumerary veins with free ends, number: (0) 4; (1) 3
28	Thorax, wing, supernumerary veins without free ends, number: (0) 0; (1) 3
29	Thorax, wing, vein bm-cu: (0) absent; (1) present
30	Abdomen, proximal tergites, stripes pattern: (0) continuousus; (1) interrupted
31	Abdomen, distal tergites, stripes pattern: (0) continuousus; (1) interrupted
32	Abdomen, distal tergites, interupted stripes, interruption position: (0) laterally; (1) medially
33	Male terminalia, aedeagus, general form: (0) non ring-like; (1) ring-like

34	Male terminalia, aedeagus, apical portion, setae: (0) absent; (1) present
35	Male terminalia, aedeagus, dorsal projection: (0) absent; (1) present
36	Male terminalia, aedeagus, ventral projection: (0) absent; (1) present
37	Aedeagus, dorsal view, basal width related to the apice: (0) equal; (1) wider
38	Male terminalia, aedeagus apodeme, general shape: (0) stick; (1) other
39	Male terminalia, adeagus apodeme, apical fork: (0) present; (1) absent
40	Male terminalia, surstyli, fused to epandrium: (0) no; (1) yes
41	Male terminalia, surstilus, preensisetae: (0) present; (1) absent
42	Male terminalia, surstyli, prensisetae, form: (0) pointed; (1) rod-shaped
43	Female terminalia, spermathecal capsule, basal width related to the apice: (0) wider; (1) narrower; (2) equal
44	Female terminalia, spermathecal capsule, spinules: (0) absent; (1) present
45	Head, front, color pattern: (0) unicolor; (1) bicolor
46	Head, front, ventral portion, color: (0) brownish; (1) yellow; (2) brown; (3) dark brown
47	Head, front, dorsal portion, color: (0) brownish; (1) brown; (2) dark brown; (3) yellow
48	Head, front, ocellar traingle, color: (0) brownish; (1) brown; (2) dark brown; (3) yellow; (4) black
49	Front, ocellar triangle, ocelli, highlighted color: (0) present; (1) absent
50	Head, face, color: (0) yellow; (1) brownish; (2) brown
51	Head, antena, first flagellomere, color pattern: (0) unicolor; (1) bicolor
52	Antena, pedicel, color: (0) brownish; (1) yellow; (2) brown; (3) dark brown
53	Thorax, scutum, color: (0) yellow; (1) brownish; (2) brown; (3) dark brown; (4) black
54	Thorax, scutum, spots color: (0) brownish; (1) dark brown; (2) brown; (3) yellow
55	Thorax, scutellum, color pattern: (0) unicolor; (1) bicolor
56	Thorax, lateral region, pleura, spots color: (0) dark brown; (1) white
57	Thorax, wing, color: (0) hyaline; (1) not-hyaline
58	First pair of legs, femur, color pattern: (0) unicolor; (1) bicolor
59	Thorax, second and third pair of legs, color pattern: (0) unicolor; (1) bicolor
60	Abdomen, dorsal region, proximal portion, color: (0) yellow; (1) brownish; (2) dark brown; (3) brown
61	Abdomen, tergites, stripes, color: (0) black; (1) brown; (2) dark brown; (3) brownish

Supp. File 10: Sixty-two discrete characters used to perform all matrixes. .xlsx file.

Supp. File 11: Matrix used to perform the analysis with the treatment *E*; .tnt file.

Supp. File 12: List of synapomorphies supporting the main monophyletic species groups obtained by the treatments *A* and *A'*. Only the clades with all component species suggested in the text are presented. *: character also supporting the clade through implied weighting; Char.: Character; CI: Consistency Index; RI: Retention Index. See characters number on Supp. File 1.

Species group	Treatment	Synapomorphies	CI	RI
a	*A*	Char. 1: 11.428-12.370 --> 13.043-13.537	0.325	0.78
		Char. 4: 0.666-0.680 --> 0.700-0.730	0.203	0.385
		Char. 14: 0.347-0.375 --> 0.333	0.337	0.305
		Char. 18: 1.733-1.888 --> 2.000	0.412	0.173
		Char. 22: 0.023-0.026 --> 0.028	0.148	0.541
		Char. 35: 0.106-0.119 --> 0.091-0.098	0.147	0.526
		Char. 36: 0.659-0.662 --> 0.647	0.174	0.499
		Char. 46: 11.325-11.538 --> 11.764	0.177	0.519
		Char. 104: 0 --> 1	0.231	0.714
		Char. 105: 0 --> 3	0.25	0.591
		Char. 106: 0 --> 3	0.308	0.654
		Char. 110: 0 --> 1	0.188	0.629
		Char. 111: 1 --> 0	0.222	0.6
b	*A*	Char. 0: 3.500-3.575 --> 3.168-3.431	0.239	0.712
		Char. 1: 11.428-12.370 --> 10.170-10.901	0.325	0.78
		Char. 4: 0.647-0.680 --> 0.747-0.800	0.203	0.385
		Char. 17: 2.285-2.500 --> 2.181-2.222	0.192	0.198
		Char. 21: 0.063 --> 0.065-0.073	0.169	0.556
		Char. 22: 0.023-0.026 --> 0.032-0.036	0.148	0.541
		Char. 29: 1.739-1.817 --> 1.687-1.706	0.162	0.258
		Char. 35: 0.123-0.132 --> 0.102-0.106	0.147	0.526
		Char. 37: 1.285-1.317 --> 1.318-1.335	0.304	0.29
		Char. 40: 2.500-2.543 --> 2.691-2.786	0.327	0.604
		Char. 54: 9.271-9.718 --> 9.054*	0.268	0.302
		Char. 57: 0.916-1.000 --> 0.909	0.556	0.418
		Char. 58: 0 --> 1*	0.25	0.667
		Char. 69: 0 --> 2*	0.6	0.833
		Char. 71: 0 --> 1*	0.2	0.75
b	*A'*	Char. 0: 3.500 --> 3.168-3.431	0.228	0.694
		Char. 1: 11.428-13.043 --> 10.170-10.901	0.294	0.746
		Char. 4: 0.647-0.676 --> 0.747-0.800	0.202	0.382
		Char. 17: 2.300-2.400 --> 2.181-2.222	0.197	0.224
		Char. 21: 0.053-0.063 --> 0.065-0.073	0.151	0.492
		Char. 22: 0.023-0.028 --> 0.032-0.036	0.148	0.541
		Char. 29: 1.817-1.923 --> 1.687-1.706	0.165	0.271
		Char. 35: 0.123 --> 0.102-0.106	0.159	0.57
		Char. 36: 0.665-0.687 --> 0.697	0.177	0.51

		Char. 37: 1.287-1.317 --> 1.318-1.335	0.404	0.543
		Char. 40: 2.543-2.592 --> 2.691-2.786	0.346	0.638
		Char. 49: 20.558-21.312 --> 21.710-23.534	0.273	0.537
		Char. 57: 1.000 --> 0.909	0.557	0.421
c	*A*	Char. 22: 0.017-0.020 --> 0.013-0.014*	0.148	0.541
		Char. 34: 2.210-2.234 --> 2.143	0.146	0.397
		Char. 77: 0 --> 1*	0.143	0.4
c	*A*′	Char. 14: 0.320-0.339 --> 0.303	0.42	0.512
		Char. 25: 0.500-0.510 --> 0.574	0.255	0.569
		Char. 34: 2.156-2.234 --> 2.143	0.153	0.428
		Char. 47: 0.493-0.500 --> 0.487	0.376	0.449
		Char. 104: 2 --> 1	0.2	0.657
d	*A*′	Char. 19: 0.095-0.097 --> 0.086-0.088	0.224	0.378
		Char. 45: 2.678-2.927 --> 1.875-2.439	0.248	0.461
		Char. 83: 0 --> 1	1.000	1.000
e	*A*	Char. 1: 11.428-12.370 --> 10.937	0.324	0.780
		Char. 4: 0.691-0.696 --> 0.676	0.203	0.385
		Char. 8: 1.866-2.425 --> 1.750	0.167	0.439
		Char. 22: 0.022-0.026 --> 0.021	0.148	0.541
		Char. 25: 0.756-0.791 --> 0.312	0.249	0.553
		Char. 102: 0 --> 1	0.500	0.667
		Char. 111: 1 --> 2	0.222	0.600
f	*A*	Char. 6: 1.000-1.061 --> 1.106	0.202	0.454
		Char. 8: 2.281-2.363 --> 2.200	0.167	0.439
		Char. 36: 0.684-0.722 --> 0.666	0.174	0.499
		Char. 37: 1.323-1.365 --> 1.282	0.304	0.291
		Char. 42: 0.552-0.553 --> 0.539	0.226	0.299
		Char. 78: 1 --> 0	0.250	0.800
		Char. 80: 1 --> 0	0.250	0.800
f	*A*′	Char. 2: 1.056 --> 0.960-1.000	0.226	0.596
		Char. 45: 2.678-4.000 --> 2.285-2.307	0.248	0.461
		Char. 60: 0 --> 1	0.143	0.667

Supp. File 13: List of synapomorphies supporting the main monophyletic species groups obtained by the treatments *B* and *B'*. Only the clades with all component species suggested in the text are presented. *: character also supporting the clade through implied weighting; Char.: Character; CI: Consistency Index; RI: Retention Index. See characters number on Supp. File 3.

Species group	Treatment	Synapomorphies	CI	RI
a	*B*	Char. 3: 0.421-0.437 --> 0.420	0.292	0.788
		Char. 70: 0.476-0.477 --> 0.460	0.332	0.645
		Char. 137: 0 --> 1	0.2	0.818
		Char. 150: 0 --> 1	0.214	0.686
		Char. 151: 0 --> 3	0.2	0.455
		Char. 152: 0 --> 3	0.308	0.654
		Char. 156: 0 --> 1	0.2	0.657
e	*B*	Char. 12: 0.201-0.220 --> 0.200	0.203	0.602
		Char. 27: 0.100-0.120 --> 0.140	0.243	0.638
		Char. 54: 0.711-0.717 --> 0.730-0.740	0.290	0.760

Supp. File 14: List of synapomorphies supporting the main monophyletic species groups obtained by the treatments *C* and *C'*. Only the clades with all component species suggested in the text are presented. *: character also supporting the clade through implied weighting; Char.: Character; CI: Consistency Index; RI: Retention Index. See characters number on Supp. File 1.

Species group	Treatment	Synapomorphies	CI	RI
a	*C*	Char. 1: 1.094-1.126 --> 1.147-1.162	0.28	0.704
		Char. 4: 0.221-0.225 --> 0.230-0.238	0.199	0.382
		Char. 8: 0.443-0.457 --> 0.477	0.175	0.47
		Char. 14: 0.129-0.138 --> 0.124	0.28	0.366
		Char. 18: 0.436-0.460 --> 0.477	0.335	0.184
		Char. 22: 0.010-0.011 --> 0.012	0.152	0.547
		Char. 35: 0.043-0.048 --> 0.038-0.040	0.145	0.529
		Char. 36: 0.219-0.220 --> 0.216	0.172	0.498
		Char. 46: 1.090-1.098 --> 1.106	0.174	0.537
		Char. 104: 0 --> 1*	0.231	0.714
		Char. 105: 0 --> 3*	0.25	0.591
		Char. 106: 0 --> 3*	0.308	0.654
		Char. 110: 0 --> 1*	0.188	0.629
		Char. 111: 1 --> 0*	0.222	0.6
a	*C'*	Char. 1: 1.070-1.076 --> 1.147-1.162	0.271	0.689
		Char. 4: 0.224-0.225 --> 0.230-0.238	0.203	0.397
		Char. 8: 0.428-0.443 --> 0.477	0.161	0.414
		Char. 14: 0.129-0.140 --> 0.124	0.331	0.502
		Char. 17: 0.506-0.530 --> 0.500	0.201	0.244
		Char. 18: 0.436-0.441 --> 0.477	0.356	0.258
		Char. 22: 0.010-0.011 --> 0.012	0.173	0.61
		Char. 35: 0.048-0.050 --> 0.038-0.040	0.153	0.56
		Char. 36: 0.220-0.221 --> 0.216	0.173	0.5
		Char. 38: 0.477-0.492 --> 0.464	0.191	0.47
		Char. 46: 1.083-1.090 --> 1.106	0.17	0.526
b	*C*	Char. 0: 0.653-0.660 --> 0.620-0.646	0.235	0.709
		Char. 1: 1.094-1.126 --> 1.048-1.075	0.28	0.704
		Char. 4: 0.216-0.225 --> 0.242-0.255	0.199	0.382
		Char. 17: 0.516-0.544 --> 0.502-0.508	0.192	0.2
		Char. 21: 0.026 --> 0.027-0.030	0.164	0.551
		Char. 22: 0.010-0.011 --> 0.013-0.015	0.152	0.547
		Char. 29: 0.437-0.449 --> 0.429-0.432	0.16	0.265
		Char. 35: 0.050-0.053 --> 0.042-0.043	0.145	0.529
		Char. 40: 0.544-0.549 --> 0.567-0.578	0.263	0.619
		Char. 54: 1.011-1.030 --> 1.002	0.233	0.307
		Char. 57: 0.282-0.301 --> 0.280	0.531	0.432
		Char. 58: 0 --> 1	0.25	0.667

		Char. 69: 0 --> 2*	0.6	0.833
		Char. 71: 0 --> 1	0.2	0.75
b	*C*′	Char. 4: 0.224-0.227 --> 0.255-0.258	0.203	0.397
		Char. 29: 0.437-0.449 --> 0.429-0.432	0.156	0.238
		Char. 36: 0.220-0.221 --> 0.229-0.237	0.173	0.5
		Char. 40: 0.537-0.549 --> 0.578	0.283	0.655
		Char. 44: 0.139-0.140 --> 0.135-0.136	0.19	0.283
		Char. 68: 1 --> 0	0.91	0.444
c	*C*	Char. 22: 0.007-0.008 --> 0.005-0.006*	0.152	0.547
		Char. 34: 0.506-0.509 --> 0.497	0.145	0.388
		Char. 77: 0 --> 1*	0.143	0.4
c	*C*′	Char. 14: 0.120-0.124 --> 0.114	0.331	0.502
		Char. 34: 0.499-0.509 --> 0.497	0.144	0.381
		Char. 47: 0.174-0.176 --> 0.172	0.329	0.396
		Char. 104: 2 --> 1	0.214	0.686
d	*C*′	Char. 0: 0.837-0.845 --> 0.892	0.238	0.714
		Char. 8: 0.531-0.543 --> 0.549	0.161	0.414
		Char. 20: 0.312-0.317 --> 0.319	0.5	0.386
		Char. 30: 0.307-0.308 --> 0.312	0.314	0.458
		Char. 38: 0.505-0.536 --> 0.685	0.191	0.47
		Char. 39: 0.118-0.119 --> 0.109	0.288	0.51
		Char. 40: 0.391-0.410 --> 0.377	0.283	0.655
		Char. 46: 0.879-0.921 --> 0.851	0.17	0.526
		Char. 47: 0.174-0.176 --> 0.357	0.329	0.396
		Char. 49: 1.278 --> 1.186	0.307	0.58
		Char. 79: 1 --> 0	0.333	0.6
		Char. 88: 0 --> 1	1	1
		Char. 88: 1 --> 0	0.125	0.5
		Char. 101: 2 --> 0	0.154	0.154
		Char. 103: 0 --> 1	0.083	0.522
		Char. 111: 1 --> 2	0.182	0.486
e	*C*	Char. 0: 0.653-0.660 --> 0.648	0.235	0.709
		Char. 1: 1.094-1.126 --> 1.076	0.280	0.704
		Char. 8: 0.443-0.457 --> 0.439	0.175	0.470
		Char. 25: 0.241-0.244 --> 0.118	0.233	0.550
		Char. 102: 0 --> 1	0.500	0.667
		Char. 111: 1 --> 2	0.222	0.600
f	*C*	Char. 6: 0.301-0.314 --> 0.323	0.202	0.450
		Char. 8: 0.516-0.526 --> 0.505	0.175	0.470
		Char. 36: 0.226-0.236 --> 0.221	0.172	0.498
		Char. 37: 0.366-0.373 --> 0.358	0.272	0.318
		Char. 38: 0.486-0.505 --> 0.477-0.483	0.193	0.474

