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Desvendando a fauna do Bioma Pampa no Rio Grande do Sul: inventário da fauna de Drosophilidae (Insecta, Diptera).

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SUMÁRIO

Desvendando a fauna do Bioma Pampa no Rio Grande do Sul: inventário da fauna de Drosophilidae (Insecta, Diptera).

Resumo: A família Drosophilidae teve sua primeira espécie descrita em 1787 quando Fabricius descreveu *Musca funebris,* que mais tarde foi reclassificada como *Drosophila funebris*, espécie tipo da família que atualmente é composta por cerca de 4.170 espécies descritas. Os drosofilídeos são popularmente conhecidos como "mosca da fruta", mas além de frutos em decomposição, também podem ser encontrados em flores, guano de morcego, cladódios de cactos, carcaças de insetos e outros animais, fluxos de seiva e material vegetal em decomposição, e em muitos outros recursos. Por estas moscas serem muito abundantes, apresentarem curto ciclo de vida, e serem facilmente coletadas, tornaram-se bons modelos em estudos de genética, e nas décadas mais recentes estão também se tornando bons modelos ecológicos. Embora muito estudadas nos diversos biomas do mundo, poucos estudos foram desenvolvidos nos Campos Sulinos, ambiente que tem sofrido com a degradação em decorrência da agricultura e pecuária no estado do Rio Grande do Sul. Nos últimos anos o bioma Pampa vem recebendo maior atenção do Ministério do Meio Ambiente, com isso espera-se que este seja mais valorizado e receba maior atenção científica, contribuindo para sua conservação. No presente estudo, mostragens sazonais de drosofilídeos foram realizadas em uma área natural, na região do município de Bossoroca (28° 45'024"S 54° 56'729"W) entre Abril de 2011 e Abril de 2012, com iscas de banana e recursos naturais encontrados em campo. Nas coletas foram considerados os ambientes de campo aberto, borda e interior das manchas de mata que compõem o Pampa. Além do acompanhamento da variação sazonal das populações de Drosophilidae, também foi analisada a variação de medidas de diversidade como, por exemplo, o índice de heterogeneidade de Shannon-Wiener (H') e o de equitabilidade de Smith-Wilson (E_{var}),

entre campo, borda e interior de mata ao longo das estações. Novas espécies foram propostas para o gênero *Rhinoleucophenga: R. pampeana* sp. nov., *R. missionera* sp. nov. e *R. pampeana* sp. nov. Através de uma ampla revisão da literatura, também foi realizado um levantamento das espécies de Drosophilidae registradas no Bioma Pampa, incluindo toda sua extensão, entre Brasil, Uruguai e Argentina. Como resultado das novas amostragens, *Drosophila briegeri* Pavan & Breuer, *D. fuscolineata* Duda, *Rhinoleucophenga obesa* Loew, *R. punctulata* Duda, *R. subradiata* Duda e *Zygothrica orbitalis* Sturtevant foram pela primeira vez registradas neste bioma, e dentre as mais abundantes estiveram *Drosophila simulans* Sturtevant, *D. willistoni* Sturtevant, *D. mediopunctata* Dobzanhsky & Pavan, *D. buzzatii* Patterson & Wheeler, *D. mercatorum* Patterson & Wheeler, *D. maculifrons* Duda, *D. immigrans* Sturtevant, *D. hydei* Sturtevant e *Zaprionus indianus* Gupta. De uma maneira geral, tanto espécies neotropicais quanto exóticas foram mais concentradas na borda e no interior das manchas de mata, sendo esta tendência influenciada pela variação sazonal. Consequentemente, medidas de diversidade também variaram ao longo das estações entre o campo aberto e o interior das manchas de mata. Logo, a sazonalidade parece ser o principal componente capaz de explicar a variação da diversidade de Drosophilidae no Pampa durante o período amostrado, sendo as manchas de mata fundamentais para a manutenção dessa diversidade. O Pampa brasileiro revelou uma grande riqueza de Drosophilidae, mais do que o dobro dos registros atuais para o Pampa uruguaio e argentino. Apesar disso, os três países apresentam amplas áreas do bioma Pampa ainda totalmente desconhecidas quanto a fauna de Drosophilidae. O conjunto dessas informações ressalta a importância e a necessidade de mais estudos para desvendar a fauna de Drosophilidae no bioma Pampa.

1. CAPÍTULO I

1.1. INTRODUÇÃO

1.1.1. A família Drosophilidae

Possivelmente ¾ das espécies de animais metazoários descritos ao redor do planeta Terra são insetos (Ruppert & Barnes, 1996), os quais provavelmente originaram-se nas latitudes equatoriais e então se espalharam para as demais regiões da Terra (Erwin, 1981). Porém, são muitas as hipóteses para tentar explicar essa diversificação bem sucedida dos insetos, que vão desde o surgimento e dobramento das asas até o desenvolvimento das peças bucais e mudanças nos hábitos alimentares, passando por rápidos períodos de desenvolvimento e "aperfeiçoamento" genético (Mayhew, 2007).

Dentro dessa megadiversa classe Insecta, a ordem Diptera, a qual compreende de 10 a 15% de toda a biodiversidade mundial (Yeates *et al.*, 2007), divide-se em duas sub-ordens, sendo uma delas a sub-ordem Brachycera onde encontra-se a família Drosophilidae, a qual segundo Throckmorton (1975) teve sua origem nas regiões tropicais, há cerca de 50 milhões de anos.

O gênero mais estudado da família Drosophilidae é *Drosophila*, o qual teve sua primeira espécie descrita em 1787 quando Fabricius descreveu *Musca funebris*, que mais tarde foi reclassificada como *Drosophila funebris*, espécie tipo da família Drosophilidae (Bächli, 2006).

