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Diversidade e padrões ecológicos em comunidades de Drosophilidae (Diptera): integrando diversidade taxonômica e funcionalidade

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Diversidade e padrões ecológicos em comunidades de Drosophilidae (Diptera): integrando diversidade taxonômica e funcionalidade

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NannyeDias

SUMÁRIO

Diversidade e padrões ecológicos em comunidades de Drosophilidae (Diptera): integrando diversidade taxonômica e funcionalidade

Resumo

Buscar entender as funções desempenhadas pelas espécies nas comunidades de Drosophilidae é uma importante contribuição sobre a dinâmica das comunidades e seus efeitos no funcionamento do ecossistema. Deste modo, diversidade e padrões ecológicos dentro das comunidades podem ser melhores compreendidos, não somente pela perspectiva taxonômica como também quanto à funcionalidade. Com a ampla diversidade e distribuição do grupo foi possível proporcionar maiores informações em algumas lacunas ecossistêmicas, como efeito de borda, técnicas de amostragem e funcionalidade (Capítulos II, III, IV e V). No capítulo II analisamos os padrões de distribuição temporal da abundância, riqueza e composição de Drosophilidae em um fragmento de mata em função da distância da borda. Observamos que algumas espécies apresentam distribuição assimétrica no fragmento, sendo mais restritas à borda e outras ao interior de mata. Usando armadilhas coloridas pela primeira vez, no capítulo III conduzimos a identificação de indivíduos capturados em três ambientes do Rio Grande do Sul. Os resultados demonstraram que as armadilhas coloridas com uma eficiência marcante na amostragem de espécies de Drosophilidae, principalmente de espécies antofílicas, e algumas outras espécies capturadas acessoriamente em flores na região Neotropical, como *D. melanogaster* e *D. willistoni*. No capítulo IV realizamos uma revisão de atributos funcionais, nos concentrando apenas em estudos da história natural e ecologia de Drosophilidae o que poderiam ajudar a explicar aspectos funcionais do grupo. Sugerimos três categorias (percepção do habitat, o desempenho de voo e respostas ecofisiológicas) para compreender o padrão funcional em Drosophilidae. A

revisão proporciona um protocolo/guia prático para melhor correlacionar métricas funcionais para questões ecológicas do grupo. Testamos esse protocolo no capítulo V em um levantamento de fauna em áreas de vegetação florestais e vegetações campestres, onde avaliamos a riqueza e funcionalidade da comunidade. Os índices funcionais não diferiram significativamente demonstrando que as espécies possuem uma variação dos atributos mensurados (o tamanho do olho, comprimento da gena, comprimento do flagelômero e escapo, tamanho da asa, tamanho do tórax e tamanho do corpo) que na verdade são compartilhadas entre elas independente da vegetação. No geral, os trabalhos realizados nesta tese reforçam diversidade taxonômica e composição funcional em Drosophilidae como importantes ferramentas para entender padrões e o funcionamento dos ambientes gerando dados com maior precisão quanto à distribuição e papel ecológico dos grupos, partindo de diferentes métricas amostrais, como aqui realizadas.

Diversity and ecological patterns in Drosophilidae (Diptera) communities: integrating taxonomic diverstiy and functionality

Abstract

Understanding the functions performed by species in Drosophilidae communities is an important contribution to the dynamics of communities and their effects on ecosystem functioning. In this way, diversity and ecological patterns within communities can be better understood, not only from a taxonomic perspective but also in terms of functionality. With the wide diversity and distribution of the group, it was possible to provide more information on some ecosystem gaps, such as edge effect, sampling techniques and functionality (Chapters II, III, IV and V). In chapter II we analyze the patterns of temporal distribution of abundance, richness and composition of Drosophilidae in a forest fragment as a function of distance from the edge. We observed that some species present asymmetric distribution in the fragment, being more restricted to the edge and others to the interior of the forest. Using colored traps for the first time, in chapter III we conducted the identification of individuals captured in three environments in Rio Grande do Sul. The results showed that the colored traps are efficiency in the sampling of Drosophilidae species, mainly anthophilic species, and some other species caught by the way in flowers in the Neotropical region, such as D. melanogaster and D. willistoni. In chapter IV we carried out a review of functional attributes, focusing only on studies of the natural history and ecology of Drosophilidae, which could help to explain functional aspects of the group. We suggest three categories (habitat perception, flight performance and ecophysiological responses) to understand the functional pattern in Drosophilidae. The review provides a practical protocol to

better correlate functional measurements to ecological issues in the group. We tested this protocol in Chapter V in a survey of fauna in areas of forest and grassland vegetation, where we evaluated the richness and functionality of the community. The functional indices wasn't differ significantly, demonstrating that the species have a variation of the measured attributes (eye size, gena length, flagellomere and scape length, wing size, thorax size and body size) that are actually shared between they are independent of vegetation. Overall, the work carried out in this thesis reinforces taxonomic diversity and functional composition in Drosophilidae as important tools to understand patterns and the functioning of environments, generating data with greater precision regarding the distribution and ecological role of groups, starting from different sample metrics, as performed here.

1. CAPÍTULO I

1.1Introdução geral

1.1.1 Família Drosophilidae

Drosophilidae é uma família cosmopolita que possui representantes em praticamente todas as partes do mundo, em diferentes ecossistemas. Algumas espécies são endêmicas de determinadas regiões, outras são cosmopolitas devido à sua grande capacidade de dispersão. Atualmente, existem aproximadamente 4.500 espécies de Drosophilidae conhecidas distribuídas em 76 gêneros pertencentes a duas subfamílias: Steganinae e Drosophilinae (Bächli, 2021; Toda, 2021). Em particular, a família inclui a espécie *Drosophila melanogaster* Meigen, 1830, organismo modelo chave usado em muitos estudos biológicos até os dias atuais.

Basicamente, alimentam-se de leveduras e bactérias, que se desenvolvem sobre substratos orgânicos em decomposição (Bächli, 2021; Dobzhansky e Pavan, 1950) No geral, suas espécies podem utilizar um ou poucos sítios de alimentação e oviposição, enquanto outras espécies são mais generalistas, uma das características que favoreceu a muitas espécies da família ter se constituído como organismos modelo para os mais diferentes estudos ecológicos e evolutivos (Markow e O’Grady, 2007; Miller et al., 2017). Nos últimos anos, houve um aumento no número de estudos taxonômicos e bigeográficos com uso do modelo Drosophilidae, processo fundamental para conhecer a fauna local e avaliar o grau de degradação de ambientes naturais, uma vez que a família é extremamente sensível às mudanças nas condições do se habitat (Bizzo et al., 2010; Ferreira e Tidon, 2005; Mata et al., 2008).

Para captura do grupo é essencial o uso de técnicas adequadas para uma cobertura fiel nos diferentes ambientes, e/ou intercalar mais de um método, pois um pode suprir a deficiência amostral do outro. Tradicionalmente, estudos de fauna de Drosophilidae utilizam armadilhas com atrativo de banana fermentada capturando preferencialmente o gênero *Drosophila* (Bizzo et al., 2010; Duarte et al., 2018; Emerich et al., 2012; Garcia et al., 2012; Gottschalk et al., 2007, 2008; Hochmüller et al., 2010; Mateus et al., 2018; Poppe et al., 2012). Recentemente, alguns outros métodos apropriados vêm sendo utilizados para pesquisas de insetos do grupo, como armadilhas de atração, uso de redes entomológicas, coleta em flores (Cordeiro et al., 2020; Schmitz e Valente, 2019), coleta de recursos de fungos macroscópicos (Gottschalk et al., 2009; Valer et al., 2016) e armadilhas coloridas (Mendes et al., 2021a).

Como resultado, essas diferentes técnicas permitem a captura de indivíduos com diferentes características ecológicas e evolutivas na tentativa de suprir lacunas no conhecimento de outros gêneros (Cordeiro et al., 2020; Schmitz e Valente, 2019; Valer et al., 2016).

1.1.2 O Pampa Brasileiro

O bioma Pampa é uma região campestre subtropical-temperada que apresenta invernos com temperaturas negativas e verões com registros de mais de 40°C. Possui uma extensão de aproximadamente 700.000km^2, compartilhando território com Argentina, Uruguai e o estado do Rio Grande do Sul (RS) do Brasil (Bilenca e Miñarro, 2004). Segundo o mapeamento da cobertura vegetal no bioma existem três tipos de formações vegetais: a campestre, que representa 23,03% da área total no Pampa, a Florestal, que representa 5,38% e a área de transição, com 12,91% (PROBIO, 2007), fitofisionomias campestres entremeadas por manchas de mata de florestas Estacional Decidual e Ombrófila Densa (MMA, 2007; Boldrini et al., 2010). Devido a sua heterogeneidade ambiental e climática, o Pampa é considerado um bioma com condições ideais para o desenvolvimento de estudos ecológicos, porém tem sofrido grande perda de biodiversidade e de habitats.

Uma das maiores ameaças ao Pampa é o acelerado processo de expansão agrícola iniciado nos anos 1970, e têm se agravado recentemente pelos planos para conversão de extensas áreas de campos em monoculturas florestais, de acordo com o Censo Agropecuário (Almeida et al., 2006; IBGE, 2006; MMA 2007). Atualmente, restam apenas pequenos fragmentos de Pampa próximos a margens de rios em uma paisagem predominantemente agrícola (Risser 1997; Porto 2002). Em geral, o Pampa é o segundo bioma mais desmatado do Brasil com apenas 11% sem nenhum tipo de influência antrópica no Rio Grande do Sul (PROBIO 2007).

Negligenciado, principalmente em relação aos outros biomas brasileiros mesmo com aproximadamente 63% presente no território nacional, apenas recentemente o bioma Pampa 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 levantamentos de fauna (MMA, 2007).

Menos de 3% da área total de Campos Sulinos que ainda existentes no RS encontra-se protegidos por Unidades de Conservação, o que é pouco para a proteção do patrimônio ecológico e genético do Pampa (Brandão et al., 2007). Conhecer a fauna e a flora do bioma é essencial para mudar esse panorama visto que a biodiversidade desempenha papel importante papel no ecossistema. Para Drosophilidae, alguns trabalhos vêm contribuindo para mudar significativamente no conhecimento da fauna em áreas de Pampa (Hochmüller et al., 2010; Poppe et al., 2012; 2013; Valer et al., 2016; Mendes et al., 2017; Cordeiro et al., 2020; Schmitz e Valente-Gaiesky, 2019), incluindo descrições de novas espécies.

1.1.3 Diversidade e padrões ecológicos

Diversidade é um conceito complexo multifacetado que inclui escalas no espaço e tempo, e entidades como espécies, atributos e unidades evolutivas (Pavoine e Bonsall, 2011; Petchey e Gaston, 2006). Estudos de comunidades tem frequentemente focado em padrões de diversidade. Contudo, diversidade é equiparada com riqueza de espécies e/ou equitabilidade, enquanto outros componentes associados com diversidade têm sido menos explorados (Magurran, 2004; Pavoine e Bonsall, 2011). Tais componentes são importantes ferramentas para entender padrões e o funcionamento dos ambientes gerando dados com maior precisão quanto à distribuição e papel ecológico dos grupos, partindo desde métricas amostrais mais adequadas (Aguirre-Gutiérrez et al., 2016; Villéger et al., 2017), até a identificação de características resposta e traços efeito (Gallé e Bátary, 2019).

Para conservar nossa biodiversidade é necessário não só conhecer as espécies que aqui habitam como também identificar qual o seu papel na manutenção dos ecossistemas (Mouchet et al., 2010; Moretti et al., 2017; Sobral et al., 2016). Neste sentido, características morfológicas possuem grande importância na conservação. Reconhecer essas funções específicas de cada comunidade significa também ser capaz de identificar como as espécies respondem aos distúrbios e/ou condições abióticas severas (Moretti et al., 2017, Sobral et al., 2016). Respostas rápidas que inclusive são alcançadas quando se utiliza organismos pequenos e com curto ciclo de vida como modelo de estudo, como é o caso de indivíduos da família Drosophilidae (Mata et al., 2008; Mata et al., 2010).

Atualmente, existe também a possibilidade de combinar características morfológicas e relações filogenéticas em estudos de biodiversidade que mais tarde servem como ponto de partida para a formulação de hipóteses sobre a importância de processos ecológicos, históricos e biogeográficos que influenciam a organização das comunidades (Cavenderbares et al., 2009; Pavoine e Bonsall, 2011). Como as espécies e suas adaptações ao ambiente não são independentes na história evolutiva dos clados, espécies filogeneticamente relacionadas tendem a compartilhar tanto atributos morfológicos quanto requerimentos ecológicos (Turcker et al., 2016; Webb et al., 2002).

Embora as análises de diversidade funcional utilizem características que podem capturar adequadamente funções específicas de determinados táxons na comunidade, o que é chave para a componente diversidade (Mouillot et al., 2013, Sobral et al., 2016), pois podem afetar direta e indiretamente o funcionamento do ecossistema, o mais comum na literatura é avaliar a diversidade taxonômica (Cianciaruso et al., 2009; Diaz e Cabido, 2001), a qual não investiga o papel ou contexto ambiental das espécies (Ulrich et al., 2012). A diversidade taxonômica, porém, nos limita em como entender a resposta das espécies aos distúrbios e estratégias quando enfrentam condições abióticas severas, e como os processos podem variar na escala espacial separando diversidade funcional dentro de uma comunidade e/ou entre comunidades (Diaz e cabido, 2001; Petchey e Gaston, 2006).

Caracteres morfológicos tornam-se atributos funcionais quando ilustram características morfológicas, fisiológicas, comportamentais, entre outras, que podem influenciar o estabelecimento, o *fitness* e a sobrevivência das espécies (Gallé e Batáry 2019; Mendes et al., 2021b; Reich et al., 2003; Sobral et al., 2016). As variações desses atributos podem definir padrões de distribuição das espécies e também respostas dessas espécies a diferentes ambientes ou alterações no seu ambiente natural (Sobral et al., 2016; Westoby et al., 2002). A manutenção da riqueza de espécies sozinha não é o melhor enfoque para a conservação da diversidade, e sim a manutenção da integridade funcional do ecossistema (Walker, 1992), uma abordagem baseada no uso de grupos funcionais onde é importante conhecer as características das espécies (Cornelissen et al., 2003; Reich et al., 2003).

Os atributos funcionais quando analisados dependem das características da comunidade e também das funções que se pretende identificar (Pendry et al., 2007; Sobral et al., 2016). Alguns estudos demonstram importantes variações fenotípicas como resposta adaptativa a estresses naturais e/ou artificiais (Rocha et al., 2009; Tidon, 2006). Em Drosophilidae, por exemplo, Machida et al., (2021) demonstraram resposta fenotípica à variável temperatura em espécies neotropicais distantes, é o caso, por exemplo, de *Drosophila mercatorum* e *D. willistoni* que possuem correlação positiva das características para esta variável. Mais recentemente, alguns novos trabalhos vêm sendo publicados com avaliação de características morfológicas de espécies, mas raramente essas características são avaliadas em comunidades.

1.2. Objetivos

1.2.1 Objetivo geral

Ampliar as informações referentes à diversidade de Drosophilidae aprimorando o conhecimento de padrões ecológicos de distribuição e outras lacunas ecossistêmicas não exploradas, como efeito de borda, novas técnicas de amostragem e funcionalidade a partir de métricas taxonômicas e funcionais em áreas de bioma Pampa, Brasil.

1.2.2 Objetivos específicos

1. Buscar entender o impacto do efeito de borda na composição, abundância e riqueza de espécies de Drosophilidae em um fragmento no Bioma Pampa (Capítulo II);

2. Identificar se existe sensibilidade de Drosophilidae aos efeitos de borda, e se difere entre as estações do ano (Capítulo II);

3. Testar a eficiência de metodologias pan traps e malaise para Drosophilidae, identificando a composição de espécies em diferentes habitats do Brasil (Capítulos III e V);

4. Propor um protocolo padronizado para seleção e medição de características funcionais para Drosophilidae (Capítulos IV e V);

5. Descrever, avaliar e comparar diversidade e composição funcional de espécies em vegetações florestal e campestre no Bioma Pampa, buscando entender os processos que levam à montagem de comunidades Drosophilidae (Diptera) a partir de abordagens taxonômicas e funcionais, em escalas locais e regionais (Capítulos II, IV e V);

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2. CAPÍTULO II

(Manuscrito publicado no periódico *Neotropical Biology and Conservation*)

Temporal edge effects structure the assemblages of Drosophilidae (Diptera) in a Restinga forest fragment in Southern Brazil

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Temporal edge effects structure the assemblages of Drosophilidae (Diptera) in a Restinga forest fragment in Southern Brazil

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Abstract

Anthropogenic habitat fragmentation directly affects ecological processes, leading to negative biodiversity impacts for insects and other biota. Increased edge effects are one consequence of fragmentation, and may alter the composition or abundance of species in the remaining habitat fragments. Understanding the ways in which edge effects impact upon the biota is essential for conservation decision-making in fragmented landscapes. Therefore, the aim of this study was to analyze the seasonal patterns of abundance, richness, and composition of Drosophilidae in a Restinga forest fragment in the extreme south of Brazil, as a function of the distance from the border to the interior of the fragment. The data were analyzed using SIMPER analyses, which showed that the edge and the forest interior were most dissimilar during winter, followed by spring, autumn and summer. An NMDS and the SIMPER analyses showed that the lower dissimilarity between the edge and interior in spring, autumn and summer, compared to winter, is driven by immigration of individuals from outside of the forest fragment. Furthermore, some species were asymmetrically distributed in the fragment, with some species restricted to the edge of the fragment and others to the interior. This information aids in the understanding of the functioning and dynamics of fragmentation, which is fundamental for the maintenance and integrity of environments and their fauna.

Keywords: Drosophilinae, environmental detectors, exotic species, habitat fragmentation, Neotropical forest, species composition

Introduction

Habitat fragmentation, loss, and degradation are the most important causes of declining insect assemblages, as a result of impacts on both ecological patterns, such as species diversity, and ecological processes, such as dispersal (Murcia 1995; New et al. 1995; New 1997). The impacts of habitat fragmentation on biodiversity include reduced dispersal rates, decreased effective population size, loss of genetic variability, invasion of exotic species and increases in the total area subject to edge effects (Fahring 2003; Ewers and Didham 2007; Laurence et al. 2007). Edge effects are characterized by an increase in the proportion of forest edge relative to the proportion of forest interior, which causes structural changes and increases the exposure of organisms that are inside the fragment to the conditions of a different ecosystem (Murcia 1995). Which means that we can define edge as the line used to demarcate two adjacent ecosystems where both are separated by an abrupt transition also referred to as ecotone (Murcia 1995; Lennon et al. 1997).

The strength of edge effects is related to the distance to the edge of the fragment, and can differ between environmental variables. For example, the increases in solar radiation levels, temperature variations, relative humidity and wind, are variables that have a stronger influence on the microenvironment (Kapos 1989; Bierregaard et al. 1992). These changes modify the composition of biotic communities and affect biological processes, including interactions between species (Laurence 2000).

Many insect species are used as ecological indicators, such that changes in their abundance are considered indicative of degradation in different habitats (Neto et al. 1995; Ries and Debinski 2001; Wink et al. 2005, Gadelha et al. 2009; Oliveira et al. 2015). Drosophilidae flies are especially sensitive to anthropogenic actions and as such are frequently used to monitor habitat degradation (Martins 1989; Ferreira and Tidon 2005; Gottschalk et al. 2007; Mata et al. 2008; Penariol and Madi-Ravazzi 2013, Cavasini et al. 2014). There are currently 76 described Drosophilidae genera, with more than 4200 described species (Bächli 2021). In the Neotropical region, Sturtevant (1921) studied the distribution of Drosophilidae species and found 82 species distributed across 13 genera. The family is morphologically diverse, and has diversified its use of trophic resources (mainly fruits, flowers and macroscopic fungi) (Powell 1997; Wheeler 1957, 1987), such that species of varying sizes and dispersal abilities are found within each biogeographical region.

The present study aims to examine how Drosophilidae assemblages are impacted by edge effects in a Restinga forest fragment, and how these impacts vary seasonally. To the best of our knowledge, this is the first study to examine these dynamics. Specifically, we seek to understand: (i) the impact of edge effects on the composition, abundance and species richness of Drosophilidae in a forest fragment, and (ii) whether the sensitivity of Drosophilidae to edge effects differs between seasons.

Materials and methods

Study area

The study was conducted in the Horto Botânico Irmão Teodoro Luís (HBITL), a Restinga forest fragment located in the municipality of Capão do Leão (-31.815124S, -52.432228W, elevation about 16 m) in the state of Rio Grande do Sul, Brazil (Fig. 1, according to Google Earth, 2001). The HBITL has a total area of approximately 25 hectares (Guerra et al. 2015), lies approximately 50 km inland from the coast, and is a permanent preservation area. In the study region, the predominant climate is humid subtropical "Cfa", according to the Köppen classification (Alvares et al. 2013), and is made up of four distinct seasons (Spring: October to December, Summer: January to March, Autumn: April to June, and Winter: July to September). Seasonal variation is marked by a reduction in temperatures in winter, while rainfall and relative humidity remain almost constant throughout the year (IBGE 1997). The environmental data used in this study (daily average temperature, cumulative precipitation and relative humidity in each season) were collected at the Agroclimatological Station of Pelotas (1988) between February/2013 and January/2014 (see figure 2).

The study area is composed of a mosaic of Restinga forest surrounded by wetlands and anthropogenic habitats, including pastures and a few low buildings. The forest consists of different strata: trees, shrubs, and herbaceous plants, with xeromorphic, succulent and thorny vegetation (Rodrigues 2005). Large fig trees are common at the canopy level, which has a height of approximately eight meters.

>Fig. 1

Restinga features and landscape data

Geologically, the most accepted definition for Restinga refers strictly to recent and unstable sandy strips in the coastal region, with practically no vegetation covering the sand, or with only undergrowth (Souza et al. 2005). However, in plant ecology, the coastal vegetation of Restinga is defined by different forms of vegetation established on sandy soils that occur in the region of the coastal plain (Azevedo et al. 2014). These soils originate predominantly through marine deposition during the sea-level retreat phases in different geological periods, not only recent depositions (Souza et al. 2005).

The established vegetation is not homogeneous in the coastal plain since there are different factors defining the environmental conditions and, consequently, the type of vegetation that is established. Among these factors is the distance from the sea, as salinity, wind strength, and temperature varies along this gradient. Another factor is the topography of the terrain, which is associated with the processes of deposition and removal of soil in these regions (Araújo 1984; Souza et al. 2005; Scarano 2009; Azevedo et al. 2014). In addition, the vegetation itself, when established, changes the environmental conditions and allows for the occurrence of vegetative succession, such that organisms can select microhabitats in which to live (Araújo 1984; Ricklefs 2003; Scarano 2009).

In this study, we described the vegetation structure at each compass point at a distance of 30 m from each trap (composition and landscape configuration) recorded for February 2013 to January 2014, which were defined according to Restinga ecology established in resolutions 07/1996 and 417/2009 of the National Environmental Council (CONAMA) (Table 1).

>Table 1

Sampling and Statistical Analysis

Data were collected monthly between February 2013 and January 2014, inside the forest fragment. Adult Drosophilidae were sampled using 12 retention traps, according to Tidon and Sene (1988), hung 1.5 m high, and spaced 60 m apart, over three days each month (Mendes et al. 2017). Each trap contained 150 grams of smashed banana mixed with 25 grams of fresh baker’s yeast (*Saccharomyces cerevisiae*). The shortest distance between each trap and the edge of the fragment was measured in the field using a measuring tape, and was later verified from satellite images captured in February 2013 (using Google Earth and Geographic Information System software). Unfortunately, we were not able to consider 0m from the edge of the fragment in this study, due to anthropogenic activities around the fragment.

The sampled individuals were fixed in 70 % ethanol and prepared according to Wheeler and Kambysellis (1966) and Kaneshiro (1969). The individuals were identified to species level based on external morphology and male genitalia, using an Olympus CL 6000 stereomicroscope, and following identification keys (Freire-Maia and Pavan 1949; Wheeler and Kambysellis 1966, Grimaldi 1987). Females of sibling species were identified by external morphology and, when possible, the species level was determined according to the quantities of males in each trap for analysis purposes. Voucher specimens of all recorded species were deposited in the Drosophilidae Collection of the Museum of Natural Sciences Carlos Ritter (MCNCR) of the Universidade Federal de Pelotas, in the state of Rio Grande do Sul, in Brazil.

The number of species (S) and total abundance of individuals (N) sampled per month were recorded for each trap. To increase the robustness of the data, the total S and N of the traps in the same geographical coordinate of all collection events was added and analyzed by season of the year. Most inventoried insect assemblages show temporal variation in abundance and species richness (Haridas et al. 2016; Heimonen et al. 2013; Poppe et al. 2013; Mateus et al. 2018), including those sampled in this study, thus we sampled three times per season. As such, we had larger sample coverage and, at the same time, did not overestimate the distance relations to the edge, replicating the traps inside the fragment.

When working with gridded biodiversity data there is always the potential for spatial autocorrelation, and as such we used Mantel tests (Mantel 1967) with Jaccard and Morisita indices (Krebs 1999; Jaccard 1900) to check for spatial autocorrelation of the species composition between the traps in each season of the year . In each analysis, the significance level was calculated with 999 permutations. To perform the tests, geographic coordinates in UTM (Universal Transverse Mercator) were determined for the position of each trap. The distances between traps were calculated based on the Euclidean distance (Saito and Toriwaki 1994). We also tested spatial dependence of total N and S with Moran's I tests (Moran 1950) with 999 permutations. These analyses were conducted in program R version 3.1.2 (R Core Team, 2013) using the vegan and ade4 packages (Dray and Dufour 2007; Oksanen et al. 2008, from Ecological Data Analysis, 2015). As we did not detect differences between our observed data and a random pattern (with one exception in the spring samplings using a Mantel test with the Morisita index), the traps were used as the statistical sample unit for analysis (Suppl. material 1).

To compare species composition between sample units as a function of the distance to the edge of the fragment, a non-metric Multidimensional Scaling (NMDS) analysis was performed using the Bray-Curtis similarity index, after a logarithmic transformation of the absolute abundance in each sample unit. Shepard's plots (scatter plots of the distances between data points), with stress values (that reflect how well the ordination summarizes the observed distances among the samples), were plotted. Subsequently, the values of the first two axes resulting from the analysis were compared with the distance between each trap and the edge of the fragment, using a Spearman correlation analysis.

Finally, a SIMPER test was performed using the Bray-Curtis similarity index with the absolute abundances, where the distances were categorized as interior (> 100 m) and edge (< 100 m), to verify which taxa most contribute to explain the difference in species composition between the traps (Vickery 2002). These statistical analyses were conducted using the software PAST version 2.17c (Hammer et al. 2001).

Results

Over the study period, a total of 25,081 adult specimens were sampled (Suppl. material 2), distributed in 46 species belonging to five genera (*Drosophila* Fallen 1823, *Zygothrica* Wiedemann 1830, *Hirtodrosophila* Duda 1923, *Leucophenga* Mik 1886 and *Zaprionus* Coquillett 1901). Eighteen species were restricted to a particular microenvironment (Suppl. material 2), with approximately 80 % of the species being found in both the interior and the edge of the fragment (Suppl. material 3).

In winter, both species richness and absolute abundance of Drosophilidae were significantly positively correlated with distance to the border of the fragment (Suppl. material 3, Fig. 3, $p < 0.05$), such that both increase with increasing distance from the border. The correlation is highest for absolute abundance where the points are closest to the trend line (Figure 3). In all other seasons, species richness and absolute abundance did not fluctuate as a function of the distance from the border of the fragment. Overall abundance and species composition did not vary between the edge and the interior of the forest fragment, however, this pattern varied between seasons. In winter, both species richness and absolute abundance of Drosophilidae were significantly positively correlated with distance to the border of the fragment.

>Fig. 3

The results of the NMDS analysis differed between seasons, with a clear segregation of the interior and edge points only in winter (Fig. 4), when the distance to the edge of the fragment was one of the most important factors for the species composition of the Drosophilidae assemblage. Distance to the edge of the fragment was highly correlated with component 1 in winter (Table 2). The species composition of edge and interior assemblages did not differ significantly in the other seasons, nor was there a relationship between the components obtained in the NMDS analysis and the distance to the edge of the fragment (Table 2).

The SIMPER analysis also showed that the dissimilarity between the assemblages at the edge and in the interior of the forest was highest in winter (71.32 %), followed by Spring (46.73 %), Autumn (33.09 %) and Summer (21.98 %). The two most important species explaining the difference in species composition between the edge and interior assemblages in winter were the native species *Zygothrica orbitalis* (Sturtevant, 1916) and *Drosophila paraguayensis*, contributing 33.9 % and 17.9 %, respectively, to the dissimilarity between the two environments. These species are followed by three further native species: *Drosophila mediopunctata* Dobzhansky and Pavan, 1943, *Drosophila ornatifrons* Duda, 1927 and *Drosophila griseolineata* Duda, 1927, all of which were more abundant in the interior of the forest, and two exotic species: *Drosophila simulans* Sturtevant, 1919 and *Drosophila immigrans* Sturtevant, 1921, which were both more abundant at the edge of the forest fragment (Suppl. material 4).

>Table 2

In the other seasons (Suppl. material 5, 6 and 7), the assemblage was dominated by *D. simulans* Sturtevant, 1919 and the *Drosophila willistoni* subgroup. In spring and autumn, *D. simulans* was the most abundant and contributed the most to the dissimilarity between the border and the interior of the forest (55.3 % and 59.1 %, respectively), being more abundant at the edge of the forest than in the interior in both seasons (Suppl. material 5 and 7). In Summer, the *D. willistoni* subgroup was the most abundant and contributed the most (71.9%) to the dissimilarity between the interior and the edge of the forest (Suppl. material 6). *Drosophila polymorpha* Dobzhansky and Pavan, 1943 is the third most abundant species in spring, summer and autumn, however its abundance does not vary with distance from the edge of the fragment. It should also be noted that overall, in Spring, Summer, and Autumn, the differences between the border and the interior of the forest are small.

Discussion

Studies have shown that some species of Drosophilidae can be used as indicator species for monitoring environmental degradation (Ferreira and Tidon 2005; Gottschalk et al. 2007; Mata et al. 2008). However, although studies have verified the edge effect in forest areas (Döge, Valente and Hofmann 2008; Furtado and Martins 2018), few have tried to identify which species are associated with edge habitats (Penariol and Madi-Ravazzi 2013). Our results suggest that some species present habitat use strategies, at least in the winter, leading to a significant difference in abundance and species composition between the edge and interior of the fragment. However, this difference did not exist in other seasons, where the fauna in the interior of the forest fragment is similar to that of the edge.

While previous studies have shown an association between dominance and temporal variation in species abundance and different environmental variables in distinct environments (Saavedra et al. 1995; Torres and Madi-Ravazzi 2006; De Toni et al. 2007; Poppe et al. 2013; Mateus et al. 2018; Mendes et al. 2017), here we show that other factors determine the distribution of Drosophilidae species in Restinga fragments, and that the edge effect is seasonal.

As Penariol and Madi-Ravazzi (2013) had previously found, our results show that the abundance of both *Drosophila simulans* Sturtevant, 1919 and the *D. willistoni* subgroups are affected by the distance to the edge of the fragment, with the former being more abundant at the fragment border and the latter in the interior. However, the differences in abundance were only significant in the winter. Among the exotic species present in the assemblage, *Drosophila suzukii* Matsumura, 1931 was the only one able to more frequently use the interior of the forest and was also more likely to use areas of pristine vegetation than the other exotic species (*Drosophila ananassae* Doleschall, 1858, *D. buscki* Coquillett, 1901, *D. immigrans* Sturtevant, 1921, *D. melanogaster* Meigen, 1830 and *D. simulans* Sturtevant, 1919), which were restricted to the sample plots near the edge of the forest fragment. *Drosophila suzukii* Matsumura, 1931 is an invasive species that only recently arrived in Brazil and in recent years has considerably expanded its distribution in the American continent, establishing in habitats with different physiognomies (Hauser 2011; Cine et al. 2012; Deprá et al. 2014; Dos Santos et al. 2017).