		Char. 42: 0.191 --> 0.187	0.216	0.298
		Char. 78: 1 --> 0	0.250	0.800
		Char. 80: 1 --> 0	0.250	0.800
f	*C*′	Char. 1: 1.156-1.264 --> 1.502	0.271	0.689
		Char. 2: 0.315-0.318 --> 0.301	0.214	0.584
		Char. 6: 0.318-0.319 --> 0.323	0.195	0.427
		Char. 9: 1.204 --> 1.172	0.313	0.616
		Char. 21: 0.018-0.022 --> 0.017	0.159	0.534
		Char. 24: 0.011-0.012 --> 0.010	0.145	0.481
		Char. 26: 0.049 --> 0.047	0.201	0.297
		Char. 27: 0.031-0.032 --> 0.028	0.175	0.417
		Char. 36: 0.226-0.227 --> 0.221	0.173	0.500
		Char. 42: 0.191-0.193 --> 0.187	0.220	0.315
		Char. 45: 0.699-0.713 --> 0.516-0.519	0.264	0.455
		Char. 46: 0.929-0.951 --> 0.854-0.910	0.170	0.526
		Char. 60: 0 --> 1	0.125	0.611
		Char. 111: 1 --> 2	0.182	0.486

Supp. File 15: List of synapomorphies supporting the main monophyletic species groups obtained by the treatments *D* and *D'*. Only the clades with all component species suggested in the text are presented. *: character also supporting the clade through implied weighting; Char.: Character; CI: Consistency Index; RI: Retention Index. See characters number on Supp. File 3.

Species group	Treatment	Synapomorphies	CI	RI
a	*D*	Char. 70: 0.169 --> 0.164	0.302	0.648
		Char. 137: 0 --> 1	0.2	0.818
		Char. 150: 0 --> 1	0.231	0.714
		Char. 151: 0 --> 3	0.2	0.455
		Char. 152: 0 --> 3	0.308	0.654
		Char. 156: 0 --> 1	0.2	0.657
c	*D*	Char. 1: 0.470-0.477 --> 0.451-0.462	0.308	0.795
		Char. 6: 0.164-0.170 --> 0.152-0.158	0.326	0.736
		Char. 7: 0.107-0.110 --> 0.099	0.238	0.562
		Char. 11: 0.086-0.107 --> 0.085	0.464	0.668
		Char. 20: 0.054-0.056 --> 0.048	0.357	0.753
		Char. 23: 0.110 --> 0.097-0.104	0.242	0.66
		Char. 24: 0.099-0.100 --> 0.087	0.29	0.689
		Char. 31: 0.062-0.064 --> 0.049	0.232	0.582
		Char. 40: 0.041-0.047 --> 0.035	0.218	0.488
		Char. 62: 0.181-0.182 --> 0.158-0.170	0.256	0.735
		Char. 79: 0.221-0.225 --> 0.213	0.268	0.777
		Char. 139: 0 --> 1	0.2	0.692
		Char. 149: 0 --> 1	0.083	0.522
		Char. 164: 1 --> 0	0.231	0.615
e	*D'*	Char. 12: 0.084-0.086 --> 0.073-0.079	0.219	0.647
		Char. 16: 0.100 --> 0.107	0.284	0.779
		Char. 25: 0.181 --> 0.187-0.191	0.402	0.823
		Char. 26: 0.100-0.110 --> 0.113	0.299	0.706
		Char. 38: 0.086 --> 0.093-0.095	0.305	0.744
		Char. 60: 0.158 --> 0.167	0.329	0.794
		Char. 62: 0.113 --> 0.126-0.127	0.268	0.751
		Char. 76: 0.158 --> 0.167-0.170	0.176	0.591
		Char. 102: 0.033 --> 0.029	0.499	0.440
		Char. 111: 0 --> 1	0.111	0.385

Supp. File 16: List of synapomorphies supporting the main monophyletic species groups obtained by the treatments *E* and *E'*. Only the clades with all component species suggested in the text are presented. *: character also supporting the clade through implied weighting; Char.: Character; CI: Consistency Index; RI: Retention Index. See characters number on Supp. File 5.

Species group	Treatment	Synapomorphies	CI	RI
a	*E*	Char. 31: 0 --> 1	0.056	0.19
		Char. 33: 0 --> 1	0.063	0.318
		Char. 36: 0 --> 1	0.111	0.2
		Char. 46: 0 --> 1*	0.15	0.514
		Char. 47: 0 --> 3*	0.158	0.273
		Char. 48: 0 --> 3*	0.211	0.423
		Char. 50: 1 --> 0	0.095	0.424
		Char. 52: 0 --> 1	0.158	0.543
		Char. 53: 1 --> 0*	0.148	0.343
		Char. 54: 1 --> 3	0.25	0.182
		Char. 61: 2 --> 1	0.2	0.333
a	*E'*	Char. 46: 0 --> 1	0.273	0.771
		Char. 47: 0 --> 3	0.200	0.455
		Char. 48: 0 --> 3	0.286	0.615
		Char. 53: 1 --> 0	0.200	0.543
c	*E*	Char. 19: 0 --> 1*	0.125	0.3
		Char. 46: 0 --> 1	0.15	0.514
c	*E'*	Char. 46: 2 --> 1	0.273	0.771
d	*E'*	Char. 25: 0 --> 1	1.000	1.000

Supp. File 17: Table of unweighted SPR distances. Topological comparison tests among the most parsimonious trees generated by each treatment performed to the data set. Bellow the diagonal: SPR similarity indices, higher values mean higher topology congruency. Above the diagonal: SPR moves, lower values mean higher topology congruency. PDF file.

Treatment	*A*	*B*	*C*	*D*	*E*	*A'*	*B'*	*C'*	*D'*	*E'*
A	-	38	2	37	0	20	54	22	65	23
B	0.4154	-	31	8	4	34	28	30	33	30
C	0.9692	0.5231	-	37	0	20	55	21	60	23
D	0.4308	0.8769	0.4308	-	3	36	28	31	33	28
E	1	0.9385	1	0.9538	-	7	20	4	21	1
A'	0.6923	0.4769	0.6923	0.4462	0.8923	-	46	24	60	69
B'	0.1692	0.5692	0.1538	0.5692	0.6923	0.2923	-	39	9	37
C'	0.6615	0.5385	0.6769	0.5231	0.9385	0.6308	0.4	-	56	18
D'	0	0.4923	0.0769	0.4923	0.6769	0.0769	0.8615	0.1385	-	37
E'	0.6462	0.5385	0.6462	0.5692	0.9846	0.5538	0.4308	0.7231	0.4308	-

9. CAPÍTULO IX

(Manuscrito aceito para publicação no peródico *Annals of the Entomological Society of America*)

Poppe et al.: assemblages' structure of Drosophilidae in the pampas.

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9.1. Changes in the assemblages' structure of Drosophilidae (Diptera) associated to contrasting environments in the pampas biome across temporal and spatial scales.

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9.1.1. Abstract

The Pampas biome is a subtropical-temperate grassland region presenting striking climatic characteristics, with negative records of temperature in the winter up to 40°C during the summer. Long sampling periods are necessary to best understand the structure of Drosophilidae assemblages through the current climatic conditions in the heterogeneous landscape of the pampas. Samples of drosophilids were taken in natural and urban areas during 12 climatic periods. The environmental influence on the assemblages' structure was temporally and spatially analyzed through nMDS, IndVal and PERMANOVA. The following hypotheses were tested: (i) the structure of drosophilids assemblages vary through different habitats from a same area, and (ii) the presence and abundance of Drosophilidae species in an environment is regulated by the interaction among phytophysionomy and climatic traits of each local. The pampas assemblages were dominated by few species, although it is a highly diverse biome concerning the Drosophilidae species. The environmental heterogeneity was confirmed as a determinant factor on the assemblages' structures, explaining 8.86% of the assemblages' composition. Nonetheless, the interaction between temperature and humidity levels was pointed as the environmental component more influent on the Drosophilidae assemblages, explaining 37.28% of all variation noticed. Together, environmental type and climatic elements were able to explain 56.45% of the assemblages' variation. Open and close environments, such as the grassland and the forest patches, respectively, present peculiar composition of Drosophilidae assemblages and must be equally considered in the pampas preservation strategies.

Keywords: biodiversity, conservation, *Drosophila*, temperature, bioindicator.

9.1.2. Resumo

O Bioma Pampa é uma região campestre subtropical-temperada que apresenta características climáticas marcantes, invernos com temperaturas negativas e verões com registros de mais de 40°C. Longos períodos de amostragem são necessários para o melhor entendimento da estrutura das assembleias de Drosophilidae na paisagem heterogênea do Pampa. Coletas de drosofilídeos foram realizadas em áreas naturais e urbanas do Pampa durante 12 períodos climáticos. A influência ambiental sobre a estrutura das assembleias foi temporal e espacialmente analisada por meio de nMDS, IndVal e PERMANOVA. As seguintes hipóteses foram testadas: (i) a estrutura das assembleias de drosofilídeos variam através de diferentes habitats de uma mesma área e, (ii) a presença e a abundancia das espécies de Drosophilidae em um ambiente é regulada pela interação da fitofisionomia e características climáticas de cada local. As assembleias do Pampa foram dominadas por poucas espécies, embora este seja um bioma muito diverso com relação à fauna de Drosophilidae. A heterogeneidade ambiental foi um fator determinante na estrutura das assembleias, explicando 8,86% da composição das mesmas. No entanto, a interação entre temperatura e os níveis de umidade foi indicada como o componente ambiental mais influente sobre as assembleias de Drosophilidae, explicando 37,28% de toda a variação percebida. O tipo de ambiente amostrado e os componentes climáticos juntos explicaram 56,45% da variação nas assembleias. Ambientes abertos e fechados, campos e matas, respectivamente, apresentaram uma composição própria para as assembleias de Drosophilidae e, devem ser igualmente considerados em estratégias de preservação do Pampa.

Palavras-chave: biodiversidade, conservação, *Drosophila*, temperatura, bioindicador.

The seasonal population dynamics of drosophilids has been studied for some years (Dobzhansky and Pavan 1950, Saavedra et al. 1995, Tidon 2006, Bizzo et al. 2010), as well as the habitat preference of many species (Sene et al. 1980, Vilela et al. 1983, Martins 1987, Mata et al. 2010). However, the knowledge of the species' response to the current climatic conditions from different localities is very important to understand the regional diversity pattern of Drosophilidae assemblages (Parsons 1991, Tidon 2006, Bizzo et al. 2010, Rohde et al. 2010, Poppe et al. 2013), mainly in environments less studied as the pampas.

Neotropical forest environments historically have received higher attention than grassland areas in Drosophilidae fauna studies (Dobzhansky and Pavan 1950, De Toni et al. 2007), possibly because grassland and savannah areas seemed to be less attractive to drosophilids when compared to forest habitats (Pavan 1959). However, in the last decade studies performed in grasslands and savannah environments revealed high diversity and interesting ecological patterns of drosophilids and other arthropods (Mata et al. 2008a, Silva et al. 2011, Medeiros et al. 2012, Mata and Tidon 2013, Poppe et al. 2014).

The pampas biome represents the South American grasslands; it presents striking climatic characteristics: negative records of temperature in the winter up to temperatures around 40°C during the summer, with constant levels of air humidity around 70%. Once Drosophilidae is a speciose taxon and very sensitive to changes in the habitat conditions, the species of that family are good model organisms to evaluate the climatic and environmental influence over the biodiversity (Dobzhansky and Pavan 1950, Tidon 2006, Mata et al. 2008b).

Recently, evidences of diversity loss in an urban area in the pampas region was noticed in comparison to a forest fragment, under strong influence of the temperature (Poppe et al. 2012). After that, a high diversity of drosophilids was found in a relatively well-conserved natural area (Poppe et al. 2014). A more detailed study showed evidences for interaction between climatic conditions and habitat choice for drosophilid species (Poppe et al. 2015a). But a temporally more comprehensive study is necessary to a better understanding of the Drosophilidae phenology in the pampas, which could also generate best comprehensions of the insects diversity patterns in worldwide subtropical grassland areas.

The present study represents the highest sample efforts to evaluate the environmental influences on the Drosophilidae assemblages in the subtropical-temperate grasslands in the South America. New records of species are expected since the pampas have been presented recently as high diverse environment concerning to the Drosophilidae fauna (Poppe et al. 2014, 2015b); thus, best understand the role of environmental factors to regulate the presence and abundance of drosophilids in that biome is important to propose conservation strategies of local diversity. The following hypotheses were tested: (i) the structure of drosophilids assemblages vary through different habitats from a same area, and (ii) the presence and abundance of Drosophilidae species in an environment is regulated by the interaction among phytophysionomy and climatic traits of each local.

9.1.4. Material and Methods

Study Area

The Pampa biome is limited between latitudes of 28°–38°S, with subtropical-temperate weather and rain every month. The surveyed region is classified as “Cfa” according to the Köppen climatic classification, having maximum temperatures higher than 22°C and minimum temperatures between -3 and 18°C, without remarkable periods of dry.