Atualmente *Drosophila* conta com aproximadamente 1.165 espécies descritas (Bächli, 2012). Este número só tende a aumentar, uma vez que estimativas da fauna desconhecida se baseiam nas taxas de novas descrições, sendo que a taxa de descrição de novas espécies de insetos é muito alta. Segundo Wilson (1999), no período de 1980 a 1998, cerca de 7.700 novas espécies de insetos foram formalmente descritas por ano. E no que se refere especificamente a Drosophilidae, no ano de 2006, de acordo com os dados apresentados por Schmitz *et al*. (2007) haviam cerca de 3.800 espécies de drosofilídeos descritas, em 2010 esse número passou para cerca de 4.000 espécies descritas, como foi apresentado por Mata *et al*. (2010) e atualmente existem aproximadamente 4.170 espécies descritas (Bächli, 2012). Ou seja, nos últimos seis anos o número de drosofilídeos descritos aumentou em aproximadamente 66 espécies por ano.

Apesar de os drosofilídeos serem popularmente conhecidos como “mosca da fruta”, as moscas desta família não se alimentam dos frutos, mas sim das leveduras que crescem na matéria orgânica em decomposição (Carson, 1971). Além disto, embora a grande maioria das espécies seja coletada em frutos em decomposição, muitas espécies do gênero *Drosophila* e de outros gêneros da família Drosophilidae já foram encontrados em flores (Schmitz & Hofmann, 2005; Schmitz *et al*., 2009), guano de morcego (Tosi *et al*., 1990), cladódios de cactos (Carson, 1971; Mizuguchi, 1978; Vilela *et al*., 1983), carcaças de insetos e carne (Lachaise & Tsacas, 1983), fluxos de seiva e material vegetal em decomposição (Carson, 1971) ou exibem comportamento predatório (de hemípteros ou larvas aquáticas de mosquitos), comensal (de caranguejos, aranhas, abelhas), parasitário (de lagartas de lepidópteros) e até de canibalismo, no caso de larvas que se encontram em um recurso super povoado (Carson, 1971; Ashburner, 1981; Lachaise & Tsacas, 1983).

No Brasil, importantes estudos com drosofilídeos foram realizados pelo pesquisador alemão Oswald Duda no início do século XX, e tomaram grande impulso a partir da década de 1940 com as visitas do geneticista e evolucionista Theodosius Dobzhansky, (Freire-Maia & Pavan, 1949; Strickberger, 1962). Porém, por ser amplamente estudada por geneticistas e por ser um organismo relativamente fácil de coletar na natureza e também de fácil manutenção em laboratório, *Drosophila* tornou-se um organismo modelo em genética, sendo *Drosophila melanogaster* Meigen seu representante mais famoso. Segundo Pavan (1959) nenhum outro animal além do homem foi alvo de tantos estudos como esta mosca. Assim, uma série de outros estudos foram desenvolvidos nos anos subsequentes, principalmente estudos filogenéticos (Throckmorton, 1975; Grimaldi, 1990; Remsen & O'Grady, 2002; Brisson *et al*., 2006), ecológicos (Atkinson, 1979; Parsons, 1989, 1991; Bachli & Burla, 1992; Martins, 2001; Silva *et al*., 2005; Schmitz & Hofmann, 2005; Tidon, 2006; De Toni *et al*., 2007; Schmitz *et al*., 2007, 2010; Mata *et al*., 2008; Döge *et al*., 2008; Hochmüller *et al*., 2010; Bizzo *et al*., 2010; Garcia *et al*., 2012; Poppe *et al*., 2012) e obviamente moleculares e genéticos (Cunha *et al*., 1984; Bonorino *et al*., 1993; Loreto *et al*., 1998; Valente *et al*., 2001; Robe *et al*., 2005; Colares *et al*., 2006; Cordeiro *et al*., 2008; Deprá *et al*., 2009, 2010).

Porém, apesar de o gênero *Drosophila* ser muito estudado, ainda há uma grande carência de conhecimento referente a outros gêneros, um bom exemplo é o gênero *Rhinoleucophenga* do qual a maior parte dos registros é oriunda de um estudo de Malogolowkin (1946) e muitas das descrições das espécies são pouco informativas e carecem de melhores análises, apesar de algumas espécies já terem sido melhor analisadas por Vilela & Bächli (2009) (D. Grimaldi e M. Gottschalk, comunicação pessoal).

Portanto, ainda há vários aspectos biológicos para se conhecer sobre a família Drosophilidae. E para isso é necessário uma relação entre diferentes níveis do conhecimento, ou seja, o conhecimento genético deve ser posto no contexto ecológico. Uma vez que a genética de *Drosophila* é muito conhecida e estudada, mas sua ecologia ainda precisa ser mais explorada (Chaves & Tidon, 2008), principalmente em biomas pouco estudados como o bioma Pampa (Gottschalk *et al*., 2008).

1.1.2. Ecologia de *Drosophila*

Os primeiros trabalhos clássicos não relacionados à genética de *Drosophila* no Brasil datam das décadas de 1940 e 1950: Dobzhansky & Pavan (1943), Freire-Maia & Pavan (1949), Dobzhansky & Pavan (1950), Frota-Pessoa (1954) e Pavan (1959), os quais incluem descrições de um grande número de espécies, grandes levantamentos taxonômicos, chaves de identificação e diversas abordagens ecológicas. Esses estudos foram reflexos da vinda do famoso evolucionista Theodosius Dobzhansky para o Brasil na década de 1940.

Porém, entre as décadas de 1960 e 1980, pouca atenção foi voltada para estudos ecológicos com *Drosophila* no Brasil, as poucas referências deste período são voltadas apara a taxonomia de drosofilídeos, ainda com algum enfoque genético, predominando a descrição de muitas espécies e alguns poucos comentários de ecologia (Cordeiro, 1963, 1964; Malogolowkin, 1963; Mourão & Bicudo, 1967; Mourão & Gallo, 1967; Gallo, 1973; Vilela & Sene, 1977; Vilela & Pereira, 1982; Val, 1982; Vilela, 1983) salvo poucas referências como Bélo & Filho (1976), Brncic & Valente (1978), Araújo & Valente (1981), que abordaram alguns aspectos da relação espécies – ambiente.