The seasonal edge effect observed in this study may suggest two scenarios. Firstly, the vegetation of the forest fragment may be less permeable for species associated with the matrix in periods of lower temperature. The species that invade degraded, high stress environments, such as *Drosophila suzukii* Matsumura, 1931, *D. ananassae* Doleschall, 1858, *D. buscki* Coquillett, 1901, *D. immigrans* Sturtevant, 1921, *D. melanogaster* Meigen, 1830 and *D. simulans* Sturtevant, 1919 are, in their majority, exotic to the Neotropical region (Saavedra et al. 1995; Ferreira and Tidon 2005; Silva et al. 2005; Gottschalk et al. 2007). Gottschalk et al. (2007) have previously suggested this scenario based on their study of Drosophilidae along an environmental gradient associated with urbanization in the south of Brazil. These authors observed that exotic species associated with the urban environments, like *D. simulans* Sturtevant, 1919, were not collected in non-urban or less urbanized areas during the winter, even when the species were present in the highly urbanized areas. However, in the other seasons of the year, they were highly abundant in all areas.

The second possible scenario to explain the seasonal edge effect observed is that abundances of exotic species are generally lower during the cooler seasons of the year, such that probability of capture/detection is lower and the pattern encountered during warmer times cannot be detected due to lower sample size. This variation in the abundance of Drosophilidae species is well documented for the state of Rio Grande do Sul (Franck and Valente 1985; Saavedra et al. 1995; Silva et al. 2005; Poppe et al. 2013). In addition, winter is the only season wherein species abundance and richness are greater inside the forest fragment, and is the only time of the year that the assemblage is dominated by native species. Thus, vegetation fragments may be functioning as a refuge for native species at these times of the year. Moreover, the ecological niche of Drosophilidae is not stable throughout the year, and their abundance and occurrence depend on climatic variations that influence the behavior of flies and the phenology of plants which, in turn affect the availability of food resources and oviposition sites (Valente and Araújo 1991).

In conclusion, the results of the present study show that Drosophilidae are sensitive to edge effects in the Restinga fragment studied, and that their overall abundance is significantly higher in the interior than at the edge of the fragment in the winter. In terms of species composition, we show that most of the species sampled use both environments near the edge and environments in the interior of the forest fragment, but with different intensities. Finally, we show that some species were restricted to edge or interior environments, suggesting that certain species have different habitat use strategies. Our results provide important direction for future research, and have broad implications for the conservation of Drosophilidae. Small Restinga forest fragments, such as the one studied here, can contribute to the persistence of assemblages, thus improving the habitat quality of small fragmented forests that may be important for the maintenance of biodiversity.

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Author contributions

All authors participated in writing the manuscript. MFM, MLB, MSG and LAS collected the insects and data collect. MFM and MSG identified the insects. MFM, MLB, VSV and MSG performed the analyses and wrote the first draft of the manuscript as part of MFMs PhD thesis at the Universidade Federal do Rio Grande do Sul, Brazil. All authors have approved the manuscript and there are no conflicts of interest to declare.

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Figure captions

Figure 1. Map data 2013 of South America highlighting Brazil (in gray) and the location of the Horto Botânico Irmão Teodoro Luís in Restinga Forest (HBITL - red dot) in the state of Rio Grande do Sul (in dark gray). Blue dots - indicate the location of each trap in the HBITL. According Google Earth, accessed in http://www.google.com.br/earth/

Figure 2. Average A: mean daily temperature (º C), B: cumulative precipitation (mm) and C: relative humidity (%) of the seasons during the study (spring (Spr): October to December, summer (Sum): January to March, autumn (Aut): April to June, and winter (Win): July to September). In the box plots, the boundary of the gray box indicates the 50% central percentile, the black line within the box marks the median, and the whiskers above and below the box indicate the lower and higher 25 % percentiles.

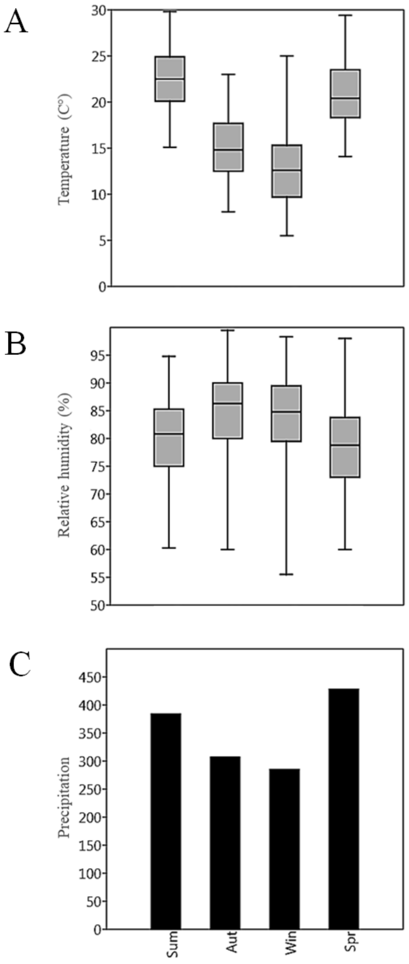

Figure 3. Relationship between species richness (A) and absolute abundance (B) of Drosophilidae and the distance to the edge of a Restinga forest fragment in southern Brazil. Four seasons of the year were sampled and are represented by different colors - Blue line: winter; red line: spring; yellow line: summer; gray line: autumn.

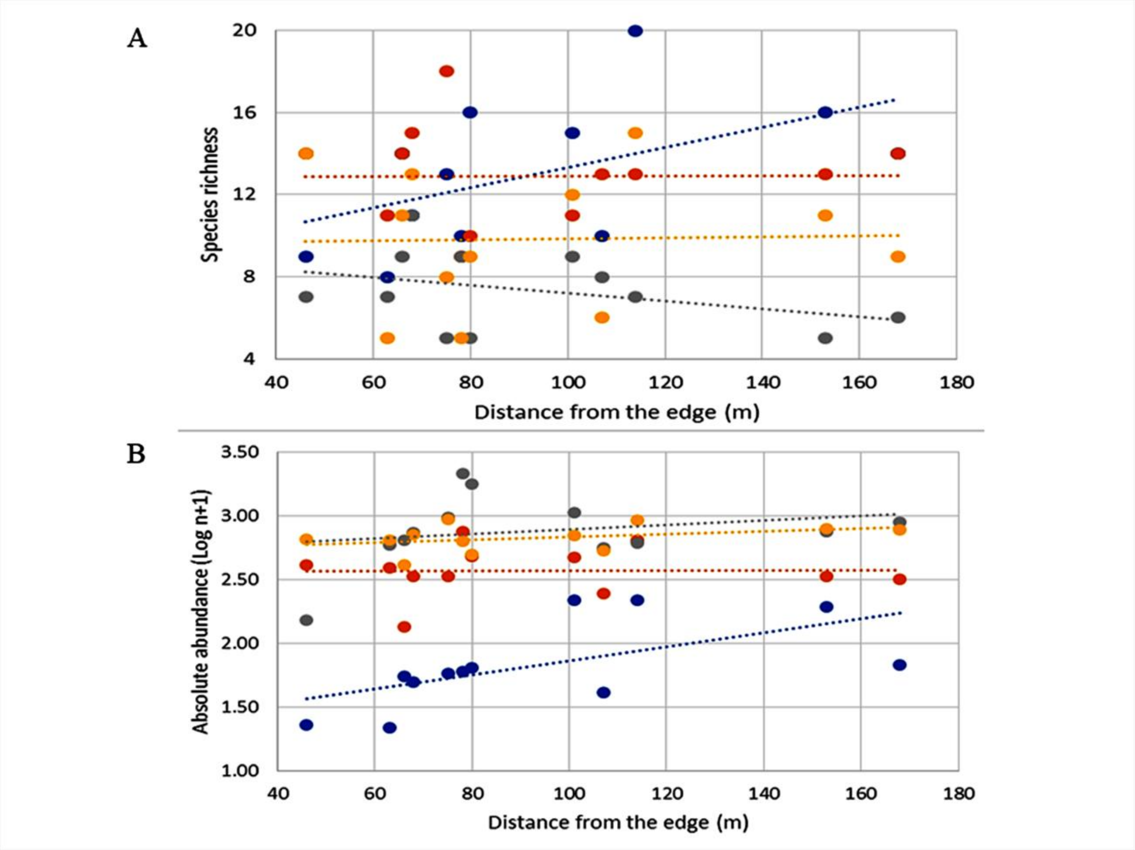

Figure 4. NMDS ordering analysis with the Bray-Curtis similarity index for the Drosophilidae assemblages sampled in a Restinga fragment in southern Brazil for the four seasons of the year. A-B: winter; C-D: spring; E-F: summer; G-H: autumn. A, C, E, F: Coordinates 1 and 2 generated in the NMDS analysis and plotted. B, D, F, H: Shepard's plots with the stress values of each analysis.

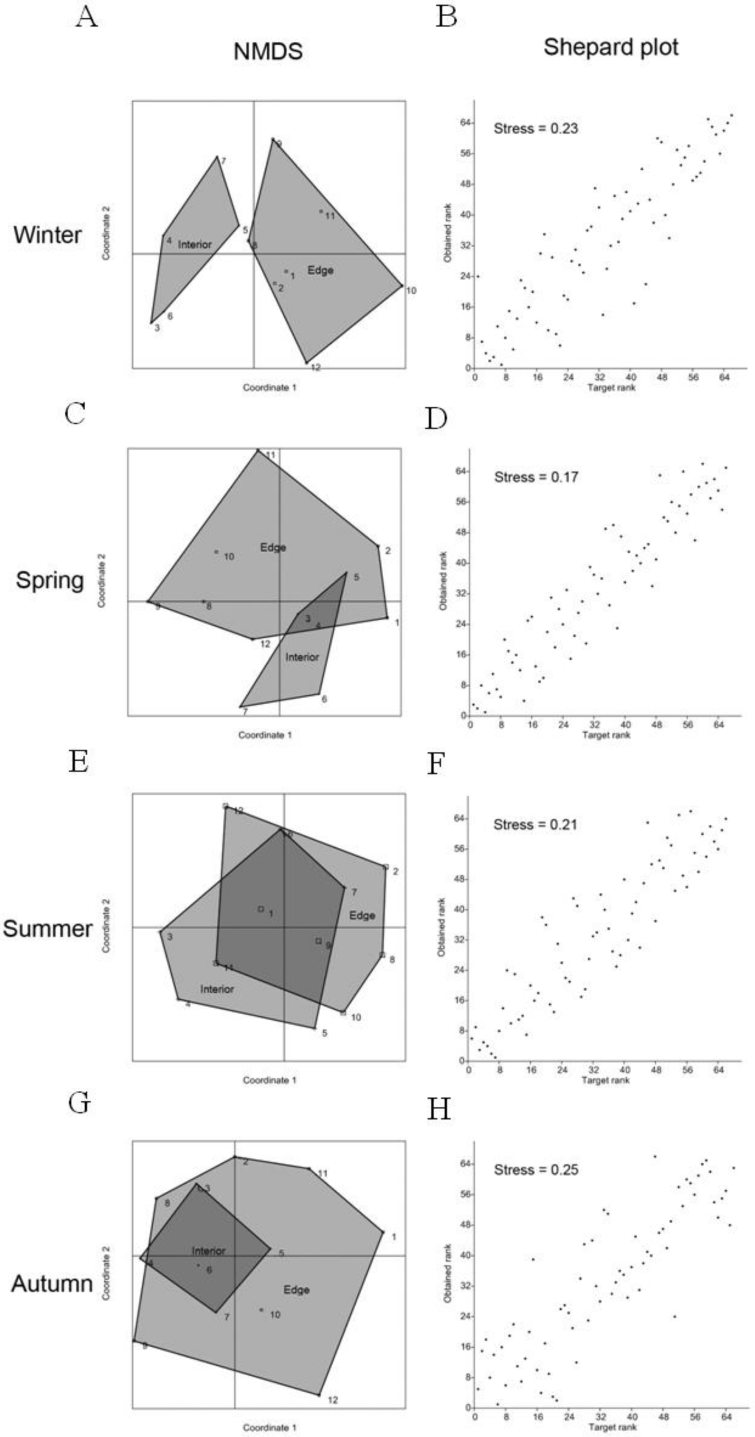
A
B
NMDS
Shepard plot
Winter
Interior
Edge
Coordinate 1
Coordinate 2
Stress = 0.23
Obtained rank
Target rank
C
D
Spring
Interior
Edge
Stress = 0.17
E
F
Summer
Interior
Edge
Stress = 0.21
G
H
Autumn
Interior
Edge
Stress = 0.25
0
8
16
24
32
40
48
56
64

Table captions

Table 1. Characterization of the vegetation around the twelve sampled points from the interior to the edge of a Restinga fragment in Southern Brazil, from February 2013 to January 2014, following Azevedo *et al* 2014.

Table 2. Spearman’s correlation index values (Rs) and significance (p) of the correlations between coordinates 1 and 2 obtained in the NMDS analysis and the distance to the border of a Restinga fragment in the South of Brazil, for the four seasons of the studied year.

Table 1

Trap	**Sites**	**Latitude**	**Longitude**	**Distance**	**Phytophysiognomy**
Point 1	edge	-31.815987S	-52.432165W	68 m	Arboreal with open canopy, high presence of exotic plant
Point 2	edge	-31.815124S	-52.432228W	75 m	Arboreal with closed canopy
Point 3	interior	-31.814578S	-52.432152W	114 m	Dense vegetation with arboreal phytophysiognomy
Point 4	interior	-31.814299S	-52.431723W	101 m	Dense vegetation with arboreal phytophysiognomy
Point 5	interior	-31.814106S	-52.432241W	168 m	Dense vegetation with arboreal phytophysiognomy
Point 6	interior	-31.813892S	-52.432732W	153 m	Dense vegetation with arboreal phytophysiognomy
Point 7	interior	-31.813410S	-52.433035W	107 m	Arboreal with closed canopy
Point 8	edge	-31.813077S	-52.432518W	80 m	Arboreal with open canopy, flooded
Point 9	edge	-31.813302S	-52.431736W	78 m	Arboreal with open canopy, flooded
Point 10	edge	-31.813860S	-52.433489W	63 m	Arboreal with closed canopy
Point 11	edge	-31.814267S	-52.433704W	46 m	Arboreal with closed canopy, high presence of exotic plant
Point 12	edge	-31.814588S	-52.433527W	66 m	Arboreal with closed canopy, high presence of exotic plant

Table 2

	Winter		**Spring**		**Summer**		**Autumn**	
	Rs	**p**	**Rs**	**p**	**Rs**	**p**	**Rs**	**p**
Coordinate 1	-0.888	0.0001	0.224	0.484	-0.021	0.948	-0.455	0.138
Coordinate 2	0.0559	0.863	-0.434	0.159	-0.014	0.966	-0.028	0.931

Supplementary material 1

Mantel and Moran's I tests

	Mantel (Jaccard index)		**Mantel (Morisita index)**		**Moran's I (S)**		**Moran's I (N)**	
	R obs	**p**	**R obs**	**p**	**I (obs, expected at random)**	**p**	**I (obs, expected at random)**	**p**
Summer	0.14	0.15	-0.05	0.58	-0.12; -0.51	0.78	-0.32; -3.98	0.99
Spring	0.08	0.26	0.28	0.04*	-0.15; -0.99	0.85	-0.11; -0.33	0.70
Winter	0.09	0.24	0.16	0.14	-0.15; -0.92	0.84	-0.20; -1.70	0.93
Autumn	-0.14	0.85	-0.13	0.89	-0.05; 0.68	0.26	-0.08; 0.17	0.53

Supplementary material 2

Species	**Restinga forest**		**Total**
	Interior	**edge**	
Drosophila genus			
Dorsilopha subgenus			
D. busckii group			
D. busckii Coquillett, 1901	1	4	5
Drosophila subgenus			
D. annulimana group			
D. arassari Cunha and Pavan, 1947	11	16	27
D. schineri Pereira and Vilela, 1987	21	11	32
D. cardini group			
D. cardini Sturtevant, 1916	2	*	2
D. neocardini Streisinger, 1946	1	*	1
D. polymorpha Dobzhansky and Pavan, 1943	826	761	1,587
D. coffeata group			
D. fuscolineata Duda, 1925	*	1	1
D. guarani group			
D. griseolineata Duda, 1927	49	25	74
D. maculifrons Duda, 1927	9	*	9
D. ornatifrons Duda, 1927	81	61	142
D. immigrans group			
D. immigrans Sturtevant, 1921	153	221	374

D. pallidipennis group			
D. pallidipennis Dobzhansky and Pavan, 1943	2	*	2
D. repleta group			
D. buzzatii Patterson and Wheeler, 1942	2	1	3
D. hydei Sturtevant, 1921	2	8	10
D. mercatorum Patterson and Wheeler, 1942	62	108	170
D. nigricuria Patterson and Mainland, 1943	*	1	1
D. onca Dobzhansky and Pavan, 1943	60	69	129
D. papei Bächli and Vilela, 2002	*	1	1
D. repleta Wollaston, 1958	3	4	7
D. tripunctata group			
D. bipunctata Patterson and Mainland, 1943	*	1	1
D. cuaso Bächli, Vilela and Ratcov, 2000	2	1	3
D. mediopicta Frota-Pessoa, 1954	*	1	1
D. mediopunctata Dobzhansky and Pavan, 1943	64	43	107
D. mediosignata Dobzhansky and Pavan, 1943	12	1	13
D. mediostriata Duda, 1925	2	1	3
D. nappae Vilela, Valente and Basso-da-Silva, 2004	6	2	8
D. paraguayensis Duda, 1927	237	133	370
D. montevidensis Goñi and Vilela, 2016	*	1	1
Siphlodora subgenus			
D. flexa Loew, 1866	1	*	1
Sophophora subgenus			

D. melanogaster group			
D. ananassae Doleschall, 1858	*	2	2
D. melanogaster Meigen, 1830	6	17	23
D. simulans Sturtevant, 1919	3,742	7,448	11,090
D. suzukii Matsumura, 1931	60	40	100
D. saltans group			
D. neosaltans Pavan and Magalhães, 1950	28	29	57
D. prosaltans Duda, 1927	11	25	36
D. sturtevanti Duda, 1927	4	12	16
D. willistoni group			
D. nebulosa Sturtevant, 1916	*	1	1
D. willistoni subgroup	4,730	5,606	10,336
Hirtodrosophila genus			
Hirtodrosophila sp.	1	2	3
Leucophenga genus			
Leucophenga sp.5	*	1	1
Zaprionus genus			
Za. vittiger group			
Za. indianus Gupta, 1970	1	9	10
Zygothrica genus			
Zy. ptilialis Burla, 1956	4	*	4
Zy. dispar group			
Zy. dispar (Wiedemann, 1830)	2	*	2

Zy. prodispar Duda, 1925	3	*	3
Zy. orbitalis group			
Zy. orbitalis (Sturtevant, 1916)	304	3	307
Zy. vittimaculosa group			
Zy. vittimaculosa Burla, 1956	5	*	5
Total			**25,081**

Supplementary material 3

Spearman's correlation index (Rs) values and significance (p) of the correlations between species richness and absolute Drosophilidae abundance and distance for the edge of the analyzed vegetation fragment, for the four seasons of the studied year.

	Species Richness		**Absolute Abundance**	
	Rs	**p**	**Rs**	**p**
Winter	0.654	0.021	0.748	0.005
Spring	-0.171	0.596	-0.077	0.812
Autumn	-0.322	0.308	0.308	0.331
Summer	0.0316	0.922	0.371	0.236

Supplementary material 4

Results of the SIMPER analysis comparing the Drosophilidae assemblages from the interior and edge of a fragment of Restinga in southern Brazil, during Winter. Mean dissimilarity values of each taxon, species contribution to observed dissimilarity (%), cumulative contribution of species to observed dissimilarity (%), mean absolute abundance per trap in the interior and edge of the Restinga fragment. *exotic species in the Neotropical region.

Taxon	**Average dissimilarity**	**Contribution %**	**Cumulative contribution %**	**Average abundance**	
				Interior	**Edge**
Zy. orbitalis	24.18	33.90	33.90	61.20	0.43
D. paraguayensis	12.79	17.93	51.83	27.20	4.71
*D. simulans**	5.93	8.31	60.14	9.60	13.60
D. mediopunctata	4.56	6.39	66.53	10.80	2.71
D. ornatifrons	3.72	5.21	71.74	7.00	2.86
*D. immigrans**	2.75	3.86	75.60	4.00	4.43
D. griseolineata	2.46	3.45	79.06	4.80	0.43
Other species	14.94	20.94	100.00	22.60	17.42

Supplementary material 5

Results of the SIMPER analysis comparing the Drosophilidae assemblages from the interior and edge of a fragment of Restinga in southern Brazil, in the Spring season. Mean dissimilarity values of each taxon, species contribution to observed dissimilarity (%), cumulative contribution of species to observed dissimilarity (%), mean absolute abundance per trap in the interior and edge of the Restinga fragment. *exotic species of the Neotropical Region.

Taxon	**Average dissimilarity**	**Contribution %**	**Cumulative contribution %**	**Average abundance**	
				Interior	**Edge**
*D. simulans**	25.84	55.30	55.30	438.00	751.00
D. willistoni subgroup	9.34	19.98	75.28	149.00	112.00
D. polymorpha	7.27	15.56	90.84	124.00	81.30
D. paraguayensis	0.94	2.01	92.85	9.00	11.30
*D. immigrans**	0.73	1.56	94.41	10.40	18.60
D. onca	0.59	1.26	95.67	9.20	6.43
*D. suzukii**	0.47	1.02	96.68	12.00	5.57
Other species	1.55	3.32	100.00	22.20	17.72

Supplementary material 6

Results of the SIMPER analysis comparing the Drosophilidae assemblages from the interior and border of a Restinga fragment in the South of Brazil, during Summer. Mean dissimilarity values of each taxon, the contribution of the species to observed dissimilarity (%), contribution cumulative species for observed dissimilarity (%), mean absolute abundance per trap in the interior and edge of the Restinga fragment. *exotic species of the Neotropical Region.

Taxon	**Average dissimilarity**	**Contribution %**	**Cumulative contribution %**	**Average abundance**	
				Interior	**Edge**
D. willistoni subgroup	15.81	71.90	71.90	608.00	530.00
*D. simulans**	3.86	17.58	89.48	111.00	86.70
D. polymorpha	0.65	2.96	92.44	15.00	10.90
D. mercatorum	0.39	1.79	94.23	3.80	5.71
D. griseolineata	0.19	0.87	95.10	2.60	0.86
D. prosaltans	0.17	0.78	95.88	1.40	2.14
D. neosaltans	0.11	0.49	96.37	1.60	1.43
Other species	0.80	3.63	100.00	8.00	6.43

Supplementary material 7

Results of the SIMPER analysis comparing the Drosophilidae assemblages from the interior and edge of a Restinga fragment in southern Brazil, during Autumn. Mean dissimilarity values of each taxon, species contribution to observed dissimilarity (%), cumulative contribution of species to observed dissimilarity (%), mean absolute abundance per trap in the interior and edge of the Restinga fragment. *exotic species of the Neotropical Region.

Taxon	**Average dissimilarity**	**Contribution %**	**Cumulative contribution %**	**Average abundance**	
				Interior	**Edge**
*D. simulans******	19.56	59.11	59.11	190.00	205.00
D. willistoni subgroup	10.45	31.59	90.70	187.00	171.00
D. polymorpha	1.34	4.04	94.74	20.00	20.90
D. mercatorum	0.48	1.44	96.18	4.20	1.86
D. paraguayensis	0.35	1.07	97.25	1.20	2.57
D. mediopunctata	0.17	0.50	97.75	0.40	1.14
*Za. indianus******	0.16	0.47	98.22	0.20	1.29
Other species	0.59	1.78	100.00	2.20	3.29

3. CAPÍTULO III

(Manuscrito publicado no periódico da *Revista Brasileira de Entomologia*)

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First report of colored pan traps to capture Drosophilidae (Diptera)

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ABSTRACT

The use of robust sample methodologies to estimate the highest number of species with different ecological requirements and traits is essential to the knowledge construction of the biodiversity and to establish wildlife assessment and monitoring programs. Our aims were to study the performance of colored *pan traps* in the capture of Drosophilidae (Diptera), a method never used for sampling this taxon. During six months, colored pan traps (blue, yellow and white) were tested in three areas in Southern Brazil. We captured 375 individuals of 30 species belonging to four genera of Drosophilidae. The most abundant species were *Drosophila lutzii* (n=215) p=0.58, *Scaptomyza* sp. (n=55) p=0.15 and *D. bromelioides* (n=17) p=0.04, all of them, anthophilous species. All colored pan traps captured a high quantify species of Drosophilidae, mainly anthophilous species.

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Introduction

Drosophilidae (Insecta, Diptera) is considered the ideal model for studying different ecological issues, such as edge effect and human disturbance, for example (Mata et al., 2008; Mata et al., 2010; Penariol and Madi-Ravazzi 2013). One of the main reasons behind this practice is its ability to use a wide variety of resources as substrate (mainly fruits, flowers and macroscopic fungi) (Carson, 1971; Grimaldi, 1987; Markow and O'Grady, 2008; Mendes et al. 2017; Schmitz and Valente 2019; Valer et al., 2016).

Some different methods are common to insect surveys, such as the banana attractive trap (Bizzo et al., 2010; Duarte et al., 2018; Emerich et al., 2012; Garcia et al., 2012; Gottschalk et al., 2007; Hochmüller et al., 2010; Mateus et al., 2018; Poppe et al., 2012), use of entomological nets, collection in flowers (Cordeiro et al., 2020; Schmitz and Valente, 2019), collection in macroscopic fungus resources (Gottschalk et al., 2009; Valer et al., 2016) and pan traps (Whestphall et al., 2008, Halinski et al., 2018). However, although historically the use of different methodologies for capturing individuals with different ecological and evolutionary behaviors seems to be clear to measure the fauna of the natural environments, analyzing articles that present local researches of Drosophilidae reveals that the way the individuals are captured is little variable from one study to another.

In this context, there are several techniques to capture Drosophilidae in the wild, all of which with their positives and negatives aspects. For instance, banana attractive trap has been widely used to capture Drosophilidae, but is biased due to a significant collection of species of the genus *Drosophila*, while sub-sampling other groups (Mendes et al., 2017; Penariol et al., 2008). Besides that, some methods, such as active resource collection or banana/orange attractive traps (Cordeiro et al., 2020; Valer et al., 2016), are excellent for capturing a large number of dominant species, and others are less efficient in sampling species richness but are excellent for their ability to capture underrepresented species (rare species), such as malaise traps for example (Frankie et al., 2002; Agosti and Alonso, 2003). In general, the most used approaches for collecting adults are based on capturing individuals attracted by bait (Medeiros and Klackzo, 2004; Penariol et al., 2008). Thus, the use of traps with banana baits, for example, is one of the most used techniques in Drosophilidae fauna surveys.

The use of the colored pan traps has intensified considerably since the last decade, mostly associated with floral resources, due to its efficiency in capturing a wide range of floral visitors (Wilson et al., 2008; Westphal et al., 2008; Halinski et al., 2015, 2018; Tuell and Isaacs, 2009; Vrdoljak and Samways, 2012). According to the literature, this technique also has no collector bias of insects (Westphal et al., 2008). It is a good method to capture flower dwelling Drosophilidae, but it is biased in this direction (it favors flower dwelling Drosophilidae). Moreover, different color pan traps seem to attract different frequencies of species, another bias. It is a potential method of monitoring diversity of capturing insects, since this technique does not depend on odors' attraction or eliminates the collector (Almeida et al., 1998; Campbell and Hanula, 2007; Wilson et al., 2008).

The colored traps have been used to capture many different types of insects (Campbell and Hanula, 2007). For example, some yellow traps have been used to catch a wide variety of phytophagous insects (Kirk, 1984), predators (Leksono et al., 2005), and pollinators (Halinski et al., 2015); blue pan traps catch predominantly Hymenoptera (Aguiar and Sharkov, 1997; Halinski et al., 2018), and white or yellow traps catch mostly Diptera (Disney et al., 1982; Halinski, 2018).

In this sense, to know the environments' biodiversity, it is always advisable to use different collection methods, estimating the largest possible number of groups with different ecological and behavioral characteristics (Schauff, 1986). Here, we tested the efficiency of pan traps methodology for the Drosophilidae and identified the species composition in different agricultural habitats in Southern Brazil.

Material and methods

Drosophilidae sampling

The study using colored pan traps was conducted in three environments in an ecoclimatic region in the state of Rio Grande do Sul, municipality of Estrela, Brazil (29°30'07”S, 51°57'57”W). The region is characterized by pasture areas, fragments of forest, and annual crops (canola, soy, corn, and wheat) (Maluf and Westphalen, 1994). According to the Köppen classification, the study area is considered cfa with humid subtropical climate, after a prior classification of Köppen-geiger (Alvares et al., 2013). The municipality of Estrela is in the Rio Grande do Sul Central-oriental mesoregion and belongs to the Northeast Lower Hillside. The average temperature is 19.3°C, the average relative humidity is 75%, annual precipitation is 1.547mm, an altitude of 52m, and predominance of subtropical forest (IBDF, 1983; IBGE, 1992; Maluf and Westphalen, 1994).

To test colored pan traps in Drosophilidae, with one collection each month, the individuals were sampled with blue, yellow, and white pan traps exposed for 24 hours in each environment (adapted from Westphal et al., 2008), from August to December 2010 and July 2011. These traps were placed in three areas associated with oilseed crops (*Brassica napus* – Hyola 61- canola crops). In the period of blooming of the crop

(August to October), three plots were collect inside the crop, inside the forest fragments and near the plantation.

Description of the colored traps

The traps consist of colored pots (white, yellow, and blue) of 11cm diameter and 4mm high painted with ultraviolet spray to maximize insects' attraction. The pots were filled with water and neutral liquid detergent to break the surface tension, thus making the captured material sink (Almeida et al., 1998; Krug and Alves-dos-Santos, 2008; Teixeira, 2012; Williams et al., 2001) (Figure 1). The plot configuration used 15 traps divided into three equilateral triangles (with three colors traps) with sides measuring three meters totalizing 270 traps (45 for each color; to 270: 15 x 3 = 45; 45 x 6 = 270). The triangles were distributed in a way that they were 15m apart (adapted from FAO, 2010). The traps were adjustable in the supports matching the height of the vegetation.

>>>Figure 1

Species identification

The individuals removed from the traps were fixed in absolute alcohol and identified based on external morphology, using taxonomic keys and species descriptions according to specialized literature (Burla, 1956; Grimaldi, 1987; Grimaldi, 2016; Schmitz and Valente, 2019; Vilela and Bächli, 1990). Individuals of cryptic species were prepared and dissected for later identification of terminalia (females species were identified to external morphology and associated with males, respectively) (Bächli et al., 2004). Voucher specimens of all the recorded species were deposited in the Entomological Collection of the Museum of Science and Technology, at Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS).

Statistical analysis

The absolute and relative abundances of each species (*n* and *p*, respectively) and the species richness (number of species in the sample, S) were used to characterize the assembly captured. Sampling effort was estimated by coverage of Drosophilidae species represented in these three areas associated with oilseed crops along the year with colored pan traps. Thus, the number of individuals was used and an accumulation curve was constructed in the R environment, using the “iNEXT” package (Hsieh et al., 2016).

In the iNEXT analysis, individual-based abundance data were used to select a diversity order of q, based on species richness (q = 0) (Chao et al., 2016). Significance level was calculated with 999 permutations with Chao1, an estimator based on abundance that underestimate true richness at low sample size based on the number of singletons (species captured once) and doubletons (species captured twice). Besides that, iNEXT can interpolate and extrapolate species richness by taking into account a measure of sample coverage (Chao and Jost, 2012; Chao et al., 2016).

Results

A total of 375 individuals were captured, belonging to four genera and 30 taxa (Table 1). Among the sampled genera, *Drosophila* presented the highest species richness (S=13), followed by the *Scaptomyza* (S=7), which was recorded for the first time in the Pampa Biome, in Brazil. *Drosophila lutzii* was the dominant species in the two areas evaluated (*n* = 215; *p* = 57.9%), followed by *Scaptomyza* sp. (*n* = 55; *p* = 14.8%) and *D. bromelioides* (*n* = 17; *p* = 4.6%).

>>> Table 1

>>> Figure 2

The individual rarefaction curve shows the taxonomic differences between the sampled areas. However, the sample coverage curves do not indicate a trend towards asymptote (Figure 2), suggesting that a greater number of species could be sampled; however, indicating the need for increasing sampling occasions in these sites. Furthermore, Chao1 estimator predicted 71 and 43 species to canola crops and forest fragments. Results suggest that we can collect more species in this location with this type of baited trap. In grassland vegetation the curves indicated the inflection point, with Chao1 estimator predicting 12 species, close to the species richness values observed (Table 2).