The collecting areas are in the South of Brazil, Rio Grande do Sul State. The natural Pampas area (28°45’01”S; 54°56’55”W, 200 m) is a mosaic composed by forest patches (deciduous seasonal forest) and mainly by grasses characterized by *Aristida jubata* (Poaceae) and rhizomatous grasses (family Poaceae), next to the small municipality of Bossoroca. The urban area is classified as a medium urbanized area based on the percentage of vegetation cover (Gottschalk et al. 2007), it is located in the downtown of the municipality of São Luiz Gonzaga (28°24’21”S; 54°57’06”W, 200 m) and it is approximately 45 kilometers from the sampled natural area. Surrounding the urban and natural sampled areas there is a mosaic of forest patches, natural grasslands and agricultural areas used mainly for soybean cultivation.

In order to better represent the environmental heterogeneity, the natural area was divided into three different environments according to their phytophysionomy: open grassland, edge of forest (transitional environment) and inner of forest patch.

Climatic data and Species sampling

The samples were performed with a total of 420 banana baited traps (Tidon and Sene 1988) in 12 periods from 2011 to 2014 (Table 1). During the collections the traps

remained on field for three days, at least a distance of approximately 30-40 meters from each other. The specimens caught were preserved in 96% ethanol to further identification in the laboratory. The use of banana baited traps is a very common collecting technique in Drosophilidae studies.

Climatic data for each sampling period were obtained from direct measures on field through a digital thermohygrometer Hikari® HK T240; the measures were taken to each natural and urban environment. The rainfall levels and the average of relative humidity were daily obtained at a climate station maintained by a local agricultural company (COOPATRIGO–Cooperativa Tritícola Regional). The rainfall level considered to the performed analyzes was represented by the sum of the weekly rainfall during each moment of sampling.

The identification of specimens was performed based on external morphology and the male terminalia according to specialized literature. The analysis of male terminalia was conducted according to Bächli et al. (2004). Certain individuals belonging to the species groups of *Drosophila repleta* Wollaston, *D. tripunctata* Loew, *D. cardini* Sturtevant, *D. saltans* Sturtevant and *D. annulimana* Duda that remained unidentified at the species level were not included in the statistical analysis of the species abundance and richness measures. However, they were included in the total number of individuals (*N*).

Data analyses

In order to verify the assemblages' structure fluctuation in the sampled area, the total number of individuals (*N*), observed richness (*Sobs*) and species richness estimated by rarefaction method (*Srar*) were performed through each sampled environment and period.

Species richness estimated by rarefaction method (*Srar*) was performed in order to compare the species richness in each site without the effects of different total number of collected specimens, for each sampled period, since each period presented specific combination of climatic variables. The analyses were performed considering a minimal common *N* specific to each period. Significant differences of *Srar* among the sites were verified through a Kruskal-Wallis test.

Differences in the composition and abundance of drosophilids among the assemblages of each environment were examined using the non-Metric Multidimensional Scaling (nMDS), based on group averaging and Bray-Curtis similarity measures, for each sampled period. Furthermore, Indicator Value (IndVal) method was performed in order to identify characteristic species of a particular habitat. It combines measurements of the degree of specificity and fidelity of a species to a habitat type (Dufrene and Legendre 1997), providing a percentage of relationship of that species with a specific environment. It is also useful to identify the principal species responsible for either the similarities between groups within a specific environment or the differences between groups from different environments.

The interaction and influence of temperature, humidity, rainfall and environment type on the species abundance and, thus on the drosophilids assemblages' structure was analyzed by a Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson 2001). The abundance data were fourth-root transformed to reduce the weight of common species and Bray-Curtis similarities were used to measure the dissimilarities between samples (Mata and Tidon 2013). The data of traps in each site were grouped, thus a micro climatic analyze was not performed to access the climatic conditions in each trapped-point. Five main vectors were performed to the PERMANOVA analysis (Table 2).

The analyses of *Srar* and nMDS were performed in the software PAST v., 1.94b (Hammer et al. 2001). The IndVal and PERMANOVA analyzes were performed in the software R 3.1.1 (R Development Core Team, 2013) using the *labdsv* and the *Vegan* packages, respectively.

9.1.5. Results

A total of 55,860 drosophilids were collected, belonging to 62 species of *Drosophila*, 13 of *Rhinoleucophenga*, eight of *Zygothrica*, two of *Amiota*, two of *Leucophenga* and one of *Zaprionus*. In the total of collected species, 26 remained as not assigned to any known species, and probably some of them are not described yet. *Drosophila senei* Vilela, *D. suzukii* Matsumura, *D. trapeza* Heed & Wheeler and *Zy. dispar* Wiedemann were for the first time recorded in the pampas, and the natural area represents the new southernmost locality to the last two species. Most of the species were recorded in the inner and in the edge of forest patch, while the lowest richness was recorded in the urban area (Supp. Table S1).

From 88 species sampled, only 11 species presented abundance higher than 1% and were responsible for more than 83% of specimens collected: *Drosophila simulans* Sturtevant (38.13%), *D. willistoni* Sturtevant (12.01%), *Zaprionus indianus* Gupta (8.77%), *D. mercatorum* Patterson & Wheeler (7.69%), *D. busckii* Coquillett (5.07%), *D. polymorpha* Dozhansky & Pavan (3.29%), *D. immigrans* Sturtevant (3.04%), *D. mediopunctata* Dobzhansky & Pavan (1.91%), *D. maculifrons* Duda (1.35%), *D. buzzatii* Patterson & Wheeler (1.26%) and *D. hydei* Sturtevant (1.05%). The dominance of each species was alternated according to each environment and period sampled;

furthermore, the forest patch was the unique environment dominated by neotropical species (62.3% of individuals) (Supp. Table S1).

The influence of dominant species on the assemblages' similarity is linked to the climatic conditions of the pampas and to the local phytophysionomy of each sampled habitat, since the assemblages' composition fluctuated among the periods and environments sampled (Fig. 1). The relationship of the species with each environment was determinant to the similarity among assemblages. *Drosophila buzzatii* and *D. hydei* were commonly found in the grassland (IndVal = 0.42 and 0.35, respectively; p = 0.001), although they had also been present in the urban area and forest patch in some periods. Nevertheless, mainly those species and *D. busckii* (IndVal = 0.29; p = 0.001) contributed for the differentiation of the grassland assemblages from the others. In the same way the neotropical species *D. willistoni*, *D. mediopunctata* and *D. maculifrons* were well associated with the forest patch (IndVal = 0.82, 0.46 and 0.33, respectively; p = 0.001). Differently, the urban assemblage was well characterized by the dominance of exotic and cosmopolite species *Z. indianus, D. simulans*, *D. busckii*, *D. immigrans* and the neotropical *D. mercatorum* (IndVal = 0.78, 0.51, 0.29, 0.50 and 0.56, respectively; p = 0.001), those species highly contributed to the elevated number of total individuals sampled in the urban area (Supp. Table S1; Table 5).

The environment type was confirmed as a determinant factor on the assemblages' structures by the PERMANOVA test (Table 3), explaining alone 8.86% of the assemblages' composition. Nonetheless, the interaction between temperature and humidity levels was pointed as the environmental component more influent on the Drosophilidae assemblages, explaining 37.28% of all variation noticed. Together, those elements were able to explain 56.45% of the assemblages' variation.

The temperatures ranged from 3°C in the coldest period (July) to 36°C in the hottest periods (December), and the average of air relative humidity was never lower than 60-65%. Through the environments, the forest patch presented milder thermal conditions than the grassland and urban area mainly, which were the most hostile ones (Table 4). So, in most of periods the edge and the inner of forest were indistinct to the number of species recorded (Table 5), however, the assemblage from the edge of forest was strongly affected by the lowest (3-17°C) and high (16-28°C) temperatures in July 2011 and February 2014, respectively, strictly decreasing the total of sampled individuals and richness in that environment. On the other hand, in the rainiest period (240 mm), August 2014, a general tendency of the assemblages increase was noticed (Table 5), as well as a high similarity among urban and natural assemblages (Fig. 1).

The grassland and urban area normally presented the lowest richness (Table 5); furthermore, the assemblages from those environments presented the species relative abundance and composition more negatively influenced by the periods of lowest and highest temperatures (Supp. Table S1). However, immediately after stressing periods the grassland assemblages start to be recovered in richness and abundance of specimens (Table 5). In a period of 21-35°C associated with ca. 70 mm of rainfall (December 2012 and 2013) the assemblages from the grassland and the urban area were similar in the relative abundance and composition of species, increasing the similarity among those environments (Fig. 1). Differently, the richness of the assemblage from the inner of forest presents a tendency of decreasing in the periods of temperature around 16-27°C, as notice in April 2012 and October 2013 (Table 5). So, the assemblages presented a general tendency of lowest similarity during periods of very low temperatures (3-17°C in July 2011 and 12-22°C in August 2013), severe heat (19-33°C, December 2011 and 2012) or high rainfall (170 mm, April 2011) (Fig. 1); but, in some periods of high

temperatures and moderate rainfall (15-35°C and 67 mm, December 2013 and February 2014) the similarities among the assemblages increased. Thus, the interaction among the environmental factors is reinforced as a regulator to the structure of Drosophilidae assemblages in the pampas.

9.1.6. Discussion

Assemblages dominated by few species are commonly noticed in studies of Drosophilidae (Tidon 2006, De Toni et al. 2007, Poppe et al. 2014), as well as the influence of that species in the similarity among assemblages from different environments. The environment type was determinant to the assemblages' structure in the pampas, as hypothesized. In most of the samples, the edge and the inner of forest patch were very similar considering species richness, and also assemblages' composition; in addition, the forest assemblages differ more pronounced among the grassland and urban environments. The habitat preference of some Drosophilidae species is well documented (Sene et al. 1980, Vilela et al. 1983, Martins 1987, Poppe et al. 2012), so environments with different phytophysionomy tend to be inhabited by different and specific species, as well as different levels of habitat disturbance affects the assemblage composition (Gottschalk et al. 2007). Additionally, the high dissimilarity among urban and natural assemblages may be related to geographic distance between the sampled areas (Nekola and White 1999) that are surrounded by a heterogeneous landscape of forest patches, grassland and agricultural patches. On the other hand, there are many possible factors to contribute to the highest richness in the forest patch, such as (i) higher number of microhabitats – Shorrocks and Sevenster (1995) highlighted the spatial heterogeneity as the main mechanism maintaining

diversity of *Drosophila* communities; (ii) higher number of available breeding and feeding resources – some authors (Kimura et al. 1977, Toda 1977, Bizzo et al. 2010) mentioned that many drosophilids are able to explore various resources, but most species mainly depend on only one breeding site and many times it is seasonal, reflecting in the species abundance and distribution pattern in an area (Mata et al. 2015); and (iii) climatic stability – Parsons (1989) pointed inner of forests as mesotherms environments, presenting highest Drosophilidae diversity during stressing climatic periods (Tidon 2006, Poppe et al. 2015a).

The structure and diversity of insect assemblages are directly linked to spatial and temporal elements (Parsons 1991, Bryant et al. 2002). The climatic interaction with environment type was confirmed as a determinant factor to the structure of the Drosophilidae assemblages in the pampas, mainly by the interaction among environment types, temperature and humidity level. Complementary, regular rainfalls guarantee the constant environment humidity during hot periods in the sampled area, which was determinant to the maintenance of drosophilids diversity in the region. Although the habitat preference of some Drosophilidae species have been noticed in previous ecological studies in other environments (Dobzhansky and Pavan 1950, Sene et al. 1980, Vilela et al. 1983), the interaction between the particular phytophysionomy of the pampas and the current regional climatic conditions revealed comprehensive patterns of interaction among the species and the habitat types in that biome.

The assemblages' similarity highly fluctuated over the time. Immediately after stressing periods the grassland assemblages start to be re-established in richness and relative abundance of specimens. The role of forest patches as refuge to drosophilids during stressing periods was hypothesized by some authors in the pampas and in the Brazilian savannah (Tidon 2006, Poppe et al. 2015a). According to Wallner (1987) and

Parsons (1989, 1991) the migratory behavior of the flies is based on temperature/humidity relationships, thus insect population from dry areas (grassland) tend to fluctuate more than those from wet areas (inner of forest), avoiding desiccating environmental conditions, reflecting on the similarity indices through the time. Furthermore, the migratory pattern may cause a reproductive debt in the origin areas (Roff 1977); consequently, it could intensify the discrepancy among the assemblages from the grassland and the forest patch in some periods.

Some species of *D. repleta* group were abundantly found in the grassland in most samples; but during climatic stressing periods their relative abundance increased in the forest patches. Thus, that species presented moderate IndVal to the grassland environment, and they are pointed as able to move to closed areas avoiding stressing conditions (Parmesan 1996, Bryant et al. 2002, Tidon 2006). The migratory pattern of species from *D. repleta* group is not a tested hypothesis here. It could be related to the intrinsic traits of that species; according to Throckmorton (1982) the ancestor of the *D. repleta* species group was proposed as a forest species, subsequently moved into arid habitats. Thus, the species of *D. repleta* group are mostly found in semiarid regions with open vegetation (Pavan 1959, Sene et al. 1980, Poppe et al. 2014) and theoretically evolutionarily adapted to move into the forest patches in stressing conditions.

Neotropical species with high to moderate indicator values to a specific area, such as *D. willistoni*, *D. mediopunctata*, *D. maculifrons* and *D. ornatifrons* Duda, were negatively affected by high climatic oscillations, for being species highly specific to the forest and thus theoretically unable to move to other environments. According to Parsons (1989) stressing conditions favor the dominance of common and exotic species, decreasing the number of rare or specialist species, as noticed in the urban area. However, stressing climatic periods seems to inhibit the dominance of the exotic species

Z. indianus in natural areas during periods of cold temperatures in the pampas (Poppe et al. 2015a). The new exotic species recorded in the pampas, *D. suzukii*, was few abundant and frequent in the samples; however, it represents a recent invasion in the South America continent (Deprá et al. 2014), so, only further studies in the pampas can confirm its ability to establish in that biome.

Therefore, temperature and humidity are very important ecological factors in the studied area, causing temporal fluctuation in the species occurrence and abundance, in all the sampled environments. On the other hand, the cyclic interaction among climatic elements and the spatial heterogeneity is important to the diversity maintenance of Drosophilidae in the sampled region of pampas. However, 16.52% of the assemblages' fluctuation was not explained by the environmental factors considered here, and can be motivated by intrinsic traits of species or by the analysis of different environmental variables (Throckmorton 1982, Shorrocks and Sevenster 1995, Roque et al. 2013). Nonetheless, both open and close environments, such as the grassland and the forest patches, respectively, present peculiar composition of Drosophilidae assemblages and must be equally considered in the pampas preservation strategies.

9.1.7. Acknowledgments

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9.1.8. References

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9.1.9. Tables

Table 1: Strategy of sampling in the natural and urban areas, 420 banana-baited traps were used. The numbers indicate the amount of traps used by environment during each sampled period. - : not sampled.