Aos poucos, a partir da década de 1990, os estudos ecológicos com drosofilídeos estão se tornando mais comuns. Mata *et al*. (2008) reforçou a importância ecológica de drosofilídeos como organismos bioindicadores, justamente em função da Taxonomia bem definida e Evolução e Genética bem conhecida destas moscas, além do baixo custo e fácil amostragem, a grande abundancia e distribuição na natureza e também fácil estocagem e cultivo em laboratório. Essa importante característica bioindicadora já foi aplicada por muitos pesquisadores tanto em ambientes naturais (Tidon-Sklorz & Sene, 1999; Martins, 2001; Tidon *et al*., 2003; Mata *et al*., 2008) quanto em ambientes urbanos (Lucchese *et al*., 2002; Gottschalk *et al*., 2007).

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

Consequentemente, sendo o Brasil um país de grande extensão territorial e também de grande variação climática, com clima Tropical ao norte e Temperado ao sul (Stumpf *et al*., 2009), torna-se de grande relevância científica o conhecimento das assembléias de Drosophilidae nos diferentes biomas e habitats brasileiros como a Mata Atlântica (Medeiros & Klaczko, 2004; De Toni *et al*., 2007; Gottschalk *et al*., 2007; Döge *et al*., 2008), Cerrado (Tidon *et al*., 2003; Mateus, 2006; Tidon, 2006; Mata *et al*., 2008, 2010), Manguezal (Schmitz *et al*., 2007, 2010), Caatinga (Mizuguchi, 1978; Tidon-Sklorz & Sene, 1995) Pantanal (Val & Marques, 1996) Amazônia (Martins, 1987, 1995, 2001), Mata de Araucária (Saavedra *et al*., 1995), Restinga (Bizzo & Sene, 1982; Bizzo *et al*., 2010), Pampa (Costa *et al*., 2003; Silva *et al*., 2005; Poppe *et al.*, 2012; Garcia *et al*., 2012).

Desse modo, aos poucos as lacunas existentes no conhecimento ecológico de Drosophilidae estão sendo preenchidas por estudos desenvolvidos por Ecólogos, Taxonomistas e Geneticistas, apresentando *Drosophila* também como um organismo modelo em ecologia.

1.1.3. O Pampa

Com uma área de aproximadamente 700.000 km^2, o Pampa é compartilhado entre Brasil, Uruguai e Argentina (Bilenca & Miñarro, 2004). No território brasileiro, o bioma é exclusivo da região sul e abrange cerca de 176.000 km^2, equivalendo a 63% do território do estado do Rio Grande do Sul e a 2.1% do território nacional (Collares, 2006).

Portanto, este bioma limita-se a altas latitudes, onde o clima de acordo com a classificação de Köppen corresponde ao tipo “CF”, temperado, com chuva em todos os meses. Dentro do tipo “CF” ocorrem dois subtipos: “Cfa”, subtropical, com temperatura média das máximas superior a 22ºC e a média das mínimas variando entre –3º e 18ºC; “Cfbl”, subtropical, com a média das máximas inferior a 22ºC e a média das mínimas oscilando entre –3 e 18ºC (Nimer, 1977). A ausência de período seco pronunciado é o principal motivo que, segundo Marchiori (2004), inviabiliza o uso do termo Savana para o para os Campos sulinos.

No Rio Grande do Sul, os campos são a paisagem dominante principalmente na metade sul do estado, e apesar de aparentemente uniformes abrigam uma grande biodiversidade, embora negligenciada tanto pela comunidade científica quanto pela sociedade em geral. Deste modo, apenas uma mínima parcela dos campos está em unidades de conservação (Boldrini *et al*., 2010).

As paisagens campestres do Pampa são naturalmente invadidas por contingentes arbóreos representantes das florestas Estacional Decidual e Ombrófila Densa, notadamente nas partes norte e leste do Rio Grande do Sul, caracterizando um processo de substituição natural das estepes por formações florestais (MMA, 2007). Porém, segundo Lindman (1906) originalmente, os campos no Rio Grande do Sul não eram caracterizados por uma paisagem completamente desprovida de elementos arbóreos e/ou arbustivos. Esta característica foi descrita pelo autor no final do século XIX, quando visitou inúmeras áreas campestres numa época em que havia reduzida atividade antrópica, descrevendo os campos do seguinte modo:

"*... seria certamente difícil encontrar uma só milha quadrada em que não encontrasse na paisagem um grupo de árvores ou uma parte florestal...*".

É possível dividir fitofisionomicamente as formações campestres do Estado do Rio Grande do Sul em Campos de solos rasos (fronteira oeste), Campos de solos profundos (região sudoeste), Campos dos areais (região centro-oeste), Vegetação savanóide (Serra do Sudeste – planalto sul-rio-grandense), Campos do centro do estado (região entre o planalto sul-brasileiro e o planalto sul-rio-grandense), Campos litorâneos (região litorânea entre as latitudes de 30º e 33° Sul) por fim, e mais importante por se tratar da nossa região de coleta, os Campos barba-de-bode (região noroeste do Rio Grande do Sul). Este último é composto principalmente por gramíneas do tipo C4, constituído por uma dupla estrutura de vegetação, o estrato superior é caracterizado por *Aristida jubata* Herter (Poaceae) e o inferior por gramíneas rizomatosas da família Poaceae (*Axonopus jesuiticus* Araújo, *Paspalum notatum* Herter, *Paspalum lepton*

Schult e *Axonopus affinis* Chase) podendo haver uma alteração na composição dessas espécies dependendo de condições de solo (Boldrini *et al*., 2010).

Estimativas conservadoras apontam 2.200 espécies vegetais campestres para o estado (Boldrini *et al*., 2010). E esse valor pode ser extremamente alto quando se considera que os campos sulinos passam por invernos rigorosos e verões escaldantes além da ação antrópica. Muitos drosofilídeos, como as espécies do grupo *Drosophila bromeliae*, utilizam recursos florais como sítios de ovoposição e alimentação (Schmitz & Hofmann, 2005; Schmitz *et al*., 2010) o que torna a diversidade da flora dos campos fundamental na manutenção da sua fauna.

Historicamente a agricultura comercial no Estado teve início no decorrer do século XVIII, com o plantio de trigo pelos colonos açorianos. Até esse ponto, a agricultura presente no Estado estava ligada apenas a práticas de subsistência pelos povos indígenas. O primeiro registro de cultivo de soja no Brasil data de 1914 no município de Santa Rosa (região noroeste do Rio Grande do Sul), porém, somente na década de 1970 é que a soja se consolidou como a principal cultura do agronegócio brasileiro, onde 80% do volume produzido na época se concentrava nos três estados da Região Sul do Brasil (EMBRAPA, 2005).