Absolute abundance and species richness observed of Drosophilidae regarding the trap colors and sampled areas, showed higher abundance and species richness in the Canola crops area for almost all the trap colors (except the white trap). This results could be observed for abundance and species richness by trap color between the sampled areas (except for white color in Grassland) (Table 1).

>>> Table 2

Discussion

We know that Drosophilidae species can use a wide variety of resources as a substrate (Carson, 1971; Markow and O'Grady, 2008), which are used for feeding and/or as courting sites for reproduction. However, although the family occupies several niches, fauna surveys focus on a few techniques for capturing species, privileging certain taxonomic or ecological groups (Carson, 1971; De Toni et al., 2007; Gottschalk et al., 2009; Hochmüller et al., 2010; Mitsui et al., 2010; Garcia et al., 2012; Poppe et al., 2012; Valer et al., 2016).

The sample coverage values in the collected traps have shown superiority in the abundance and species richness of Drosophilidae captured in Canola crops, a pattern also observed for other groups of insects (Le Feón et al., 2016; Halinski et al., 2015, 2018), and similar to that pointed out by Prado et al. (2017). The use of pan traps is recommended for insect inventories in open landscapes with flowers, since only a few taxonomical groups have distribution restricted to closed landscapes (Prado et al., 2017). In massive flowering, the methodology of pan traps supports the collection of almost all groups of insects, especially those that use floral resources like pollen and nectar. Because of canola's open flower, all insects, including Drosophilidae, are potential pollinators and this could explain why we found such flies in our samples, but further studies are needed.

Accumulation curves indicate a higher coverage with pan traps in the Canola crops and grassland vegetation areas. But the values are also high in the forest area, revealing the importance of micro-habitats and their influence on communities' structuring. The different efficiency profile of the trap in the open Canola crops suggests that these changes can be attributed to the floral biology of the extant plants in the sampled environment, as well as the higher incidence of light on the trap, that could attract more individuals (Azevedo et al., 2015; Campbell and Hanula, 2007; Gollan et al., 2011; Vrdoljak and Samways, 2012).

In addition, the color of the traps influenced the efficiency of capturing Drosophilidae, as it is possible to observe by the higher species-richness and absolute abundance in the blue and yellow pan traps. Different results were obtained from other groups of insects, where only the yellow pan trap was identified as better for capturing individuals (Azevedo et al., 2015; Leong and Thorp, 1999; Ramíres-Freire et al., 2014). However, blue pan traps can also catch different species or, as in our study, more individuals of *D. lutzii.* Here, yellow pan traps attract more flies in the *Brassica napus* (canola crops) period, where the canola flowers are yellow. Such as the genus *Scaptomyza*, collected only in the canola environment in September, which corresponds to the peak of the canola blooming period. Thus we hypothesized that the different colors in the area (such as yellow pan traps similar to the color of canola crops) could be the reason to attract different fauna, and very useful to know the environment's diversity. However, these results showed possible consequences on the estimated diversity of the canola environment capturing uncommon or rare species, not collected for other techniques and/or sub sampled other species groups.

Drosophila proved to be the most representative genus of fauna associated with colored pan traps, with the dominance of the species of the *Phloridosa* subgenus and *bromeliae* group in all the sampled months. Among them are *D. lutzii*, *D. bromelioides*, *D. bromeliae*, and *D. denieri*, which have a wide distribution in the Neotropical region and are found in a wide variety of plants (Blauth and Gottschalk, 2007; Cordeiro et al., 2020; Da silva and Martins, 2004; De Toni et al., 2007; Frota-Pessoa, 1952; Gottschalk et al., 2007; Grimaldi, 2016; Petersen, 1960; Schmitz and Valente, 2019). In recent studies, Cordeiro et al. (2020) and Schmitz and Valente (2019) investigated the Drosophilidae fauna associated with flowers in Brazil and demonstrated that these species are the most representative, corroborating the efficiency of pan traps for capturing anthophilous Drosophilidae.

Concerning the knowledge of *Scaptomyza*, the second most abundant genus present in this study, the literature mentions that its species are mainly saprophagous (Brncic, 1983; Hackman, 1959; Markon and O'Grady, 2008). In addition to this habit, some species of *Scaptomyza* are randomly sampled using other breeding sites, such as spider egg bags, mustard leaf-miners (Brassicaceae), and some are predators (Lapoint et al., 2013; Montague and Kaneshiro, 1982; Magnacca et al., 2008; Markon and O'Grady, 2008; Schmitz and Valente 2019).

In Brazil, the genus is virtually ignored with just seven described species recorded, five of them from Pampa Biome (Gottschalk et al., 2008; Santos and Vilela, 2005; Schmitz and Valente 2019; Poppe et al., 2016), and many waiting to be described, as those sampled in this study. The results show significant taxonomic gaps in the knowledge of the genus for the Neotropics, which can be attributed to the sampling difficulty. For these reasons, we consider that the pan traps are efficient and recommend them for future studies of *Scaptomyza*. Other benefits of this method are the absence of resource bias and the low cost, since human resources for collections can be reduced.

Considering the diversity of species and knowing part of their hosts, both in the larval and adult stages, it is assumed that *Zygothrica* is predominantly mycophagous (Burla, 1956; Grimaldi, 1987). Even so, some species also use flowers as a trophic resource, such as *Zygothrica dispar* and *Z. prodispar* sampled here, once again reinforcing the use of this technique for sampling anthophilous species. Besides, we captured *Z. venustipoeyi*, which represents a new record of occurrence and expands its distribution to the south (29° 30' 07”S, 51° 57' 57”W) (Bächli, 2019; Gottschalk et al., 2008; Poppe et al., 2016).

Fauna surveys in natural environments based on banana, flower, and fungi baits in Pampa biome, for example, have sampled between 9 to 46 species in temporal studies (Cordeiro et al., 2020; Hochmüller et al., 2010; Mendes et al., 2017; Poppe et al., 2012; Schmitz and Valente, 2019; Valer et al., 2016). The species richness obtained in this study, compared to those studies, praises colored pan traps as a good alternative to assess sample coverage in different environments, regardless of abundance since this technique has found species that are not usually detected by traditional surveys.

This colored traps demonstrated a marked efficiency in sampling Drosophilidae species (375 individuals), mainly anthophilous and some are bycatch in flowers in the Neotropical region, such *D. melanogaster* and *D. willistoni* (Brncic, 1983; Frota-Pessoa, 1952; Grimaldi, 1987; Pipkin, 1964; Schmitz and Hofmann, 2005; Schmitz and Valente, 2019). Besides that, others groups of insects were sampled on this study with colored traps such as wasps (814 individuals), bees (350 individuals), Diptera taxa (1.110 individuals), coleopterans (116 individuals) and butterflies (58 individuals), corroborating its efficiency in capturing a wide range of floral visitors (Halinski et al., 2015, 2018).

The positive results obtained with this technique bring new implications for biodiversity conservation for this group of entomofauna, in addition to proposing the method as a new alternative for capturing adult anthophilous species that can yield a sufficiently representative portion of species richness in different habitats (Grootaert et al., 2010; Hagler and Jackson, 2001; Wilson et al., 2008). Robust methodologies help to sample the largest possible number of species with different ecological and behavioral characteristics, perhaps on the future insert, can be considered to capture biological indicator species monitoring the effects anthropogenic activities, for example. Furthermore, having knowledge about new methodologies is essential to help establish fauna assessment take off one and, ex. to monitoring programs in the environments.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

MFM and RH contributed with the experimental design and collected field; MFM, MSG, RH and VLSV contributed with manuscript writing; MFM, MSG, HRM and CD contributed with species identification and data analysis. All authors read, revised and approved the manuscript.

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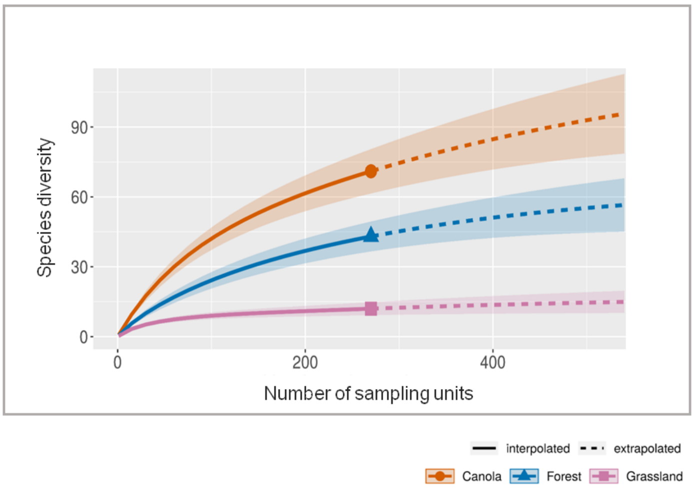
Figure captions

Figure 1 Representation of colored pan traps model used in study areas, the municipality of Estrela, Rio Grande do Sul State, Southern Brazil.

Figure 2 Drosophilidae richness (q = 0) estimated by rarefaction (solid curves) and extrapolation (dashed curves) based on sample size, with corresponding 95% of confidence intervals (shaded areas).



Tables captions

Table 1 Absolute abundance of Drosophilidae species collected from August to December 2010 and July 2011 in three areas of the municipality of Estrela, Rio Grande do Sul State, Southern Brazil, with pan traps with three colors (yellow - Y, blue - B, and white - W), the relative abundance of species in all sample, and the months in that each species was collected (p, relative abundances, a, p <0.01). Abbreviations: July: S, August: A, September: S, October: O, November: N, December: D.

Table 2 Indices of diversity and richness estimators for Drosophilidae in three habitats located in Estrela, Rio Grande do Sul State, Southern Brazil.

Table 1 IN

Species	Canola crops			Forest fragments			Grassland vegetation			n	p	Sampling month
	Y	B	W	Y	B	W	Y	B	W			
Drosophila bandeirantorum Dobzhansky and Pavan, 1943		1								1	a	A
Drosophila bromeliae Sturtevant, 1921	3				2	1			1	7	0.02	J, A, S
Drosophila bromelioides Pavan and Cunha, 1947	1	8	1		1		1	1	4	17	0.04	J, S
Drosophila buscki Coquillett, 1901	1	2					1			4	a	A, S
Drosophila denieri Blanchard, 1916					4	1			1	6	0.01	J, A
Drosophila flexa Loew, 1866									1	1	a	J
Drosophila griseolineata Duda, 1927						1				1	a	S

Drosophila lutzii Sturtevant, 1916	30	74	7		32	3	8	14	47	215	0.58	J, A, S, O
Drosophila maculifrons Duda, 1927				2						2	a	S
Drosophila mediopicta Frota-Pessoa, 1954									1	1	a	J
Drosophila mediopunctata Dobzhansky and Pavan, 1943	3									3	a	S
Drosophila melanogaster Meigen, 1830				1						1	a	S
Drosophila pallidipennis Dobzhansky and Pavan, 1943	2	4	1				1			8	0.01	J, A, S
Drosophila polymorpha Dobzhansky and Pavan, 1943		1								1	a	A
Drosophila sp.1		1								1	a	S
Drosophila sp.2							1			1	a	S
Drosophila TIPO III (Schmitz and Valente, 2019)		1								1	a	S

Drosophila willistoni Sturtevant, 1916	4									4	0.01	A, S
Leucophenga sp.5 (Mendes et al., 2017)						1				1	a	S
Zygothrica sp.1		1	1							2	a	N
**Zygothrica venustipoeyi* Burla, 1956									1	1	a	O
Zygothrica prodispar Duda, 1925		2		3	6	1			1	13	0.03	S, D
Zygothrica zygopoeyi cf.					1					1	a	S
Scaptomyza sp.1	1									1	a	S
Scaptomyza sp.2	2		1							3	a	S
**Scaptomyza adusta* Loew, 1852	4	2	2							8	0.02	S
**Scaptomyza pipinna* Goñi and Vilela, 2016	3	2	1							6	0.01	S
Scaptomyza sp.5	3	3	1							7	0.02	S
Scaptomyza sp.6	1		1							2	a	S

Scaptomyza sp.7	10	3	2	28	9	3				55	0.15	S
Total	68	105	18	34	55	11	12	15	57	375	1	

p, relative abundances, a, $p < 0.01$
*new record Pampa Biome

Table 2

	Canola crops	**Forest fragments**	**Grassland vegetation**
Richness obs (S)	20	11	11
Absolute abundance (n)	191	100	84
Dominance	0.826	0.829	0.951
Singletons	5	5	9
Doubletons	3	1	0
Chao 1	71	43	12

4. CAPÍTULO IV

(Manuscrito publicado no periódico *Community Ecology*)

Title page

Title:

Functional traits for ecological studies: a review of characteristics of Drosophilidae (Diptera)

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Abstract

Studies in the area of functional ecology not only aid in our understanding of the dynamics and structure of communities, but also help elucidate the mechanistic consequences of habitat changes and their implications for species conservation, the nature of biological interactions and the provision of ecosystem services. However, trait selection and measurement are usually not standardized between studies, even for groups that have been widely studied, such as the Drosophilidae (Diptera). In order to suggest a standardized framework for trait selection and measurement for the Drosophilidae, here we reviewed the literature published between September 2018 and June 2021. The review returned 52 relevant papers, and based on the traits presented we organized three categories of functional traits which we suggest need to be analysed in order to understand functional pattern of the Drosophilidae: habitat perception, flight performance and ecophysiological responses. Habitat perception includes those traits that provide the ability to recognize an environment and access its available resources. Flight performance includes those traits related to flight and mobility, while ecophysiological responses include those traits related to energy allocation, survival, and reproductive efficiency. Based on these categories, we projected the expected results for a broad application of the traits we selected in studies with Drosophilidae in natural environments. For example, using the functional approach for a comprehensive diagnosis of the relationship between Drosophilidae and their environments. We encourage a wide use of our framework for both basic and applied studies of Drosophilidae, particularly for those that seek to test hypotheses regarding community structure.

Keywords: morphological characters, Drosophilidae species composition, environmental conditions, functional diversity, functional morphology.

Introduction

Interest in the processes that lead to the structuring of communities is increasing, as an understanding of these processes can lead to a deeper understanding of how environmental variations shape patterns of biodiversity, ecosystem functions and services (Flynn et al. 2009; Ulrich et al. 2012). This understanding is deepened by analyses of functional attributes that are related to morphological, physiological and behavioral characteristics that influence the establishment, performance, fitness and survival of species (Nock et al. 2016; Reich et al. 2003). Furthermore, variations in these traits can define patterns of distribution and responses of these species to different environments or changes in their natural environment (Westoby et al., 2002).

As such, robust conservation strategies require a focus not only the maintenance of species richness, but also on the functional integrity of ecosystems, and this requires the use of a functional groups approach. Such an approach requires knowledge of species functional traits (Cornelissen et al. 2003; Pla et al. 2012; Reich et al. 2003; Walker 1992). Functional trait selection for any given study depends on both the characteristics of the community and the ecosystem functions to be identified (De Bello et al. 2010, Podgaiski et al. 2011; Pendry et al. 2007).

Functional diversity analyses use traits of individuals that can capture specific functions of certain taxa in the community. However, the most common approach in the literature is still limited to assessments of taxonomic diversity (Cianciaruso et al. 2009; Diaz and Cabido 2001), which do not investigate the role or the response of each species in the environment (Gerisch et al. 2012). This is because diversity analyses restricted to the taxonomic dimension limit the inferences regarding responses to environmental disturbances and which ecological strategies of the organisms when facing severe abiotic conditions (Diaz and Cabido 2001; Petchey and Gaston 2006). As an example we could cite generalist and specialist species, once they have different behavior that allows their survival in distinct habitats.

A functional ecology approach is key to understanding biodiversity patterns and processes, as changes in functional richness and diversity can directly and indirectly affect the functioning of the ecosystem (Cianciaruso et al. 2009). Measuring and understanding functional traits allow for a better understanding of ecological processes, which can be more useful for informing conservation and restoration actions. However, databases of functionally important traits, especially of less studied invertebrate taxa, are scarce and usually incomplete (Moretti et al. 2017). As such, the development of such trait databases according to standardized protocols across taxa would represent an important advance for both theoretical understanding of functional ecology and the formation of new hypotheses.

Organisms have different distributional limits owing to different ecophysiological tolerances, thermal tolerances and habitat requirements (Honek 1996; Verberk 2011). However, not all species are equally affected by environmental conditions that cause pronounced changes in an ecosystem (Gottschalk et al. 2007; Poppe et al. 2013), as they respond differently based on a set of traits. While many taxa are very sensitive to direct disturbances and their side effects, and may even disappear from large areas, others remain stable or achieve high dominance in altered habitats (Gottschalk et al. 2007; Mata et al. 2008, 2010). Some arthropods groups including ants, bees, beetles, butterflies, and spiders, have different functional relationships with the ecosystem, as a result of niche filtering along environmental gradients (e.g. climatic, structural, and land-use gradients) and by abiotic and biotic disturbances (e.g. fire, floods and biological invasions) (Wong, Guénard and Lewis 2019).

Functional ecology and phylogenetic relationships in biodiversity studies

Organisms can be grouped based on phylogenies inferred using molecular and morphological information. As such, it is possible to combine functional traits and phylogenetic relationships in biodiversity studies, allowing for the testing of hypotheses regarding the importance of ecological, historical, and biogeographical processes for the organization of communities (Cavender-Bares 2009; Pavoine and Bonsall 2011). As species and their adaptations to the environment are not independent of their evolutionary histories, phylogenetically related species share both morphological attributes and ecological requirements (Grimaldi 1990; Webb et al. 2002). This phenomenon is well documented in African cichlids and several other groups, including drosophilid flies (Michalak et al. 2001). Generally, quantitative variations in morphological traits are enough to distinguish sibling species (Franco et al. 2006; Moraes et al. 2004; Spaniol et al. 2019), and these variations can be associated with functional differences.

Therefore, patterns of coexistence in communities based on functional and phylogenetic similarities reflect different structuring forces, which may be attributed to environmental filters and ecological interactions (Webb et al. 2002) or be independent of phylogenetic and functional relationships between taxa (Hubbell 2006; Spaniol et al. 2019). As such, including functional ecology may be crucial to improve our understanding of which selective pressures drive the adaptations of different organisms, as expressed in their traits (Flynn et al. 2009). However, given the actions of natural selection, in functional ecology it is necessary to provide measurement protocols of the functional characteristics of highly studied organisms and to encourage their application in potentially new approaches.

Traits for use in functional ecology studies of Drosophilidae

One benefit of using small organisms with a short life cycle, such as the Drosophilidae, as study models is that results can be obtained relatively rapidly. The Drosophilidae originated in tropical regions approximately 50-60 million years ago and currently comprises approximately 4,400 described species (Throckmorton 1975; Powell 1997; Bächli 2021). Yeasts and bacteria, which grow on decomposing organic substrates (fruits, fungus and flowers, for example) are essential diet requirements for several species. Due to this habit, Drosophilidae can be easily captured in the field using standardized methodologies that can be carried out simultaneously in different locations (Bächli 2021; Dobzhansky and Pavan 1950).

Some Drosophilidae species are considered environmental detectors, especially because they are sensitive to anthropogenic actions (Avondet et al. 2003; Martins 1989; Ferreira and Tidon 2005; Gottschalk et al. 2007; Mata et al. 2008; Penariol and Madi-Ravazzi 2013; Cavasini et al. 2014). Nutritional variation, temperature, population density, presence of polluting substances, and habitat fragmentation are strong factors that can influence the phenotypic expression some morphological characteristics. Furthermore, these factors could directly affect the stability of Drosophilidae species (Bochdanovits and Jong 2003; Moller and Swaddle 1997; Sevenster and Van Alphen 1993).

In recent years, there has been an increase in surveys of Drosophilidae fauna worldwide, improving our knowledge of their occurrence and distribution in different ecosystems and biomes (Ferreira and Tidon 2005; Gottschalk et al. 2007; Mateus et al. 2006; Mendes et al. 2017). However, studies using traditional measures of diversity are poorly predictive of the structure and the functioning of communities when compared with studies using functional measures. Number of hard and soft traits have been identified, or traits values already obtained, for a wide variety of species (e.g. from terrestrial and aquatic communities, as seen by Nock et al. 2016). However, it is uncommon to have studies associating functional diversity and distribution in Drosophilidae. This is probably due to body length, differences in resource use and wide niche occupation between species.

Drosophilidae species can reflect their environment since species have different environmental quality requirements (Ferreira and Tidon 2005; Gottschalk et al. 2007; Mata et al. 2010), and by key traits that enable to assess the functional dimensions of communities (Furtado and Martins 2018; Mateus et al. 2006; Saavedra et al. 1995). These traits include morphological structures, as well as behavioral and habitat use aspects, which are easy to determine, measure and use, thus revealing life habits or potential ecological interactions.

Accordingly, in our review, we mainly focus on studies of the natural history and ecology of Drosophilidae that may help explain functional aspects of the species. Based on a bibliographic search on indexing platforms (Scielo, Google Scholar and Web of Science). We used the following keywords: “functional traits” + Drosophilidae, “morphological characters” + Drosophilidae + wing or terminalia or eyes or habitat perception, “species composition” + Drosophilidae, “functional diversity” + Drosophilidae, and “functional morphology” + Drosophilidae. A total of 85 papers were returned by the search up to June 2021, of which 52 were included in our review (papers published between September 1962 and March 2020). The 33 papers excluded from the review had speculative or uninformative data.

Thus, as has been previously done for functional traits in other insect taxa (Arnan et al. 2014; Cornelissen et al. 2003; Newbold et al. 2013; Tscharntke et al. 2008; Weischer et al. 2012). There is a lack of compilation traits in Drosophilidae, with only studies that used one or two functional traits. Here we suggest a framework of functional traits to be used in studies on the family Drosophilidae, and we organized into three different categories to encompass all relevant ecological functions, as well as to relate this information to different Drosophilidae assemblages based on our data compilation (Table 1).

The three trait categories we suggest are: i) habitat perception, ii) flight performance, and iii) ecophysiological responses. Habitat perception includes those traits related to visual capacity and habitat use, including eye measurements, habitat specificity (generalist/specialist species), and feeding sites in larvae and adults. Flight performance includes those traits related to locomotion, movement, and flight capacity, reflecting the performance of the individual while exploring the environment. These characteristics are obtained through morphological measurements of the body and the wings. Ecophysiological response traits include mechanisms associated with breeding, and thermoregulation (Figure 1 and 2).

>> Figure 1- Illustrations of functional trait measurements suggested for Drosophilidae (Diptera) (blue and red). (A) Drosophilidae, lateral and dorsal view; (B) Head, lateral and frontal view; (C) Thorax, dorsal and lateral view. Abbreviations: ar, arista; vb s, vibrissa; pc orb s, proclinate orbital seta; arc orb s, anterior reclinate orbital seta; p rc orb, posterior reclinate orbital seta; poc s, postocellar setae; sbvb s, subvibrissal setulae; pprn s,postpronotal setae; anepst, anepisternum; kepst, katepisternum; npl, notopleuron; spal s, supra-alar seta; anepm, anepimeron; mr, meron; acr s, achrostichal setulae; a dc s, anterior dorsocentral setae; p dc s, posterior dorsocentral setae; b sclt s, basal scutellar setae; ap sctl s, apical scutellar setae; ap sctl s, postopronotal setae; hlt, halter; i vt s, inner vertical seta; o vt s, outer vertical setae.

>> Figure 2 – Illustrations of functional trait measurements suggested for Drosophilidae (Diptera) (blue and red). (A) Wing (with venation names, proposed by Cumming and Wood 2010) and positions of landmarks; (B) Female terminalia, lateral and dorsal view. Abbreviations: ovscp, oviscapt valve; spmth, spermathecal capsule; T8, abdominal tergites; S8, abdominal sternites; Epiprct, epiproct; Hyprct, hypoproct.

i. Habitat perception traits

This set of characteristics allows us to test how Drosophilidae habitat perception acts to shape species distributions, and can help to explain spatio-temporal patterns.

Eye size - This characteristic mainly represents the visual capacity and environmental perception of adult individuals (Götz 1970), though for some species it can be related to mating success and thus sexual dimorphism, such as in *Zygothrica* species (Grimaldi 1987). The surface area of compound eyes can affect the visual field through changes in the eye's structure, leading to differences in sensitivity and visual acuity, thus, eye size variation, together with variation in other parts of the head can influence behavior (Gaspar et al. 2020; Götz 1970). For example, *D. melanogaster* and other species in this subgroup exhibit substantial variation in eye size caused by differences in the diameter and/or number of ommatidia (Gaspar et al. 2020; Ramaekers et al. 2019). Greater sensitivity is important when light is low, while greater visual acuity increases the ability to recognize co-specific characteristics and vegetation (Götz 1970). Among closely related *Drosophila* species, variation in the size of the visual system occurs mainly between species with high courtship, mate selection, and host competition pressures, suggesting that speciation may be linked to visual abilities (Keesey et al. 2019).

Habitat specialists - This concerns the level of specialization of Drosophilidae species in a particular habitat or condition, which, in this case, can be perceived by sensory parts such as the arista and the flagellomere (Burnet et al. 1971; Shanbhag et al. 1999; Stocker 1994). Forests can be perceived as environmental mosaics, where specialization to particular habitats can restrict the occurrence of species. This can lead to increased species turnover when species have more stringent ecological requirements (Ferreira and Tidon 2005; Mata et al. 2008; Poppe et al. 2013). Thus, habitat specialist species have a stronger response to environmental characteristics (e.g. structural complexity or connectivity of habitat patches), and thus may be more strongly impacted by either natural variations or anthropogenic impacts than are more generalist species (Ferreira and Tidon 2005; Mata et al. 2008; Penariol and Madi-Ravazzi 2013).

Specificity of trophic resources - refers to the diversity of trophic resources used by species. Although most Drosophilidae species are collected on decaying fruits, other habits have been described, including: fungi and flower breeding, as well as some predatory/parasitic species (Carson 1971; Dobzhansky and Pavan 1950; Lachaise and Tsacas 1983; Mata et al. 2015). Species are classified as specialists when they exclusively use a single resource, while generalists can use two or more resources (Ricklefs 2003). Thus, monophagous species can be classified as specialists when using a single resource, oligophagous species are generalists that use two resources, and polyphagous species are generalists that use several resources (Magnacca et al. 2008). In addition, larvae may or may not have different resources available to them, and this could influence habitat specialization in adults (Dobzhansky and Pavan 1950; Grimaldi 1987; Pipkin 1964). This trait should however take into account the degree of knowledge of the trophic resources used by the species studied, as a poorly sampled species can be mistakenly considered to be a specialist.

ii. Flight performance traits

This category includes characteristics related to flight mode, which suggest that there is a trade-off between the allocation of mass and allometry (Coyne and Beecham 1987; Bitner-Mathé and Klaczko 1999; Fartyal et al. 2017; Moraes et al. 2004). Studies have shown that wings are structures that respond to natural selection and, in the case of species with spotted wings, also to sexual selection (Beldade et al. 2002; Flower 1964; Frankino et al. 2005; Lewin 1985; Thomas 1993). Wing traits are highly correlated with body size and do not vary with age, nutrition, or reproductive status of the adult (Grimaldi 1990; Kennington et al. 2003; Lehmann 2001; Pitchers et al. 2019). For example, the strength of a muscle varies with the size of its transverse area or with the square of its width. Whereas what the muscle moves (the mass of the body) varies with the cube of the linear dimension. Therefore, as a body becomes smaller, the muscles become relatively stronger (Triplehorn and Johnson 2011). Assuming that individuals have different responses to different biotic and abiotic conditions, this should lead to the expression of different phenotypes in which natural selection has acted or is acting.

Body measurements - Represented by measurements of the wing, the general size of the body and its two-dimensionality, in its entirety or for specific parts of the body such as the thorax and the femur of the forelegs. Thorax measurements can be considered characteristics associated with movement, because the flight muscles are located in the thorax (Dickinson and Lighton 1995; Lehmann 2001; Triplehorn and Johnson 2011). Wing size seems to be an important descriptor of the organism's mobility since the wing does not vary with adult age, or its reproductive state (Dickinson and Lighton 1995; Moraes et al. 2004; Sayad and Yassin 2019; Triplehorn and Johnson 2011). Width, anterior length of the thorax, and wingspan are cited as directly correlated to flight speed or other measures of Drosophilidae (Cavicchi et al. 1981; Dickinson and Lighton 1995; Lehmann 2001; Robertson 1962; Sokoloff 1966). Beyond this, increased wing to thorax size ratio (or reduced wing loading) has been repeatedly proposed to be adaptive for flight at cold temperatures (Starmer and Wolf 1989; David et al. 1994).

Size relationship - This is used to characterize the wing shape through a total wing length/ thorax size ratio (Azevedo et al. 1998; Triplehorn and Johnson 2011). Sometimes, qualitative categories like small, medium and large and this ratio are used in Diptera studies (Tallefer and Wheeler 2012). Higher values of the total wing length/thorax ratio are found in Drosophilidae, which are related to longer lasting and energy efficient flights (Dickinson and Lighton 1995; Grimaldi 1990).

iii. Ecophysiological responses traits

This set of characteristics encompasses the allocation of energy to vital functions and to reproductive characteristics in Drosophilidae. Here we consider characteristics related to environmental factors that regulate metabolic activity.

Thermoregulation - refers to an organism's ability to maintain its vital functional activities in unfavorable temperature conditions. Greater melanization increases the absorption of radiant heat and, under low temperatures, allows ideal body temperature to be reached quickly (Honek 1996; Parkash et al. 2011; Rajpurohit et al. 2008). Beyond this, light colors may be more efficient in preventing desiccation in places where overheating is most likely (Huey and Pascual 2009; Kutch et al. 2014; Pipkin 1962; Rajpurohit et al. 2008). If the body temperature is out of the ideal range, homeostasis is unbalanced and the performance of individuals may decrease or basic life functions may even cease (Huey and Kingsolver 1989; Huey and Pascual 2009; Rajpurohit et al. 2008). According to the literature, quantitative traits such as body melanisation vary due to genetic attributes and their interaction with environmental factors (Sabath et al. 1973; Wittkopp et al. 2003). Increased melanisation at lower temperatures is thought to be adaptive for thermoregulation and to improve metabolic activity (Capy et al. 1988; David et al. 1990; Gilbert et al. 1996, 1998). Thus, darker individuals can better absorb solar radiation to keep the body warm for various functions (flight, foraging and mating) under colder climatic conditions. On the contrary, in lowland populations and other warmer environments, lighter individuals would be less prone to overheating (Parkash et al. 2011).

Reproductive organs - Insect terminalia are among the most diverse and complex morphological structures. The great variation in the length of the female terminalia suggests that morphological differences may be one of the factors resulting in reproductive isolation between Diptera species. An example, are, individuals that actively take part in reproduction and oviposition to dispersion more (Iwaizumi et al. 1997; Peluffo et al. 2015). This category comprises the size and shape of the female terminalia, including the oviscapt and eggs. The size and shape of terminalia can be affected by environmental characteristics, including resource availability (Atallah et al. 2014; Grimaldi 1987; Klug et al. 1974; Peluffo et al. 2015; Yassin 2013).

Functional traits and Drosophilidae assemblages

Several characteristics discussed in this review can vary between individuals or were associated with certain environments. Others are new suggestions that have only been previously tested in laboratory settings, and thus need to be directly observed in nature or tested in field experiments. Characteristics associated with flight performance and wing morphometry are highly used in community assembly studies of other animal groups such as bees, butterflies and hoverflies (Balmford et al. 1993; Beldade et al. 2002; Aguirre-Gutiérrez et al. 2016; Thomas 1993), because they can provide information regarding migration, colonization, and habitat fragmentation, which can be useful for biodiversity conservation (Araújo et al. 2004). Given that body measurements are relatively easy to take, they are often used to understand the associations between functional traits and habitat use (Bitner-Mathé and Klaczko 1999; Hofmann et al. 2005; Furtado and Martins 2018). Positive correlations between morphological traits and ecological processes have been found for *Drosophila* species (e.g. *D. melanogaster, D. simulans*), including: head structures (e.g. eye size, ommatidia and gena) and habitat perception (by Gaspar et al. 2020; Ramaekers et al. 2019); sensory parts (e.g. antennal sensilla) and habit specialism (Gao et al. 2020), including adult and larvae feeding habit (Atkinson et al. 1977; Carson 1971; Lachaise and Tsacas 1983; Mata et al. 2015, Pipkin et al. 1966); and body size (e.g. wing and thorax); and dispersal ability or flight perfomance (Coyne and Beecham 1987; Bitner-Mathé and Klaczko 1999; Dickinson and Lighton 1995; Fartyal et al. 2017; Lehmann 2001; Moraes et al. 2004; Triplehorn and Johnson 2011).