	Sampled Area			
Sampled period	**Grassland**	**Edge of forest**	**Forest**	**Urban**
April 2011	10	10	10	-
July 2011	10	10	10	-
October 2011	10	10	10	-
December 2011	10	10	10	-
April 2012	10	10	10	-
December 2012	10	10	10	10
April 2013	10	10	10	-
August 2013	10	10	10	10
October 2013	10	10	10	10
December 2013	10	10	10	10
February 2014	10	10	10	10
August 2014	10	10	10	10

Table 2: Five main vectors performed to the PERMANOVA analysis: Minimum Temperature; Maximum Temperature; Humidity; Rainfall; Environment type. The states stipulated to the matrix analyses and their respective descriptions are provided.

PERMANOVA Vectors	States	Description
Minimum Temperature	0	3°C
	1	12-15°C
	2	16-17°C
	3	18-19°C
	4	20-21°C
Maximum Temperature	0	17°C
	1	22-24°C
	2	25-26°C
	3	27-28°C
	4	30°C
	5	33-35°C
Humidity	0	60-65%
	1	70-80%
	2	81-89%
	3	90-100%
Rainfall	0	0-20 mm
	1	60-79 mm
	2	80-119 mm
	3	120-240 mm
Environment type	1	Grassland
	2	Edge of forest
	3	Forest
	4	Urban area

Table 3: PERMANOVA results for drosophilids assemblage structure, showing the variance partitioning values for the main environmental factors and their interactions. Local: environment type; Hum: humidity; Max: maximum temperature; Min: minimum temperature, Rain: rainfall.

Environmental variable	d.f.	Sum of Squares	Mean Square	F Model	R2 (%)	*p*
Local	1	0.7752	0.77524	6.4347	8.863	0.001
Local:Hum	1	0.2885	0.2885	2.3946	3.298	0.019
Local:Max:Hum	1	0.3114	0.31142	2.5849	3.56	0.016
Local:Min:Max	1	0.3022	0.3022	2.5084	3.455	0.011
Min	1	0.4443	0.44433	3.688	5.08	0.002
Max	1	0.3727	0.37273	3.0938	4.261	0.006
Min:Max	1	0.4766	0.47658	3.9557	5.448	0.002
Rain	1	0.432	0.43196	3.5854	4.938	0.001
Min:Hum	1	0.2296	0.22961	1.9058	2.625	0.047
Max:Hum	1	0.4299	0.4299	3.5683	4.915	0.002
Min:Rain	1	0.2984	0.29837	2.4765	3.411	0.016
Min:Max:Rain	1	0.2539	0.25393	2.1077	2.903	0.036
Min:Hum:Rain	1	0.3241	0.32414	2.6905	3.706	0.008
Total					56.463	
Residual	12	1.4457	0.12048		16.528	
Not singnificant interactions					27.009	
Total					100	

Table 4: Climatic data of each sampled period in the Urban area (UA), Grassland (G), Edge of forest (E), and Inner of forest (F). -: not measured.

Sampled period	Average of Minimum Temperature (°C)				Average of Maximum Temperature (°C)				Average of Humidity (%)				Weekly Rainfall (mm)			
	UA	G	E	F	UA	G	E	F	UA	G	E	F	UA	G	E	F
April 2011	-	18	18	18	-	27	25	25	-	90	90	90	-	170	170	170
July 2011	-	3	3	3	-	17	17	17	-	85	85	85	-	117	117	117
October 2011	-	14	14	14	-	24	24	24	-	90	90	90	-	80	80	80
December 2011	-	19	19	19	-	33	30	30	-	65	65	75	-	10	10	10
April 2012	-	18	16	15	-	30	28	25	-	60	60	70	-	0	0	0
December 2012	20	20	20	20	33	33	30	30	63	63	63	80	79	79	79	79
April 2013	-	15	15	15	-	27	25	25	-	77	77	85	-	93	93	93
August 2013	12	12	12	12	22	22	22	22	70	70	70	85	12	12	12	12
October 2013	15	15	15	15	26	26	26	25	75	75	75	75	20	20	20	20
December 2013	21	21	21	20	35	35	34	30	60	60	60	80	71	71	71	71
February 2014	17	18	16	15	30	30	28	26	70	70	70	85	67	67	67	67
August 2014	14	14	14	14	24	24	24	25	85	85	85	85	240	240	240	240

Table 5: Total number of collected individuals (*N*), observed richness (S*obs*) and species richness estimated by rarefaction method (S*rar*) for each sampled period and environment. Different letters in S*rar* column indicate significant richness differences among the environments (Kruskal-Wallis test, $p < 0.05$); Standard *N*: Maximum number of collected individuals shared among all environments in each sampled period. UA: Urban area; G: Grassland; E: Edge of forest; F: Inner of Forest. SD: Standard Deviation; -: not sampled.

	N					***Sobs***					***Srar* ± SD**				
	UA	**G**	**E**	**F**	**Total**	**UA**	**G**	**E**	**F**	**Total**	**Standard *N***	**UA**	**G**	**E**	**F**
April 2011	-	483	560	480	1523	-	9	20	18	27	230	-	8.44 ± 0.64 (A)	15.38 ± 1.57 (B)	14.00 ± 1.46 (B)
July 2011	-	5	49	1171	1225	-	3	13	21	27	30	-	-	9.73 ± 1.22 (A)	4.91 ± 1.23 (B)
October 2011	-	506	893	1223	2622	-	9	18	19	24	230	-	7.86 ± 0.84 (A)	12.40 ± 1.55 (B)	13.99 ± 1.55 (B)
December 2011	-	6	248	775	1029	-	4	16	28	30	230	-	- (A)	15.87 ± 0.34 (B)	17.33 ± 2.11 (B)
April 2012	-	357	114	291	762	-	9	10	13	16	90	-	5.58 ± 0.96 (A)	9.22 ± 0.77 (B)	9.29 ± 1.27 (B)
December 2012	691	246	1816	2439	5192	15	12	26	23	41	230	7.87 ± 1.57 (B)	11.71 ± 0.51 (A)	10.93 ± 1.84 (AC)	8.93 ± 1.55 (BC)
April 2013	-	1259	1170	6336	8765	0	7	17	31	35	230	-	5.62 ± 0.73 (A)	11.78 ± 1.61 (B)	11.28 ± 1.60 (B)
August 2013	6632	143	984	1516	9275	18	13	36	35	49	120	7.91 ± 1.27 (A)	11.35 ± 0.74 (B)	18.15 ± 2.18 (C)	17.47 ± 2.12 (C)
October 2013	2936	461	424	240	4061	8	18	17	13	25	230	5.85 ± 0.86	12.54 ± 1.38	11,10 ± 1.28	11.76 ± 0.46

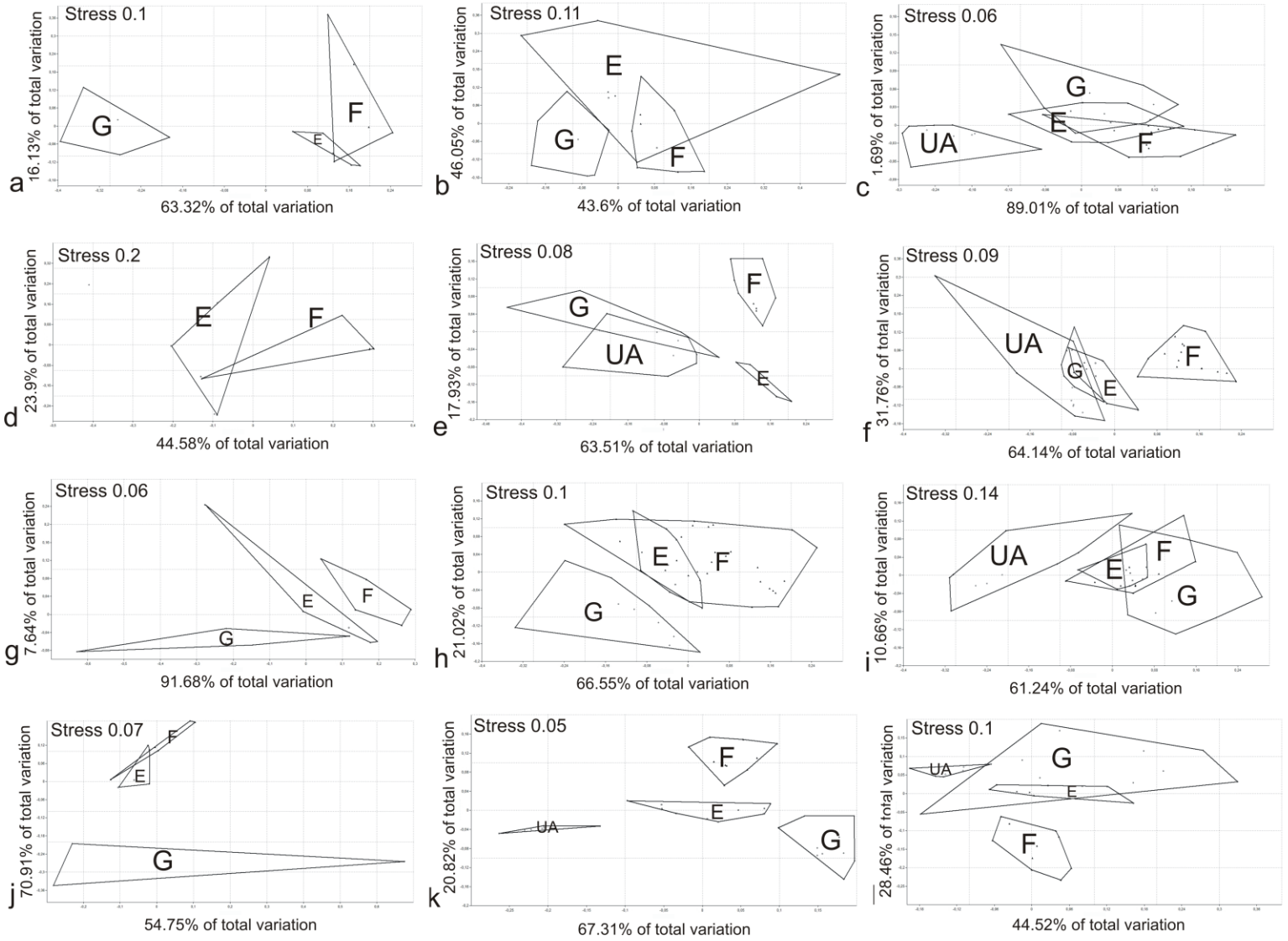
												(A)	(B)	(B)	(B)
December 2013	1256	657	926	2332	5171	19	18	17	27	35	230	10.65 ± 1.66	13.17 ± 1.41	10.70 ± 1.45	11.54 ± 1.73
												(BC)	(A)	(BC)	(AC)
February 2014	3360	143	221	272	3996	8	9	8	13	18	140	3.19 ± 0.91	8.74 ± 0.47	7.06 ± 0.77	10.26 ± 1.22
												(B)	(AC)	(C)	(A)
August 2014	6269	1017	1661	3292	12239	27	18	33	38	53	230	11,85 ± 1.40	10.67 ± 1.54	16.45 ± 2.11	21.29 ± 2.06
												(A)	(A)	(B)	(C)

9.1.10. Supplementary Table

Supplementary Table S1: Absolute abundance of Drosophilidae species collected during 12 sampled periods in the South of Brazil, Rio Grande do Sul State. UA: Urban area (municipality of São Luiz Gonzaga). The natural area G: Grassland; E: Edge of forest; F: Inner of Forest. *: exotic species. HTML file.

9.1.11. Figures

Fig. 1. nMDS results, comparisons of drosophilid assemblages among sampled environments in each period. a: April 2011; b: April 2012; c: October 2013; d: July 2011; e: December 2012; f: December 2013; g: October 2011; h: April 2013; i: February 2014; j: December 2011; k: August 2013; l: August 2014. G: Grassland; E: Edge of forest; F: Inner of forest; UA: urban area. Axis *x*: Coordinate 1; Axis *y*: Coordinate 2.


a
Stress 0.1
16.13% of total variation
63.32% of total variation
G
F
E
b
Stress 0.11
46.05% of total variation
43.6% of total variation
E
G
F
c
Stress 0.06
1.69% of total variation
89.01% of total variation
G
E
UA
F
d
Stress 0.2
23.9% of total variation
44.58% of total variation
E
F
e
Stress 0.08
17.93% of total variation
63.51% of total variation
F
G
UA
E
f
Stress 0.09
31.76% of total variation
64.14% of total variation
UA
G
E
F
g
Stress 0.06
7.64% of total variation
91.68% of total variation
E
F
G
h
Stress 0.1
21.02% of total variation
66.55% of total variation
E
F
G
i
Stress 0.14
10.66% of total variation
61.24% of total variation
UA
E
F
G
j
Stress 0.07
70.91% of total variation
54.75% of total variation
F
E
G
k
Stress 0.05
20.82% of total variation
67.31% of total variation
F
E
UA
G
Stress 0.1
28.46% of total variation
44.52% of total variation
UA
G
E
F

10. CAPÍTULO X

(Manuscrito aceito para publicação no periódico Drosophila ***Information Service*****)**

10.1. The diversity of Drosophilidae in the South American pampas: update of the species records in an environment historically neglected.

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10.1.1. *Introduction*

In the last decades many faunal surveys of Drosophilidae have been done in different Neotropical environments in Brazil, such as Atlantic rainforest (De Toni *et al*., 2007), Cerrado (Mata *et al*., 2008), mangrove swamps (Schmitz *et al*., 2007), Caatinga (Tidon-Sklorz and Sene, 1995), Pantanal (Val and Marques, 1996), Amazonian rainforest (Martins, 1987), Araucarian forest (Saavedra *et al*., 1995; Cavasini *et al*., 2014), Restinga (Bizzo *et al*., 2010). However, the Pampas biome, which is the southernmost environment, was neglected by the researchers mainly for being an open grassland environment and apparently lacking breeding and feeding resources to drosophilids.

The pampas covers southernmost Brazil, all of Uruguay, and the central region of eastern Argentina. It is a heterogeneous landscape, with a matrix of natural grasses and small patches of forest. The seasons are well defined, and the annual temperature range is extremely pronounced, ranging from negative values in the winter up to 40°C in the summer. This temperature range has been pointed as determinant to the presence and maintenance of Drosophilidae species in the region (Poppe *et al*., 2013, 2015).

Only recently Poppe *et al*. (2014) highlighted the high diversity of drosophilids in this environment. The same is noticed in Uruguay (Goñi *et al*., 1998, 2002, 2012). While in Argentina most of information comes from the studies focused predominantly on genetics and/or autecology (Wheeler and Magalhães, 1962; Hale and Singh, 1991). Thus, it is evident the poor knowledge of the Drosophilidae fauna in the South grasslands of South America.