Desde então o Bioma Pampa tem sofrido grande perda de biodiversidade e de habitats devido ao acelerado processo de expansão agrícola iniciado nos anos 1970, e agravado recentemente pelos planos para conversão de extensas áreas de campos em monoculturas florestais, de acordo com o Censo Agropecuário (MMA, 2007), restando, muitas vezes, apenas pequenos remanescentes em uma paisagem predominantemente agrícola (Risser, 1997; Porto, 2002; Bencke, 2003). Apenas 11.7% do Pampa permanece sem nenhum tipo de influência antrópica no Rio Grande do Sul (PROBIO, 2007), e por isso vem recebendo atenção especial do Ministério do Meio Ambiente como uma área prioritária para a conservação da biodiversidade (Hasenack, 2007) e práticas de inventários (MMA, 2007).

1.1.4. Inventários enquanto há tempo

Quantificar o número de espécies de um dado ambiente é a base da ecologia de comunidades onde os valores de riqueza de espécies são uma das medidas mais diretas da diversidade Magurran (1988), e o conhecimento das espécies que constituem uma comunidade é importante não somente para comparar diferentes ambientes mas também para a criação de propostas de conservação ambiental (Kruger, 2006). Desse modo, levantamentos contínuos de fauna são essenciais para o monitoramento do ambiente (Brown Jr., 1996).

Embora o estado do Rio Grande do Sul seja um dos mais bem estudados do Brasil em relação à fauna de drosofilídeos, grande parte dos estudos concentra-se em localidades pertencentes ao Bioma Mata Atlântica (Petersen, 1960; Franck & Valente, 1985; entre outros), enquanto que o Bioma Pampa tem sido grandemente negligenciado, sendo um dos mais inexplorados do Brasil, como notado por Gottschalk *et al*., (2008). Os únicos inventários da diversidade de Drosophilidae realizados neste bioma estão restritos a localidades na cidade de Porto Alegre e proximidades: na porção leste do estado em uma área florestada no Parque Estadual de Itapuã (Valente & Araújo, 1991), em uma área de campo em Guaíba (Saavedra *et al*., 1995) e nas regiões urbanas de Porto Alegre (Silva *et al*., 2005; Garcia *et al*., 2008; Garcia *et al*., 2012). E ainda em uma área de transição entre o Pampa e a Mata Atlântica na região central do estado (Hochmüller *et al*., 2010), onde foi encontrada uma inesperada riqueza de espécies, ou seja, sete espécies do gênero *Drosophila* e mais duas dos gêneros *Leucophenga* e

Rhinoleucophenga foram pela primeira vez registradas no estado do Rio Grande do Sul, destacando a diversidade local. E na região noroeste do estado há um único levantamento de espécies realizado por Poppe *et al*. (2012).

Além da carência de estudos no Pampa, outro fator que salienta a importância da realização de inventários nesse bioma é a pouca representatividade dos Campos Sulinos no Sistema de Unidades de Conservação, de modo que apenas 2.58% da área total de campos naturais ainda existentes no Estado encontram-se protegidos por UCs, sendo insuficiente para a proteção do patrimônio ecológico e genético do Pampa (Brandão *et al.*, 2007), onde inventários da fauna e da flora poderiam desempenhar um papel importante na proposta de ampliação das áreas de preservação.

Todavia, os campos sulinos, mesmo aqueles que se encontram em melhores estados de conservação, sofrem com um processo histórico de descaracterização devido à introdução de cultura de grãos e pecuária no Rio Grande do Sul, sendo que na nossa região de coleta isso se deve ao fato de que os solos férteis, que necessitam de pouca intervenção agrícola, estimulam os agricultores e causam a desconfiguração dos campos.

Segundo a Lista das Espécies da Flora Ameaçada de Extinção no Rio Grande do Sul, 213 táxons pertencentes a 23 famílias estão ameaçados de extinção, sendo que 146 destes táxons encontram-se no bioma Pampa (SEMA, 2002). Kivinen (2007) ressalta que na Finlândia ¼ de todas as espécies em risco de extinção estão associadas a áreas de agricultura. Jacques & Nabinger (2006), apontam para uma conversão de cerca de 130 mil ha/ano de campo nativo em diferentes culturas agrícolas (introdução de espécies forrageiras exóticas, cultivos de soja e arroz, e silvicultura), e os autores ainda salientam que se esta taxa de degradação se mantiver, muito provavelmente o Pampa desaparecerá até o fim deste século.

Sendo assim, apesar dos esforços dos pesquisadores brasileiros para amostrar a diversidade de drosofilídeos, mesmo em biomas bem estudados como a Mata Atlântica ainda há muitas espécies para serem descritas (Medeiros & Klaczko, 2004). E em todo o território nacional ainda existem muitas regiões praticamente desconhecidas, e inevitavelmente, com o estado de degradação de alguns biomas, muita informação já se perdeu com a extinção de espécies. Amplas áreas endêmicas estão sumindo tão depressa que os pesquisadores não tem mais a oportunidade de estudá-las satisfatoriamente (Döge *et al*., 2004; Blauth & Gottschalk, 2007).

Consequentemente, percebe-se a necessidade de realizar um levantamento de espécies de drosofilídeos visando ampliar o conhecimento e as informações taxonômicas mais específicas ao bioma Pampa brasileiro, antes que este bioma se torne mais devastado do que já está. O levantamento da fauna de Drosophilidae no Pampa fornecerá a base para muitas outras abordagens da questão Biodiversidade (considerando também os aspectos genéticos desses insetos) que requerem um rigoroso levantamento de espécies, de seus locais de criação e alimentação bem como das suas associações neste bioma tão importante quanto pouco explorado.

1.2.Objetivos

1.2.1. Objetivo geral:

Conhecer as espécies de Drosophilidae já registradas no bioma Pampa brasileiro, uruguaio e argentino assim como as regiões mais amostradas em cada país e buscar expandir os registros de ocorrência de espécies em ambiente natural do Pampa brasileiro, observando as influências que o ambiente exerce sobre as assembléias de Drosophilidae.