Drosophilidae color has been treated quantitatively and qualitatively more and more in recent years, mainly in laboratory experiments. However, how color and diversity patterns respond to different ecological patterns remains poorly understood (Brisson et al. 2005, 2006; Grimaldi and Jaenike 1984; Hofmann et al. 2005; Pipkin 1953). Body color, as well as the size of the egg and the oviscapt, have been associated with activity, thermoregulation and reproductive strategy in, for example, *Drosophila subobscura*, *D. yakuba* and *D. santomea* (by Huey and Pascual 2009; Peluffo et al. 2015, respectively). Ecophysiological traits are unresolved in Drosophilidae, requiring a description of the relationship between larvae and host resources (Bochdanovits and Jong 2003; Grimaldi, 1987), as well as aspects of adult survival and reproduction in different environments. In *Drosophila*, we already know that pigmentation influences courtship and is likely to be subject to sexual selection in species with spotted wings (Hegde et al. 2005; Prud'homme et al. 2006), and that extreme head shape variation plays a role in sexual selection in stalked-eyed *Zygothrica* flies (Grimaldi 1987).

Details on Drosophilidae's visual systems and how to measure them were successfully proposed and used by Götz (1970). However, it is still unclear how visual information guides Drosophilidae's behavior in nature, how human impact can change these contexts and how to extend them to the community level. Greater visual sensitivity may be necessary for species that are subject to the low luminosity inside a forest (larger eyes, larger facets and a higher number of ommatidia), in order to increase visual recognition of signs and threats. Based on previous research, we hypothesized that a combination of functional traits (body, wing and eye size) can characterize Drosophilidae assemblage's responses to the environment. Given the wide variety of environments exploited by the family, color diversity may be one strategy likely to be unique to the species in each environment.

According to the literature, community compositions can change along a gradient of urbanization and this is usually associated with vegetation, scarcity of resources, land cover, temperature and humidity (Avondet et al. 2003; Ferreira and Tidon 2005; Gottschalk et al. 2007). We already know that temperature has a direct environmental effect on ectotherm body size such that lower growth temperatures usually result in larger adults. At the same time, temperature dependent larval resource allocation may shape adult body size (Atkinson 1994; Bochdanovits et al. 2003), while humidity may explain features of latitudinal clines not attributable to temperature (Kennington et al. 2003).

Species with few and large eggs, as a reproductive strategy, could be associated with larger size of the terminalia (valves of oviscapt and last abdominal segments modified to oviposition) and less specificity of trophic resources exploited in the larval stage, given the constant conditions and resources for the growth of the larvae. Because of environmental constancy, we can predict that the species would have a specialized diet during the larval phase. Beyond this, the limitation in trophic resources for drosophilid larvae could influence competitive interactions between them (Krijger et al. 2001).

Corroborating some laboratory results of single traits, and community structure in natural habitats may show the following functional patterns: short/small bodies, associated with larger wings, which allows faster strokes and greater acceleration. The diversity of colors can be diminished as there is less variation in background color in environments with short vegetation. Regarding the use of habitat and food resources, generalist species in their larval stage should be less usually than adult stage. Drosophilidae species with better visual acuity would also be able to more successfully exploit habitats with open vegetation environments, being less susceptible to abiotic factors.

Functional traits emphasize the functional role of species in a community (Gallé and Batáry 2019). The exponential growth in the use of trait-based information has improved our understanding of ecosystem processes (Gallé and Batáry 2019). Here, we assessed how environmental gradients and trade-offs shape functional traits and subsequently ecosystem processes, to shape Drosophilidae distribution.

Concluding observations

In all three functional categories presented in this study we found studies that present key characteristics capable of determining the structure of Drosophilidae assemblages in natural habitats. Most of the suggested traits are relatively easy to measure and/or have reliable information available in the literature. Some other characteristics, such as reproduction and the relationship between generalist species and larval stages, are harder to assess for entire assemblages. Thus, increasing information on these characteristics is a key area for research effort, which would support their use in functional studies of Drosophilidae.

Nunney and Cheung (1997), explain that phenotype induced by a particular set of environmental conditions may yield a fitness gain to the species.This adaptive response would lead to differentiation among populations from different environments. Functional traits commonly characterize organism physiology, morphology or behavior (Cornelissen et al. 2003). In retrospect, we hypothesize that some traits suggested here are likely to result in the detection of important effects, such as (i) eye size and food specialization (characteristics of habitat perception); (ii) flight capacity (flight performance traits); and (iii) thermoregulation (ecophysiological response traits).

The quantification of functional traits for biodiversity research has resulted in improved understanding of ecological phenomena (Petchey et al. 2004; Nock et al.

2016), and studying the relationships between important organism traits and higher level ecosystem phenomena often affords deeper mechanistic insight in to how diversity can be important in the provision of ecosystem functions or services (Nock et al. 2016). Beyond this, trait-based data also facilitate comparisons of biodiversity effects across ecosystems in contrast to taxon-specific approaches, rendering results more amenable to being incorporated into developing general principles in ecology (McGill et al. 2006).

Here, our aim was to review and propose the characteristics of Drosophilidae that can be used to understand the ecological structure and dynamics of these insects in any natural habitat. This can also be considered as a combination of several hypotheses, generally thought of in terms of isolated traits, and suggested here to explain how Drosophilidae occupy habitats in terms of environmental characteristics. Thus, the traits suggested here can be broadly applied in Drosophilidae community studies.

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Figure caption

Fig. 1 Illustrations of functional trait measurements suggested for Drosophilidae (Diptera) (blue and red). **a** Drosophilidae, lateral and dorsal view; **b** head, lateral and frontal view; **c** thorax, dorsal and lateral view. Abbreviations: *ar* arist, *vb s* vibrissa, *pc orb s* proclinate orbital seta, *arc orb s* anterior reclinate orbital seta, *p rc orb* posterior reclinate orbital seta, *poc s* postocellar setae, *sbvb s* suvibrissal setulae, *pprn s* postpronotal setae, *anepst* anepisternum, *kepst* katerpisternum, *npl* notopleuron, *spal s* supra-alar seta, *anepm* anepimeron, *mr* meron, *acr s* achrostichal setulae, *a dc s* anterior dorsocentral setae, *p dc s* posterior drosocentral setae, *b sclt s* basal scutellar setae, *ap sctl s* apical scutellar setae, *ap sctl s* postopronotal setae, *hlt* halter, *i vt s* inner vertical seta, *o vt s* outer vertical setae.

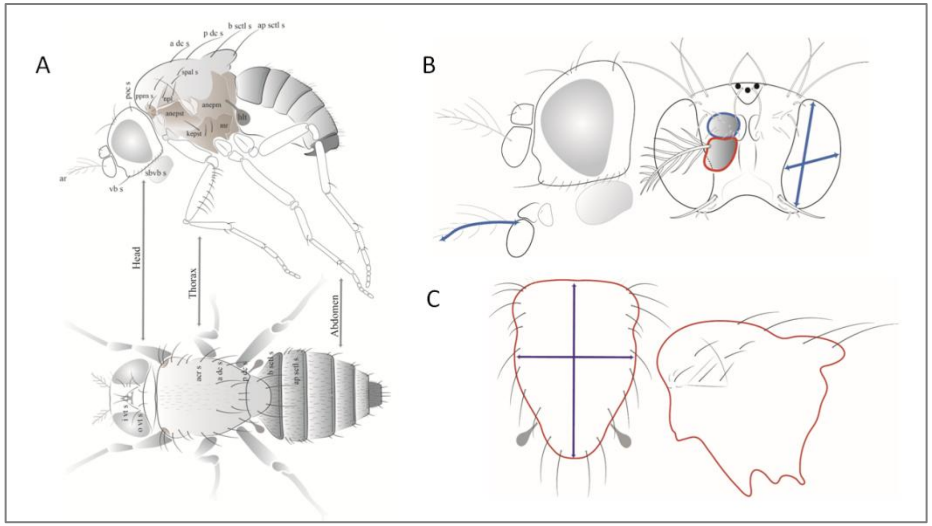

Fig. 2 Illustrations of functional trait measurements suggested for Drosophilidae (Diptera) (blue and red). **a** Wing (with venation names, proposed by Cumming & Wood, 2010) and positions of landmarks; **b** Female terminalia, lateral and dorsal view. Abbreviations: *ovscp* ovis- capt valve, *spmth* spermathecal capsule, *T8* abdominal tergites, *S8* abdominal sternites, *Epiprct* epiproct, *Hyprct* hypoproct.

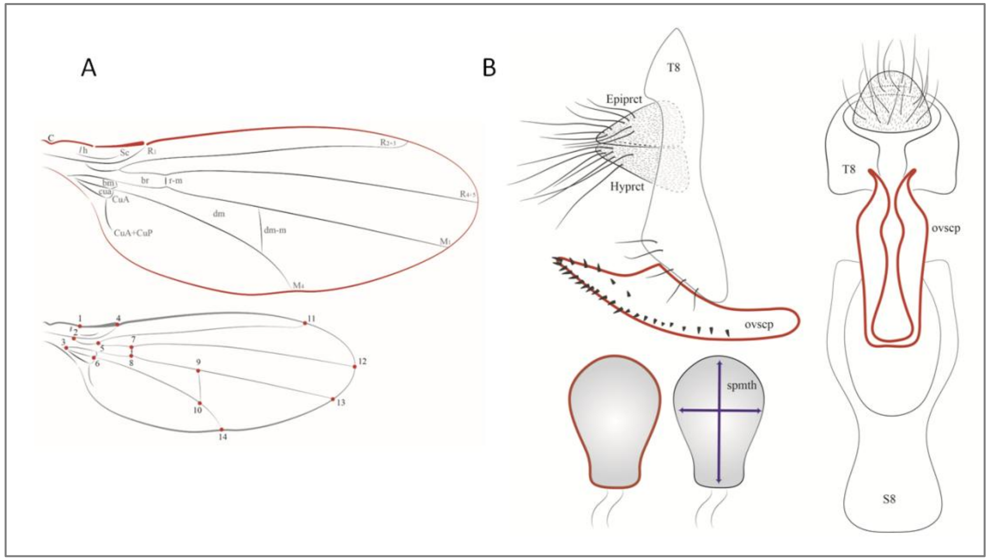

Table 1 Drosophilidae functional traits and functional responses suggested for studies in natural environments

Traits	**Type**	**How to measure?**	**Functional response**	**Reference**
Habitat perception				
Eye size / ommatidia size / gena size	Continuous	Eye length / width Eye area Number of ommatidia Gena length	Visual capacity / sensitivity/ acuity	Gaspar et al. 2020; Götz 1970; Grimaldi 1987; Keesey et al. 2019; Ramaekers et al. 2019
Habitat specialist	Categorical	Review of the natural history of the sensory parts and environmental explored by species	Habitat use/ recognition	Cavasini et al. 2014; Ferreira and Tidon 2005; Mata et al. 2008; Penariol and Madi-Ravazzi 2013; Poppe et al. 2013

Antennal sensilla	Continuous	Flagellomere, Scape and Pedicle length / width Flagellomere, Scape and Pedicle area	Habitat perception	Gao et al. 2020; Stocker 1994
Sensory parts	Continuous	Number of branches in the arista / length of arista	Habitat perception	Burnet et al. 1971; Foelix et al. 1989; Gao et al. 2020; Sayad and Yassin 2019; Shanbhag et al. 1999; Stocker 1994
Adult feeding habit	Categorical	Review of the natural history of trophic resource use by species	Specificity of trophic resources	Atkinson et al. 1977; Carson 1971; Grimaldi 1987; Lachaise and Tsacas 1983; Mata et al. 2015, Pipkin et al. 1966
Larval feeding habit	Categorical/Continuous	Review of the natural history of trophic resource use by species	Specificity of trophic resources	Carson 1971; Dobzhansky and Pavan 1950; Grimaldi 1987

Traits	**Type**	**How to measure?**	**Functional response**	**Reference**
Flight performance				
Wing size	Continuous	Wing area Distance of landmarks	Dispersal capacity, Flight speed	Cavicchi et al. 1981; Dickinson and Lighton 1995; Fartyal et al. 2017; Tantawy 1964

Wing shape	Continuous	Wing area	Flight speed, Maneuverability	Coyne and Beecham 1987; Bitner-Mathé and Klaczko 1999; Dickinson and Lighton 1995; Fartyal et al. 2017; Lehmann 2001; Moraes et al. 2004
Thorax size ratio (length)/ wing size	Continuous	Base of the most anterior humeral bristle to the posterior tip of the scutellum (thorax length) / wing area	Flight speed, Energetic efficiency	Azevedo et al. 1998; Dickinson and Lighton 1995; Fartyal et al. 2017; Tallefer and Wheeler 2012
Angle/ thorax width	Continuous	Lateral angle thorax / thorax length Lateral thorax area / thorax length	Flight speed	Dickinson and Lighton 1995; Triplehorn and Johnson 2011

Traits	**Type**	**How to measure?**	**Functional response**	**Reference**
Ecophysiological response traits				
Body color	Continuous	Quantification of the color spectrum Color description history	Activity / Thermoregulation	Brisson et al. 2005, 2006; Capy et al. 1988; David et al. 1990; Gilbert et al. 1996, 1998; Huey and Pascual 2009; Kutch et al. 2014; Pipkin 1962; Rajpurohit et al. 2008; Triplehorn and Johnson 2011
Egg size / shape	Continuous	Base of the most anterior distal to the posterior tip of the egg	Reproductive strategy	Anagnostou et al. 2010; Grimaldi 1987; Huey et al. 1995; Klug et al. 1974; Peluffo

		Egg area		et al. 2015; Vijendravarma et al. 2010
Oviscapt size / shape	Continuous	Base of the most anterior distal to the posterior tip of the oviscapt / oviscapt area (frontal, lateral view)	Reproductive strategy	Atallah et al. 2014; Atkinson 1979; Boulétreau-Merle et al. 1982; Grimaldi 1987; Yassin and Orgogozo 2013
Body size - ratio size thorax (length)/wing size	Continuous	Relation base of the most anterior humeral bristle to the posterior tip of the scutellum (thorax length) / wing area	Thermoregulation	Kutch et al. 2014; Rajpurohit et al. 2008; Triplehorn and Johnson 2011

5. CAPÍTULO V

(Manuscrito em preparação para o periódico *Journal of Insect Conservation*)

6. CAPÍTULO VI

6.1Conclusão geral e perspectivas

Embora estudos envolvendo Drosophilidae como modelo biológico tenham aumentado nos últimos anos por tratar-se de um dos grupos de insetos com biologia melhor conhecida, ainda existem lacunas no conhecimento do grupo, tais como padrões de diversidade, distribuição, estruturação, funcionalidade e métodos de amostragem em comunidades de drosofilídeos neotropicais. Mesmo o Rio Grande do Sul, que é um dos estados que possui maior conhecimento sobre levantamentos de fauna, o Pampa é um dos biomas brasileiros mais negligenciados em termos de conservação, sendo apenas nos últimos 14 anos realizados estudos que tentam compreender, padrões de diversidade, composição, ocorrência e distribuição nos diferentes ambientes e fitofisionomias do Bioma Pampa.

Neste trabalho, melhoramos o conhecimento sobre efeitos de borda sobre comunidades Drosophilidae, principalmente sobre o comportamento de alguns grupos de espécies nativas em épocas mais frias do ano (Capítulo II). Nossos resultados corroboram Drosophilidae como um grupo sensível a efeitos de borda, mostrando que sua abundância é significativamente maior no interior do que na borda do fragmento no inverno. Além disso, a maioria das espécies amostradas utiliza tanto ambientes próximos à borda quanto ambientes no interior do fragmento florestal, mas com intensidades diferentes. Apesar de nossos esforços em apresentar resultados quanto à distribuição temporal das espécies com desenho amostral aplicável principalmente em pequenos fragmentos como no Pampa, por exemplo, encorajamos também, porém, que em estudos futuros devem ser incluídas e verificadas também dados de matriz.

Propomos armadilhas coloridas como uma nova técnica de amostragem para caracterização da fauna de Drosophilidae. Esse foi o primeiro registro desta metodologia para o grupo (Capítulo III), que se mostrou eficiente principalmente na captura de espécies antofilicas incluindo gêneros raramente amostrados com outras metodologias mais convencionais. Na verdade, trabalhos anteriores já haviam demonstrado o uso de armadilhas coloridas na captura principalmente de visitantes florais, mas para outros grupos de insetos. Acreditamos que com esses resultados armadilhas coloridas aprimorem o conhecimento de espécies antofílicas e tornem-se também peça chave para captura de Drosophilidae, mesmo na ausência do recurso floral no ambiente. O maior número de espécies com diferentes características ecológicas e comportamentais, talvez no futuro, e armadilhas coloridas possa ser considerada para capturar espécies indicadoras monitorando os efeitos das atividades antrópicas, por exemplo.

Uma estrutura padronizada para seleção e medição de características morfológicas para os Drosophilidae, foi organizada em três categorias de atributos funcionais as quais sugerimos que precisam ser analisadas para compreender o padrão funcional de Drosophilidae: como a percepção do habitat, o desempenho de voo e respostas ecofisiológicas (Capítulo IV). Esperamos que a padronização de características presentes na revisão facilite a identificação de funções específicas de cada comunidade de Drosophilidae, para assim serem capazes de identificar como as espécies respondem aos distúrbios e/ou condições abióticas severas. Como uma medida teste, aplicamos o protocolo utilizando caracteres morfológicos contínuos da revisão, que se demonstraram importantes para aprimoramento sobre a funcionalidade do grupo em vegetações campestre e florestal do Bioma Pampa (Capítulo V). O desenho amostral com armadilhas malaise proposto inicialmente previa obter respostas funcionais para todos os meses do ano com indivíduos capturados, mas aqui apresentamos a resposta funcional para oito meses. Ainda assim, os dados apresentados aqui (com quinze gêneros determinados nas distintas áreas) demonstram uma análise inicial para entender como os atributos estão relacionados a determinados papeis funcionais ecossistêmicos. Reconhecer a funcionalidade de Drosophilidae ainda é um desafio em nível de comunidade, mas nossos resultados são um primeiro passo sobre potencial do grupo para mostrar processos que estruturam comunidades biológicas.

No geral, entendemos o quão é importante conhecer diferentes técnicas amostrais, pois elas contribuem para captura de grupos taxonômicos específicos, como foi com as armadilhas coloridas e malaise (primeiros registros de uso em levantamentos de fauna para o Brasil). Como perspectivas futuras, esperamos chegar até o nível de espécie todo o material amostrado, e ainda realizar uma revisão das diferentes formas de amostragem já utilizadas em levantamentos da fauna de Drosophilidae no Brasil a fim de melhorar o reconhecimento dos principais grupos de espécies capturados por cada uma. É importante conhecer o funcionamento e a manutenção dos processos das comunidades, esperamos conseguir visualizar a variação funcional temporal dos atributos que estejam relacionados com determinados papéis ecossistêmicos, com o aumento de indivíduos mensurados e a inclusão dos quatro meses faltantes aqui (maio, junho, agosto e outubro). Além disso, esperamos que seja possível avaliar os dados temporais de funcionalidade e a diversidade filogenética, medidas de comunidade em ascensão, que juntas poderiam conectar papeis funcionais incorporando também as relações filogenéticas das espécies, inferindo relacionamento das características e questões históricas da comunidade.

Por fim, a riqueza de gêneros capturada neste trabalho futuramente deverá ser explorada a níveis taxonômicos menores por especialistas dos determinados grupos para revisão e descrição de prováveis espécies novas. Em nosso grupo de pesquisa temos um interesse particular para revisão e descrição de espécies dos gêneros *Mycodrosophila*, *Paramycodrosophila, Scaptomyza* e *Zygothrica*.

7. CAPÍTULO VII

Apêndices

7.1 Apêndice I -

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Three new species of *Zygothrica* (Diptera: Drosophilidae) from the Neotropical region, Brazil

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ABSTRACT

Zygothrica (Diptera: Drosophilidae) is an almost entirely Neotropical genus, with thirteen species found in other biogeographical regions. The genus includes around 130 valid species, of which 54 occur in Brazil. There have been no new descriptions of species of this genus since the 1987s, although a large number of undescribed species are recognized in entomological collections. Here, three species of *Zygothrica* Wiedemann (1830) are described from Brazil, and figures are presented for external morphology and terminalia.

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Introduction

Zygothrica Wiedemann (1830) was first proposed as a subgenus of *Achias* (Platystomatidae: Diptera), and then elevated to genus by Loew (1873). Later, a pioneering study was carried out by Sturtevant (1920), where he reviewed all previously described Drosophilidae species and proposed a new species of *Zygothrica*. Subsequently, Duda (1925) and Burla (1956) studied and described species from Costa Rica and Brazil, respectively, increasing the number of species in the genus. Despite these studies, until the 1980s there were inconsistencies in genus delimitation. Burla (1956) proposed some diagnostic characters for *Zygothrica*, which were later, refuted by others taxonomists because they overlap with some *Hirtodrosophila* Duda 1923 characters. In 1987, a more accurate diagnosis for *Zygothrica* was proposed by Grimaldi, together with a phylogenetic hypothesis and ecological aspects of some species, in his monographic paper on the systematics and phylogeny of the genus.

To date, the genus is predominantly Neotropical and has around 130 valid species (Bächli, 2020), of which 54 occur in Brazil (Grimaldi, 1990; Gottschalk et al., 2008; Robe et al., 2014; Tidon et al., 2017). No new species descriptions of this genus have been reported to the Neotropical region since 1987, when Grimaldi described 49 new species, representing the most substantial contribution of his study. However, a large number of unidentified specimens have been deposited in entomological collections. In this paper, we describe three new species from Brazil, and present figures for external morphology and terminalia.

Materials and methods

Individuals were identified based on external morphology and the analysis of male or female terminalia, which were prepared following a protocol adapted from (Bächli et al., 2004; Mendes and Gottschalk, 2019). The terminalia were disarticulated in glycerin and mounted on temporary microscope slides with glycerin jelly (aqueous solution of 2% gelatin and 50% glycerin) (Grimaldi, 1987) for illustration and photographic records. Subsequently, the microscope slides were dismounted and the genital sclerites were placed in microtubes with glycerin and attached to the specimen.

Five individuals of *Zygothrica duovittata* sp. nov. the terminalia had previously been mounted on permanent slides with natural Canada balsam. In this case, we dismounted the slides following the protocol proposed by Vilela and Bächli (2000). As the Canada balsam in the microscope slides did not stiffen, the coverslips covering the male terminalia were easily removed without the use of xylol. The genital sclerites were transferred to a depression slide with a drop of creosote, and were observed hourly until the sclerites seemed clear. Subsequently, the sclerites were placed in 100% ethanol for 2 minutes and then in glycerine to be disarticulated and mounted on a slide with glycerine jelly (as described above) for illustration and photographic records.

The terminalia were drawn using an Olympus CL 6000 optical microscope with an attached camara lucida (1.8x magnification for the camara lucida, 20x for the objective lens and 10x for the eyepiece lens). One individuals of each species were selected and photographed with a Zeiss Discovery V.20 photomicroscope, in lateral, dorsal and frontal views. The wing was also photographed in detail. The images were then stacked using the software AxioVision 4.9.1.

The morphological nomenclature followed Cumming and Wood (2010). Measurements of body structures and color descriptions followed Grimaldi (1987) and

Vilela and Bächli (2000). Complementary information added to the original labels was obtained from the database of the Instituto Brasileiro de Geografia e Estatística and presented between brackets in the type series characterization. Species distribution information was obtained from TaxoDros v.1.04 (2019/02) (Bächli, 2020).

The three species described were already dry with hexamethy Idisilazane (Brown 1993) and pinned, except to paratypes of *Zygothrica duovitta* sp. nov. Here, species were morphologically compared with photographs of known *Zygothrica* species, which are mainly type series deposited in collections. Finally, all specimens studied still are preserved in dry way in a paper triangle on entomological pins, except to abdominal sclerites and terminalia attached in ethanol and pinned with the respective specimens. All holotypes and paratypes are deposited in the Entomological Collection of the Instituto Oswaldo Cruz (CEIOC), Rio de Janeiro, RJ, Brazil.

Results

Taxonomy

The species groups arrangement followed Grimaldi (1987) and Burla (1956).

***Zygothrica* Wiedemann**

Zygothrica Wiedemann, 1830: 16 (subgenus of *Achias* Fabricius). Type species *Achias dispar* Wiedemann, 1830 (original designation). Subsequent references: Sturtevant, 1920: 156; Burla, 1956: 215; Grimaldi, 1987: 147.

Drosophilura Hendel, 1913: 389. Type species *Drosophilura caudata* Hendel, 1913 (original designation).

Tanyglossa Duda, 1925: 189 (subgenus of *Zygothrica*). Type species *Zygothrica tenuirostris* Duda, 1925. Preoccupied by Meigen, 1803: 267 (Diptera: Tabanidae).

Diagnosis. See Grimaldi (1987).

dispar group

aldrichi subgroup

***Zygothrica japunibaensis* sp. nov.**

(urn:lsid:zoobank.org:act:DABA9659-236C-4C35-8C6E-D56405244409)

(Figures 1-3; 10A)

Type-series. Holotype #m labeled "*Zygothrica japunibaensis* sp. nov. Mendes *et al.* HOLOTYPE #m", "[Brazil], E[stado] do Rio [de Janeiro], Angra [dos Reis], Japun[í]ba, L. Trav. F; VII.[1]936; Col: L. Trav.". Abdominal sclerites and terminalia in vials with glycerine; wing was removed and mounted on a permanent microscope slide and all material was attached to the specimen.

Type locality. Japuníba, city of Angra dos Reis, State of Rio de Janeiro, Brazil.

Diagnosis. Body color mainly brown; mesonotum brown with two yellow narrow stripes in the line of the dorsocentrals, extending up to 2/3 of the length of mesonotum; acrostichal setulae in 6 lines; whitish-yellow pleurae; abdomen brown pollinate, tergites III and VI lighter; arista with 5 dorsal branches, 1 ventral and 3 short inner branches, plus terminal fork; first flagellomere ochre, hairy, length about 2.5x the length of the pedicel; facial carina prominent, ochre; legs yellow, apex of tibia darker; wings with apical spot, dM-Cu infuscated, mid-radial spot reaching R_{2+3} and proximal radial-medial spots.

Description. *#m*

Head. Brown; head width = 0.9mm, slightly wider than thorax (hypercephalic). Eyes dark red, bare. First flagellomere ochre, hairy, length about 2.5x the length of the pedicel, and about 2x the width. Aristae with 5 dorsal branches, 1 ventral and 3 short inner branches, plus the terminal fork. Orbital plates yellow. Orbital bristles dark brown. Distance between or1 and or2 about 60% of the distance between or1 and or3. Frons brown; ocellar triangle slightly darker, covering 1/3 of the length of the frons; length of the frons about 1.5x the anterior width; posterior width of the frons about 2/3 of the anterior width. Brown median frontal vitta almost reaching the ptilinal fissure; postocellar bristles convergent. Face and gena ochre. Proboscis ochre, with lighter palps.

Thorax. Mesonotum brown, with two narrow stripes in the line of the dorsocentral, extending up to 2/3 of the length of mesonotum; 6 lines of parallel acrostichal setulae. Notopleura, supra-alar area and postalar callus yellow. Scutellum brown, marginally yellow. Basal scutellar setae converging. Pleurae yellow. Legs yellow, apex of tibia darker. Thorax length = 1.04mm. Thorax width = 0.66mm.

Wings. Membrane with color pattern as follows: apical spot, dM-M infuscated, mid-radial spot reaching R_{2+3} and proximal radial-medial spots. R_{2+3} straight toward C, R_{4+5} and M slightly convergent, both slightly curved posteriorly. Halters yellow. Indices: C = 1.80; ac = 3.87; hb = 0.70; 4c = 0.93; 4v = 1.22; 5x = 1.00; M = 0.25; prox x = 0.43. Wing length = 2.13mm.

Abdomen. Light brown; tergites I, II, V and VI darker than tergites III and IV.

Terminalia. Epandrium rounded not microtricose, longer than hypandrium; ventral lobes small with 4 bristles. Cerci free, microtricose, with long bristles. Hipoproctal plate (*sensu* Grimaldi, 1987) present. Ventral cercal lobes (*sensu* Grimaldi, 1987) membranous. Surstyli rounded, with 10-14 prensisetae arranged in crescent format. Subepandrial sclerite previously projected ahead. Postgonites (gonopods *sensu* Grimaldi, 1987) square-shaped, with one prominent bristle and fused to the hypandrium. Pregonites (paraphysis *sensu* Grimaldi, 1987) fused to postgonites and without obvious bristles. Hypandrium V-shaped. Phallus (basiphallus + distiphallus *sensu* Grimaldi, 1987) tubular, with basiphallus narrower than distiphallus, with an indent in the apex, bare; length of the phallus about 3x the width of distiphallus; basiphallus fused to the phallapodeme (aedeagal apodeme *sensu* Grimaldi, 1987). Phallapodeme shorter than phallus.

#*f*. Unknown.

Geographic distribution. Known only from type locality.

Etymology. The species epithet refers to the type locality of Japuníba, situated in the city of Angra dos Reis, State of Rio de Janeiro, Brazil.

Comments: The phallus is similar to *Z. nigropleura* Grimaldi 1987, but the distiphallal scales are absent and the length of the phallapodeme is smaller in relation to the phallus in *Z. japunibaensis* sp. nov.

orbitalis group

***Zygothrica duovittata* sp. nov.**

(urn:lsid:zoobank.org:act:BA6C2E5B-6378-4C6A-B858-2DAACA3D02D4)

(Figures 4-6; 10B)

Type series. Holotype: #m labeled "*Zygothrica duovittata* sp. nov. Mendes *et al.* HOLOTYPE #m", "Brasil, Paraná, Diamantina do Norte, Est[ação]. Ecol[ógica]. do Caiuá; 22°49'38"S 45°39'45"W; 30.IV.2011; Col: J. P. Junges". Paratypes: 14 #m labeled "*Zygothrica duovittata* sp. nov. Mendes *et al.*; PARATYPE 01 #m" "Brasil, São Paulo, Teodoro Sampaio, P.E. Morro do Diabo; 03.V.2011; Col: J. P. Junges"; 01 #m labeled "*Zygothrica duovittata* sp. nov. Mendes *et al.*; PARATYPE ♂", "Brasil, [Pará], Belém do Pará, Utinga; 22.VI.[19]65; Col: H.S. Lopes". Abdominal sclerites and the terminalia were stored in microvials with glycerine, one wing was removed and prepared on a permanent microscope slide and all materials were attached to the specimens. Finally, the material type was fixed in ethanol 70% .

Type locality. Estação Ecológica do Caiuá, city of Diamantina do Norte, State of Paraná, Brazil (22°49'38"S, 45°39'45").

Diagnosis. Predominantly yellow body; scutum light brown, with four dark brown bands between dorsocentral bristle lines, the central pair darker and wider (the intensity of the coloration could vary); 8-9 irregular lines of acrostichal setulae between dc; scutellum light brown, slightly lighter margin; pleura yellow; abdomen yellow, with medial black bands on tergites I, II, III and IV; aristae with 5 dorsal, 1 ventral and 4 short inner branches, plus the terminal fork; first flagellomere light brown, length about 2.5x the width, and about 2x the length of the pedicel; carina facial light brown; legs yellow; wings hyaline, without spots; R_{2+3} straight, R_{4+5} slightly convergent toward M.

Description. *#m*

Head. Brown; width = 1.10 (0.96-1.13) mm. Eyes red, bare. Pedicel and scape brown, flagellomere brownish yellow, length about 2.5x the width, and about 2x the length of the pedicel. Aristae with 5 dorsal branches, 1 ventral and 4 short inner branches, plus the terminal fork. Orbital plates brown. Orbital bristles brown. Distance between or1 and or2 = 0.06 (0.05-0.08) mm, between and1 to or3 = 0.10 (0.9-0.11) mm and between or2 and or3 = 0.04 (0.04-0.07) mm. Front ochre. Ocellar triangle brown, occupying approximately 1/2 of the frontal length; anterior frontal width equal to length, posterior width slightly smaller than anterior. Face ochre. Gena yellow. Proboscis, palps and labellum yellow.