Poppe *et al*. (2014) mentioned the record of 95 species in the grasslands of Brazil, Uruguay and Argentina. After that, some studies have been performed in pampas pointing the record of more species, including the new invasion of *D. suzukii*. Thus, the present report is an update of the list of recorded Drosophilidae species in the South American pampas.

10.1.2. *Material and Methods*

A comprehensive literature search of species recorded in the pampas of Brazil, Uruguay and Argentina was performed, including not only taxonomic studies, but also genetic, evolutionary and ecological ones. Some species records are from unpublished samples performed by us in the Brazilian pampas (28°45'01"S 54°56'55"W; 30°20'44"S 54°19'32"W). These data updated the species list presented by Poppe *et al*. (2014) to that biome.

10.1.3. *Results and Discussion*

Thirteen species were included in the pampas species list proposed by Poppe *et al*. (2014): *Drosophila senei* Vilela, *D. suzukii* Matsumura, *D. trapeza* Heed and Wheeler, *Hirtodrosophila levigata* (Burla), *H. mendeli* (Mourão, Gallo and Bicudo), *H. morgani* (Mourão, Gallo and Bicudo), *Mycodrosophila projectans* (Sturtevanti), *Paraliodrosophila antennata* (Wheeler), *Rhinoleucophenga joaquina* Schmitz, Gottschalk and Valente, *R. punctuloides* Poppe, Schmitz and Valente, *Zygothrica poeyi* (Sturtevanti), *Z. prodispar* Duda and *Z. dispar* Wiedemann (Table 1). Except to the two first species, the pampas represents the southernmost record region to the other ones.

Rhinoleucophenga was the genus presenting most new records of species in the pampas, beyond the two species previously mentioned other four species are under description process by J.L. Poppe (data not shown).

Poppe *et al*. (2014) highlighted the presence of 10 exotic species in the pampas. Deprá *et al*. (2014) pointed the first record of *D. suzukii* in the South America continent,

after that, the respective species was recorded in many localities in the pampas increasing to 11 the number of exotic species in this environment.

New areas were included as sampled sites to the Brazilian pampas: São Gabriel (30°20'44"S, 54°19'32"W), Santiago (Robe *et al*., 2014) (29°11′09″S, 54°53′50″W), Pelotas (Robe *et al*., 2014) (31°48′58″S, 52°25′55″W) and Rio Grande (Robe *et al*., 2014) (32°32′25″S, 52°32′34″W). A total of 108 Drosophilidae species are now known from the Brazilian, Uruguyan and Argentinian pampas (Table 1). Twelve of 13 new recorded species were found only in the Brazilian pampas; only *D. suzukii* is widespread by the Brazilian and Uruguayan pampas (B. Goñi pers. comm.). Despite it is probably still a gross underestimate of pampas diversity, since most of this biome is still not intensively sampled, the presented data indicate the high diversity of Drosophilidae in the South America grasslands, an environment historically neglected by the researchers due its "poor diversity" appearance.

10.1.4. *Acknowledgments*

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10.1.5. *References*

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10.1.6. *Table*

Table 1: List of Drosophilidae flies recorded in the Pampas. *: new species added in the list presented by Poppe *et al*. (2014). New record localities, 1: 30°20'44"S 54°19'32"W; 2: 29°11'09"S 54°53'50"W (Robe *et al*., 2014); 3: 31°48'58"S 52°25'55"W (Robe *et al*., 2014); 4: 32°32'25"S 52°32'34"W (Robe *et al*., 2014); 5: 28°45'01"S 54°56'55"W.

Genus	Group	Species
Cladochaeta	*bomplandi*	*C. bomplandi* (Malloch)
Drosophila	*annulimana*	*D. annulimana* Duda
		D. arassari da Cunha & Frota-Pessoa
		D. schineri Pereira & Vilela
	bromeliae	*D. bromelioides* Pavan & da Cunha
	busckii	[1]*D. busckii* Coquillett
	calloptera	*D. quadrum* (Wiedemann)
	canalinea	*D. piratininga* Ratcov & Vilela
	cardini	*D. cardini* Sturtevant
		D. cardinoides Dobzhansky & Pavan
		D. neocardini Streisinger
		[1]*D. polymorpha* Dobzhansky & Pavan
	coffeata	*D. fuscolineata* Duda
		D. pagliolii Cordeiro
	dreyfusi	*D. briegeri* Pavan & Breuer

flavopilosa	*D. cestri* Brncic
	D. cordeiroi Brncic
	D. flavopilosa Frey
	D. incompta Wheeler &Takada
guarani	*D. alexandrei* Cordeiro
	D. griseolineata Duda
	[1]*D. maculifrons* Duda
	D. ornatifrons Duda
immigrans	*D. immigrans* Sturtevant
melanogaster	*D. ananassae* Doleschall
	D. kikkawai Burla
	D. malerkotliana Parshad & Paika
	D. melanogaster Meigen
	[1]*D. simulans* Sturtevant
	[1,5]**D. suzukii* Matsumura
mesophragmatica	*D. gaucha* Jaeger & Salzano
obscura	*D. subobscura* Collin in Gordon
pallidipennis	*D. pallidipennis* Dobzhansky & Pavan
repleta	*D. aldrichi* Patterson
	D. antonietae Tidon-Sklorz & Sene
	D. buzzatii Patterson & Wheeler
	D. hydei Sturtevant
	D. koepferae Fontdevila & Wasserman
	[1]*D. mercatorum* Patterson & Wheeler
	D. meridionalis Wasserman
	D. nigricruria Patterson & Mainland
	D. onca Dobzhansky & Pavan
	D. paranaensis Barros
	D. repleta Wollaston
	[5]**D. senei* Vilela
	D. serido Vilela & Sene
	D. zottii Vilela
saltans	*D. prosaltans* Duda
	D. pulchella Sturtevant
	D. sturtevanti Duda
sticta	*D. sticta* Wheeler
tripunctata	*D. angustibucca* Pavan
	D. bandeirantorum Dobzhansky & Pavan
	D. cuaso Bächli, Vilela & Ratcov
	D. mediopicta Frota-Pessoa
	[1]*D. mediopunctata* Dobzhansky & Pavan
	D. mediosignata Dobzhansky & Pavan
	D. mediostriata Duda
	D. mediovittata Frota-Pessoa
	D. nappae Vilela, Valente & Basso-da-Silva
	D. neoguaramunu Frydenberg
	D.paraguayensis Duda

		D. paramediostriata Townsend & Wheeler
		D. roehrae Pipkin & Heed
		[5]**D. trapeza* Heed & Wheeler
		D. trifilum Frota-Pessoa
	virilis	*D. virilis* Sturtevant
	willistoni	*D. bocainensis* Pavan & da Cunha
		D. capricorni Dobzhansky & Pavan
		D. fumipennis Duda
		D. nebulosa Sturtevant
		D. parabocainensis Carson
		D. paulistorum Dobzhansky & Pavan
		D. willistoni Sturtevant
	Ungrouped	*D. caponei* Pavan & da Cunha
		D. denieri Blanchard
		D. flexa Loew
		D. lutzii Sturtevant
		D. serenensis Brncic
Hirtodrosophila	*glabrifrons*	[3]**H. levigata* (Burla)
	hirticornis	[2,3]**H. mendeli* (Mourão, Gallo and Bicudo)
		[3]**H. morgani* (Mourão, Gallo and Bicudo)
Leucophenga	*Ungrouped*	*L. maculosa* Coquillett
Mycodrosophila	*Ungrouped*	[3,4]**M. projectans* (Sturtevanti)
Paraliodrosophila	*Ungrouped*	[2]**P. antennata* Wheeler
Rhinoleucophenga	*Ungrouped*	*R. gigantea* (Thomson)
		[5]**R. joaquina* Schmitz, Gottschalk & Valente
		R. missionera Poppe et al.
		R. obesa (Loew)
		R. pampeana Poppe *et al*.
		R. punctulata Duda
		[5]**R. punctuloides* Poppe, Schmitz & Valente
		R. subradiata Duda
		R. sulina Poppe et al.
Scaptomyza	*mesoscaptomyza*	*S. striaticeps* Wheeler & Takada
		S. nigripalpis Malloch
	parascaptomyza	*S. pallida* (Zetterstedt)
	scaptomyza s. str.	*S. graminum* (Fallén)
	Ungrouped	*S. spinipalpis* Seguy
Zaprionus	*armatus*	*Z. indianus* Gupta
Zygothrica	*atriangula*	[3]**Z. poeyi* (Sturtevanti)
	bilineata	[3]*Z. bilineata* (Williston)
	dispar	[3,5]**Z. dispar* (Wiedemann)
		[3]**Z. prodispar* Duda
	hypandriata	*Z. hypandriata* Burla
	Orbitalis	*Z. orbitalis* (Sturtevant)

vittimaculosa	*Z. vittimaculosa* Burla
Ungrouped	[3]*Z. ptilialis* Burla

11. CAPÍTULO XI

11.1. Principais Conclusões

11.1.1. Redescrições e novas espécies de *Rhinoleucophenga*

Minimizamos as chances de erro na identificação de espécies de *Rhinoleucophenga* em trabalhos futuros com Drosophilidae, através da redescrição de algumas espécies que haviam sido descritas na primeira metade do século XX (Capítulos III e IV), e ainda descrições complementares das fêmeas de *R. joaquina* Schmitz, Gottschalk & Valente e *R. punctulata* Duda (Capítulo II). As novas descrições foram enriquecidas com fotos dos espécimes, chaves dicotômicas ilustradas, desenhos das estruturas reprodutivas e apresentação de novos caracteres diagnósticos que muito provavelmente facilitarão a identificação das espécies de *Rhinoleucophenga*.

A descrição de 17 novas espécies representa um acréscimo de cerca de 70% para o atual número de espécies em *Rhinoleucophenga* (Capítulos II, III, IV e V), e ainda contribui para o conhecimento da diversidade e padrão de distribuição do gênero na região Neotropical. Além disso, a correta identificação das espécies é fundamental para a geração de dados de distribuição e para o desenvolvimento e aprimoramento de estudos biogeográficos e evolutivos.

11.1.2. Revisão de *Rhinoleucophenga obesa* sensu Malogolowkin (1946) e Lima (1935)

Dados morfológicos e moleculares são complementares na diferenciação de *R. obesa* sensu Malogolowkin (1946) e *R. gigantea* (Thomson) (Capítulo VI). Os espécimes descritos por Lima (1935) e Malogolowkin (1946) como *R. obesa* representam a mesma espécie, e a separação entre estes espécimes e *R. gigantea* foi facilitada pela identificação de caracteres no epêndrio dos machos e na espermateca das fêmeas, os quais são mais facilmente percebidos do que as diferenças sutis presentes nos edeagos dos machos.

Não resolvemos totalmente o problema relacionado à identidade de *R. obesa* (Loew), uma vez que os espécimes da região Neártica e o holótipo não foram revisados (apenas algumas informações disponíveis na literatura), mas damos um importante

passo para isso, uma vez que na região Neotropical as referências mais utilizadas para a definição da espécie são Malogolowkin (1946) e Lima (1935).

11.1.3. Variação morfológica e molecular entre populações de *Rhinoleucophenga punctulata* na região Neotropical

Dados morfológicos e moleculares são complementares e atualmente indispensáveis para o entendimento dos padrões de diversidade e distribuição de *R. punctulata* na região Neotropical (Capítulo VII).

A variação intraespecífica percebida entre as populações de *R. punctulata* pode estar relacionada às características de cada bioma onde a espécie foi registrada, sendo a influência de fatores abióticos sobre a morfologia das espermatecas uma hipótese a ser testada em estudos futuros.

11.1.4. Relações filogenéticas de *Rhinoleucophenga* com base em dados morfológicos

Caracteres morfológicos contínuos mostraram-se importantes para o aprimoramento da filogenia proposta para *Rhinoleucophenga* (Capítulo VIII). Embora o tratamento empregado sobre os conjuntos de caracteres exerça influência sobre os resultados gerados, o gênero *Rhinoleucophenga* mostrou-se parafilético em relação à *Pararhinoleucophenga* na maioria das análises realizadas.

Seis agrupamentos monofiléticos de espécies foram repetidamente obtidos na maioria dos métodos de busca desenvolvidos. Essa repetibilidade mostra confiabilidade em nossos resultados, mesmo que os valores de suporte de alguns desses agrupamentos tenham sido relativamente baixos em algumas análises.

A hipótese filogenética gerada a partir de dados contínuos tratados como razões, *log-transformados*, re-escalonados e com aplicação de pesagem foi preferida em relação às demais, por apresentar individualmente maiores valores de suporte à maioria dos agrupamentos propostos e, sendo teoricamente a mais confiável hipótese filogenética para *Rhinoleucophenga* disponível até o momento.

A utilização de dados moleculares não foi possível para a maioria das espécies, devido ao fato de que muitas espécies são raras e/ou presentes somente em coleções entomológicas. Estudos futuros, usando dados moleculares e ecológicos, associados às

informações morfológicas, podem propiciar o aprimoramento da proposta evolutiva do gênero *Rhinoleucophenga*, assim como corroborar os resultados apresentados.

11.1.5. Influência de fatores ambientais sobre as assembleias de drosofilídeos

As características climáticas da região subtropical-temperada, onde se localiza o bioma Pampa, oscilam acentuadamente ao longo do ano afetando fortemente animais ectotérmicos como drosofilídeos. Desse modo, as oscilações de temperatura e umidade associadas com a heterogeneidade do ambiente constituíram o principal fator ambiental atuando sobre a estrutura das assembleias de Drosophilidae no Pampa (Capítulo IX). Aparentemente, temperaturas extremas inibem a estabilização e dominância de algumas espécies exóticas, tais como *Zaprionus indianus* e *Drosophila suzukii*, na região estudada; porém, a estabilidade da última espécie no Pampa deve ser verificada em estudos futuros.

A mancha de mata foi o ambiente que apresentou maior riqueza, e o único ambiente dominado por espécies neotropicais. No entanto, algumas espécies mostraram-se mais relacionadas ao campo (principalmente espécies do grupo da *D. repleta*); assim, tanto a proteção das matas quanto dos campos são fundamentais para a preservação da diversidade de Drosophilidae no bioma Pampa.

Uma pequena parcela da diversidade de Drosophilidade no Pampa não pode ser explicada pelos componentes abióticos analisados, podendo ter ocorrido em função de alguns aspectos como interações intra e interpopulacionais, condições microclimáticas, distribuição vertical das populações ou talvez ainda por influência dos métodos de coleta, porém, esses fatores não foram analisados no desenvolvimento desta Tese.