1.2.2. Objetivos específicos:

- Inventariar uma área natural de bioma Pampa, até então desconhecida quanto a diversidade de Drosophilidae. E também ampliar o conhecimento taxonômico referente à região sul do Brasil, mais especificamente o estado do Rio Grande do Sul, através de novos registros de espécies tanto para a região sul quanto para o bioma Pampa. (Capítulo II)
- Descrever novas espécies de drosofilídeos coletados em área natural do bioma Pampa. (Capítulo II)
- Realizar uma revisão da literatura sobre as espécies que compõem as assembléias de Drosophilidae no bioma Pampa, incluindo Brasil, Uruguai e Argentina e ainda apontar as regiões onde se concentram os maiores esforços amostrais em cada país. (Capítulo II)
- Caracterizar a estrutura e a dinâmica das assembléias de drosofilídeos presente no Bioma Pampa, na região noroeste do Rio Grande do Sul, através da abundancia de espécies, riqueza de espécies coletadas e equitabilidade dessas assembléias entre os ambientes de campo aberto, borda e interior das manchas de mata, no decorrer das estações do ano. (Capítulo III)

1.3.Resultados Gerais:

1.3.1. Do Pampa brasileiro, uma área natural na região noroeste do Rio Grande do Sul.

No total 7.164 drosofilídeos pertencentes a 51 espécies foram coletados, incluindo membros das subfamílias Drosophilinae e Steganinae, dos quais 36 espécies pertencem ao gênero *Drosophila*, duas espécies ao gênero *Amiota*, dez espécies ao gênero *Rhinoleucophenga*, uma espécie ao gênero *Zaprionus* e duas espécies são pertencentes ao gênero *Zygothrica*.

O gênero *Drosophila* dominou as amostras com 97% dos espécimes coletados, sendo assim, as espécies mais abundantes foram: *Drosophila simulans* Sturtevant (45.13%), *D. willistoni* Sturtevant (7.69%), *D. mediopunctata* Dobzanhsky & Pavan (5.93%), *D. buzzatii* Patterson & Wheeler (5.40%), *D. mercatorum* Patterson & Wheeler (4.40%), *D. maculifrons* Duda (4.13%), *D. immigrans* Sturtevant (1.80%), *D. hydei* Sturtevant (1.14%) e além do gênero *Drosophila*, ainda tivemos *Zaprionus indianus* Gupta como uma das espécies mais abundantes (1.68%).

Algumas espécies foram registradas pela primeira vez no bioma Pampa: *Drosophila briegeri* Pavan & Breuer, *D. fuscolineata* Duda, *Rhinoleucophenga obesa* Loew, *R. punctulata* Duda, *R. subradiata* Duda e *Zygothrica orbitalis* Sturtevant. Três espécies foram descritas para o gênero *Rhinoleucophenga*: *Rhinoleucophenga pampeana* sp. nov., *Rhinoleucophenga missionera* sp. nov. e *Rhinoleucophenga sulina* sp. nov. o que representa um aumento de aproximadamente 15% para o número de espécies deste gênero.

Apesar do registro do grupo da *Drosophila willistoni*, representado por *D. nebulosa* Sturtevant, *D. bocainensis* Pavan & da Cunha e pela própria *D. willistoni*, a ausência de *D. paulistorum* Dobzhansky & Pavan que já vem sendo observada por alguns autores (Dobzhansky & Pavan, 1950; Goñi *et al.*, 1998; Poppe *et al*., 2012) também foi percebida aqui.

A comunidade estudada foi composta principalmente por espécies Neotropicais (45 espécies), porém, em termos de abundância, dominada pela exótica *Drosophila simulans*.

Entre as espécies mais abundantes (com pelo menos 1% de abundância relativa) as exóticas estiveram em todos os ambientes analisados (campo aberto, borda e interior de mata), algumas das Neotropicais, tais como *D. willistoni*, *D. maculifrons* e *D. mediopunctata* nunca foram presentes no campo aberto. Porém, as Neotropicais do grupo da *D. repleta* Sturtevant demonstraram preferência pelo campo aberto, principalmente no período de outono.

De uma maneira geral, tanto espécies Neotropicais quanto exóticas foram mais concentradas na borda e no interior das manchas de mata, sendo esta tendência influênciada pela variação sazonal.

Outras espécies, tais como *D. flexa* Loew, *D. kikkawai* Burla, *D. mediostriata* Duda e *R*. lp10, embora menos abundantes nas amostras, apresentaram alta especificidade para a borda de mata, assim como *D. briegeri*, *D. capricorni* Dobzhansky & Pavan, *D. nebulosa*, *D. piratininga* Ratcov & Vilela, *D*. sp.2, *D*. sp.3, *R. sulina* sp. nov., *R*. lp3, *R*. lp5 e *Zygothrica ptilialis* Burla apresentaram alta especificidade para o interior de mata.

Analisando o padrão de distribuição das espécies entre os locais e através das estações, também foi possível perceber variações nas medidas de diversidade: índice de heterogeneidade de Shannon-Wiener (H’), índice de equitabilidade de Smith-Wilson

(E_{var}), riqueza observada de espécies (S_{obs}), riqueza de espécies estimada por rarefação (S_{rar}) e a abundância total de espécimes coletados em cada estação (N).

Desse modo, no ambiente de campo aberto o maior valor para o índice de heterogeneidade (H') foi no período de Abril/2011 (outono) e este mesmo índice decaiu drasticamente neste ambiente nos períodos de Julho e Dezembro, inverno e verão, respectivamente. Na borda de mata este índice foi também baixo no período de inverno. Por outro lado, no interior das manchas de mata não houve variação deste índice durante as estações, mas o índice de equitabilidade de Smith-Wilson (E_{var}) foi baixo na mata no período de Julho (inverno).

Nos três ambientes, a abundância total de espécimes coletados foi mais elevada no período de Outubro (primavera) e a riqueza observada de espécies (S_{obs}) no campo aberto a na borda da mata foi mais elevada no período de Abril/2011 (outono), bem como no interior da mata, o maior valor de Sobs foi no período de Outubro (primavera). A mesma tendência se manteve para a riqueza estimada por rarefação (S_{rar}), porém no interior da mata o maior valor foi em Dezembro (verão).