Thorax. Brown, with four dark brown bands, the two central are more intense and wider; 8-9 irregular lines of acrostichal setulae between the dorsocentral bristles; scutellum brown and slightly lighter in the margins; yellow pleura; 2 katepisternal setae, posterior about 2x the length of the anterior. Legs yellow. Thorax length = 1.07 (1.07-1.29) mm, width = 0.75 (0.84-0.98) mm.

Wings. Hyalines, without distinct spots; veins R_{2+3} straight, R_{4+5} slightly curved towards M; bM-Cu absent; halters yellow. Indices: C = 2.63 (1.87-3.20); ac = 3.15 (2.20-3.76); hb = 0.58 (0.52-0.63); 4c = 0.82 (0.60-0.88); 4v = 1.39 (1.24-1.48); 5x = 1.44 (1.18-1.82); M = 0.35 (0.29-0.42); prox. x = 0.34 (0.32-0.40). Wing length = 2.74 (2.17-2.46) mm.

Abdomen. Yellow, with medium black bands on tergites II, III and V, extending from anterior to posterior margin and not extending laterally. Note: The color intensity of the tergites may vary.

Terminalia. Epandrium with U-shaped form, ventral lobes small. Cerci elongated posteriorly, with long bristles and microtricose (except in the inner margin). Hipoproctal plate present, with two median-ventral elongated processes and one pair of bristles. Surstyli rounded, with 10-16 prensisetae arranged in two rows. Prominent and previously designed Subepandrial Sclerite. Large postgonites (gonopods *sensu*

Grimaldi, 1987), attached to the hypandrium and containing a median bristle on the inner margin. Large pregonites (paraphysis *sensu* Grimaldi, 1987) fused to gonopods and with three obvious bristles. Hypandrium V-shaped, as long as the epandrium and with many growth lines. Phallus (aedeagus *sensu* Grimaldi, 1987) with margin ornamented by scales fused to the phallapodeme (aedeagal apodeme sensu Grimaldi, 1987) with irregular/wavy margin, with two apical projections, broad in the apex, with scales in the ventral portion and small bristles laterally. Phallapodeme shorter than phallus and tapered in lateral view.

#f. Unknown.

Geographic distribution. The holotype and paratype specimens were collected in the Estação Ecológica do Caiuá, Diamantina do Norte, Paraná, Brazil (22°49’38”S 45°39’45”W); Teodoro Sampaio, São Paulo, Brazil; and Utinga, Belém, Pará, Brazil.

Etymology. The specific epithet derives from the Latin "duo" which means two and was proposed in reference to two darker longitudinal stripes in the middle of the thorax of the specimens.

vittatifrons group

***Zygothrica grajau* sp. nov.**

(urn:lsid:zoobank.org:act:348F7C30-2441-4CC0-A74A-0E05D8F2E690)

(Figures 7-9; 10C)

Type. Holotype #m labeled with with the information "*Zygothrica grajau* sp. nov. Mendes *et al.* HOLOTYPE #m", "Brasil, [Rio de Janeiro], Rio de Janeiro, Grajau; 21.II.1965; Col: H.S. Lopes". Abdominal sclerites and the terminalia were stored in a microvial with glycerine, the wing was removed and prepared on a permanent slide with Canada balsam. The material was attached to the specimen.

Type locality. Grajaú, Rio de Janeiro, Rio de Janeiro, Brazil.

Diagnosis. Body color predominantly yellow. Thorax yellow, with three pairs of dark brown longitudinal stripes in the scut, a pair of stripes between dorsocentral bristles, a pair along the dorsocentral and a pair laterally to the dorsocentral, interrupted in the transverse suture; scutellum almost entirely dark brown with three yellow bands, one median and two laterally; convergent basal scutellar bristles; pleura yellow. Abdomen yellow; tergites I yellow, II and III with dark brown bands extending laterally without reaching the margin, tergites IV to VI with brown rounded spots in the median region; aristae with 5 dorsal, 2 ventral and 3-4 short inner branches, plus terminal fork; flagellomere yellow; prominent facial carina, slightly whitish-yellow; legs yellow. Wings hyaline; lappet and bM-Cu absent; brown spot on the apical portion of the wing and extending from the final portion of C to just before M. R_{4+5} slightly curved. Halters yellow.

Description. *#m*

Head. Yellow. Width = 0.68 mm. Eyes dark red, with interfacetal setulae. Escape yellow, pedicel brown and flagellomere yellow, slightly whitish; length of the flagellomere approximately 2x the length of the pedicel. Aristae with 5 dorsal, 2 ventral and 3-4 short inner branches, plus terminal fork. Orbital plates yellow. Orbital bristles dark brown. Distance between or1 and or2 = 0.06mm, between or1 and or3 = 0.13mm and between or2 and or3 = 0.07mm. Frons with dark brown frontal vittae, and orbital plates yellow. Ocellar triangle yellow, corresponding to more than 2/3 of the length of the forehead; region between ocelli dark brown; convergent postocellar bristles; anterior width of the frons equivalent to the length, posterior width slightly smaller than the anterior. Facial carina large, yellow. Gena yellow, slightly whitish. Proboscis, palpus, and labellum yellow.

Thorax. Yellow, with three pairs of dark brown dorsal longitudinal bands; a pair between dorsocentral bristles, a pair along the dorsocentral bristle lines and a pair laterally of the dorsocentral bristle lines and interrupted in the transverse suture; scutellum dark brown with three yellow longitudinal stripes, one median and two lateral; basal scutellar bristles convergent; pleura yellow, 2 katerpisternal bristles, of which the second is 1,5x longer than the first. Length = 0.88mm, width = 0.70mm. Legs yellow.

Wings. Hyalines; lappet and bM-Cu absent; brown apical spot extending in third and fourth sections of C. Vein R_{4+5} slightly curved posteriorly. Halters yellow. Indices: C = 3.04; ac = 3.30; hb = 0.55; 4c = 0.74; 4v = 1.65; 5x = 1.35; M = 0.32; prox. x = 0.37. Length = 1.98mm.

Abdomen. Yellow, tergite I yellow, tergite II yellow with a black band in posterior region not extending laterally, tergite III with black band reaching tergite II and extending laterally, tergite IV with medium black band touching the previous tergite, tergite V-VI with black rounded median spot not touching the previous tergite.

Terminalia. Epandrium rounded and ventral lobes small. Cerci free, elongated posteriorly; with long setae at the apex and microtricose at lateral margin and base. Hipoproctal plate present, with two medial ventral elongated processes. Surstylus S-shaped, with 6-8 prensisetae arranged in a half-moon. Postgonites (gonopods *sensu* Grimaldi, 1987) attached to the hypandrium and with two small setae on the inner margin. Small pregonites (paraphysis *sensu* Grimaldi, 1987) fused to gonopods. V-shaped hypandrium, shorter than the epandrium. Phallus (aedeagus *sensu* Grimaldi, 1987), broad in the apical region and with evident setulae in the ventral portion. Phallapodeme (aedeagal apodeme sensu Grimaldi, 1987) long and laterally broad.

#f. Unknown.

Geographic distribution: Known only from type locality.

Etymology: The species name refers to the type locality, Grajau, city of Rio de Janeiro, Rio de Janeiro, Brazil.

Discussion

The diagnostic characters we used to place *Zygothrica japunibaensis* sp. nov. in the *aldrichii* subgroup were: the pattern of dark spots on the wings (with an apical spot, spot in the apex of R_{2+3}, and sometimes with this spot joining with the mid-radial spot); brown-red, bare or short-bristled eyes; and a dark brown and bright ocellar triangle (the median frontal vita) extending towards the ptilinal suture. The species presents a medium level of hypercephaly and a distinct pattern of coloration on the abdomen. The abdomen color is similar to *Z. nigropleura*, but *Zygothrica japunibaensis* sp. nov. presents tergites III and VI lighter while in *Z. nigropleura* all tergites are entirely black-brown. The phallus is also similar to *Z. nigropleura*, but the distiphallal scales are absent and the length of the phallapodeme is smaller in relation to the phallus in *Z. japunibaensis* sp. nov.

There are a number of consistent diagnostic characters for the *vittatifrons* group, and these were used to assign *Z. grajau* sp. nov. to this group (Burla, 1956). The characters are: a yellow to brown thorax with 4 longitudinal dark brown to black stripes; the presence of another dark band laterally, which is interrupted by a transverse suture; and slightly brownish wings with dark spots. These later two characters are consistent and were the basis for us to assign *Z. grajau* sp. nov. to this group (Burla, 1956). *Zygothrica grajau* sp. nov. is similar to *Z. vittipoecila* and *Z. vittisecta*, both of which are already registered in Brazil but which have not been assigned to any group. They largely coincide in body morphology, especially in terms of the pattern of coloration of the thorax and wings. Comparing *Z. grajau* sp. nov. with the original descriptions of *Z. vittipoecila* and *Z. vittisecta* (Burla, 1956), we note the following distinctions: (1) differences in the color of the abdomen, as the yellow tergites II and III have a black band on the posterior margin of the tergite, extended laterally but not reaching the margin, and the other tergites have a dark band only in the median region of tergites; (2) slightly straighter and narrower phallapodeme when compared to *Z. vittipoecila*; (3) the phallapodeme is similar in length to the phallus, the angle between the ventral rod and the phallus is small and the surstyli have a smaller number of prensisetae, while in *Z. vittisecta* the cerci are more elongated, the phallapodeme is shorter than the phallus and the angle between the ventral rod and the phallus is bigger, and the prensisetae in the surstyli are more numerous.

Zygothrica duovittata sp. nov. and *Z. orbitalis* (Sturtevant, 1916) are cryptic species within the *orbitalis* group, and the most evident differences are in male terminalia. Both *Z. orbitalis* and *Z. duovittata* sp. nov. have red eyes, acrostichal setulae of between 8-10 lines, brown bristles and dark longitudinal stripes on the thorax. Furthermore, there is no distinction between the color intensity of these bands in the descriptions of the two species (Burla, 1956; Sturtevant, 1916). However, the phallus differs between the species. In *Z. duovittata* sp. nov. it is more rounded distally, with a more pronounced presence of scales and with a more elongated projection of the apex of aedeagus than that of *Z. orbitalis*.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution statement

All authors participated in writing the manuscript and wrote the first draft of the manuscript as part of MFM’ M.Sc dissertation at the Universidade Federal de Pelotas, RS, Brazil. M.Sc MFM and Dr. MSG identified the insects.

In this study, we identify specimens collected in several localities from Brazil that have been deposited in the Entomological Collection of the Instituto Oswaldo Cruz (CEIOC), RJ, Brazil.

Ethics approval consent to participate

This research followed the guidelines specified by the research permits from the Entomological Collection of Instituto Oswaldo Cruz (CEIOC), RJ, Brazil.

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Figure caption

Figure 1 *Zygothrica japunibaensis* sp. nov., Holotype #m. Lateral view. Scale bar: 1.0 mm.

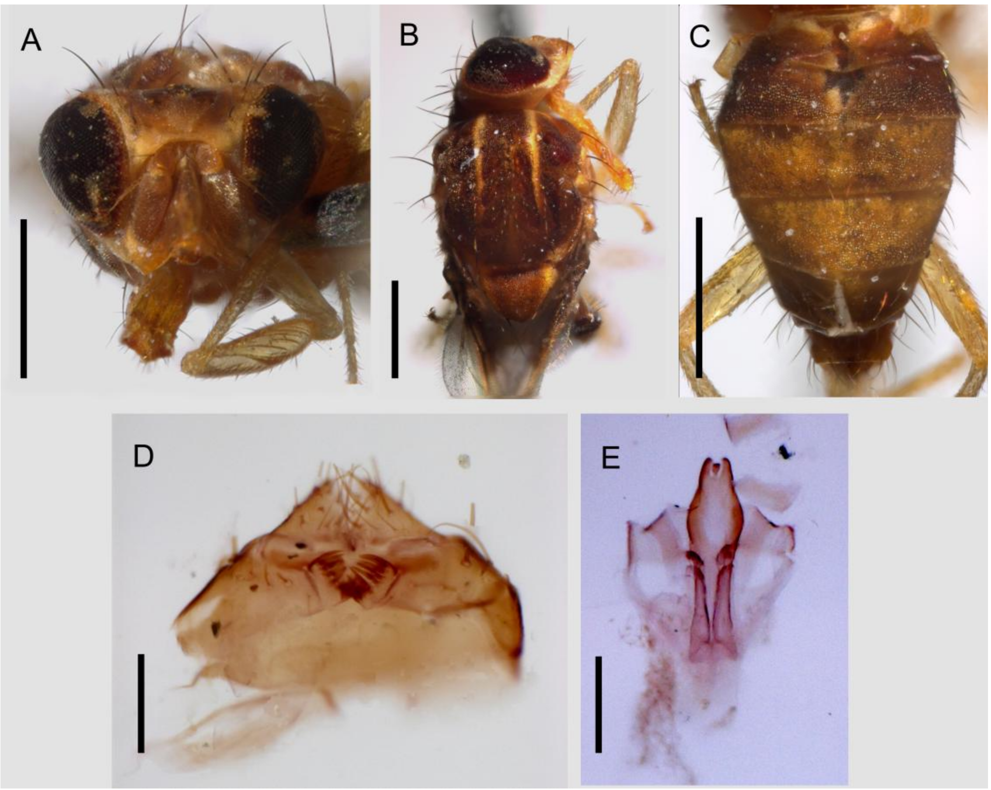

Figure 2 *Zygothrica japunibaensis* sp. nov., Holotype #m. a: head, front view; b: thorax, dorsal view; c: abdomen, dorsal view; d: epandrium, cerci, surstyli, and subepandrial sclerite, posteroventral view; e: phallus, phallapodeme, hypandrium, pregonites, and postgonites, ventral view. Scale bars: a, b, c: 0.5 mm; d, e: 0.1 mm.

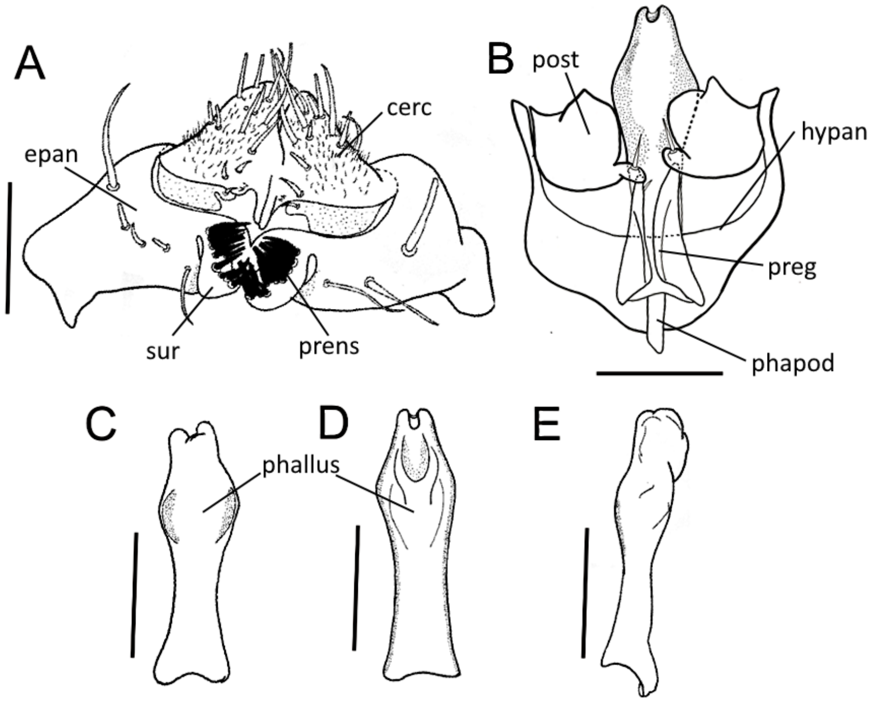

Figure 3 *Zygothrica japunibaensis* sp. nov., Holotype #m. Male terminalia. a: epandrium, cerci, surstyli, and subepandrial sclerite, posterior view; b: phallus, phallapodeme, hypandrium, pregonites, and postgonites, ventral view; c-e: phallus and phallapodeme, dorsal, ventral, and oblique lateral views, respectively. Scale bars: 0.1 mm. *Abbreviations*: cerc, cercus; epan, epandrium; post, postgonite; hypan, hypandrium; phapod, phallapodeme; sur, surstylus; prens, prensisetae.

Figure 4 *Zygothrica duovittata* sp. nov., Holotype #m. Lateral view. Scale bars: 1.0 mm.

Figure 5 *Zygothrica duovittata* sp. nov., Holotype #m. a: head, frontal view; b: thorax, dorsal view; c: epandrium, cerci, surstyli and subepandrial sclerite, ventroposterior view; d: phallus, phallapodeme, and postgonites, lateral view. Scale bars: a-b: 0.5 mm; c-d: 0.1 mm.

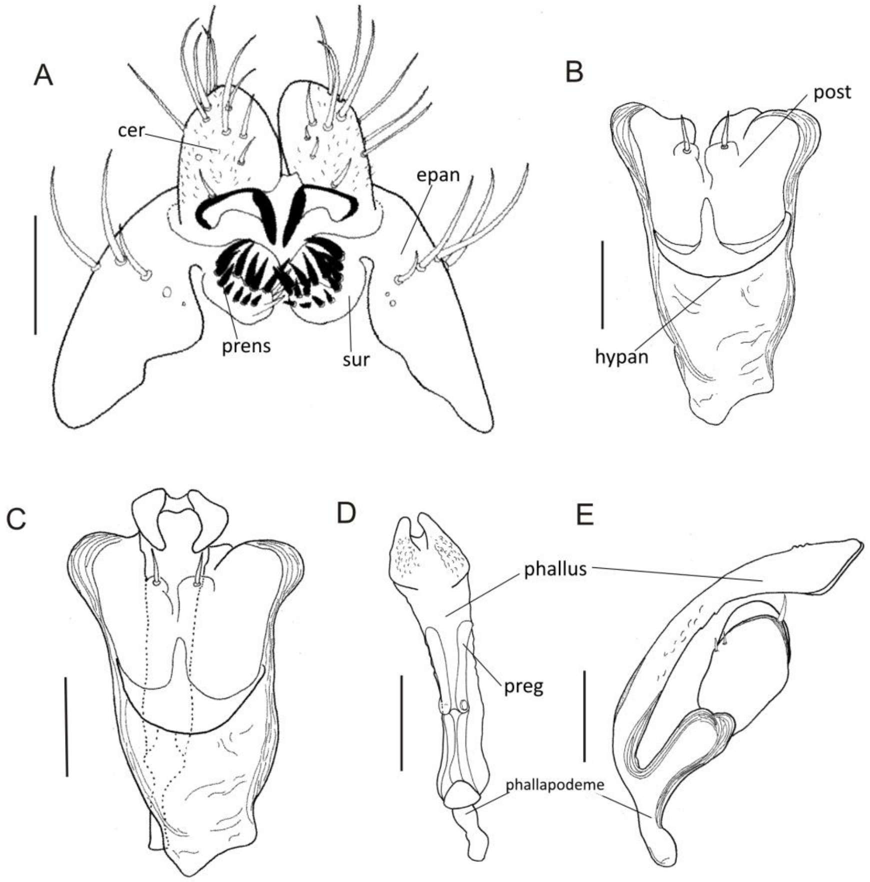

Figure 6 *Zygothrica duovittata* sp. nov., Holotype #m. Male terminalia. a: epandrium, cerci, surstyli, and subepandrial sclerite, posterior view; b-c: phallus, phallapodeme, hypandrium, pregonites, and postgonites, ventral view; d-e: phallus and phallapodeme, ventral and lateral views, respectively. Scale bars: 0.1 mm. *Abbreviations*: cerc, cercus; epan, epandrium; post, postgonite; preg, pregonite; hypan, hypandrium; sur, surstylus; prens, prensisetae.

Figure 7 *Zygothrica grajau* sp. nov., Holotype #m. Lateral view. Scale bars: 1.0 mm.

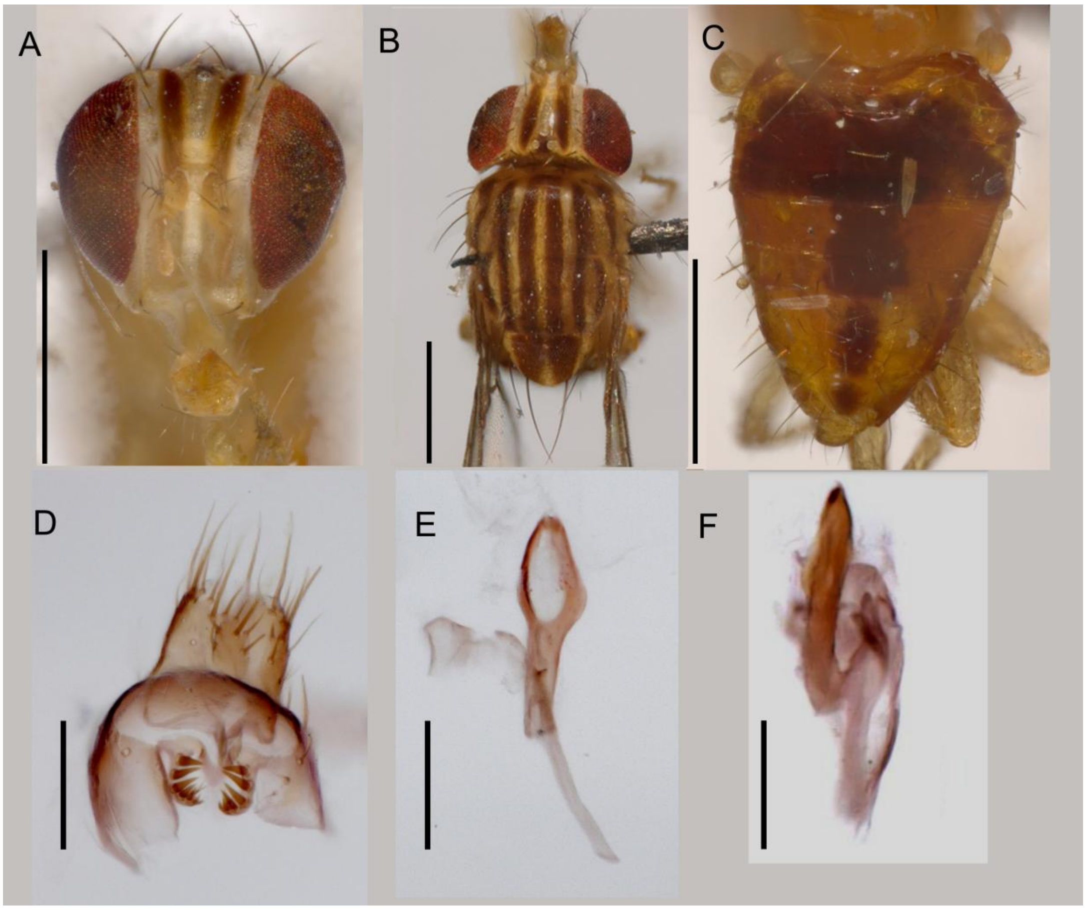

Figure 8 *Zygothrica grajau* sp. nov., Holotype #m. a: head, frontal view; b: thorax, dorsal view; c: abdomen, dorsal view; d: epandrium, cerci, surstyli, and subepandrial

sclerite, posteroventral view; e-f: phallus, phallapodeme, and postgonites, ventral view; f: phallus, phallapodeme, and postgonites, ventral view. Scale bars: a-c: 0.5 mm; d-f: 0.1 mm.

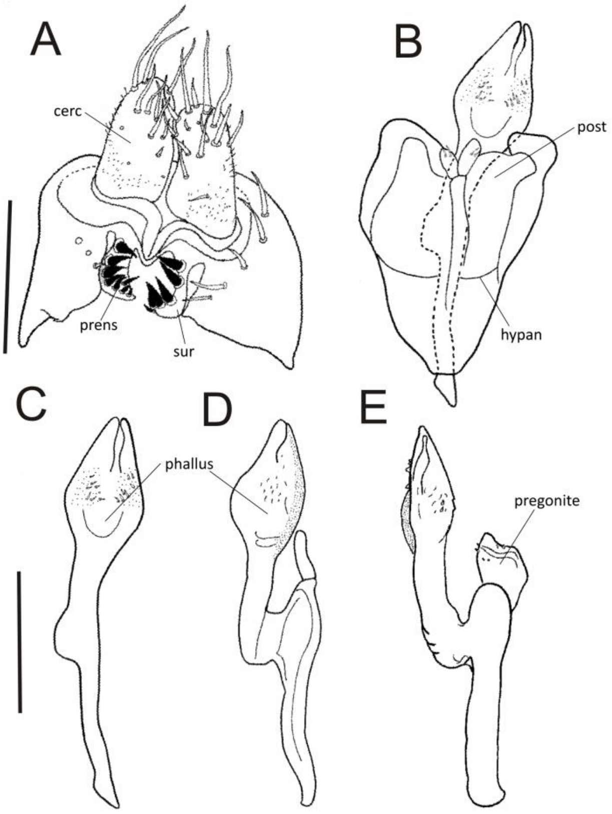

Figure 9 *Zygothrica grajau* sp. nov., Holotype #m. Male terminalia. a: epandrium, cerci, surstyli, and subepandrial sclerite, posteroventral view; b: hypandrium, postgonites, pregonites, phallus and phallapodeme, ventral view; c-e: phallus, phallapodeme, and postgonites in frontal, lateral, and oblique lateral view. Scale bars: 0.1 mm. *Abbreviations*: cerc, cercus; post, postgonite; hypan, hypandrium; sur, surstylus; prens, prensisetae.

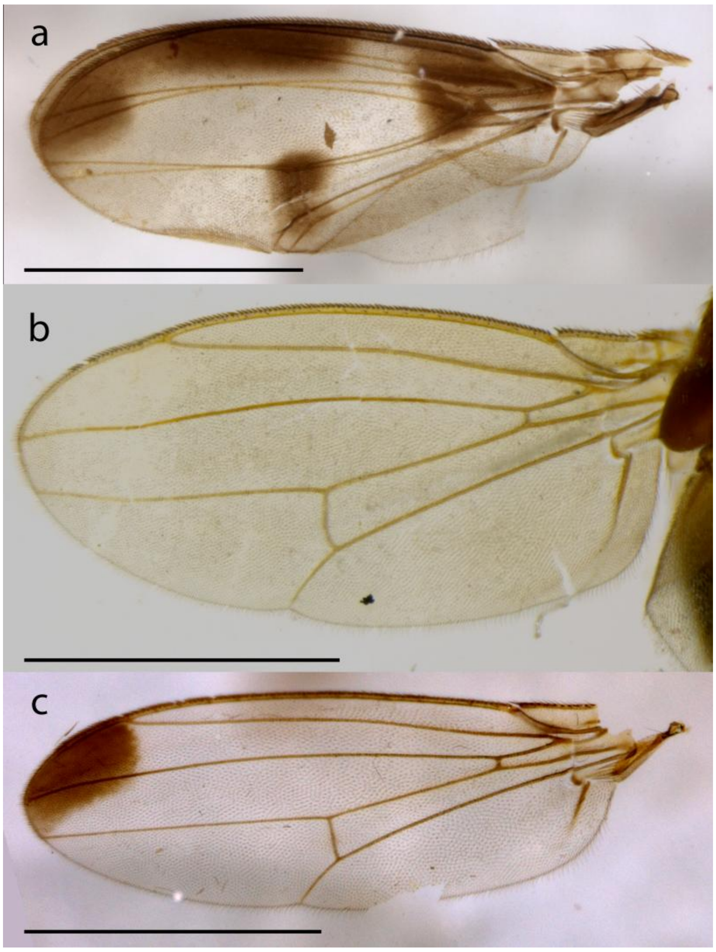

Figure 10 Wings of the new *Zygothrica* species described. a: *Zygothrica japunibaensis* sp. nov. Holotype #m.; b: *Zygothrica duovittata* sp. nov. Holotype #m.; c: *Zygothrica grajau* sp. nov. Holotype #m. Scale bars: 1.0 mm.

7.2 Apêndice II

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Two new species and a new species group of *Mycodrosophila* Oldenberg, 1914 (Diptera: Drosophilidae) from the Amazon

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Abstract

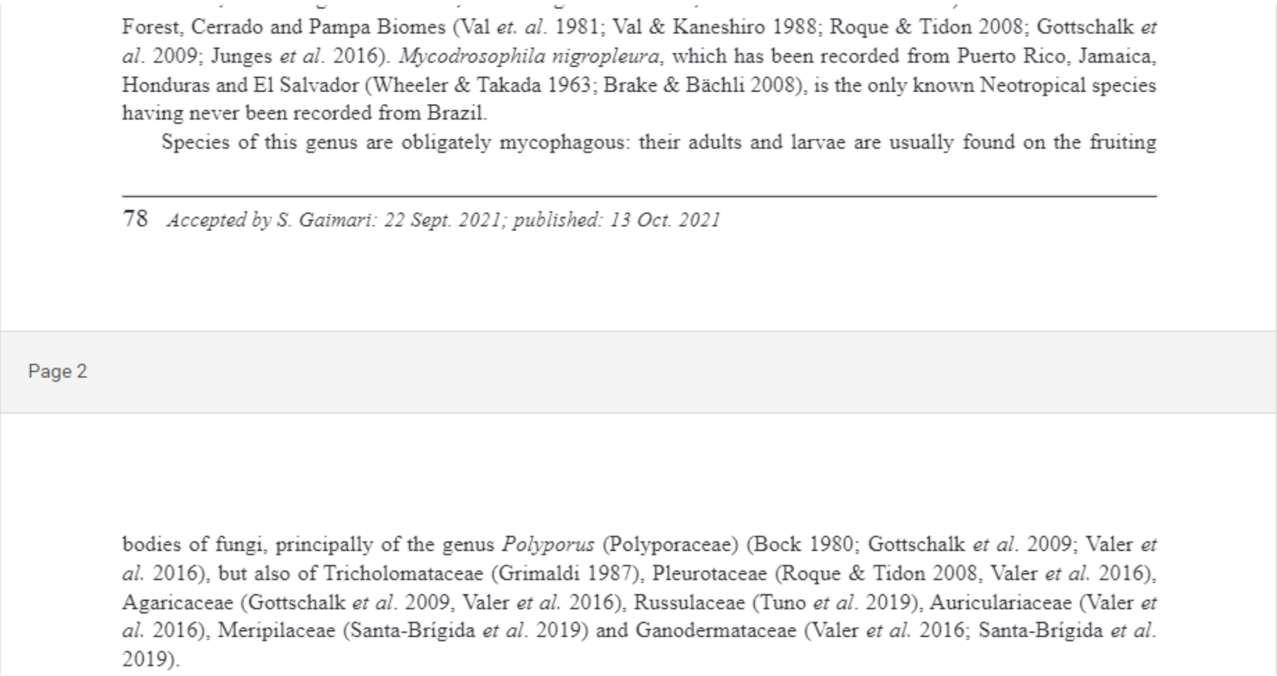
There are 130 described species in the genus *Mycodrosophila* Oldenberg, 1914, distributed across all biogeographic regions. Most of these species show essential mycophagy. Currently, ten species are known from the Neotropical Region, nine of which are found in the Amazon, Atlantic Forest, Cerrado and Pampa biomes of Brazil. In this study, we describe the adult external morphology and structures of male and/or female terminalia for two new species from the Amazon Biome of Brazil. In addition, we propose a new species group, the *Mycodrosophila neoprojectans* group, encompassing the two new species described here, together with three previously described species from the Neotropics.

Key words: Insect, Drosophilinae, taxonomy, terminalia, Neotropical Region

Introduction

There are 130 described species in the genus *Mycodrosophila* Oldenberg, 1914 (Bächli 2021), which are generally described as small flies measuring approximately 2 mm in length, and having the head with silvery frons, the dark notum, a pair of posterior dorsocentral setae (Oldenberg 1914; Wheeler & Takada 1963; Okada 1986b; Grimaldi 2010; Mendes & Gottschalk 2019), and the dark costal lappet developed on the wing in the subgenus *Mycodrosophila* but not in subgenus *Promycodrosophila* (Okada 1986b). Species of this genus occur on several continents, with a greater number of species in the Australian-Oceania Regions, SE Asia and Africa (Burla 1954; Okada 1956, 1965, 1968, 1986a, b; Bock 1980; McEvey & Polak 2005). In the Neotropical Region, ten species have been recorded: *M. brunnescens* Wheeler & Takada, 1963, *M. cornuta* Mendes & Gottschalk, 2019, *M. dimidiata* (Loew, 1862), *M. elegans* Wheeler & Takada, 1963, *M. hofmanni* Junges *et al*., 2016, *M. neoprojectans* Wheeler & Takada, 1963, *M. nigropleura* Wheeler & Takada, 1963, *M. projectans* (Sturtevant, 1916), *M. pseudoprojectans* Wheeler & Takada, 1963, and *M. valentae* Junges *et al*., 2016 (Bächli 2021).