11.1.6. Atualização da lista de espécies de Drosophilidae com registros no Pampa

A diversidade de Drosophilidae no bioma Pampa tem ganhado projeção nos últimos anos, assim como a grande diversidade de *Rhinoleucophenga* neste ambiente. Provavelmente, com a ampliação do número de áreas amostradas essa riqueza, que atualmente é de 108 espécies (Capítulo X), pode aumentar, uma vez que as amostras concentram-se em poucas regiões do bioma; por exemplo, os nossos trabalhos (J.L. Poppe e colaboradores) foram concentrados na região noroeste do Rio Grande do Sul, nas proximidades dos municípios de Bossoroca e São Luiz Gonzaga. Além disso, novos métodos de coleta podem revelar uma nova diversidade de drosofilídeos nesse bioma, já

que a grande maioria das coletas foi realizada com a utilização de iscas de banana fermentada e muitos gêneros de Drosophilidae, como *Rhinoleucophenga*, apresentam hábitos peculiares de alimentação, podendo ter sido subestimados com iscas de banana.

Após o aceite para a publicação do nosso manuscrito de revisão da lista de espécies de Drosophilidae com registros no bioma Pampa, João Junges e colaboradores publicaram na Revista Brasileira de Entomologia um manuscrito propondo *Mycodrosophila valentae* sp. nov., com registros no Pampa (Junges J, Gottschalk MS, Loreto ELS & Robe LJ (2015) Two new species of *Mycodrosophila* (Diptera, Drosophilidae) proposed by molecular and morphological approaches, with a key to American species. *Revista Brasileira de Entomologia* 60, 30-39). Assim o número de espécies registradas neste bioma já foi elevado para 109.

12. CAPÍTULO XII

ANEXOS

12.1. Normas de formatação do periódico *Zootaxa*.

Preparation of manuscripts

1) *General.* All papers must be in English. Authors whose native language is not English are encouraged to have their manuscripts read by a native English-speaking colleague before submission. Nomenclature must be in agreement with the *International Code of Zoological Nomenclature* (4th edition 1999), which came into force on 1 January 2000. Author(s) of species name must be provided when the scientific name of any animal species is first mentioned (the year of publication needs not be given; if you give it, then provide a full reference of this in the reference list). Authors of plant species names need not be given. Metric systems should be used. If possible, use the common font New Times Roman and use as little formatting as possible (use only **bold** and *italics* where necessary and indentions of paragraphs except the first). Special symbols (e.g. male or female sign) should be avoided because they are likely to be altered when files are read on different machines (Mac versus PC with different language systems). You can code them as m# and f#, which can be replaced during page setting. The style of each author is generally respected but they must follow the following general guidelines.

2) The **title** should be concise and informative. The higher taxa containing the taxa dealt with in the paper should be indicated in parentheses: e.g. A taxonomic revision of the genus *Aus* (Order: family).

3) The **name(s) of all authors** of the paper must be given and should be typed in the upper case (e.g. ADAM SMITH, BRIAN SMITH & CAROL SMITH). The address of each author should be given in *italics* each starting a separate line. E-mail address(es) should be provided if available.

4) The **abstract** should be concise and informative. Any new names or new combinations proposed in the paper should be mentioned. Abstracts in other languages may also be included in addition to English abstract. The abstract should be followed by a list of **key words** that are not present in the title. Abstract and key words are not needed in short correspondence.

5) The arrangement of the **main text** varies with different types of papers (a taxonomic revision, an analysis of characters and phylogeny, a catalogue etc.), but should usually start with an **introduction** and end with a list of **references**. References should be cited in the text as Smith (1999), Smith & Smith (2000) or Smith *et al.* (2001) (3 or more authors), or alternatively in a parenthesis (Smith 1999; Smith & Smith 2000; Smith *et al*. 2001). All literature cited in the text must be listed in the references in the following format (see a sample page here in PDF).

A) **Journal paper**:
Smith, A. (1999) Title of the paper. *Title of the journal in full*, volume number, page range.

B) **Book chapter**:
Smith, A. & Smith, B. (2000) Title of the Chapter. *In*: Smith, A, Smith, B. & Smith, C. (Eds), *Title of Book*. Publisher name and location, pp. x–y.

C) **Book**:
Smith, A., Smith, B. & Smith, C. (2001) *Title of Book*. Publisher name and location, xyz pp.

D) **Internet resources**
Author (2002) Title of website, database or other resources, Publisher name and location (if indicated), number of pages (if known). Available from: http://xxx.xxx.xxx/ (Date of access).

Dissertations resulting from graduate studies and non-serial proceedings of conferences/symposia are to be treated as books and cited as such. Papers not cited must not be listed in the references.

Please note that:

(1) **journal titles must be written in full (not abbreviated)**

(2) **journal titles and volume numbers are followed by a ","**

(3) **page ranges are connected by "n dash", not hyphen "-", which is used to connect two words.**

For websites, it is important to include the last date when you see that site, as it can be moved or deleted from that address in the future.

On the use of dashes: (1) Hyphens are used to link words such as personal names, some prefixes and compound adjectives (the last of which vary depending on the style manual in use). (2) En-dash or en-rule (the length of an 'n') is used to link spans. In the context of our journal that means numerals mainly, most frequently sizes, dates and page numbers (e.g. 1977–1981; figs 5–7) and also geographic or name associations (Murray–Darling River; a Federal–State agreement). (3) Em-dash or em-rule (the length of an 'm') are used far more infrequently, and are used for breaks in the text or subject, often used much as we used parentheses. In contrast to parentheses an em-dash can be used alone; e.g. What could these results mean—that Niel had discovered the meaning of life? En-dashes and em-dashes should not be spaced.

6) Legends of **illustrations** should be listed after the list of references. Small illustrations should be grouped into plates. When preparing illustrations, authors should bear in mind that the journal has a matter size of 25 cm by 17 cm and is printed on A4 paper. For species illustration, line drawings are preferred, although good quality B&W or colour photographs are also acceptable. See a guide here for detailed information on preparing plates for publication.

7) **Tables**, if any, should be given at the end of the manuscript. Please use the table function in your word processor to build tables so that the cells, rows and columns can remain aligned when font size and width of the table are changed. Please do not use Tab key or space bar to type tables.

8) **Keys** are not easy to typeset. In a typical dichotomous key, each lead of a couplet should be typed simply as a paragraph as in the box below:

1 Seven setae present on tarsus I ; four setae present on tibia I; leg I longer than the body; legs black in color ... Genus A
- Six setae present on tarsus I; three setae present on tibia I; leg I shorter than the body; legs brown in color ... 2
2 Leg II longer than leg I ... Genus B
- Leg II shorter than leg I ... Genus C

Our typesetters can easily convert this to a proper format as in this PDF file.

Deposition of specimens

Whenever possible, authors are advised to deposit type specimens in national or international public museums or collections. Authors are also advised to request registration numbers of deposited material in advance of the acceptance of papers to avoid unnecessary delay of publication. Some countries (e.g. Australia) require that primary type specimens be deposited in collections of the country of origin; authors are advised to take this into consideration.

Submission

Please follow the above basic guidelines and check if your manuscript has been prepared according to the style and format of the journal. Authors are encouraged to submit manuscripts by e-mail as attachments to the subject Editors responsible for your taxa or subject areas; manuscripts on small insect orders without subject editors should be submitted to Dr **Ernest Bernard** (ebernard@utk.edu); manuscripts on other invertebrate taxa without subject editors should be submitted to the Chief editor.

Prior to submitting a manuscript and figures to an editor, please check our website if there are two or more editors per subject, and then contact one of these to announce your intention to submit a manuscript for review. Please indicate the size of the manuscript, the number of figures and the format of these files. Your editor can then respond with special instructions, especially for the submission of many image files.

When you submit your manuscript to your editor, it will be more expedient to the review process if you offer the names of three or more potential reviewers with their complete postal and email addresses. It is also important to include the following statements in your cover letter:

1) All authors agree to its submission and the Corresponding author has been authorized by co-authors; 2) This Article has not been published before and is not concurrently being considered for publication elsewhere (including another editor at Zootaxa); 3) This Article does not violate any copyright or other personal proprietary right of any person or entity and it contains no abusive, defamatory, obscene or fraudulent statements, nor any other statements that are unlawful in any way.

Otherwise, your manuscript will not be processed.

For manuscripts with numerous illustrations, which might be saved as separate TIFF or JPG files, for the purpose of review, it will be easier and more efficient for the subject

editors and reviewers to have the figures converted into one larger PDF (Portable Document Format) file, instead of requiring the subject editor to save many files, cutting and copying these into a string of messages/files to the reviewers. You should retain the original figures in a higher resolution format for the final production of the accepted paper. For the text, PDF file along with RTF (Rich Text format) files are preferred. The advantage of submitting a rtf file for the text part of the manuscript is that the reviewers can emend the manuscript electronically. If you cannot prepare PDF files, then submit text in RTF and the figures in TIFF (line drawing scanned at 600 dpi and half tone at 300 dpi; please use LZW compression, if you can, to reduce the size of e-files for easy transmission); if halftone TIFF files are too big (exceeding 2 MB), then submit them in jpeg. See here for detailed information on preparing plates for publication.

Vector files (charts, maps etc) are best submitted as EMF.

If you do not have access to e-mail, you can send three copies of the manuscript by post. Please double space your ms and leave ample margins for printed manuscripts.

Authors of accepted papers will be asked to submit an electronic version of the manuscript so that the publisher needs not to re-key or scan the ms. At this stage, the text part of the ms must be submitted as RTF or MS Word files and figures as TIFF files. Authors please be aware that line drawings must be scanned at 600 or 900 dpi as line art (=1 bit); they must NOT be scanned as 8 bit or full colour images. Please read details here.

In submitting the final version of revised manuscript to editors, authors are asked to provide the following information to all proper typesetting and indexing of the manuscript:

1) Corresponding author name and email
2) Author last name and running title (<40 characters; to be used in footer)
3) Number of plates and cited references
4) High taxon name (i.e. taxon section in Zootaxa website) and number of new taxa described in the paper

Authors need to complete and return an Assignment of Copyright form when paper is accepted for publication. Authors of institutions that do not allow transfer of copyrights to publishers (e.g. government institutions such as USDA, CSIRO) should attach a copyright waiver or similar documents.

Review process

When a manuscript is received by the Editor, he/she will have it reviewed by at least two peers qualified to evaluate the manuscript and he/she normally asks the reviewers to complete the review in one month. However, the reviewing process will normally take longer, depending on the length of the manuscript and reviewer's responses.

Publication

Once the manuscript is accepted by your subject editor, final files, produced according to Zootaxa requirement, will be forwarded by your subject editor to the chief editor, who will then link with author and the printer to ensure that the paper is published

without unnecessary delay. Normally the proof will be sent to the author for checking 1 to 3 weeks after the final files are accepted. The paper will usually be published with two weeks (for larger papers it will take longer) once the corrections to the proof are received.

Page charge and colour plates. There is **no page charge** for publishing with *Zootaxa*. Publication of **colour figures/photographs** in online edition is also free of charge (print version in black and white). If colour plates in the print edition are desired, authors will be asked to contribute towards the full cost. Current rates: 300 USD for the first colour page; 200 USD for each additional colour page.

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Recommendations about nomenclature for papers submitted to Zootaxa

Guide (one page PDF) for preparing final files for publication

Quick guide (one page PDF) for reference style

EndNote output style for Zootaxa (prepared by Paulo Petry)

Order form for reprints, open access, colour plates and links

Recommended form for listing corrections to proof

Copyright form

Important links

International Code of Zoological Nomenclature (4th edition 1999)

Nomenclator Zoologicus

ZooBank

ISSN 1175-5326 (Print Edition) & ISSN 1175-5334 (Online Edition)
Published by Magnolia Press, Auckland, New Zealand

12.2. Normas de formatação do periódico *Entomological Science*.

Author Guidelines

Authors wishing to submit to *Entomological Science* should read these instructions carefully before preparing their manuscripts. They are also recommended to consult the following reference: *Scientific Style and Format: The CBE Manual for Authors, Editors, and Publishers*, Sixth Edition, CBE Style Manual Committee, 1994, Cambridge University Press.

AIMS AND SCOPE
Entomological Science is the official English language journal of the Entomological Society of Japan. The Journal publishes original research papers and reviews from any entomological discipline or from directly allied field in ecology, behavioral biology, physiology, biochemistry, development, genetics, systematics, morphology, evolution and general entomology. Papers on techniques or applications will be considered for publication if they significantly advance the field of entomological science in the opinion of the Editors and Editorial Board.

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Authors should submit their manuscripts to *Entomological Science* online to facilitate even quicker and more efficient processing. Please log onto the sitedirectly at:http://mc.manuscriptcentral.com/ens
All manuscripts submitted to the Journal must comply with these Author Guidelines. Failure to do so will result in return of the manuscript and possible delay in publication.
Manuscripts should be written so that they are intelligible to the professional reader who is not a specialist in the particular field. They should be written in English, in a clear, concise, direct style. Where contributions are judged as acceptable for publication on the basis of scientific content, the Editor and the Publisher reserve the right to modify typescripts to eliminate ambiguity and repetition and improve communication between author and reader.

Pre-submission English-language editing
Authors for whom English is a second language may choose to have their manuscript professionally edited before submission. A list of independent suppliers of editing services can be found at
http://authorservices.wiley.com/bauthor/english_language.asp.
All services should be paid for and arranged by the author, and the use of these services does not guarantee acceptance or preference for publication.

MANUSCRIPT CATEGORIES AND STANDARD
Manuscripts may be submitted as research papers or review articles. Research papers should be the product of original scientific research or observation, and are classed as either short or long communications. Short communications should have the main body of the text no longer than three printed pages.
All manuscripts, including short communications, should include a discussion of the significance of the results and their relationship to other work, and will be reviewed by at least two referees. Manuscripts will be considered for publication only if they have not been published or submitted for publication elsewhere.

FORMAT AND PREPARATION

File formats and page settings

Manuscripts must be on A4 size pages, in 12 pt font size, with 24 to 28 lines per page, and with all-round margins of 30 mm. The manuscript has to be submitted as Microsoft Word or Rich Text Format.

Manuscript composition

The manuscript should start with the title of the paper, followed by the names of the authors and membership number (in case author is a member of the Entomological Society of Japan), the addresses of the institutions at which the work was carried out, an abstract, key words and the main body of text. The abstract should be a concise summary (250 words or fewer) of the manuscript's significant content. There should be no more than seven key words, which should not duplicate words in the title and should be arranged in alphabetical order, and preferably include the order and family names of the major organism(s) considered, if not appearing in the title.

The IMRAD format ("Introduction", "Materials and methods", "Results" and "Discussion") is recommended for long manuscripts, although following this format is at the author's discretion. The text of manuscripts submitted as short communications should be undivided.

Character and paragraph formats

Words to be italicized should be typed in italics but should not be underlined, and capitalization should be avoided in all cases except for headings, abbreviations of depositories and/or institutions, and technical terms. Hard returns should be used only at the end of a paragraph and words should not be hyphenated. Other formatting, such as headings and subheadings, should follow that in the latest issue of *Entomological Science*.