Apesar dessa interação entre local e estação, a sazonalidade parece ser o principal componente, sendo capaz de explicar 48.57% da variação do índice de heterogeneidade, enquanto que o local foi responsável por 38.82% e apenas 12.61% não foi explicado por nenhum desses dois componentes.

Além de iscas de banana, também foram trazidas para o laboratório algumas espécies de flores coletadas em campo: *Zephyranthes* sp. (Amaryllidaceae), *Elephantopus mollis* Kunth (Asteraceae), *Hypoxis decumbens* L. (Hypoxidaceae), *Ruellia morongii* Britton (Acanthaceae), *Indigofera asperifolia* Bong. (Fabaceae), *Aspilia montevidensis* Spreng. (Asteraceae), *Tibouchina gracilis* Bonpl.

(Melastomataceae), *Glandularia peruviana* L. (Verbenaceae), *Calendula arvensis* L. (Asteraceae), *Achyrocline satureioides* Lam. (Asteraceae), *Baccharis trimera* Less. (Asteroideae), *Hypochaeris radicata* L. (Asteraceae), *Hypochaeris maculata* L. (Asteraceae), *Calliandra eriophylla* Benth. (Fabaceae), *Cyperus pseudovegetus* Steud. (Cyperaceae), *Tillandsia aeranthos* Loisel. (Bromeliaceae), *Oxalis magnifica* Rose (Oxalidaceae); e também fungos dos gêneros *Pycnoporus* (Polyporaceae) e *Psilocybe* (Strophariaceae) e esterco de gado, mas nenhum drosofilídeo emergiu destes possíveis recursos naturais utilizados para oviposição.

1.3.2. Pampa Brasileiro, Uruguaio e Argentino.

Atualmente há 92 espécies descritas de Drosophilidae já registradas para todo o bioma Pampa, das quais 83 ocorrem no Brasil (se inclusas as três espécies de *Rhinoleucophenga* descritas neste estudo, estes números mudam para 95 e 86 espécies, respectivamente), 30 no Pampa argentino e 26 no Pampa uruguaio. Embora o Pampa brasileiro apresente mais do que o dobro do número de espécies do Pampa argentino, no território argentino ocorrem seis espécies não registradas no Brasil nem no Uruguai, são elas: *Cladochaeta bomplandi* Malloch, *D. koepferae* Fontdevila & Wasserman, *D. serenensis* Brncic, *Scaptomyza pallida* Zetterstedt, *Scaptomyza spinipalpis* Séguy e *Scaptomyza striaticeps* Wheeler & Takada.

Apesar do Pampa uruguaio não apresentar nenhuma espécie exclusiva, esta região compartilha três espécies com o Pampa argentino que não tem registro para a região do bioma brasileiro: *D. subobscura* Collin, *Scaptomyza graminum* Fallén e *Scaptomyza nigripalpis* Malloch. Quando o Pampa uruguaio é associado ao Pampa brasileiro, existem sete espécies de *Drosophila* não registradas para o Pampa argentino: *D. arassari* da Cunha & Frota-Pessoa, *D. denieri* Blanchard, *D. hydei* Sturtevant, *D.*

maculifrons Duda, *D. mediovittata* Frota-Pessoa, *D. ornatifrons* Duda e *D. virilis* Sturtevant.

Quanto ao número de localidades amostradas em cada país, sem considerar o esforço amostral, o Pampa uruguaio parece ser o mais explorado, com 38 pontos amostrados, porém, a maioria destes se concentra na região da capital Montevideo, sendo destacada a imensa área inexplorada na região central do Uruguai. O mesmo problema é percebido na Argentina, onde há poucos estudos neste bioma e os poucos pontos amostrados se concentram na região de Buenos Aires e Mar Del Plata. Mas apesar do Pampa brasileiro ser o mais rico quando comparado com os outros dois países, o mesmo problema é observado, ou seja, os pontos amostrados no Pampa brasileiro são concentrados principalmente na região metropolitana de Porto Alegre.

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2. CAPÍTULO II

Manuscrito a ser submetido ao Journal of Zoological Systematics and Evolutionary Research

2.1. High Biodiversity of Drosophilidae (Instecta, Diptera) in the Pampas Biome, with Descriptions of New *Rhinoleucophenga* Species.

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Keywords: Pampa biome, Drosophilidae, *Rhinoleucophenga*.

Abstract: In the last decades many faunal surveys of Drosophilidae have been done in various environments in Brazil. But approximately 30 to 50% of the drosophilids in Brazil have not yet been described and the degradation of some biomes causes a profound loss of species diversity and information about community structure. This is the situation with the Pampa, which covers southernmost Brazil, all of Uruguay, and the central east region of Argentina. Seasonal collections were made in a natural area of Pampa within the limits of the municipality of Bossoroca in the state of Rio Grande do Sul, Brazil (28° 45’024”S 54° 56’729”W), from April 2011 to April 2012. The survey of pampas Drosophilidae diversity was conducted via an intensive literature search of species recorded in Brazil, Uruguay and Argentina, including not only taxonomic studies, but also genetic, evolutionary and ecological ones. A total of 7,164 drosophilids of 51 species were collected, being 36 species belonging to the genus *Drosophila*, two to *Amiota*, ten to *Rhinoleucophenga*, one to *Zaprionus* and two to *Zygothrica*. Some species were for the first time recorded in the Pampa biome: *Drosophila briegeri*, *D. fuscolineata*, *Rhinoleucophenga obesa*, *R. punctulata*, *R. subradiata* and *Zygothrica orbitalis*. Furthermore, three new species of genus *Rhinoleucophenga* were described: *R. pampeana* sp. nov., *R. missionera* sp. nov. and *R. sulina* sp. nov. Despite Brazilian pampa being the richest when compared with Uruguay and Argentina, the three countries presented the same problem: huge areas totally unknown about the Drosophilidae fauna. The combination of this information and the knowledge of the current state of preservation of Pampa biome stress the necessity of a continuous study to unveil the Drosophilidae diversity in this biome.