Nine species of this genus have been found in Brazil: from the Amazon biome (Wheeler & Takada 1963; Junges *et al*. 2016; Santa-Brígida *et al*. 2017; Santa-Brígida *et al*. 2019; Mendes & Gottschalk 2019) and from the Atlantic



Forest, Cerrado and Pampa Biomes (Val *et. al.* 1981; Val & Kaneshiro 1988; Roque & Tidon 2008; Gottschalk *et al.* 2009; Junges *et al.* 2016). *Mycodrosophila nigropleura*, which has been recorded from Puerto Rico, Jamaica, Honduras and El Salvador (Wheeler & Takada 1963; Brake & Bächli 2008), is the only known Neotropical species having never been recorded from Brazil.

Species of this genus are obligately mycophagous: their adults and larvae are usually found on the fruiting

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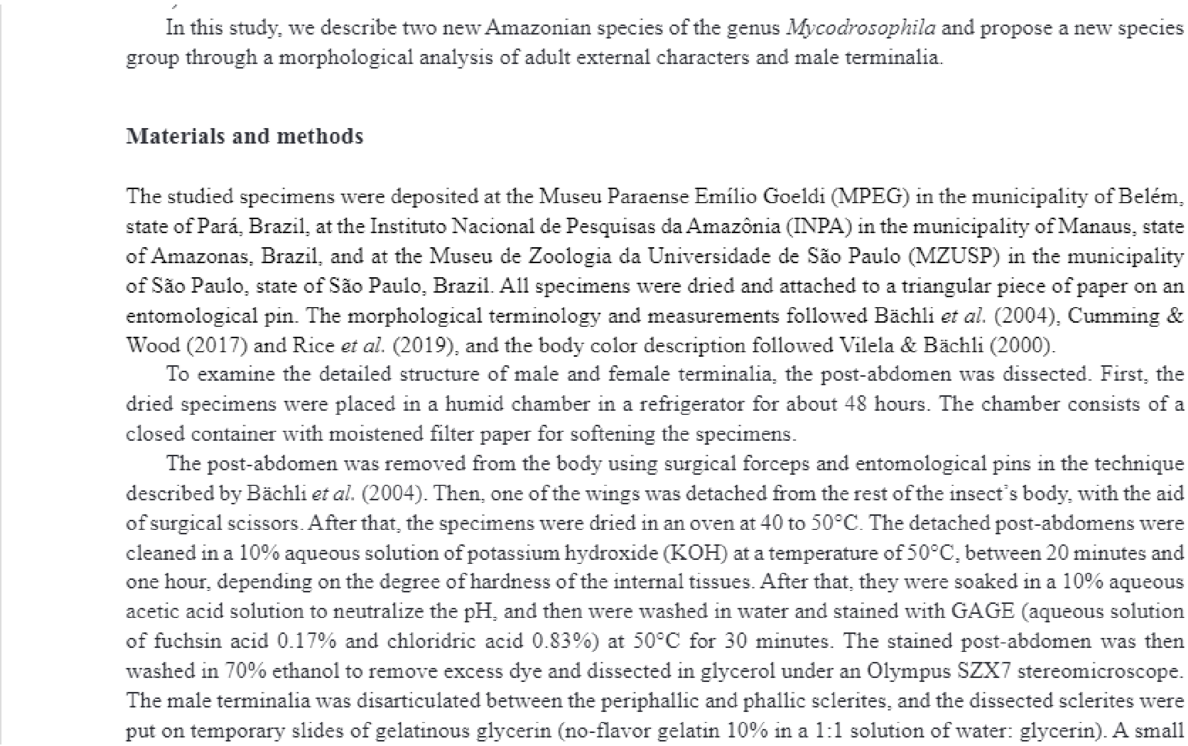
bodies of fungi, principally of the genus *Polyporus* (Polyporaceae) (Bock 1980; Gottschalk *et al.* 2009; Valer *et al.* 2016), but also of Tricholomataceae (Grimaldi 1987), Pleurotaceae (Roque & Tidon 2008, Valer *et al.* 2016), Agaricaceae (Gottschalk *et al.* 2009, Valer *et al.* 2016), Russulaceae (Tuno *et al.* 2019), Auriculariaceae (Valer *et al.* 2016), Meripilaceae (Santa-Brígida *et al.* 2019) and Ganodermataceae (Valer *et al.* 2016; Santa-Brígida *et al.* 2019).



In this study, we describe two new Amazonian species of the genus *Mycodrosophila* and propose a new species group through a morphological analysis of adult external characters and male terminalia.

Materials and methods

The studied specimens were deposited at the Museu Paraense Emílio Goeldi (MPEG) in the municipality of Belém, state of Pará, Brazil, at the Instituto Nacional de Pesquisas da Amazônia (INPA) in the municipality of Manaus, state of Amazonas, Brazil, and at the Museu de Zoologia da Universidade de São Paulo (MZUSP) in the municipality of São Paulo, state of São Paulo, Brazil. All specimens were dried and attached to a triangular piece of paper on an entomological pin. The morphological terminology and measurements followed Bächli *et al.* (2004), Cumming & Wood (2017) and Rice *et al.* (2019), and the body color description followed Vilela & Bächli (2000).

To examine the detailed structure of male and female terminalia, the post-abdomen was dissected. First, the dried specimens were placed in a humid chamber in a refrigerator for about 48 hours. The chamber consists of a closed container with moistened filter paper for softening the specimens.

The post-abdomen was removed from the body using surgical forceps and entomological pins in the technique described by Bächli *et al.* (2004). Then, one of the wings was detached from the rest of the insect's body, with the aid of surgical scissors. After that, the specimens were dried in an oven at 40 to 50°C. The detached post-abdomens were cleaned in a 10% aqueous solution of potassium hydroxide (KOH) at a temperature of 50°C, between 20 minutes and one hour, depending on the degree of hardness of the internal tissues. After that, they were soaked in a 10% aqueous acetic acid solution to neutralize the pH, and then were washed in water and stained with GAGE (aqueous solution of fuchsin acid 0.17% and chloridric acid 0.83%) at 50°C for 30 minutes. The stained post-abdomen was then washed in 70% ethanol to remove excess dye and dissected in glycerol under an Olympus SZX7 stereomicroscope. The male terminalia was disarticulated between the periphallic and phallic sclerites, and the dissected sclerites were put on temporary slides of gelatinous glycerin (no-flavor gelatin 10% in a 1:1 solution of water: glycerin). A small put on temporary slides of gelatinous glycerin (no-flavor gelatin 10% in a 1:1 solution of water: glycerin). A small amount of gelatinous glycerin was put on a slide and heated to at least 60°C. When the gelatinous glycerin melted the sclerites were positioned in the gelatin under a stereomicroscope, and covered with a coverslip before the gelatin became viscous. The detached wing was mounted in Canadian balm on a permanent slide.

Measurements of the external morphology were performed using a Zeiss Discovery V.20 stereomicroscope equipped with the *Axiohome* system. The following characters were measured for the holotypes and/or paratypes: body length (lateral view, from the face to the terminalia), wing length (from the insertion of the wing in the thorax to the apex of R_{4+5}), wing width (smaller width near the middle of C to near the apex of CUA_1), distances between orbital setae 1 and 2, between 1 and 3, and between 2 and 3; and wing indices C (CII/CIII, CII = distance between the subcostal break and the apex of the R_{2+3}; CIII = distance between the apex of R_{2+3} and the apex of the R_{4+5}), ac (CIII/CIV, CIV = distance between the apex of R_{4+5} and the apex of M_1), hb (section of CIII with hard bristles/CIII), 4c (CIII/MIII, MIII = distance between the intersection of the M_1 and r-m and the intersection between M_1 and dm-cu), 4v (MIV/MIII, MIV = distance between the intersection of the M_1 and dm-cu and the apex of the M_1), 5x (CUA_1/dm-cu, CUA_1 = distance between the apex of CUA_1 and the intersection of the CUA_1 and dm-cu; dm-cu = dm-cu length), M (CUA_1/dm-cu) and prox. x (R_{4+5}/MIII, R4+5 = distance between the intersection of R_{2+3} and R_{4+5} and the intersection of R_{4+5} and r-m). The measurements of the paratypes were present as mean ± standard deviation and, in parentheses, the lowest and highest values.

Microphotographs of the external morphology were taken with the *Axiohome* system installed in the stereomicroscope. Several images were taken at different depths of focus and later merged into a multifocal image using the program AxioVision SE64 Rel. 4.9.1. Photos of the terminalia sclerites were taken with a Samsung Galaxy S10e cell phone (model SM-970F) attached to one of the eyepieces of an Olympus BX51 microscope, with the camera configured in professional mode, auto focus turned off and maximum resolution selected (RAW file format). Several images taken at different depths of focus were merged into a multifocal image with the C method of the Helicon Focus 7.6.6 Pro program (www.heliconsoft.com).

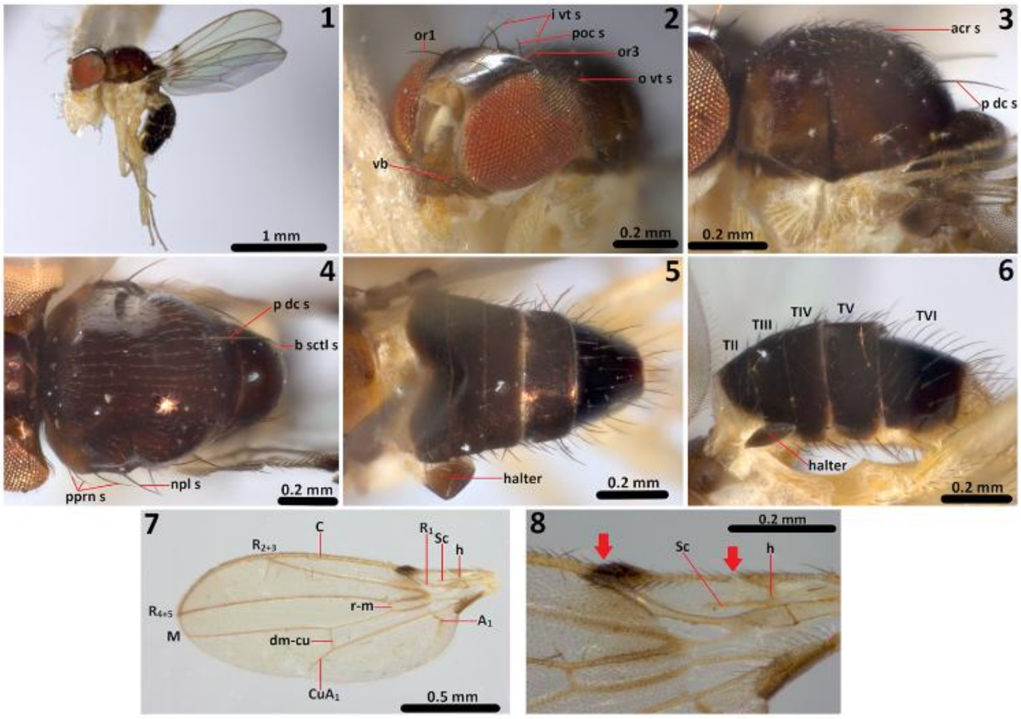
Results

Mycodrosophila (*Mycodrosophila*) *amazonica* n. sp.

(Figs 1–22)

Type material. Holotype ♂, code 03-MYCORO, labeled as follow: "Alto Paraíso, RO [= state of Rondônia], 26/X/2–XI/1985, MZ-Polonoroeste" (MZUSP). Holotype condition: head stuck to a paper triangle attached to the specimen, aristae and flagellomeres 1 missing, right and left wings removed and set between coverslips attached to the specimen, abdomen dissected, and terminalia sclerites stored in a microtube with glycerin attached to the specimen.

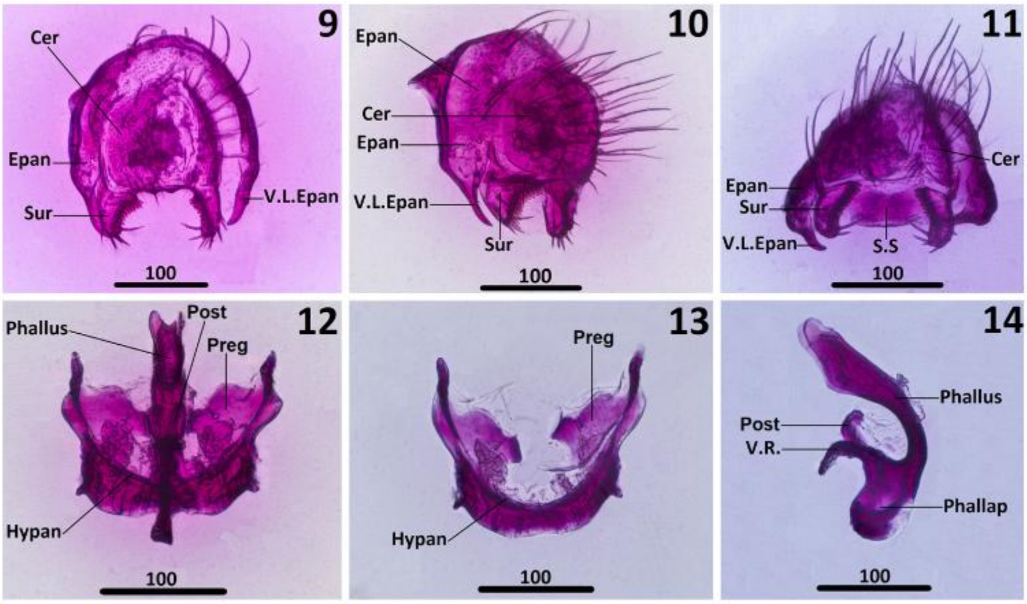
Paratypes: (06♂, 05♀), code: ♂02-MYCOPA, ♂03-MYCOPA, ♂04-MYCOPA, ♂06-MYCOPA labeled as follow: "Belém, PA [= state of Pará], Brasil, Parq. Utinga [Parque Estadual do Utinga], Tronco Podre [= rotting fallen log], 27.IX.2016, F.S. Carvalho Filho [collector]", 1°25'19.4"S, 48°26'25.4"W (MPEG); ♂08-MYCOPA, ♂10-MYCOPA, ♀12-MYCOPA labeled as follow: "Tailândia, PA [= state of Pará], Brasil, Área da Agropalma, 10.VII.2017, F.S.Carvalho-Filho [collector]" (MPEG); ♀24-MYCOPA, labeled as follow: "Barreirinhas, PA. [= state of Pará], Rio Tapajós. [= Tapajós River], Brasil, X–XI. 1970, Exp. Perm. Amaz." (MPEG); ♀06-MYCORR labeled as follow: "Brasil Roraima, Pacaraima, 5–8-III-1988, Eq. J.A.Rafael, Armadilha de Shannon [= Shannon trap]" (INPA); ♀07-MYCORR, labeled as follow: "Brasil Roraima, Pacaraima, 5–8-III-1988, Eq. J.A.Rafael" (INPA); ♀04-MYCORO, labeled as follow: "Alto Paraíso, RO [= state of Rondônia], 26/X/2–XI/1985, MZ-Polonoroeste" (MZUSP).



FIGURES 1–8. Holotype (♂03-MYCORO) of *Mycodrosophila amazonica* **n. sp.** 1, Habitus (lateral view); 2, head (fronto-lateral view); 3, thorax (lateral view); 4, thorax (dorsal view); 5, abdomen (dorsal view); 6, abdomen (lateral view); 7–8, wing. Abbreviations: **or1**, orbital seta 1; **or3**, orbital seta 3; **i vt s**, inner vertical seta; **poc s**, postocellar seta; **o vt s**, outer vertical seta; **vb**, vibrissa; **acr s**, acrostichal setula; **p dc s**, posterior dorsocentral seta; **b sctl s**, basal scutellar seta; **pprn s**, postpronotal seta; **npl s**, notopleural setae; **TII**, tergite 2; **TIII**, tergite 3; **TIV**, tergite 4; **TV**, tergite 5; **TVI**; tergite 6; **red arrows**, costal breaks.

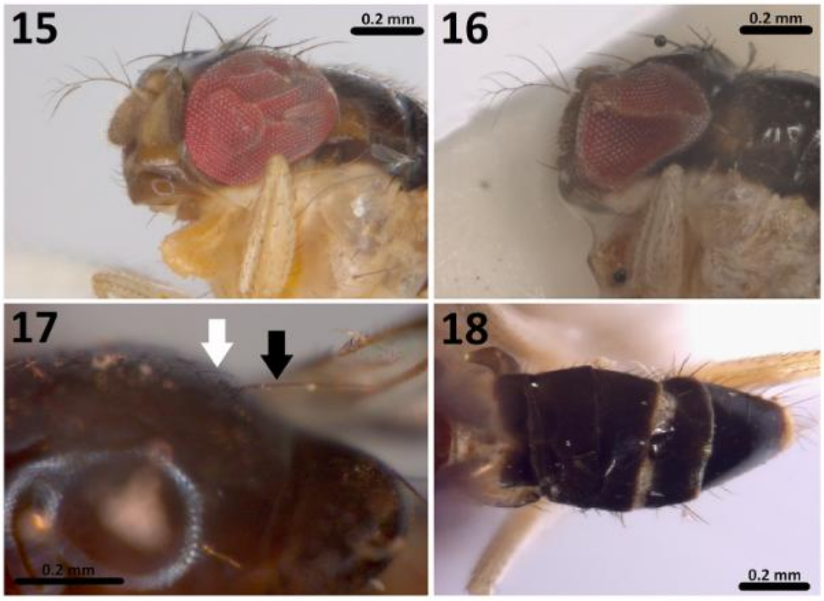
Paratype condition: ♂02-MYCOPA, right arista missing, midlegs missing, left wing removed and mounted between coverslips attached to the specimen, abdomen dissected, and terminalia sclerites stored in a microtube with glycerin attached to the specimen; ♂03-MYCOPA, aristae missing, abdomen dissected, and terminalia sclerites stored in a microtube with glycerin attached to the specimen; ♂04-MYCOPA, right arista missing, hindlegs missing, left wing removed and mounted between coverslips attached to the specimen, abdomen dissected, and terminalia sclerites stored in a microtube with glycerin attached to the specimen; ♂06-MYCOPA, head missing, right wing missing, and left wing removed and mounted between coverslips attached to the specimen; ♂08-MYCOPA, aristae missing, left wing removed and mounted between coverslips attached to the specimen, abdomen dissected, and terminalia sclerites stored in a microtube with glycerin attached to the specimen; ♂10-MYCOPA, head and right wing stuck to a paper triangle attached to the specimen, aristae missing, and legs missing; ♀12-MYCOPA, head, forelegs, and wings missing, abdomen dissected, and spermatheca and ovipositor stored in a microtube with glycerin attached to the specimen; ♀24-MYCOPA, aristae missing, left wing removed and mounted between coverslips attached to the specimen, abdomen dissected, and spermatheca and ovipositor stored in a microtube with glycerin attached to the specimen; ♀06-MYCORR, aristae missing, fore and hindlegs missing, left wing missing, abdomen dissected, and spermathecae and ovipositor stored in a microtube with glycerin attached to the specimen; ♀07-MYCORR, left arista missing; ♀04-MYCORO, head and right wing stuck to a paper triangle attached to the specimen, aristae missing, hindlegs missing, left wing removed and mounted between coverslips attached to the specimen, abdomen dissected, and spermathecae and ovipositor stored in a microtube with glycerin attached to the specimen.

Etymology. The specific epithet "*amazonica*" alludes to the wide distribution of the new species in the Amazon region.



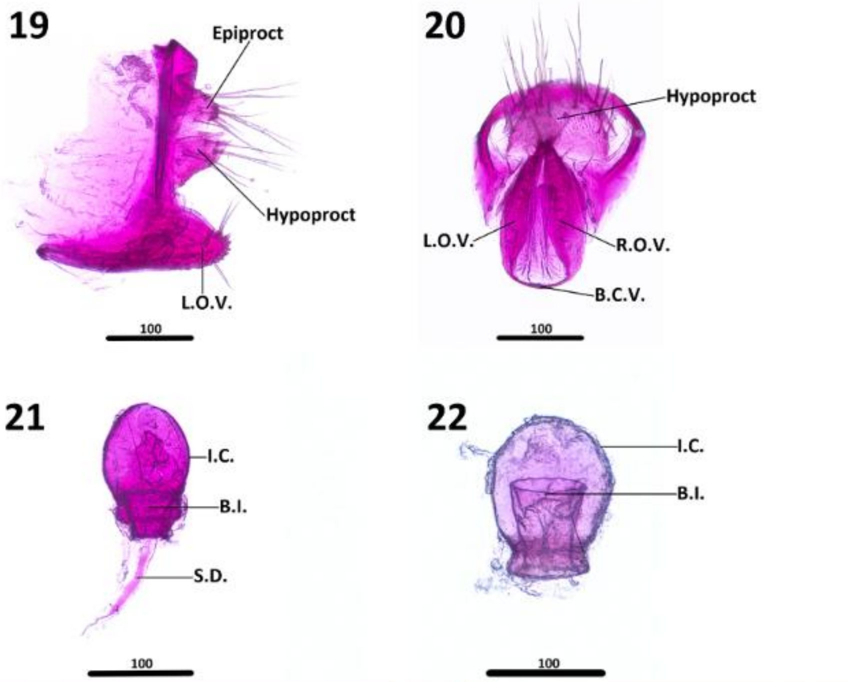
FIGURES 9–14. Holotype (♂03-MYCORO) of *Mycodrosophila amazonica* **n. sp.** Male terminalia: 9, epandrium, surstyli and cerci (posterior view); 10, ditto (postero-lateral view); 11, epandrium, surstyli, cerci and subepandrial sclerite (ventral view); 12, phallus, hypandrium, pregonites and postgonites (ventral view); 13, hypandrium and pregonites (ventral view); 14, postgonite, ventral rod, phallus and phallapodeme (lateral view). Abbreviations: **Cer**, cerci; **Epan**, epandrium; **Sur**, surstylus; **V.L.Epan**, ventral lobe of epandrium; **S.S**, subepandrial sclerite; **Hypan**, hypandrium; **Preg**, pregonite; **Post**, postgonite; **V.R.**, ventral rod; **Phallap**, phallapodeme.

Diagnosis (Figs 1–8). Combination of the following characteristics: apparently the anterior dorsocentral setae reduced; scutellum shiny; wing apex (region of intersection between C and R_{4+5}) slightly darkened; abdomen with TII–TIII and TIV–TV with a clear trapezoid-shaped light lateral spot; epandrium with 5–6 upper and 4–6 lateral setae; ventral lobe approximately the same length as the surstylus, glabrous, and presenting 3–4 long setae; surstylus connected to the epandrium, with 11 prensisetae arranged in a line, 4 inner setae and 4 outer setae; phallus tubular and projected anteriorly, 2x narrower in basiphallus than in distiphallus and the narrowed section about 1/2 of the phallus length in lateral view.



FIGURES 15–18. Paratypes of *Mycodrosophila amazonica* **n. sp.** 15—head (♀07-MYCORR, antero-lateral view); 16—head (♂02-MYCOPA, lateral view); 17—thorax: white arrow indicating anterior dorsocentral seta; black arrow, posterior dorsocentral seta (♂10-MYCOPA, dorso-lateral view); 18—abdomen (♂02-MYCOPA, dorsal view).

Description. Holotype ♂ (Code 03-MYCORO, Figs 1–14). **Head** (Fig. 1–2). Dark brown; eyes reddish and glabrous; facial carina light brown, prominent, "nose-like"; pedicel and scape light brown; flagellomeres 1 missing; arista missing; orbital plates dark brown, lighter behind orbital seta 3 (or3); occiput light brown; orbital setae dark brown; distance between or1 (proclined orbital seta*)* and or2 (anterior reclined orbital seta) = 0.03 mm, between or1 and or3 (posterior reclined orbital seta) = 0.06 mm, and between or2 and or3 = 0.03 mm; frons light brown, reflecting silver luster throughout; ocellar triangle dark brown, approximately 1/3 as long as the frons; face light brown; genae light; proboscis, labellum and palps light; post-ocellar setae dark brown, long and crossed. **Thorax** (Figs 1, 3 and 4). Dark brown; scutum shine dark brown, with 8 regular rows of acrostichal setulae; pleura pale yellow, with a dark brown region on the dorsal portion of the proepisternum, anepisternum and anepimeron, forming a confluent band with the coloration of the notum above the wing insertion; only the posterior pair of dorsocentral setae conspicuous, apparently the anterior dorsocentral setae reduced; scutellum dark brown, shiny; basal scutellar setae converging and distal ones missing; legs uniformly pale yellow. **Wings** (Figs 1, 7 and 8). Hyaline, with a dark spot on the second break of C and extending to the vein M; wing apex (region of intersection between C and R_{4+5}) slightly darkened; costal lappet dark and developed in the second break of C; vein R_{2+3} straight towards C; vein M slightly curved in the direction of R_{4+5}, close to the intersection with C; bm-cu absent. Halter with dark knob and light stem. Index: C = 1.20; ac = 4.46; hb = 0.63; 4c = 1.65; 4v = 2.2; 5x = 1.72; M = 0.54; prox. x = 0.36. Length 1.55 mm, width 0.69 mm. **Abdomen** (Figs 1, 5 and 6). Predominately dark; TI light; TII-VI dark, with light lateral margin which extends medially in the anterior and posterior margins of the tergite, being the light area more comprehensive in TII, TIII and TVI; TIV and TV with slight light indentation; sternites and intersegmental membranes light. **Body length:** 1.20 mm.

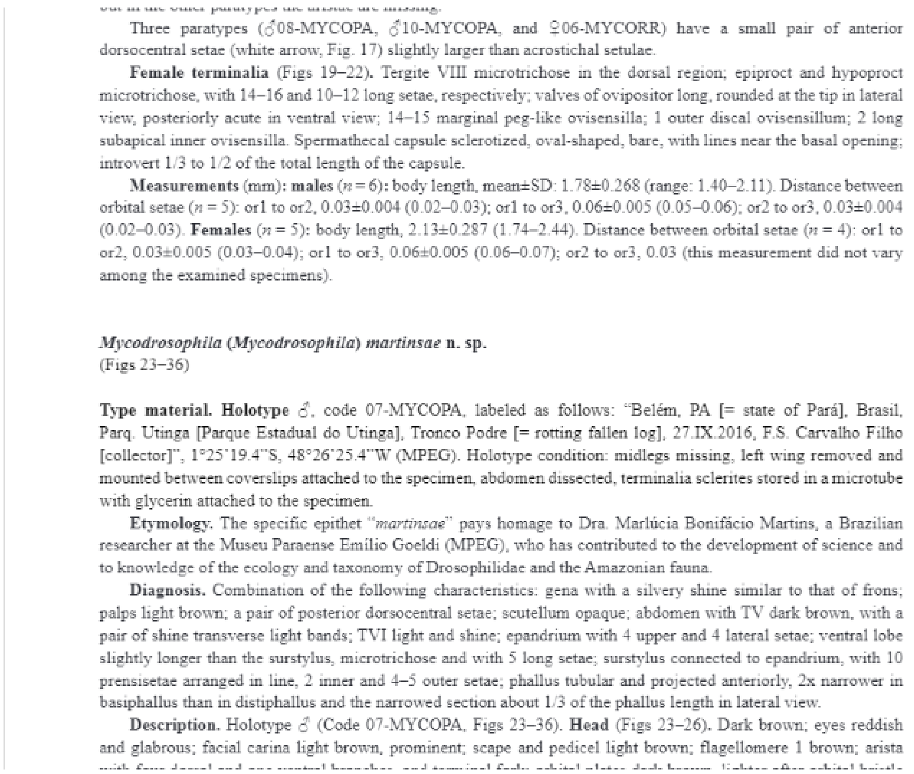


21–22, spermathecae. Abbreviations: **L.O.V.**, left oviscapt valve; **R.O.V.**, right oviscapt valve; **B.C.V.**, bridge connecting valves; **I.C.**, inner capsule; **B.I.**, basal introvert; **S.D.**, spermathecal duct.

Male terminalia (Figs 9–14). Epandrium with equal width and length, microtrichose, with 5–6 upper and 4–6 lateral setae (Epan, Figs 9–10); ventral lobe approximately the same length as the surstylus and tapered at its tip, posteriorly curved, glabrous, and presenting 3–4 long setae (V.L.Epan, Figs 9–11). Cerci free (not fused to epandrium), microtrichose and with large setae (Cer, Figs 9–11). Subepandrial sclerite wide, connecting the surstyli (S.S, Fig. 11). Surstylus connected to the epandrium, with 11 prensisetae arranged in a line, 4 inner setae and 4 outer setae (Sur, Figs 9–11). Hypandrium arc-shaped, wider than longer, almost the same length as the epandrium (Hypan, Figs 12 and 13). Pregonites large, sub-rectangular, partially fused to the hypandrium, with a seta (Preg, Figs 12 and 13). Phallus tubular and projected anteriorly, 2x narrower in basiphallus than in distiphallus (sense Grimaldi 1987), the narrowed section about 1/2 of the phallus length in lateral view; with the apical region bifurcated, indented, and bearing rows of tiny setulae its inner margin (Phallus, Figs 12 and 14). Phallapodeme flattened laterally and shorter than phallus (Phallap, Fig. 14). Ventral rod (sense Bächli *et al.* 2004) projected anteriorly and merged with

the posteromedian margin of the hypandrium (Figs 12–13). Postgonites (connected to the pregonites) elongated and anteriorly curved in lateral view, containing approximately three apical setulae, and with an internal microtrichotic surface (Post and Preg, Figs 12–14).

Remarks on male and female paratypes. Some paratypes are different from the holotype in light spots of abdominal tergites, mainly in the lateral portion of TIV and TV and/or the dorsal surface of TV (Fig. 18): eight paratypes (♂02-MYCOPA, ♂06-MYCOPA, ♂08-MYCOPA, ♂10-MYCOPA, ♀12-MYCOPA, ♀24-MYCOPA, ♀06-MYCORR, and ♀07-MYCORR) with clear dorsal patches on TV.



Three paratypes (♂08-MYCOPA, ♂10-MYCOPA, and ♀06-MYCORR) have a small pair of anterior dorsocentral setae (white arrow, Fig. 17) slightly larger than acrostichal setulae.

Female terminalia (Figs 19–22). Tergite VIII microtrichose in the dorsal region; epiproct and hypoproct microtrichose, with 14–16 and 10–12 long setae, respectively; valves of ovipositor long, rounded at the tip in lateral view, posteriorly acute in ventral view; 14–15 marginal peg-like ovisensilla; 1 outer discal ovisensillum; 2 long subapical inner ovisensilla. Spermathecal capsule sclerotized, oval-shaped, bare, with lines near the basal opening; introvert 1/3 to 1/2 of the total length of the capsule.

Measurements (mm)**: males** ($n = 6$)**:** body length, mean±SD: 1.78±0.268 (range: 1.40–2.11). Distance between orbital setae ($n = 5$): or1 to or2, 0.03±0.004 (0.02–0.03); or1 to or3, 0.06±0.005 (0.05–0.06); or2 to or3, 0.03±0.004 (0.02–0.03). **Females** ($n = 5$)**:** body length, 2.13±0.287 (1.74–2.44). Distance between orbital setae ($n = 4$): or1 to or2, 0.03±0.005 (0.03–0.04); or1 to or3, 0.06±0.005 (0.06–0.07); or2 to or3, 0.03 (this measurement did not vary among the examined specimens).

Mycodrosophila (*Mycodrosophila*) *martinsae* n. sp.

(Figs 23–36)

Type material. Holotype ♂, code 07-MYCOPA, labeled as follows: "Belém, PA [= state of Pará], Brasil, Parq. Utinga [Parque Estadual do Utinga], Tronco Podre [= rotting fallen log], 27.IX.2016, F.S. Carvalho Filho [collector]", 1°25'19.4"S, 48°26'25.4"W (MPEG). Holotype condition: midlegs missing, left wing removed and mounted between coverslips attached to the specimen, abdomen dissected, terminalia sclerites stored in a microtube with glycerin attached to the specimen.