Units and numeric values

Measurements should be expressed in SI units (but preferably avoiding cm and dm). Numbers between one and ten in the main body of the text should be written in full except dates, the numbering of figures and , numbers accompanying metrical units (such as mm, m, km, mg, g and kg) including time units (sec, min, h, day, week, month and year), and numbers used in taxonomic descriptions. Numbers greater than ten should be written as numerals. Numbers greater than 1000 should include thousand separators using thin space, e.g. 1 000. Fractions should be written as decimals.

Scientific names

All papers must conform to the latest edition of the International Code of Zoological Nomenclature. The first mention of an animal should include the full scientific name with the authority and, particularly in taxonomic papers, the year of publication. The authority and the year of publication should be separated by a comma. Genus names should not be abbreviated at the beginning of a sentence.

Research permits

If the research was carried out in areas for which research permits are necessary (e.g. nature reserves) or if it deals with organisms for which collection or import/ export permits are required (e.g. protected species), the authors must clearly detail obtaining these permits in the acknowledgments paragraph.

Taxonomic papers

In taxonomic papers, type specimens must be clearly designated and type depositories must be clearly indicated. Authors are required to deposit the name-bearing type material in an internationally recognized institution (not in private collections). For the number of segment, Roman numerals (e.g. tergum X) should be used. Body part terms specific to a given taxon should be explained in the "MATERIALS AND METHODS" or be indicated in figures. The second couplets of the key should start with a dash only (-).The list of specimens examined should be as compact as possible; for example, "Specimens examined. Japan: 1F (IUNH), Mito, 26.xi.2004, J. Kojima; 3C2F (IUNH), . . ." The holotypes (or other name-bearing types) and paratypes (or paralectotypes) are listed separately, and full label data should be given for the name-bearing types in quotation marks when they are designated in the paper.

Acknowledgments

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REFERENCES

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Gerson U, Simley R (1990) *Acarine Biocontrol Agents: An Illustrated Key and Manual*. Chapman and Hall, London.

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Include all authors' names below the title. Footnote numbers are placed outside commas in multi-authored articles.

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Abstract
On a separate page, provide an abstract of fewer than 250 words. Give scientific name and authority at first mention of the subject organism. Do not cite references, figures, tables, probability levels, or results. Refer to results only in the general sense.

Keywords
Place three to five keywords, separated by commas, on a line below the abstract. Use only singular words/nouns. Spell out scientific names (e.g., spell out *Aedes albopictus* instead of *Ae. albopictus*). Do not combine different subjects as one key word (e.g., "pesticides and grass," should be two separate keywords, "pesticide, grass." Do not use scientific names and common name at the same time as one key word [e.g., use "coffee, *Coffea Arabica*" (as 2 key words) instead of coffee (*Coffea Arabica*).

Optional foreign language abstract: All articles will have an English abstract. However, to encourage international communication, authors may include a second abstract in a language other than English. (Spanish, French, German, Russian, Portuguese, Chinese, or Japanese are accepted.) It is the author's responsibility to provide an accurate, and grammatically correct non-English version. Do not repeat the keywords.

Heading Levels
First-level headings are centered and boldfaced on their own line. Initial capital letters. Used to divide the manuscript into major sections (e.g., Materials and Methods, Results).

Second-level headings are flush left, boldface, and are also on their own line with initial capital letters. Second-level headings are rarely used except in taxonomic articles where multiple levels of headings may be necessary.)

Third-level headings are boldfaced, paragraph indented, have initial capital letters, and are followed by a period. Third-level headings are used to divide first-level sections into smaller sections.

Fourth-level headings are italicized (but not boldfaced), paragraph indented, have initial capital letters, follow immediately after a third-level heading or start a new paragraph, and are followed by a period. Fourth-level headings are used to divide third-level sections into smaller sections.

In-Text Citations

Single Author
(Smith 1993)

Two Authors
(Smith and Jones 1993)

Multiple Citations
(Smith 1996, Smith et al. 1997, Jones 1998)

Multiple Publications by Same Author(s)
(Smith et al. 1995a, 1995b, 1997; Jones 1996)

Personal Communications
(Jones 1988; L. J. Smith, personal communication). Obtain and forward (at submission) a letter of permission to use citations to personal communications (from those other than authors).

Unpublished Data
(L.J.S., unpublished data) for one author or (unpublished data) for all authors. Obtain and forward (at submission) a letter of permission to use citations to unpublished data (from those other than authors).

In Press
(Smith 1997) for in press, cite projected year of publication.
Software
(PROC GLM, SAS Institute 1999) for software user's manual.

Manufacturers
In parentheses, provide manufacturer's name and location (city, state) and model number of relevant materials and equipment. Example: (Model 3000, LI-COR, Lincoln, NE). Use generic names when possible (e.g., self-sealing plastic bags).

Reporting Requirements for Statistical Tests
All data reported (except for descriptive biology) must be subjected to statistical analysis. Descriptive biology should include information such as sample sizes and number of replications. Authors are responsible for the statistical method selected and for the accuracy of their data. Authors should be able to justify the use of a particular statistical test when requested by an editor. Results of statistical tests may be presented in the text, in tables, and in figures. Statistical methods should be described in Materials

and Methods with appropriate references. Experimental designs should also be described fully in Materials and Methods. Descriptions should include information such as sample sizes and number of replications. See specific section in this style guide for suggestions on formatting statistical results. Only *t*-tests and analyses of variance require no citation. Cite the computer program user's manual in the References Cited.

Probit/logit
When presenting results of probit/logit analysis, these columns should be included in tables (in this order, left to right); n, slope + SE, LD (or LC) (95% CL), and chi-square. When a ratio of one LD versus another is given, it should be given with its 95% CI.

Statistical tests to show what model best fits data intended to estimate the 99.9986% level of effectiveness should be presented to justify use of any model, including the probit model. Thus, we do not recommend use of the Probit 9 without tests to show that the probit model fits the data.

Analysis of Variance or *t*-test
When presenting the results of analysis of variance or a *t*-test, specify *F* (or *t*) values, degrees of freedom, and *P* values. This information may be placed in parentheses in the text. Example: ($F = 9.26$; df = 4, 26; $P < 0.001$). If readability of the text is affected by the presence of repeated parenthetical statistical statements, place them in a table.

Regression
In regressions, specify the model, define all variables, and provide estimates of variances for parameters and the residual mean-square error. Italicize variables in equations and text.

Variance and sample size
Include an estimate of the variance and sample size for each mean regardless of the method chosen for unplanned multiple comparisons. The use of Duncan's Multiple Range Test (DMRT) is not acceptable as a *mean separation test* as it is no longer commonly accepted as a method for *post hoc* mean separation analysis.

Model Analysis
At the beginning of the manuscript, authors should state clearly the goals of their model construction and analysis. Evaluation by reviewers depends upon these goals and the type of model. Authors should attempt to describe the main conclusions, limitations, and sensitivity of results to assumptions. For stochastic models, describe the variability in the results.

Modeling Guidelines
The following guidelines pertain to any mathematical model calculated for purposes other than statistical analysis. Authors must adequately describe both model structure and model analysis. Authors must explain and justify original equations and computer programs or justify the selection of a published software package used in the computation of models. Model structure and steps in the analysis must be described in the Materials and Methods section. Without presenting extensive computer code, the text must permit an understanding of the model that would allow most mathematically

inclined scientists to duplicate the work. Present all equations that represent the biology of the system being modeled. Unless their derivation is self-evident, show how the equations were derived and mention the underlying assumptions. Express how the equations are solved over time and space. Provide references for standard techniques (e.g., matrix manipulation, integration). Define all variables and parameters in each equation and describe their units (e.g., time, space, and mass). In the Materials and Methods or Results section, present the range of parameter values included in the model, and describe the uncertainty in or range of validity of these values.

Equations

Consult *Mathematics into Type* for correct formatting of equations and mathematical variables. Italicize all mathematical variables. Center more complex equations on a separate line.

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(2)

Validation or the Testing of Model Results

Authors must state why the model did not require testing (e.g., theoretical study), why it cannot be tested (e.g., lack of data), or how it was tested. Data used for testing must be independent of data used to build or calibrate the model. Describe the data and procedures in Materials and Methods. Authors should be aware that the testing of models is an important step that should be a part of most studies.

Structure of Computer Code

For models solved or simulated by computers, mention the programming language and computer used. Describe the important numerical methods used in calculating the model (e.g., integration and random number generation). Mention how the program's logic and algorithms were tested and verified. When published software is computed, provide a reference and state which procedures were used. Discuss in any section of the manuscript the limitations of the published software. Original computer programs should be made available at the request of reviewers and readers.

Gene Sequencing

Inclusion of a GenBank/EMBL accession number for primary nucleotide and amino acid sequence data is a criterion for the acceptance of a manuscript for publication. Sequences from new species and new genes must indicate the proportion of the gene sequenced and should include data from both strands. The accession number may be included in the original manuscript or the sequence may be provided for review and an accession number provided when the manuscript is revised. A manuscript will not be accepted for publication until the accession number is provided.

GenBank may be contacted at their website at http://www.ncbi.nlm.nih.gov/Genbank/submit.html. The EMBL Data Library may be contacted at their website at http://www.ebi.ac.uk/embl/Submission/index.html.

Reporting Taxonomy

Follow the *International Code of Zoological Nomenclature*, 4th ed., for taxonomic style. Center the heading that indicates the name of the taxon in bold type. Center figure numbers in parentheses under the main heading; do not use bold type. Start all

synonomies at the left margin with runovers indented. Include authors and date. References must appear in References Cited section. Use telegraphic style throughout descriptions.

Taxonomy Headings

Use only acceptable 3rd-level subheadings such as:

- Male
- Female
- Material Examined
- Type Material
- Distribution
- Etymology
- Biology
- Discussion

Avoid using Description as a subheading.

Dates

Use Roman numerals I through XII to designate month of collection. Use Arabic numerals 00 through 99 to designate collection years in the 20th century. Do not abbreviate other years, including the 21st century. Express data in this format: day-month (use a Roman numeral)-year. Example: 2-V-97.

Locality Other than Principal Types

Start with the largest area followed by successively smaller areas separated by colons. Capitalize countries. Arrange data for each locality in the following order: count of specimens and sex or stage (as applicable), city or vicinity, date, collector, and depository. Example: MEXICO: Tamaulipas: 1 male, 1 female, Ciudad Mante, 15-III-97, K. Haack; 5 females, Ciudad Victoria, 3-VII-99, C. Hughes, MCZ. Arrange localities alphabetically. Use a semicolon to separate data for different localities. Define depositories in the Materials and Methods.

Type Material

Start description with the principal type in capital letters. Follow this immediately with count and sex of specimens (use male and female symbols if possible), then place additional data in the order of locality, date, additional data, and collector. Separate these items with commas. Example: HOLOTYPE: 1 male, Locust Grove, VA, 22-X-98, on Cercis canadensis, R. H. Foote. PARATYPES: 2 males, same data.

Voucher Specimens

Voucher specimens of arthropods serve as future reference for published names used in scientific publications. Although the deposition of voucher specimens is not required as a condition for publication, authors are encouraged to deposit specimens in an established, permanent collection and to note in the published article that the expected deposition has been made and its location. Authors should contact the curator of a voucher repository before deposition concerning the procedures required for curation to ensure that the collection will accept the voucher materials. The designation and proper labeling of voucher specimens is the author's responsibility. When available, at least three specimens should be deposited. Each specimen should have the following information provided at the time of deposition:

- Standard label data that are required for the specimens collection (i.e., locality, date of collection, collector, host, ecological data, whether the specimen is from a laboratory collection, etc.).
- An identification label that includes the identifier and date of identification.
- A label that designates the specimen as "voucher."

Acknowledgments

Place the acknowledgments after the text. Organize acknowledgments in paragraph form in the following order: persons (omit all professional titles and degrees), groups, granting institutions, grant numbers, and serial publication number.

Human and Animal Use in Research and Testing

For research articles that involved the use of humans or animals, the Entomological Society of America requires that the following types of notification, as applicable, be included in the acknowledgement section of the article.

Humans. All human subjects work should reference approved Internal Review Board protocols or compliance with Health Insurance Portability and Accountability Act information policies for their organization, if the protocols are not available.

Animals. All studies should reference an approved Institutional Animal Care and Use Committee protocol or similar documents from their institutions. For trapping/collecting wild animals/birds, reference to collecting permits at the national or state level should be referenced.

Pathogens. Reference should be made to Biological Use Authorization approved by an institutional Environmental Health and Safety committee or similar body.

Sample notification: The collection and infection of wild birds with encephalitis viruses was done under Protocol 11184 approved by the Institutional Animal Care and Use Committee of the University of California, Davis, California Resident Scientific Collection Permit 801049-02 by the State of California Department of Fish and Game, and Federal Fish and Wildlife Permit No. MB082812-0. Use of arboviruses was approved under Biological Use Authorization #0554 by Environmental Health and Safety of the University of California, Davis, and USDA Permit #47901.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

Potential conflicts of interest include any relationships of a financial or personal nature between an author or coauthor and individuals or organizations within three years of submission which, in theory, could affect or bias an author's scientific judgment, or limit an author's freedom to publish, analyze, discuss, or interpret relevant data. Sources of financial support originating outside the coauthors' home institution(s) for any aspect of a study must be indicated in the Acknowledgments section of the paper. Financial support includes not only funding, but gratis provision of materials, services, or equipment. Any additional potential conflicts of interest, not covered in the acknowledgments of financial support, must be revealed to the editor at submission, and disclosed in a statement immediately following the Acknowledgments. If an author or coauthor has entered into an agreement with any entity outside that authors' home institution, including the home institution of another coauthor, giving that entity veto power over publication of the study or over presentation, analysis, discussion, or

interpretation of any results of the study, whether or not such veto power was exercised, this information must be disclosed in a statement immediately following the Acknowledgments. As a suggestion, such a statement could take the following form: "This manuscript is published with the concurrence of [*Institution / Company / Individual / etc. X*]." If no potential conflicts of interest exist, this must be stated in the cover letter to the editor at submission.

REFERENCES CITED

Cite only those articles published or formally accepted for publication (in press). Include all references mentioned in text. Include enough information to allow reader to obtain cited material (e.g., book and proceedings citations must include name and location [city and state or country] of publisher).

Abbreviate journal titles according to the most recent issue of BIOSIS Serial Sources. For non-English titled journals that are cited in the references, the title of the journal should be spelled out, and not abbreviated. Systematics-related articles may specify that all serial titles be spelled out for final publication. Citations and References should not be numbered.