INTRODUCTION

Quantifying the species composition of specific environments is fundamental to community ecology, and the richness value is one of the most direct measures of diversity (Magurran 1988). Knowledge of the species that comprise a community is important not only for comparing different environments, but also for assessing and proposing policy for environmental conservation (Kruger 2006). In this regard long-term, continuous surveys of a fauna are essential to environmental monitoring (Brown 1997).

In the last decades many faunal surveys of Drosophilidae have been done in various environments in Brazil: Atlantic rainforest (Araújo and Valente 1981; Medeiros and Klaczko 2004; De Toni et al. 2007; Döge et al. 2008; Gottschalk et al. 2009), Cerrado (Ferreira and Tidon 2005; Tidon et al. 2005; Tidon 2006; Mata et al. 2008, 2010), mangrove swamps (Schmitz et al. 2007, 2010), Caatinga (Mizuguchi 1978; Tidon-Sklorz and Sene 1995), Pantanal (Val and Marques 1996), Amazonian rainforest (Martins 1987, 1995, 2001), Araucarian forest (Saavedra et al. 1995b), restinga (Bizzo and Sene 1982; Bizzo et al. 2010), and more recently, Pampa (Silva et al. 2005; Garcia et al. 2012; Poppe et al. 2012). Despite such thorough surveying, many authors agree that 30 to 50% of the drosophilids in Brazil have not yet been described (Wheeler 1986; Val and Kaneshiro 1988; Medeiros and Klaczko 2004).

Concurrent with these biodiversity surveys, there has been the steady degradation of some biomes and what is probably a profound loss of species diversity and information about community structure. This is the situation with the Pampa, which covers southernmost Brazil, all of Uruguay, and the center east region of Argentina. Pampa was officially recognized as a Brazilian biome just in 2004 and, as a consequence, huge areas were devastated by agricultural expansion in the south of Brazil (MMA 2007). Nowadays, only little more than 11% of this biome occurs in its natural state, and only 2.58% is protected in "Conservation Units" (Brandão et al. 2007).

Information on the Drosophilidae of the Brazilian Pampa has been scarce, and in Uruguay Goñi et al. (1998, 2002, 2012) have recently developed inventories on the Drosophilidae in that region. In Argentina, most of what is known on Drosophilidae of Pampa is mainly from genetic or very focused autecological studies (Frota-Pessoa 1947; Salzano 1955; Pavan 1959; Wheeler and Magalhães 1962; Krivshenko 1963; Vilela 1990; Hale and Singh 1991; Fanara et al. 1999; Fernandez et al. 1999); few studies have been specifically about Drosophilidae assemblages or their ecology (Fernandez and Lopez 1995; Fanara et al. 1999; Montes et al. 2011). Thus there are broad areas lacking adequate investigation of the Drosophilidae fauna.

Therefore, due to the current environmental threats to Pampa and the lack of diversity studies in this biome, in the present paper we present an inventory of Drosophilidae in one locality in southern Brazil, with descriptions of three new species of *Rhinoleucophenga* genus. We also present a review of the historical records of Drosophilidae in this biome, in order to contribute toward general understanding of the occurrence of these flies in this biome.

MATERIAL AND METHODS

Study Area

The collecting area is a natural area of Pampa within the limits of the municipality of Bossoroca in the state of Rio Grande do Sul, Brazil (28° 45'024"S 54° 56'729"W) (Fig 1). According to MMA (2007) it is a priority area for conservation, with biological importance extremely high and practically unknown. The Field is compound mainly by C4 grasses, consisting a double structure of vegetation; the superior layer is characterized by *Aristida jubata* Herter (Poaceae) and the inferior layer by rhizomatous grasses of Poaceae family. Patches of forest also are part of the landscape mainly in the rivers margins. The livestock in the area allow the integrity of the natural characteristics of Pampa.

The Pampa is limited to high latitudes, with temperate weather and rain every month. The region of our collections is classified as "Cfa" according to Köppen classification, subtropical weather, presenting average of maximum temperatures higher than 22°C and the average of minimum temperatures between -3 and 18°C. The absence of outstanding dry periods is the main reason that Pampa cannot be considered as Savanna.

Collecting methods

Seasonal collections were made from April 2011 to April 2012. In each collection, thirty banana-baited traps (Tidon and Sene, 1988) were left in the field during three days, at a distance of approximately forty meters from each other. The traps were equally distributed among open field, forest edge, and inside of forest patches. The specimens caught were preserved in ethanol 96%. Substrates potentially used as breeding sites for drosophilids were collected in the field, kept in vials with vermiculite and taken to the laboratory for rearing specimens.

Description of New species

Three new species of *Rhinoleucophenga* genus, found in these field samples, are described here. Measurements and indexes for the description of new species follow Bächli et al. (2004); measurements given are averages followed by the ranges in parentheses, and they were taken with a reticle inserted into an optical stereomicroscope. Male and female terminalia were disarticulated in glycerol after treatment with 10% potassium hydroxide (KOH) and acid fuchsine (Bächli et al. 2004). Photos were taken with a digital camera coupled to an optical stereomicroscope, and drawings of the terminalia were made with a *camara lucida* attached to an optical microscope with a 10× objective lens and a 10× ocular lens. The terminology follows Grimaldi (1990), Vilela (1990) and Bächli et al. (2004). The type-series were deposited in the American Museum of Natural History (AMNH) in New York City, United States of America and in the Fundação Zoobotânica of Rio Grande do Sul (FZB/RS) in Porto Alegre, Rio Grande do Sul state, Brazil. The specimens were pinned (double-mounted) and the disarticulated terminalia kept in microvials with glycerol pinned with the respective specimens. Before pinning and taking photos, the specimens were critical-point dried with 100% ethanol.

Literature review

The survey of pampas Drosophilidae diversity was conducted via an intensive literature search of species recorded in Brazil, Uruguay and Argentina, including not only taxonomic works, but also genetic, evolutionary and ecological ones. We assumed the limits to Pampa suggested by Bilenca and Miñarro (2004) and according to i3GEO (2012). We are aware that some works possibly were not included in this revision, but feel that the great majority of references have been utilized. The sampled points referred to in each study are included in a Pampa biome map (Fig 2).