Etymology. The specific epithet "*martinsae*" pays homage to Dra. Marlúcia Bonifácio Martins, a Brazilian researcher at the Museu Paraense Emílio Goeldi (MPEG), who has contributed to the development of science and to knowledge of the ecology and taxonomy of Drosophilidae and the Amazonian fauna.

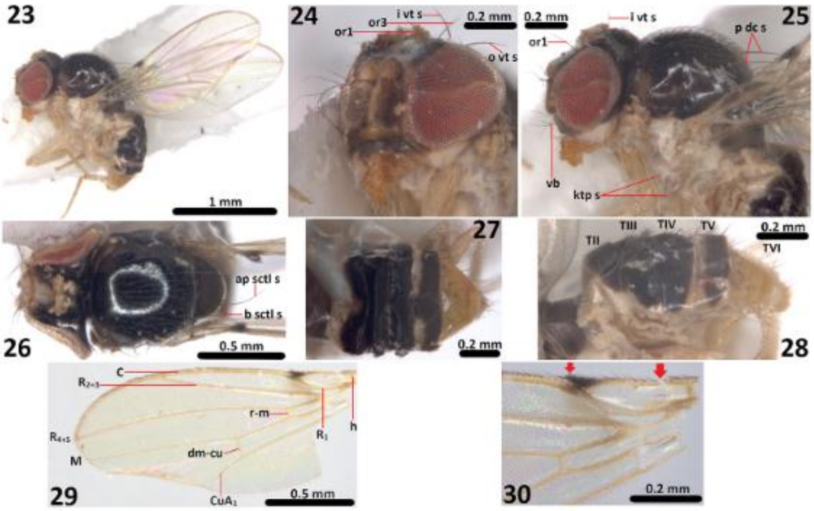
Diagnosis. Combination of the following characteristics: gena with a silvery shine similar to that of frons; palps light brown; a pair of posterior dorsocentral setae; scutellum opaque; abdomen with TV dark brown, with a pair of shine transverse light bands; TVI light and shine; epandrium with 4 upper and 4 lateral setae; ventral lobe slightly longer than the surstylus, microtrichose and with 5 long setae; surstylus connected to epandrium, with 10 prensisetae arranged in line, 2 inner and 4–5 outer setae; phallus tubular and projected anteriorly, 2x narrower in basiphallus than in distiphallus and the narrowed section about 1/3 of the phallus length in lateral view.

Description. Holotype ♂ (Code 07-MYCOPA, Figs 23–36). **Head** (Figs 23–26). Dark brown; eyes reddish and glabrous; facial carina light brown, prominent; scape and pedicel light brown; flagellomere 1 brown; arista with four dorsal and one ventral branches, and terminal fork; orbital plates dark brown, lighter after orbital bristle 3 (or3); orbital setae dark brown; distance between or1 and or2 = 0.03 mm, between or1 and or3 = 0.06 mm, and between or2 and or3 = 0.03 mm; frons light brown, reflecting silvery shine throughout; ocellar triangle dark brown, approximately 1/3 as long as the frons; occiput light brown; face light brown; gena light (with silvery brilliance similar to that on frons); proboscis and labellum light; palps light brown; postocellar setae dark brown, long and crossed. **Thorax** (Figs 23, 25 and 26). Dark brown; scutum shiny dark brown, with 8 regular rows of acrostichal setulae; pleura pale yellow, with a dark brown region in the dorsal portion of the proepisternum, anepisternum and anepimeron forming a confluent band with the notum coloration above the wing insertion; a pair of posterior dorsocentral setae; scutellum opaque with converging basal and distal scutellar setae; legs uniformly pale yellow.

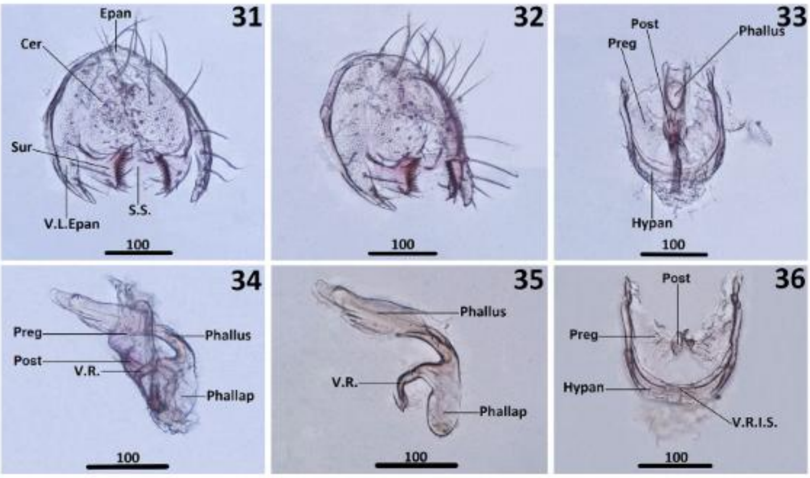
Wings (Figs 23, 29 and 30). Hyaline, stained in the second C break and extending to the vein M; costal lappet dilated and dark; vein R_{2+3} straight towards C; veins R_{4+5} and M slightly converging near the apex of the wing; bm-cu absent; halter light on the connecting stem, knob damaged; index: C = 1.22, ac = 4.35, hb = 0.60, 4c = 1.90, 4v = 2.65, 5x = 2.6, M = 0.81, prox. x = 0.59. Length 1.60 mm, width 0.72 mm. **Abdomen** (Figs 23, 27 and 28). Dark brown; TI light; TII dark brown, with slight dorsal median indentation and light trapezoidal spot on lateral portion; TIII–V dark brown with a thin light marginal spot; TIV and V with a light, right-angled triangular spot on the side, TV with a pair of dorsal shine light spots; TVI light and shine; sternites and intersegmental membranes light. **Body length:** 2.09 mm.

Male terminalia (Figs 31–36). Epandrium with equal width and length, microtricose, with 4 upper and 4 lateral setae (Epan, Figs 31 and 32); ventral lobe slightly longer than the surstylus, tapering apically, microtrichose and with 5 long setae (V.L.Epan, Figs 31 and 32). Cerci free (not fused to epandrium), microtrichose, with large setae (Cer, Figs 31 and 32). Subepandrial sclerite wide, connected to the surstyli (S.S., Fig. 31). Surstylus connected to epandrium, with 10 prensisetae arranged in line, 2 inner and 4–5 outer setae (Sur, Figs 31 and 32). Hypandrium arc-shaped, as wide as long, being almost the same length as the epandrium (Hypan, Figs 33 and 36). Pregonites large, about 1/3 of the phallus length in lateral view; the apical region forked and indented, with rows of tiny setulae on the inner margin (Phallus, Figs 33–35). Phallapodeme laterally flattened and shorter than phallus (Phallap, Figs 34 and 35). Ventral rod (sense Bächli *et al.* 2004) anteriorly projected and merged with the posteromedial margin of the hypandrium (Figs 33 and 36). Postgonites (connected to the pregonites) elongated and curved anteriorly in lateral view, bearing approximately three apical setulae, and microtrichose on the inner surface (Post and Preg, Figs 33, 34 and 36).

Female: unknown.



FIGURES 23–30. Holotype (♂07-MYCOPA) of *Mycodrosophila martinsae* **n. sp.** 23, Habitus (lateral view); 24, head (fronto-lateral view); 25, head and thorax, (lateral view); 26, ditto (dorsal view); 27, abdomen (dorsal view); 28, ditto (lateral view); 29–30, wing (red arrows indicating costal breaks). Abbreviations: **or1**, orbital seta 1; **or3**, orbital seta 3; **i vt s**, inner vertical seta; **o vt s**, outer vertical seta; **vb**, vibrissa; **p dc s**, posterior dorsocentral seta; **ap sctl s**, apical scutellar seta; **b sctl s**, basal scutellar seta; **ktp s**, katepisternal seta; **TII**, tergite 2; **TIII**, tergite 3; **TIV**, tergite 4; **TV**, tergite 5; **TVI**; tergite 6.



FIGURES 31–36. Holotype (♂07-MYCOPA) of *Mycodrosophila martinsae* **n. sp.** Male terminalia: 31, epandrium, surstyli and cerci (posterior view); 32, ditto (postero-lateral view); 33, phallus, hypandrium, pregonites and postgonites (ventral view); 34, pregonite, postgonite, ventral rod, phallus and phallapodeme (lateral view); 35, phallus, ventral rod and phallapodeme (lateral view); 36, pregonites, postgonites, hypandrium and ventral rod insertion scar (ventral view). Abbreviations: **Cer**, cerci; **Epan**, epandrium; **Sur**, surstylus; **V.L.Epan**, ventral lobe of epandrium; **S.S**, subepandrial sclerite; **Hypan**, hypandrium; **Preg**, pregonite; **Post**, postgonite; **V.R.**, ventral rod; **Phallap**, phallapodeme; **V.R.I.S.**, ventral rod insertion scar.

Mycodrosophila neoprojectans species group, new group

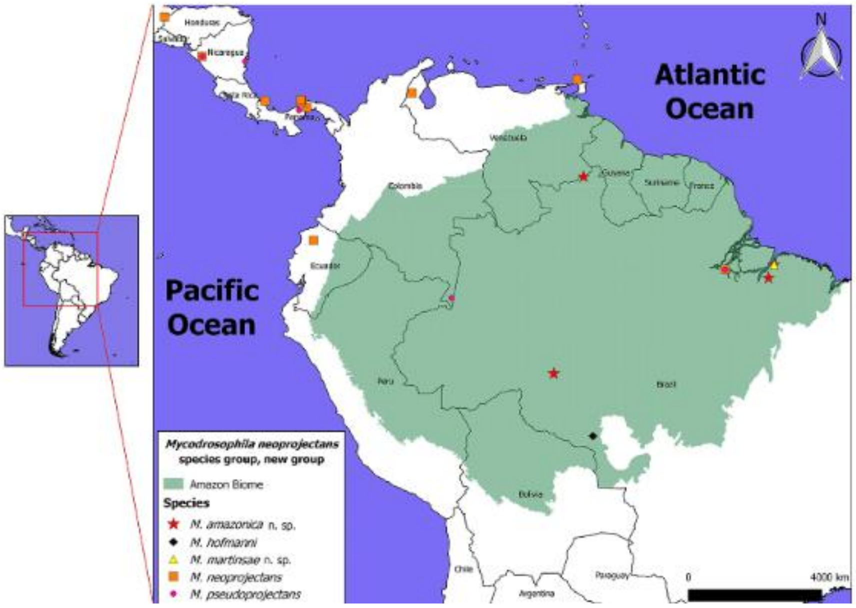
Species included and geographical distribution (Fig. 37). *Mycodrosophila neoprojectans* Wheeler & Takada, 1963: Panama, Nicaragua, Honduras, Costa Rica, Trinidad, and Ecuador (Wheeler & Takada 1963); Brazil (Amazon Biome; Santa-Brigida *et al.* 2019). *Mycodrosophila pseudoprojectans* Wheeler & Takada, 1963: Nicaragua, Panama, and Colombia (Wheeler & Takada 1963); Brazil (Amazon Biome; Santa-Brígida *et al.* 2019). *Mycodrosophila hofmanni* Junges, Gottschalk, Loreto & Robe, 2016: Brazil (Amazon Biome; Junges *et al.* 2016). *Mycodrosophila amazonica* **n. sp.**: widely distributed in the northern region of Brazil (Amazon Biome). *Mycodrosophila martinsae* **n. sp.**: Brazil (Amazon Biome).

Diagnosis. Small flies; tergites II to IV (TII–TIV) largely black but at least partly light; dark spot below the 2nd C break extending to the vein M; dark spots at the apices of R_{2+3} and R_{4+5} absent (except *M. amazonica* **n. sp.** which was slightly darkened apically), ventral lobe of the epandrium tapering apically; phallus projected anteriorly in side view, with basiphallus narrower than distiphallus in lateral view (sense Grimaldi 1987).

Key to the species of the *Mycodrosophila neoprojectans* species group

1 Tergites V and VI mostly black, except for small light spots on lateral margins; wing tip with faint darking; basiphallus 2x narrower than distiphallus and about 1/2 of the phallus length. .... *M. amazonica* **n. sp.**
\- Tergites V and/or II to VI with more or less large light area(s); wing tip without darking 2
2 Tergite VI largely black; acrostichal setulae in 10 rows; basiphallus about 1.5x narrower than distiphallus and about 1/2 of the phallus length .... *M. pseudoprojectans*
\- Tergite VI largely light; acrostichal setulae in 8 rows. 3

3 Tergite VI without a median dark spot; basiphallus 2x narrower than distiphallus and about 1/3 of the phallus length. .... *M. martinsae* **n. sp.**
\- Tergite VI with median dark spot. 4
4 Halter with dark knob and light base and stem; basiphallus about 1.5x narrower than distiphallus and about 1/2 of the phallus length; postgonite 2x longer than wider in lateral view .... *M. neoprojectans*
\- Halter fully dark; basiphallus about 1.5x narrower than distiphallus and about 1/3 of the phallus length; postgonite 3x longer than wider and backward curved in lateral view .... *M. hofmanni*



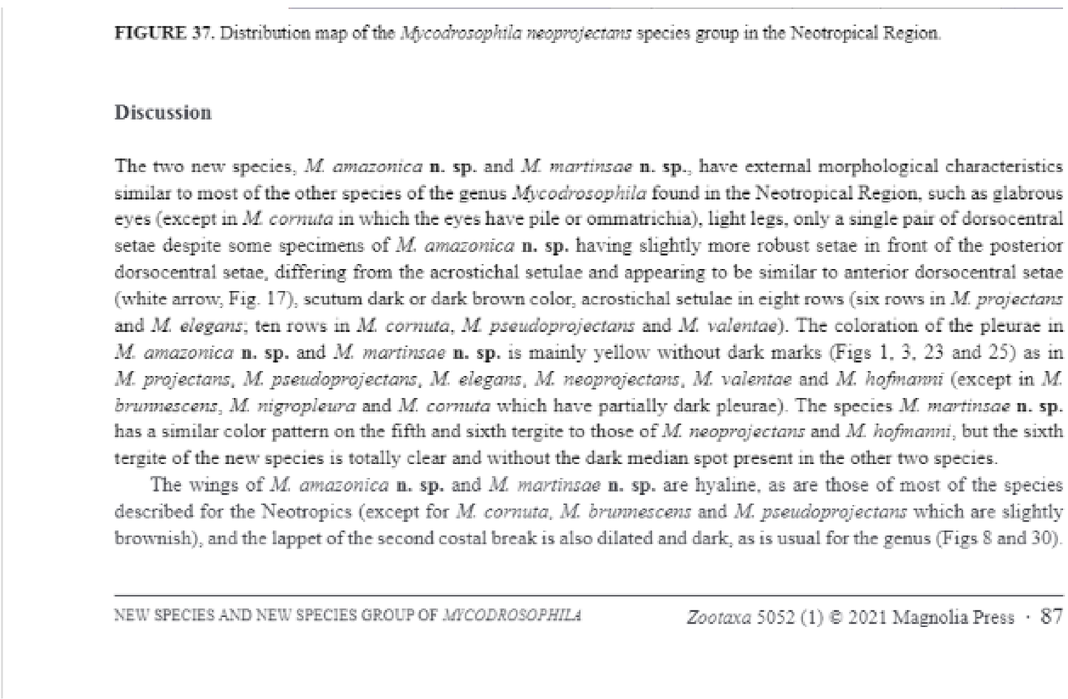
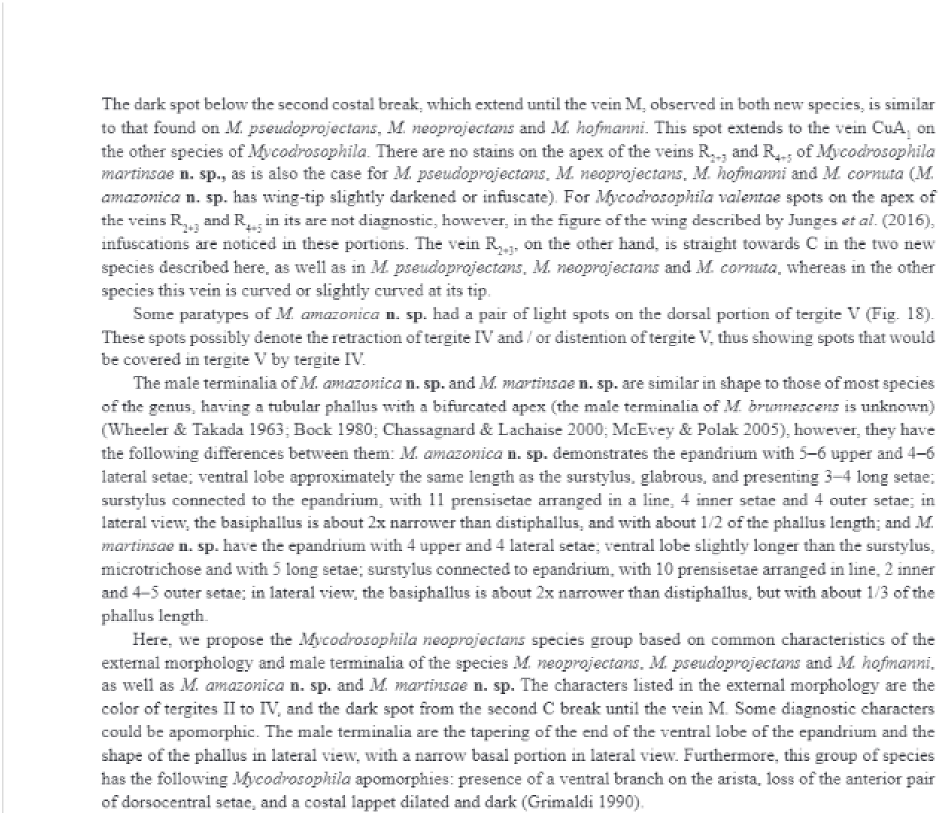


FIGURE 37. Distribution map of the *Mycodrosophila neoprojectans* species group in the Neotropical Region.

Discussion

The two new species, *M. amazonica* **n. sp.** and *M. martinsae* **n. sp.**, have external morphological characteristics similar to most of the other species of the genus *Mycodrosophila* found in the Neotropical Region, such as glabrous eyes (except in *M. cornuta* in which the eyes have pile or ommatrichia), light legs, only a single pair of dorsocentral setae despite some specimens of *M. amazonica* **n. sp.** having slightly more robust setae in front of the posterior dorsocentral setae, differing from the acrostichal setulae and appearing to be similar to anterior dorsocentral setae (white arrow, Fig. 17), scutum dark or dark brown color, acrostichal setulae in eight rows (six rows in *M. projectans* and *M. elegans*; ten rows in *M. cornuta*, *M. pseudoprojectans* and *M. valentae*). The coloration of the pleurae in *M. amazonica* **n. sp.** and *M. martinsae* **n. sp.** is mainly yellow without dark marks (Figs 1, 3, 23 and 25) as in *M. projectans*, *M. pseudoprojectans*, *M. elegans*, *M. neoprojectans*, *M. valentae* and *M. hofmanni* (except in *M. brunnescens*, *M. nigropleura* and *M. cornuta* which have partially dark pleurae). The species *M. martinsae* **n. sp.** has a similar color pattern on the fifth and sixth tergite to those of *M. neoprojectans* and *M. hofmanni*, but the sixth tergite of the new species is totally clear and without the dark median spot present in the other two species.

The wings of *M. amazonica* **n. sp.** and *M. martinsae* **n. sp.** are hyaline, as are those of most of the species described for the Neotropics (except for *M. cornuta*, *M. brunnescens* and *M. pseudoprojectans* which are slightly brownish), and the lappet of the second costal break is also dilated and dark, as is usual for the genus (Figs 8 and 30).

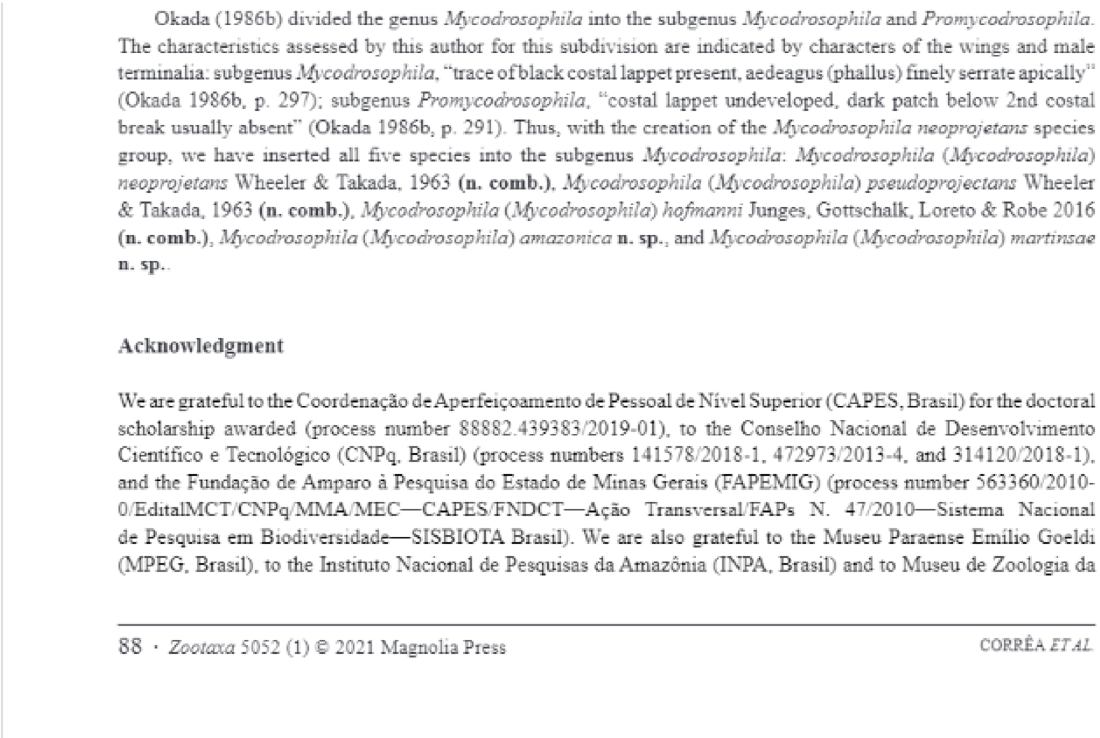


The dark spot below the second costal break, which extend until the vein M, observed in both new species, is similar to that found on *M. pseudoprojectans*, *M. neoprojectans* and *M. hofmanni*. This spot extends to the vein CuA_1 on the other species of *Mycodrosophila*. There are no stains on the apex of the veins R_{2+3} and R_{4+5} of *Mycodrosophila martinsae* **n. sp.,** as is also the case for *M. pseudoprojectans*, *M. neoprojectans*, *M. hofmanni* and *M. cornuta* (*M. amazonica* **n. sp.** has wing-tip slightly darkened or infuscate). For *Mycodrosophila valentae* spots on the apex of the veins R_{2+3} and R_{4+5} in its are not diagnostic, however, in the figure of the wing described by Junges *et al.* (2016), infuscations are noticed in these portions. The vein R_{2+3}, on the other hand, is straight towards C in the two new species described here, as well as in *M. pseudoprojectans*, *M. neoprojectans* and *M. cornuta*, whereas in the other species this vein is curved or slightly curved at its tip.

Some paratypes of *M. amazonica* **n. sp.** had a pair of light spots on the dorsal portion of tergite V (Fig. 18). These spots possibly denote the retraction of tergite IV and / or distention of tergite V, thus showing spots that would be covered in tergite V by tergite IV.

The male terminalia of *M. amazonica* **n. sp.** and *M. martinsae* **n. sp.** are similar in shape to those of most species of the genus, having a tubular phallus with a bifurcated apex (the male terminalia of *M. brunnescens* is unknown) (Wheeler & Takada 1963; Bock 1980; Chassagnard & Lachaise 2000; McEvey & Polak 2005), however, they have the following differences between them: *M. amazonica* **n. sp.** demonstrates the epandrium with 5–6 upper and 4–6 lateral setae; ventral lobe approximately the same length as the surstylus, glabrous, and presenting 3–4 long setae; surstylus connected to the epandrium, with 11 prensisetae arranged in a line, 4 inner setae and 4 outer setae; in lateral view, the basiphallus is about 2x narrower than distiphallus, and with about 1/2 of the phallus length; and *M. martinsae* **n. sp.** have the epandrium with 4 upper and 4 lateral setae; ventral lobe slightly longer than the surstylus, microtrichose and with 5 long setae; surstylus connected to epandrium, with 10 prensisetae arranged in line, 2 inner and 4–5 outer setae; in lateral view, the basiphallus is about 2x narrower than distiphallus, but with about 1/3 of the phallus length.

Here, we propose the *Mycodrosophila neoprojectans* species group based on common characteristics of the external morphology and male terminalia of the species *M. neoprojectans*, *M. pseudoprojectans* and *M. hofmanni*, as well as *M. amazonica* **n. sp.** and *M. martinsae* **n. sp.** The characters listed in the external morphology are the color of tergites II to IV, and the dark spot from the second C break until the vein M. Some diagnostic characters could be apomorphic. The male terminalia are the tapering of the end of the ventral lobe of the epandrium and the shape of the phallus in lateral view, with a narrow basal portion in lateral view. Furthermore, this group of species has the following *Mycodrosophila* apomorphies: presence of a ventral branch on the arista, loss of the anterior pair of dorsocentral setae, and a costal lappet dilated and dark (Grimaldi 1990).



Okada (1986b) divided the genus *Mycodrosophila* into the subgenus *Mycodrosophila* and *Promycodrosophila*. The characteristics assessed by this author for this subdivision are indicated by characters of the wings and male terminalia: subgenus *Mycodrosophila*, "trace of black costal lappet present, aedeagus (phallus) finely serrate apically" (Okada 1986b, p. 297); subgenus *Promycodrosophila*, "costal lappet undeveloped, dark patch below 2nd costal break usually absent" (Okada 1986b, p. 291). Thus, with the creation of the *Mycodrosophila neoprojetans* species group, we have inserted all five species into the subgenus *Mycodrosophila*: *Mycodrosophila* (*Mycodrosophila*) *neoprojetans* Wheeler & Takada, 1963 **(n. comb.)**, *Mycodrosophila* (*Mycodrosophila*) *pseudoprojectans* Wheeler & Takada, 1963 **(n. comb.)**, *Mycodrosophila* (*Mycodrosophila*) *hofmanni* Junges, Gottschalk, Loreto & Robe 2016 **(n. comb.)**, *Mycodrosophila* (*Mycodrosophila*) *amazonica* **n. sp.**, and *Mycodrosophila* (*Mycodrosophila*) *martinsae* **n. sp.**.

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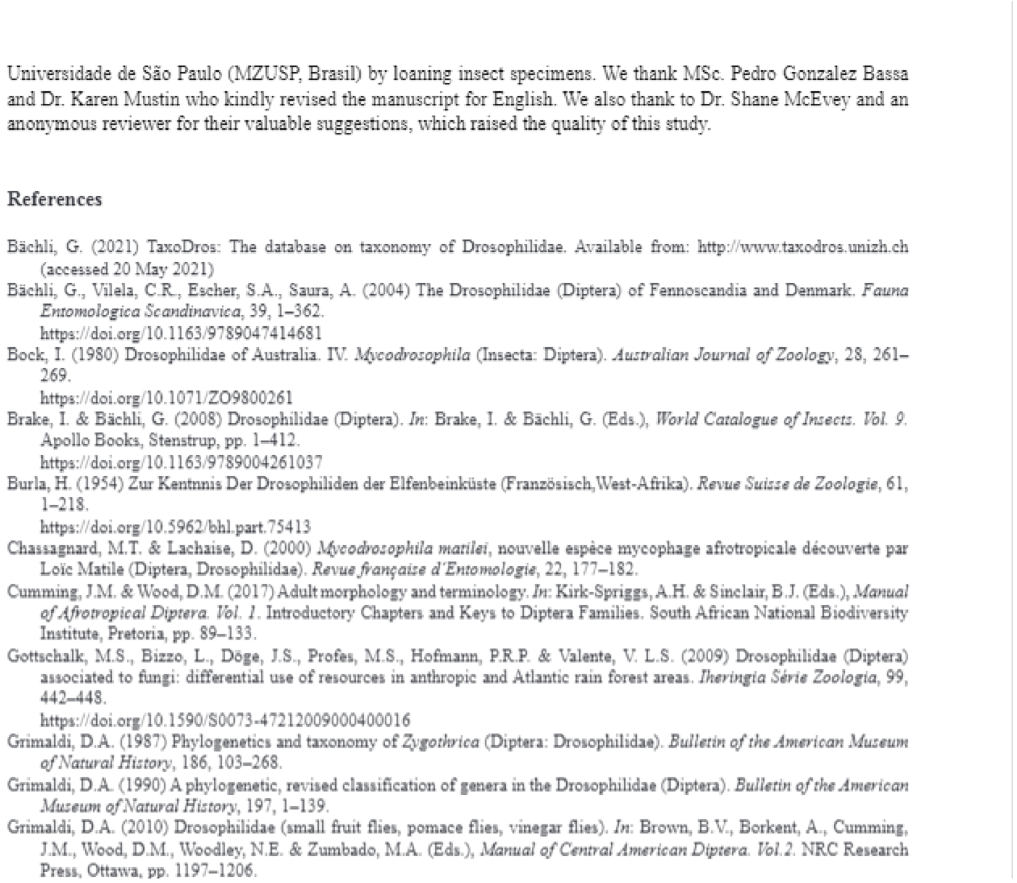

Universidade de São Paulo (MZUSP, Brasil) by loaning insect specimens. We thank MSc. Pedro Gonzalez Bassa and Dr. Karen Mustin who kindly revised the manuscript for English. We also thank to Dr. Shane McEvey and an anonymous reviewer for their valuable suggestions, which raised the quality of this study.