Alphabetical order (chronological for one author or more than two authors, and alphabetical order [by surname of second author] for two authors)

Journal Articles

Evans, M. A. 2000. Article title: subtitle (begin with lowercase after colon or dash unless first word is a proper noun). J. Abbr. 00:000–000.
Evans, M. A. 2001a. Article title. J. Abbr. 00: 000–000.
Evans, M. A. 2001b. Article title. J. Abbr. 00: 000–000.
Evans, M. A., and R. Burns. 2001. Article title. J. Abbr. 00: 000–000.
Evans, M. A., and A. Tyler. 2001. Article title. J. Abbr. 00: 000–000.
Evans, M. A., A. Tyler, and H. H. Munro. 2000. Article title. J. Abbr. 00: 000–000.
Evans, M. A., R. Burns, and A. A. Dunn. 2001. Article title. J. Abbr. 00: 000–000.

In Press

Evans, M. A. 2002. Article title. J. Econ. Entomol. (in press).

Books

Burns, R. 2001. Title (initial cap only): subtitle (no initial cap after colon). Publisher, city, state abbreviation or country.
Evans, M. A. 2001. Colorado potato beetle, 2nd ed. Publisher, city, state abbreviation or country.
Tyler, A. 2001. Western corn rootworm, vol. 2. Publisher, city, state abbreviation or country.

Article/Chapter in Book

Tyler, A. 2001. Article or chapter title, pp. 000–000. *In* T.A.J. Royer and R. B. Burns (eds.), Book title. Publisher, city, state abbreviation or country.
Tyler, A., R.S.T. Smith, and H. Brown. 2001. Onion thrips control, pp. 178–195. *In* R. S. Green and P. W. White (eds.), Book title, vol. 13. Entomological Society of America, Lanham, MD.

No Author Given

(USDA) U.S. Department of Agriculture. 2001. Title. USDA, Beltsville, MD.
(IRRI) International Rice Research Institute. 2001. Title. IRRI, City, State or Country.

Patents
Harred, J. F., A. R. Knight, and J. S. McIntyre, inventors; Dow Chemical Company, assignee. 1972 Apr 4. Epoxidation process. U.S. patent 3,654,317.

Proceedings
Martin, P. D., J. Kuhlman, and S. Moore. 2001. Yield effects of European corn borer (Lepidoptera: Pyralidae) feeding, pp. 345–356. *In* Proceedings, 19th Illinois Cooperative Extension Service Spray School, 24–27 June 1985, Chicago, IL. Publisher, City, State.

Rossignol, P. A. 2001. Parasite modification of mosquito probing behavior, pp. 25–28. *In* T. W. Scott and J. Grumstrup-Scott (eds.), Proceedings, Symposium: the Role of Vector-Host Interactions in Disease Transmission. National Conference of the Entomological Society of America,10 December 1985, Hollywood, FL. Miscellaneous Publication 68. Entomological Society of America, Lanham, MD.

Theses/Dissertations
James, H. 2001. Thesis or dissertation title. M.S. thesis or Ph.D. dissertation, University of Pennsylvania, Philadelphia.

Software
SAS Institute. 2001. PROC user's manual, version 6th ed. SAS Institute, Cary, NC.

Online Citations
Reisen, W. 2001. Title. Complete URL (protocol://host.name/path/file.name) and/or DOI (Digital Object Identifier)

Tables
Place tables after the References Cited section. Double-space and number all tables. Boldface table title. Do not repeat data already presented in text. If a table continues on more than one page, repeat column headings on subsequent page(s).
Click here to see a sample table

Title
Title should be short and descriptive. Boldface table number and title only. Include "means + SEM" in title if applicable. Do not footnote title; use the unlettered first footnote to include general information necessary to understand the title (e.g., define terms, abbreviations, and statistical tests).

Lines
Use horizontal lines to separate title from column headings, column headings from data field, and data field from footnotes. Do not use vertical lines to separate columns. All columns must have headings.

Abbreviations
Use approved abbreviations. Use abbreviations already defined in the text and define

others in the general footnote. Use the following abbreviations in the body or column headings of tables only: amt (amount), avg (average), concn (concentration), diam (diameter), exp (experiment), ht (height), max (maximum), min. (minimum), no. (number), prepn (preparation), temp (temperature), vs (versus), vol (volume), wt (weight). Use the following abbreviations for months: Jan., Feb., Mar., April, May, June, July, Aug., Sept., Oct., Nov., and Dec.

Operational Signs
Repeat operational signs throughout data field. Insert a space on either side of sign (1.42 ± 1.36).

Spacing
Leave no space between lowercase letters and their preceding values (e.g., 731.2ab).

Footnotes to Tables
Use footnotes to define or clarify column headings or specific datum within the data field. Do not footnote the title; use the unlettered first footnote to include general information necessary to understand the table (e.g., define terms, abbreviations, and statistical tests). The use of asterisks is reserved for statistical significance only.

Example:
Means within a column followed by the same letter are not significantly different ($P < 0.05$; Student *t*-test [Abbott 1925]). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant).

Use lowercase italicized superscripted letters to indicate footnotes. Footnote letters should appear in the table in consecutive order, from left to right across the table then down the page.

Figures
For review purposes, it is acceptable to include figures, whether in black and white or color, as part of the manuscript file, with each figure on a separate page. Figures should be inserted in the manuscript file in one of the following formats:

- Tagged Image File Format (.tif)* (please check settings when exporting to TIFF from the original application).
- Encapsulated PostScript (.eps)*
- Rich Text Format (.rtf)
- Editable Microsoft Word (.doc/.docx) (image files embedded into Word are often not good quality)
- Editable Microsoft PowerPoint (.ppt/.pptx) (image files embedded into PowerPoint are often not good quality).
- Microsoft Excel (.xls/.xlsx)
- Editable Portable Document Format (PDF)
- Postscript (.ps)
- Photoshop (.psd)
- Adobe Illustrator (.ai)
- Graphics Interchange Format (.gif)
- Portable Network Graphics (.png)

GIF formats, such as from websites, are not acceptable and produce poor quality printouts because of low resolution, even for peer review purposes. Charts from Excel and SigmaPlot should not be inserted unless they are in one of the above formats.

Maximum figure sizes are as follows:

- Maximum height: 240 mm (9 inches)
- Maximum width (2-column figure): 171 mm (6 inches)
- Maximum width (1-column figure): 82 mm (3 inches)

When authors are asked to submit revisions, they are also asked to provide all figures as separate, high-quality image files to allow papers to move quickly and efficiently into production upon acceptance.

For more information on preparing figures, see OUP's Author Resource Centre on figures.

Abbreviations and Symbols
Abbreviations and symbols in figures should match those in the text or be defined in legends.

Figure Captions
Type all captions double-spaced on a separate page. All captions should be in paragraph form as shown by the example below.

Fig. 1. Relationship between percentage of defoliation of oak trees and gypsy moth population density. (A) Defoliation and egg mass density. (B) Defoliation of egg density.

Letter locants on figures composed of more than one element should match those in the text (either upper- or lowercase). Do not use equal signs to define abbreviations; use commas (e.g., Ap, barometric pressure).

SUPPLEMENTAL MATERIAL
Supplemental Material may be submitted in the form of one or more (8 maximum) files to accompany the online version of an article. Such material often consists of large tables, data sets, or videos which normally are not possible or convenient to present in print media. Supplemental Material represents substantive information to be posted on the ESA journal website that enhances and enriches the information presented in the main body of a paper. However, the paper must stand on its own without the need for the reader to access the supplemental information to understand and judge the merits of the paper. Any files containing Supplemental Material must be provided at the time of manuscript submission, and will be distributed to reviewers as part of the normal peer-review process. Authors should alert the editor to the presence of Supplementary Material in their cover letter at submission. Once a paper is published, the content of accompanying Supplemental Material files cannot be altered. Although the content of any submitted Supplementary Material is subject to normal peer-review and any changes required by the editor, no copy editing will be performed by the journal's production staff. Therefore, the authors are responsible for suitable format and final appearance of Supplemental Material after acceptance of the paper.

Supplemental Material should be referenced in the body of the main paper (e.g., Supp. Table S1; Supp. Video S1), where a link will take the online reader to the file. Each supplemental file must be labeled with an appropriate title and prefaced by a short (50 words maximum) summary description of the contents. Within each file, any tables, figures, videos, or other material must be accompanied by an appropriate caption. Citations for any literature referenced within a Supplemental Material file should be listed in a References Cited section at the end of the file, even when a citation is duplicated in the main body of the paper. Videos should be brief (< 5 min) and kept to a reasonable size to facilitate downloading by readers.

NOTES ON TERMINOLOGY

Scientific Names

Scientific names and authorities must be spelled out (except for Fabricius and Linnaeus, which are abbreviated as F. and L., respectively) the first time a species is mentioned in the abstract and again in the main body of text.

Common Names

Use only those common names cited in the current *ESA Common Names of Insects & Related Organisms* online database, or those names approved by the ESA Common Names Committee. Do not use any other common name. Do not abbreviate common names (e.g., CPB for Colorado potato beetle).

Give scientific name and authority at first mention of each organism (including plants) in the abstract and again in the text.

Use of "Stadium," "Stage," and "Instar"

Manuscripts received for publication in ESA periodicals refer to arthropods and the periods of time in their development in various ways. These designations should be used consistently.

Stadium (Plural: Stadia): The period of time between two successive molts.

Stage: One of the successive principal divisions in the life cycle of an arthropod (e.g., egg, nymph, larva, prepupa, pupa, subimago, and adult).

Instar: The arthropod itself between two successive molts. For the purposes of the definition, hatching is considered a molt.

Examples of Usage:
Nymphs feed on the underside of leaves during the first stadium.

Larvae of some dermestids go through an indefinite number of stadia (or have an indefinite number of instars).

The nymphs were reared through the fifth stadium. Immature stages (e.g., eggs, larvae, and pupae; eggs and nymphs) are illustrated.

First instar of cerambycids make galleries in wood.

Some 200 first-instar spiderlings were collected. The predators fed readily on early instars of the face fly.

NOTES ON FORMATTING

Capitalization
Do not capitalize the following words in titles or subheadings: a, an, and, as, at, be, by, for, in, of, on, per, to, the.

Abbreviations
Use standard abbreviations as listed in the Council of Science Editors' *Scientific Style and Format, The CBE Manual for Authors, Editors, and Publishers*, 8th ed., or those listed in this guide. Avoid nonstandard abbreviations.

Abbreviations for Time
Use the following abbreviations for time: h (hour), min (minute), s (second), yr (year), mo (month), wk (week), d (day). Do not add "s" to create plurals (e.g., wks).

Fig./Figs.
Use "Fig." if singular and "Figs." if plural (e.g., Fig. 1; Figs. 2 and 3).

Dates
When citing dates in the text (not in tables or taxonomic reports), do not abbreviate month, and use this format: 26 January 1997.

Metric Units
Use metric units. English units may follow within parentheses only if they are of direct practical purpose.

Liter
Do not abbreviate "liter" by itself or when accompanied by a numeral.

% versus percentage
Use "%" only with numerals and in tables and figures. Close up space to numerals (e.g., 50%). Otherwise, use the word percentage (e.g., percentage of defoliation).

Per versus slash
Use "per" rather than a slash unless reporting measurements in unit to unit (e.g., insects per branch, not insects/branch; but g/cm2, not g per cm2.

Numbers
Spell out numbers at the beginning of a sentence. Spell out the numbers one through nine (10 and up are always used as numerals), unless they are used as units of measure (e.g., eight children, three dogs, 8 g, 3 ft, 0600 hours; NOT 8 children, 3 dogs, eight grams, three feet, or six o'clock am). This includes spelling out the ordinals first through ninth, along with twofold, one-way ANOVA, and one-half. Ordinals from 10 and higher are numerals, such as 10th or 51st. In some cases, such as where there is a long list of items (e.g., 8 flies, 6 mosquitoes, 4 butterflies, and 10 bees), exceptions can be made if the editor concurs. The editorial staff will have flexibility in interpreting the rule.

Zeros with P values
All numbers <1 must be preceded by a zero (e.g., $P < 0.05$).

Commas
When a number is >1,000, use a comma to separate hundreds from thousands.

Semicolon
Use a semicolon to separate different types of citations (Fig. 4; Table 2).

Repeating symbols
It is not necessary to repeat symbols or units of measure in a series (e.g., 30, 40, and 60%, respectively).

Footnotes to the Text
Avoid footnotes in the text. Use unnumbered footnotes only for disclaimers and animal use information. Place all footnotes on a separate page after References Cited. Examples of footnotes are:

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association of Laboratory Animal Care.

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Language editing, if your first language is not English, to ensure that the academic content of your paper is fully understood by journal editors and reviewers is optional. Language editing does not guarantee that your manuscript will be accepted for publication. For further information on this service, please clickhere. Several specialist language editing companies offer similar services and you can also use any of these. Authors are liable for all costs associated with such services.

ETHICS
Authors should observe high standards with respect to publication ethics as set out by ESA and the Committee on Publication Ethics (COPE). Any cases of ethical misconduct are treated very seriously and will be dealt with in accordance with ESA's

author misconduct policy and the COPE guidelines. Further information about OUP's ethical polices is available here.

12.5. Normas de formatação do periódico Drosophila ***Information Service*.**

Drosophila Information Service prints short research, technique, and teaching articles, descriptions of new mutations, and other material of general interest to *Drosophila* researchers. The current publication schedule for regular issues is annually, with the official publication date being 31 December of the year of the issue. The annual issue will, therefore, include material submitted during that calendar year. To help us meet this target date, we request that submissions be sent by 15 December if possible, but articles are accepted at any time. Receipt by 31 December is a firm deadline, due to printer submission schedules.

Manuscripts, orders, and inquiries concerning the regular annual DIS issue should be sent to James Thompson, Department of Zoology, University of Oklahoma, Norman, OK 73019. Telephone (405)-325-2001; email jthompson@ou.edu; FAX (405)-325-7560.

Submission: Manuscripts should be submitted in Word, with pictures preferably in *jpg. To help minimize editorial costs, proofs will not be sent to authors unless there is some question that needs to be clarified or they are specifically requested by the authors at the time of submission. The editor reserves the right to make minor grammatical, spelling, and stylistic changes if necessary to conform to DIS format and good English usage. Color illustrations will appear black and white in the printed version but will be in color in the electronically-accessible version on our web site (www.ou.edu/journals/dis).

Citation of References: Citation should be by name and date in the text of an article (Smith, 1989; Jin and Brown, 1990; Waters et al., 1990). At the end of the article, references should be listed alphabetically by senior author, listing all authors with initials, date, journal, volume and page numbers. Titles will not be included except for books, unpublished theses, and articles in press. An example format is:

Green, R.L., 1998, Heredity 121: 430-442.
Waters, R.L., J.T. Smith, and R.R. Brown 1990, J. Genet. 47: 123-134.

Note the initials are before each name except for the senior author.