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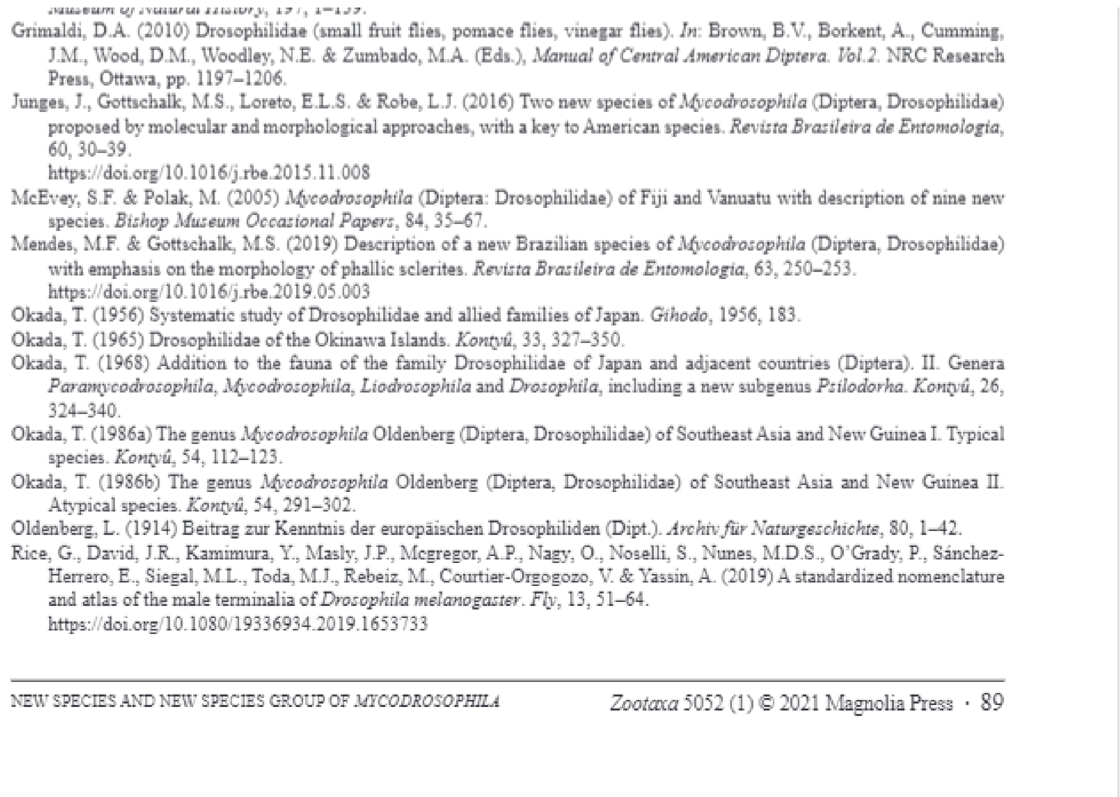

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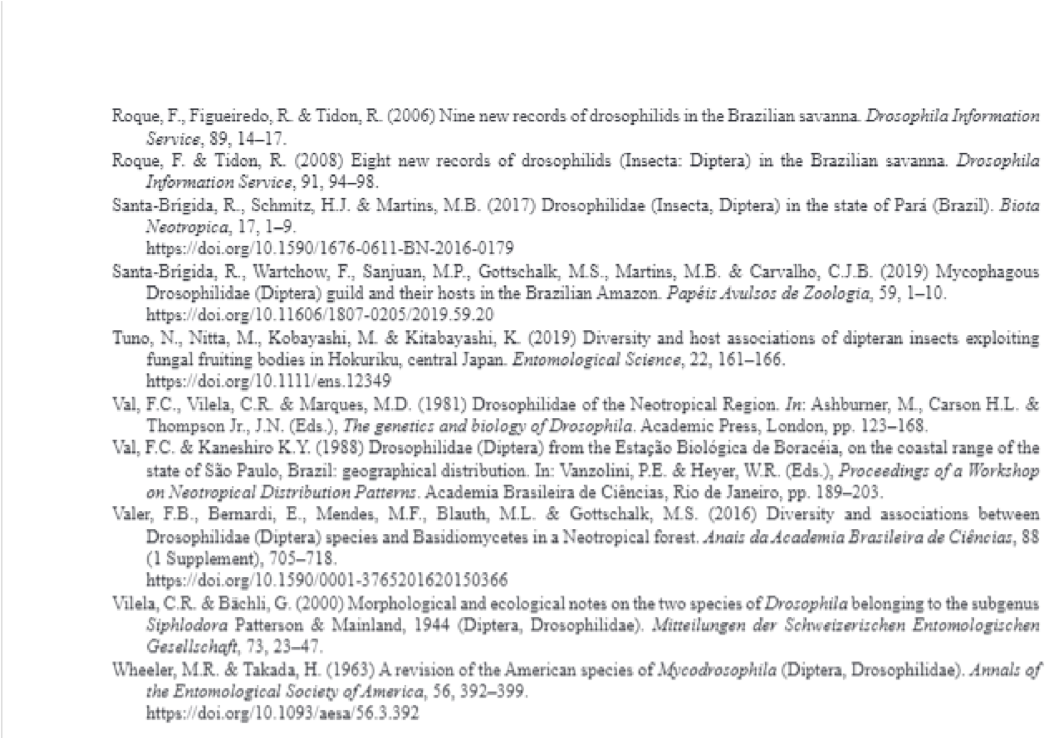

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8. CAPÍTULO VIII

Anexos

8.1 Normas de formatação do periódico *Neotropical Biology and Conservation*.

Authors Guidelines

Main Text

Title: The title should be written in sentence case (only scientific names, geographic locations, or other proper nouns should be capitalized, i.e. *Elater ferrugineus* L., Germany, etc.), and should include an accurate, clear and concise description of the reported work, avoiding abbreviations.

Authors and Affiliations: Provide the complete names of all authors, and their addresses for correspondence, including e.g., institutional affiliation (e.g. university, institute), location (street, boulevard), city, state/province (if applicable), and country. One of the authors should be designated as the corresponding author. It is the corresponding author's responsibility to ensure that the author list, and the individual contributions to the study are accurate and complete. If the article has been submitted on behalf of a consortium, all consortium members and their affiliations should be listed after the **Acknowledgements section**.

Abstract and Key Words: Please have your abstract and key words ready for input into the submission module. Key words should be in alphabetical order and ideally differ from the words used in the title. The abstract should include the following sections: introduction (stating the problem and the purpose of the study), very concise methods, results, and conclusion. The abstract should be informative (without general words), original, factful (provide a summary of the content of the article and key results of the study), written in good English, concise (between 200 and 250 words). It should be followed by up to 6–8 key words that convey the main meaning of the article.

Body Text: All papers should be in grammatically correct English. Non-native English speaking authors are required to have their manuscripts checked by a native English speaker or professional English-language editor prior to submission. Use either British/Commonwealth or American English provided that the language is consistent within the paper. A manuscript must be written with precision, clarity, and economy. The voice - active or passive - and the tense used should be consistent throughout the manuscript. Avoid the use of parenthetical comments and italics or bold for emphasis. This journal discourages the use of quotation marks except for direct quotations, words defined by the author, and words used in unusual contexts. Short quotations should be embedded in the text and enclosed in double quotation marks ("). Long quotations should be on a separate line, italicized, but without quotation marks. Single quotation marks are to be used only for a quotation that occurs within another quotation.

Spacing, Fonts, and Page Numbering: Single-space all material (text, quotations, figure legends, tables, references, etc.). Separate paragraphs with a blank line. Use a 12-point font (preferably Times New Roman or Arial).

Capitals: First capital letters should be used only in the beginning of a sentence, in proper names and in headings and subheadings, as well as to indicate tables, graphs and figure/s within the text. Software programmes should be written with capital letters (e.g., ANOVA, MANOVA, PAUP).

Italicization/Underlining: Scientific names of species and genera, long direct quotations and symbols for variables and constants (except for Greek letters), such as p, F, U, T, N, r, but not for SD (standard deviation), SE (standard error), DF (degrees of freedom) and NS (non significant) should be italicized. These symbols in illustrations and equations should be in italics to match the text. Italics should not be used for emphasis, and not in abbreviations such as e.g., i.e., et al., etc., cf. Underlining of any text is not acceptable.

Abbreviations: Abbreviations should be followed by '.' (full stop or period; for instance: i.e., e.g., cf., etc.). Note that you shouldn't add a full stop at the end of abbreviated words if the last letter of the abbreviation is the same as the last letter of the full word. For example, you should abbreviate "Eds", "Dr", "Mr" without full stop at the end. All measures, for instance mm, cm, m, s, L, should be written without full stop.

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 en-dash should be used to link numerals, sizes, dates and page numbers (e.g., 1977–1981; figs 5–7; pp. 237–258); geographic or name associations (Murray–Darling River; a Federal–State agreement); and character states combinations such as long–pubescent or red–purple. **(3)** Em-dash or em-rule (the length of an 'm') should be used rarely, only for introducing a subordinate clause in the text that is often used much as we use parentheses. In contrast to parentheses an em-dash can be used alone. En-dashes and em-dashes should not be spaced.

Footnotes: Avoid footnotes in the body text of the manuscript. It is always possible to incorporate the footnote into the main text by rewording the sentences, which greatly facilitates reading. Additionally, footnotes are not always handled well by software, and their usage may cause failures in the text processing.

Geographical coordinates: It is strongly recommended to list geographical coordinates as taken from GPS or online gazetteer, or georeferencer. Geographical coordinates must be listed in one of the following formats:

Definition: The locality consists of a point represented by coordinate information in the form of latitude and longitude. Information may be in the form of

- Degrees, Minutes and Seconds (DMS),
- Degrees and Decimal Minutes (DDM), or
- Decimal Degrees (DD).

Records should also contain a hemisphere (E or W and N or S) or, with Decimal Degrees, minus (–) signs to indicate western and/or southern hemispheres.

Examples:

- Example 1: 36°31'21"N; 114°09'50"W (DMS)
- Example 2: 36°31.46'N; 114°09.84'W (DDM)
- Example 3: 36.5243°S; 114.1641°W (DD)

- Example 4: −36.5243; −114.1641 (DD using minus signs to indicate southern and western hemispheres)

Note on accuracy: Because GPS units are very commonly used today to record latitude/longitude, many authors simply give the GPS readings for their localities. However, these readings are much too accurate. For example, a GPS unit might give the latitude in decimal seconds as 28°16'55.87"N. Since one second of latitude is about 30 m on the ground, the second figure after the decimal in 55.87 represents 30 cm, yet a typical handheld GPS unit is only accurate at best to a few metres.

We therefore recommend two ways to report GPS-based locations. If you give the GPS reading without rounding off, make sure you include an uncertainty figure as a context for the over-accurate GPS reading. We recommend the Darwin Core definition of uncertainty (http://rs.tdwg.org/dwc/terms/index.htm#coordinateUncertaintyInMeters):

"The horizontal distance (in meters) from the given decimalLatitude and decimalLongitude describing the smallest circle containing the whole of the Location."

If you only give the GPS reading, please round it off to an implied precision appropriate to the error in the measurement, or to the extent of the area sampled. We suggest rounding off

- to the nearest second in degree-minute-second format (28°16'56"N), which implies roughly ± 25-30 m at middle latitudes;
- to four decimal places in decimal degree format (28.2822°N), which implies roughly ± 10-15 m at middle latitudes;
- to two decimal places in decimal minute format (28°16.93'N), which implies roughly 15-20 m at middle latitudes.

Altitude: Many GPS users simply record the elevation given by their GPS unit. However, GPS elevation is NOT the same as elevation above sea level. GPS units record the elevation above a mathematical model of the earth's surface. The difference between this elevation and elevation above sea level can be tens of metres. In any case, the accuracy of a GPS elevation is often the same as the usual accuracy in horizontal position, so a GPS elevation such as '753 m' is much too accurate and should be rounded off to 'ca 750 m'.

We **strongly recommend** the use of Example 2 (the DDM format). The other three are also possible but will be recalculated to DDM during the process of online mapping from the HTML version of the paper.

The only restriction on format is in creating a KML (Keyhole Markup Language) file. KML latitudes and longitudes must be in the DD format shown above in Example 4.

Please also consider submitting a **table of localities** with your manuscript, either as a spreadsheet or in CSV text format. By doing so you will make your specimen localities much more easily available for use in biodiversity databases and geospatial investigations. The geospatial table will be put online as supplementary material for your paper. A minimum table will have three fields: species (or subspecies) name, latitude and longitude. A full table will have the same data for each specimen lot as appears in the text of your paper. Please check latitude/longitude carefully for each entry.

Units: Use the International System of Units (SI) for measurements. *Consult Standard Practice for Use of the International System of Units* (ASTM Standard E−380−93) for guidance on unit conversions, style, and usage.

Statistics: Use leading zeroes with all numbers, including probability values (e.g., $P < 0.001$). For every significant F−statistic reported, provide two df values (numerator and denominator). Whenever possible, indicate the year and version of the statistical software used.

Web (HTML) links: Authors are encouraged to include links to other Internet resources in their article. This is especially encouraged in the reference section. When inserting a reference to a web-page, please include the **http://** portion of the web address.

Supplementary files: Larger datasets can be uploaded separately as Supplementary Files. Tabular data provided as supplementary files can be uploaded as an Excel spreadsheet (.xls), as an OpenOffice spreadsheets (.ods) or comma separated values file (.csv). As with all uploaded files, please use the standard file extensions.

Headings and subheadings: Main headings: The body text should be subdivided into different sections with appropriate headings.

Where possible, the following standard headings should be used: **Introduction**, **Methods**, **Results**, **Discussion**, **Conclusions**, **Acknowledgements**.

References. These headings need to be in bold font on a separate line and start with a first capital letter. Please do not number headings or subheadings.

- **Introduction** − The motivation or purpose of your research should appear in the Introduction, where you state the questions you sought to answer, and then provide some of the historical basis for those questions.
- **Methods** − Provide sufficient information to allow someone to repeat your work. A clear description of your experimental design, sampling procedures, and statistical procedures is especially important in papers describing field studies, simulations, or experiments. If you list a product (e.g., animal food, analytical device), supply the name and location of the manufacturer. Give the model number for equipment used. Supply complete citations, including author (or editor), title, year, publisher, and version number, for computer software mentioned in your article.
- **Results** − Results should be stated concisely and without interpretation.
- **Discussion** − Focus on the rigorously supported aspects of your study. Carefully differentiate the results of your study from data obtained from other sources. Interpret your results, relate them to the results of previous research, and discuss the implications of your results or interpretations. Point out results that do not support speculations or the findings of previous research, or that are counter-intuitive. You may choose to include a Speculation subsection in which you pursue new ideas suggested by your research, compare and contrast your

research with findings from other systems or other disciplines, pose new questions that are suggested by the results of your study, and suggest ways of answering these new questions.

- **Conclusion** −This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.
- **References** − The list of References should be included after the final section of the main article body. A blank line should be inserted between single-spaced entries in the list. Authors are requested to include DOIs and/or links to online sources of articles, whenever possible!

Where possible, the standard headings should be used in the order given above. Additional headings and modifications are permissible.

Subordinate headings: Subordinate headings (e.g. *Field study and Simulation model or Counts, Measurements and Molecular analysis*), should be left-justified, italicized, and in a regular sentence case. All subordinate headings should be on a separate line.

Citations and References

Citations within the text: Before submitting the manuscript, please check each citation in the text against the References and vice-versa to ensure that they match exactly.

Citations in the text should be formatted as follows:

One author: Smith (1990) or (Smith 1990)

Note: The citations format depends on the way it is incorporated within the article's text:

Example:

1. According to Smith (1990), these findings…
2. These findings have been first reported in the beginning of the nineties (Smith 1990).

Two authors: Brock and Gunderson (2001) or (Brock and Gunderson 2001)

Note: When choosing between formats refer back to examples above.

Three or more authors: Smith et al. (1998) or (Smith et al. 1998)

Note: When choosing between formats refer back to examples above.

When **citing more than one source**, in-text citations should be ordered by the year of publication, starting with the earliest one:

(Smith et al. 1998, 2000, 2016; Brock and Gunderson 2001; Felt 2006).

Note: When you have a few citations from the same author but from different years (such as the case with Smith et al. above), the first year is taken into consideration when ordering the sources (in this case 1998, which is why Smith et al. come first in the list).

When having **two or more fully identical citations** (this can happen when you have more than one reference with exactly the same authors and years for one or two authors, or the same first author and year for author teams of three or more), the references are distinguished by adding the letters 'a', 'b', 'c', etc. after the years and this marking is followed in the in-text citations, respectively:

(Reyes-Velasco et al. 2018a, 2018b)

Authorship **references for species** should include a "," between author and year:

Brianmyia stuckenbergi Woodley, 2012.

References: It is important to format the references properly, because all references will be linked electronically as completely as possible to the papers cited. It is desirable to add a DOI (digital object identifier) number for either the full-text or title and abstract of the article as an addition to traditional volume and page numbers. If a DOI is lacking, it is recommended to add a link to any online source of an article.

List all authors cited in the References. For multiauthored papers, give all author names in full; the abbreviation "et al." is only allowed in the text. All journal titles should be spelled out completely and should not be italicized. Ensure that the References are complete and arranged according to name and year of publication. Personal communications and submitted manuscripts should be listed as unpublished results in the text and not listed in the References section.

Please use the following style for the reference list (or download the *Pensoft EndNote style*): here. It is also available in Zotero, when searched by journal name.

Published Papers:
Polaszek A, Alonso-Zarazaga M, Bouchet P, Brothers DJ, Evenhuis NL, Krell FT, Lyal CHC, Minelli A, Pyle RL, Robinson N, Thompson FC, van Tol J (2005) ZooBank: The open-access register for zoological taxonomy: Technical Discussion Paper. Bulletin of Zoological Nomenclature 62: 210–220.

Accepted Papers:
Same as above, but "in press" appears instead the year in parentheses.

Electronic Journal Articles:

Mallet J, Willmott K (2002) Taxonomy: Renaissance or Tower of Babel? Trends in Ecology and Evolution 18(2): 57–59. https://doi.org/10.1016/S0169-5347(02)00061-7

Paper within conference proceedings:

Orr AG (2006) Odonata in Bornean tropical rain forest formations: Diversity, endemicity and applications for conservation management. In: Cordero Rivera A (Ed.)

Forest and Dragonflies. Fourth WDA International Symposium of Odonatology, Pontevedra (Spain), July 2005. Pensoft Publishers, Sofia-Moscow, 51–78.

Book chapters:
Mayr E (2000) The biological species concept. In: Wheeler QD, Meier R (Eds) Species Concepts and Phylogenetic Theory: A Debate. Columbia University Press, New York, 17–29.

Books:
Goix N, Klimaszewski J (2007) Catalogue of Aleocharine Rove Beetles of Canada and Alaska. Pensoft Publishers, Sofia-Moscow, 166 pp.

Book with institutional author:
International Commission on Zoological Nomenclature (1999) International code of zoological nomenclature. Fourth Edition. The International Trust for Zoological Nomenclature, London.

PhD thesis:
Dalebout ML (2002) Species identity, genetic diversity and molecular systematic relationships among the Ziphiidae (beaked whales). PhD Thesis, University of Auckland, Auckland, New Zealand.

Link/URL:

BBC News: Island leopard deemed new species http://news.bbc.co.uk/

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Smith J (2018) Article Title. Journal Name 1: 1–10. https://doi.org/10.3897

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Smith J, Brock B, Gutierrez R, Gunderson A (2013) Article Title. Journal Name 1: 15–30. https://doi.org/10.3897

Smith J, Brock B, Gunderson A (2015) Article Title. Journal Name 1: 10–30. https://doi.org/10.3897

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8.2 Normas de formatação do periódico *Revista Brasileira de Entomologia*.

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Examples:

Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article.
Heliyon.

19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.

Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.

Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Reference to a website:

Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).

Reference to a dataset:

[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/ xwj98nb39r.1.

Journal abbreviations source

Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video

RBE accepts links to video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Supplementary material

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Short Communications

Short notes must report elaborated work that incorporates the same aspects required for full articles (biology, ecology, behavior, systematics, pest management, etc.). Manuscripts of anecdotal nature will not be accepted. Papers that merely report new geographic occurrences, records of species or host associations to new localities in geographical regions that they are already known will not be considered. Short Communications should be prepared as a single text and references, including also an Abstract and Keywords. Do not include subtitles (Introduction, Material and Methods, and Results and Discussion). Figures and tables will be limited to a maximum of 3 objects per manuscript.

Voucher policy

RBE encourages authors to deposit voucher and type specimens in public museums or permanent University collections. It is advisable that authors, at time of submission, clearly state in the manuscript where their material is expected to be deposited. Labeling and proper indication of voucher specimens are the authors responsibility.

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Page proofs are sent to the corresponding author and should be returned, with the necessary corrections, at the indicated deadline. Authors are entirely responsible for the scientific content of their paper, as well as for proper use of grammar. Authors are encouraged to look at the latest issues of the RBE to check current format and layout. When submitting a manuscript, authors may suggest potential reviewers. Please include the complete name and electronic address. The choice of reviewers, however, remains with the Editors.

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One set of page proofs (as PDF files) will be sent by e-mail to the corresponding author or, a link will be provided in the e-mail so that authors can download the files themselves. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. We will do everything possible to get your article published quickly and accurately. It is important to ensure that all corrections are sent back to us in one communication: please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

8.3 Normas de formatação do periódico *Community Ecology*.

Instructions authors

Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink “Submit manuscript” on the right and upload all of your manuscript files following the instructions given on the screen.

Please ensure you provide all relevant editable source files. Failing to submit these source files might cause unnecessary delays in the review and production process.

Title Page

Please make sure your title page contains the following information.

Title

The title should be concise and informative.

Author information

- The name(s) of the author(s)
- The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
- A clear indication and an active e-mail address of the corresponding author
- If available, the 16-digit ORCID of the author(s)

If address information is provided with the affiliation(s) it will also be published.

For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

For life science journals only (when applicable)

Trial registration number and date of registration

Trial registration number, date of registration followed by “retrospectively registered”

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations'.

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

To be used for all articles, including articles with biological applications

Funding (information that explains whether and by whom the research was supported)

Conflicts of interest/Competing interests (include appropriate disclosures)

Availability of data and material (data transparency)

Code availability (software application or custom code)

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

Ethics approval (include appropriate approvals or waivers)

Consent to participate (include appropriate statements)

Consent for publication (include appropriate statements)

Please see the relevant sections in the submission guidelines for further information as well as various examples of wording. Please revise/customize the sample statements according to your own needs.

Text

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX. We recommend using Springer Nature's LaTeX template.

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

References

Citation

Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).
- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat et al. 1995a, b; Kelso and Smith 1998; Medvec et al. 1999, 2000).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

Reference list entries should be alphabetized by the last names of the first author of each work. Please alphabetize according to the following rules: 1) For one author, by name of author, then chronologically; 2) For two authors, by name of author, then name of coauthor, then chronologically; 3) For more than two authors, by name of first author, then chronologically.

If available, please always include DOIs as full DOI links in your reference list (e.g. “https://doi.org/abc”).

- Journal article

  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

  Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

  Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

ISSN LTWA

If you are unsure, please use the full journal title.

Tables

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Supplementary Information (SI)

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Before submitting research datasets as Supplementary Information, authors should read the journal's Research data policy. We encourage research data to be archived in data repositories wherever possible.

Submission

- Supply all supplementary material in standard file formats.
- Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
- To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.
- High resolution (streamable quality) videos can be submitted up to a maximum of 25GB; low resolution videos should not be larger than 5GB.

Audio, Video, and Animations

- Aspect ratio: 16:9 or 4:3
- Maximum file size: 25 GB for high resolution files; 5 GB for low resolution files
- Minimum video duration: 1 sec
- Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Captions

- For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

- Supplementary Information (SI) will be published as received from the author without any conversion, editing, or reformatting.

8.4 Normas de formatação do periódico *Jornal of Insect Conservation*.

Instructions for Authors

Journal of Insect Conservation accepts the submission of Research articles, Short Communications and Review articles. Word limits for each of these article types are as follows (word limits exclude tables, figures and supplementary material):

- Research article – 8000 words
- Short communication - 2500 words
- Review – 12000 words

New abstract structure now required: -

Abstracts should be structured with a background, main body of the article and short conclusion. For research articles, the abstract structure should follow the format of a traditional scientific article and would typically have the following sections: Introduction, Aims/Methods, Results, Discussion. For reviews and short notes, the inclusion of abstract sections is up to the individual authors, although changes might be requested. All manuscripts (irrespective of article type) submitted to the Journal of Insect Conservation must end their abstract with a subsection titled ‘Implications for insect conservation’, in which authors are expected to provide a brief (1 – 2 sentences) description on the conservation implications that their article addresses.

Example conservation summary: “Implications for insect conservation: Our results show that native cover crops enhance foliar arthropod diversity over and above exotic cover crops and contributes to farm-scale compositional heterogeneity. It therefore has potential to reduce arthropod diversity loss within farmlands and to contribute to more resilient vineyard agroecosystems.”

Author guidelines: If your research includes a conservation intervention, it is strongly recommended that you provide a minimum of one sentence in the introduction of your article to summarize the evidence related to the intervention. If no related evidence has previously been published, please state this in the introduction. Please consult relevant conservation databases and/or alternative sources to check for any existing evidence relating to conservation interventions.

Reviewer guidance: If the authors research includes a conservation intervention, it is strongly recommended that you check that they have not overlooked any evidence related to interventions in the relevant literature. Please consult relevant conservation databases and/or alternative sources to check for any existing evidence relating to conservation interventions.

Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink “Submit manuscript” on the right and upload all of your manuscript files following the instructions given on the screen.

Please ensure you provide all relevant editable source files. Failing to submit these source files might cause unnecessary delays in the review and production process.

Additional request

Upon submission, the e-mail addresses of all authors will be requested. At the end of the submission process, the corresponding author will receive an acknowledgement e-mail and all co-authors will be contacted automatically to confirm their affiliation to the submitted work.

Title page

Title Page

Please make sure your title page contains the following information.

Title

The title should be concise and informative.

Author information

- The name(s) of the author(s)
- The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
- A clear indication and an active e-mail address of the corresponding author
- If available, the 16-digit ORCID of the author(s)

If address information is provided with the affiliation(s) it will also be published.

For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

For life science journals only (when applicable)

Trial registration number and date of registration

Trial registration number, date of registration followed by "retrospectively registered"

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations'.

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

To be used for all articles, including articles with biological applications

Funding (information that explains whether and by whom the research was supported)

Conflicts of interest/Competing interests (include appropriate disclosures)

Availability of data and material (data transparency)

Code availability (software application or custom code)

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

Ethics approval (include appropriate approvals or waivers)

Consent to participate (include appropriate statements)

Consent for publication (include appropriate statements)

Please see the relevant sections in the submission guidelines for further information as well as various examples of wording. Please revise/customize the sample statements according to your own needs.

Text

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX. We recommend using Springer Nature’s LaTeX template.

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Important note:

- **All authors are requested to use the continuous line numbering function for their manuscripts.**

Scientific style

Please always use internationally accepted signs and symbols for units (SI units).

Scientific style

Nomenclature: Insofar as possible, authors should use systematic names similar to those used by Chemical Abstract Service or IUPAC.

References

Citation

Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).
- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat et al. 1995a, b; Kelso and Smith 1998; Medvec et al. 1999, 2000).

Reference list

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If available, please always include DOIs as full DOI links in your reference list (e.g. "https://doi.org/abc").

- Journal article

  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

  Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

  Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

ISSN LTWA

If you are unsure, please use the full journal title.

Tables

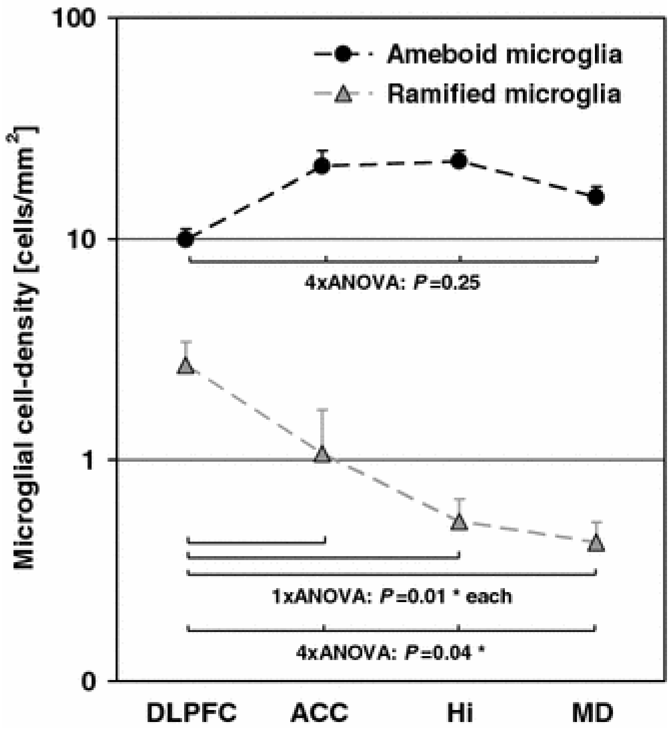
- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

Electronic Figure Submission

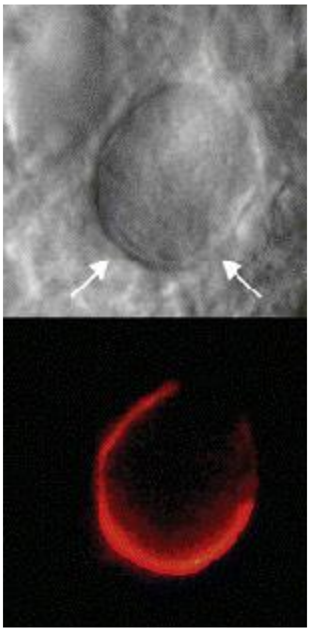
- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art



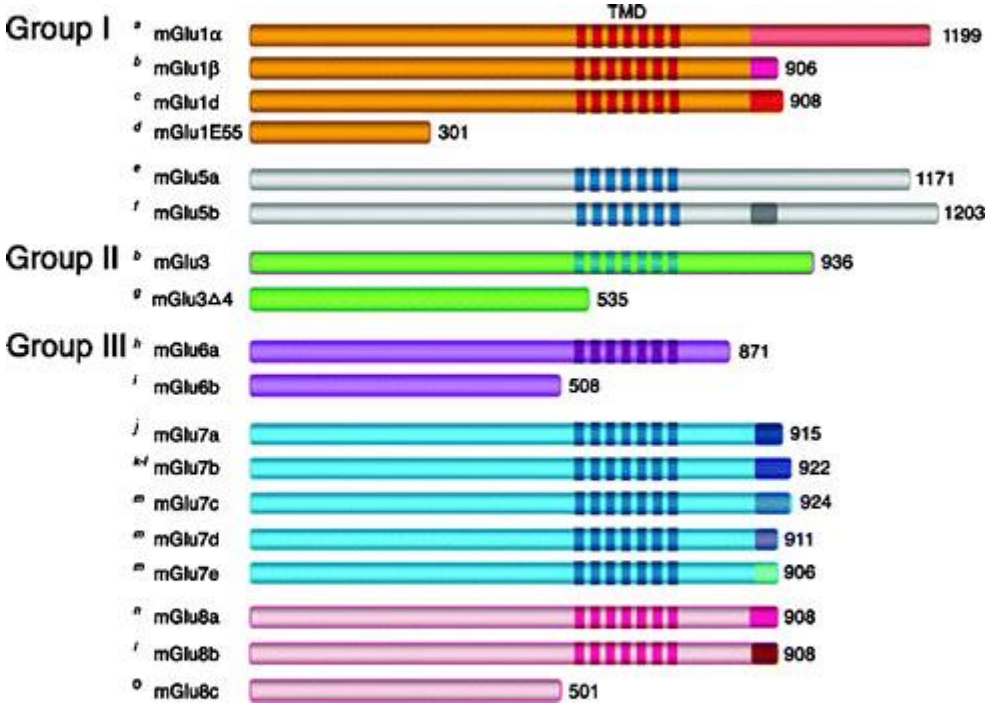
- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art



- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

Combination Art



- Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
- Combination artwork should have a minimum resolution of 600 dpi.

Color Art

- Color art is free of charge for online publication.
- If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
- If the figures will be printed in black and white, do not refer to color in the captions.
- Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).

- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

Figure Numbering

- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,"A1, A2, A3, etc." Figures in online appendices [Supplementary Information (SI)] should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

- Figures should be submitted separately from the text, if possible.
- When preparing your figures, size figures to fit in the column width.
- For large-sized journals the figures should be 84 mm (for double-column text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm.
- For small-sized journals, the figures should be 119 mm wide and not higher than 195 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

- All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)
- Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)
- Any figure lettering has a contrast ratio of at least 4.5:1

Supplementary Information (SI)

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Before submitting research datasets as Supplementary Information, authors should read the journal’s Research data policy. We encourage research data to be archived in data repositories wherever possible.

Submission

- Supply all supplementary material in standard file formats.
- Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
- To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.
- High resolution (streamable quality) videos can be submitted up to a maximum of 25GB; low resolution videos should not be larger than 5GB.

Audio, Video, and Animations

- Aspect ratio: 16:9 or 4:3
- Maximum file size: 25 GB for high resolution files; 5 GB for low resolution files
- Minimum video duration: 1 sec

- Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Text and Presentations

- Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
- A collection of figures may also be combined in a PDF file.

Spreadsheets

- Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).

Specialized Formats

- Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

- It is possible to collect multiple files in a .zip or .gz file.

Numbering

- If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
- Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.
- Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

- For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

- Supplementary Information (SI) will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

- The manuscript contains a descriptive caption for each supplementary material
- Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

Ethical Responsibilities of Authors

This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.

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8.5 Protocolo de dissecção

Novo Protocolo de Dissecção

- Retirar os dois últimos (3 para fêmeas) tergitos do abdômen e adicionar KOH 10% novo por 10-15min;

- Ajustar o banho Maria para 40-60 graus Celsius (40 para indivíduos claros e 60 para indivíduos escuros);

Observação: KOH 10% (10gr de ácido acético + 100ml H_2O destilada)

Atenção

Dê uma olhada na peça com 10 minutos de ação do KOH

A terminália não deve ficar transparente

- Após o banho Maria retirar a peça e dar um banho rápido (coloca e tira) em Ácido Acético;

- Retirar do Ácido Acético e colocar no Álcool comercial 96%

- Colocar a peça em Glicerol para dissecar;

- NÃO é necessário Corar a peça.

Preparação da lâmina

- A peça deve passar por dois banhos em álcool absoluto e um banho de Xilol (nesta ordem);

- Prepara a lâmina com Bálsamo do Canadá e adicionar a peça;

- Se não houver tempo para preparar a lâmina, a peça pode ser acondicionada (temporariamente) em ependorfe pequeno com glicerina.