RESULTS AND DISCUSSION

New field samples

A total of 7,164 drosophilids of 51 species were collected in Bossoroca, including members of the two subfamilies Drosophilinae and Steganinae. Of these, 36 species belonged to the genus *Drosophila*, two to *Amiota*, ten to *Rhinoleucophenga*, one to *Zaprionus* and two to *Zygothrica* (Table 1).

The high richness of the genus *Drosophila* was expected since it is the most diverse genus in the Drosophilidae with 1,163 described species (Bächli 2012); the genus is also the best studied and the most attracted to banana-baited traps, reflected by research on species from the Pampa biome and other biomes (Dobzhansky and Pavan 1950; Pavan 1959; Martins 2001; Schmitz et al. 2007; De Toni et al. 2007; Mata et al. 2008; Bizzo et al. 2010; Garcia et al. 2012).

Indeed, our samples were dominated by species such as *Drosophila simulans* Sturtevant (45.13%), *D. willistoni* Sturtevant (7.69%), *D. mediopunctata* Dobzhansky & Pavan (5.93%), *D. buzzatii* Patterson & Wheeler (5.40%), *D. mercatorum* Patterson & Wheeler (4.40%), *D. maculifrons* Duda (4.13%), *D. immigrans* Sturtevant (1.80%), *D. hydei* Sturtevant (1.14%) and also one species of the genus *Zaprionus*: *Z. indianus* Gupta (1.68%) (Fig 3).

Also noticeable were some absences, such as *D. paulistorum* Dobzhansky & Pavan, whose absence was noted in other regions of the Pampa as Uruguay (Goñi et al. 1998) and the northwest region of the Rio Grande do Sul state, Brazil (Poppe et al. 2012). Cordeiro and Winge (1995) also stressed the low abundance of *D. paulistorum* and the dominance of *D. willistoni* in the Pampa biome, in the northeast region of Rio Grande do Sul state, near of the Porto Alegre city. Dobzhansky and Pavan (1950), collecting in the municipality of Santo Ângelo, an Atlantic rainforest environment close to our present collecting point, stressed the same situation: the presence of only *D. willistoni*, highlighting the preference of *D. paulistorum* for hot and very humid areas as in the tropical areas in northern Brazil. In a transition area between Pampa and Atlantic rainforest, in the central region of Rio Grande do Sul State, Hochmüller et al. (2010) found a sharp difference in the composition of the *D. willistoni* group, being 95% and 1%, respectively for the relative abundance of *D. willistoni* and *D. paulistorum* individuals. In the northern Pampa, region of Porto Alegre city, Garcia et al. (2012) recorded a dominance of 86% of *D. wllistoni* against just 2.26% of *D. paulistorum* among the species of *D. willistoni* group. Thus, *D. paulistorum* indeed seems to be more limited to Tropical regions.

In contrast, some species were recorded for the first time in the Pampa biome. *Drosophila briegeri* Pavan & Breuer, which is a Neotropical species that is rare in Brazil, had been collected in the south and southeast of the country (in the Atlantic rainforest) (Breuer and Pavan 1950a; Pavan and Breuer 1954; Medeiros and Klaczko 2004; De Toni et al. 2007; Gottschalk et al. 2007; Döge et al. 2008), but only now this species was recorded in the Pampa, being the first record of the *D. dreyfusi* group in this biome. In the same way, *D. fuscolineata* Duda, a Neotropical species of the *D. coffeata* group, had been collected in other biomes such as Cerrado (Tidon 2006; Mata et al. 2008) and Atlantic rainforest (Döge et al. 2008), and for the first time in the Pampa. But species of *D. coffeata* group, such as *D. pagliolii* Cordeiro had already been collected in the Pampa by Cordeiro (1963) in the municipalities of Santa Maria and Eldorado do Sul.

About the *Rhinoleucophenga* genus, *R. obesa* Loew has been collected in the Atlantic rainforest (De Toni et al. 2007; Hochmüller et al. 2010) and in the Cerrado (Blauth and Gottschalk 2007; Chaves and Tidon 2008) but only now in the Pampa biome. Other species are *R. punctulata* Duda and *R. subradiata* Duda, the first species according to Vilela and Bächli (2009) has been found in the Brazilian Cerrado and in the Argentinean and Bolivian Chaco. So, Bossoroca represents the new southernmost record for the species. *R. subradiata* was previously known only for the Bolivian Chaco, province of Santa Cruz, being recorded in Brazil for the first time, also representing its new southernmost locality.

Another Neotropical species commonly found in Brazil is *Zygothrica orbitalis* Sturtevant (Schmitz et al. 2007; Döge et al. 2007; De Toni et al. 2007) but it was the first record of this species in the Pampa biome. Its rarity here is probably due to its preference for fungi (Grimaldi 1987, 1990), being very uncommon in fruit baits.

Although the Pampa biome had already been considered by some researches as an environment inappropriate to find drosophilids, our findings about these species highlight the high diversity of Drosophilidae in this biome.

The use of banana baited traps is a very common technique in Drosophilidae studies (Dobzhansky and Pavan 1950; Bélo and Filho 1976; Tidon 2006; De Toni et al. 2007; Mata et al. 2008; Hochmüller et al. 2010; Garcia et al. 2012, and many others), and to make accurate comparisons possible we used this sampling regimen. However, to improve our sampling efforts and locate those species not attracted to fruit-baited traps, some species of flowers were collected in the field and brought to the laboratory, as follows: *Zephyranthes sp*. (Amaryllidaceae), *Elephantopus mollis* Kunth (Asteraceae), *Hypoxis decumbens* L. (Hypoxidaceae), *Ruellia morongii* Britton (Acanthaceae), *Indigofera asperifolia* Bong. (Fabaceae), *Aspilia montevidensis* Spreng. (Asteraceae), *Tibouchina gracilis* Bonpl. (Melastomataceae), *Glandularia peruviana* L. (Verbenaceae), *Calendula arvensis* L. (Asteraceae), *Achyrocline satureioides* Lam. (Asteraceae), *Baccharis trimera* Less. (Asteroideae), *Hypochaeris radicata* L. (Asteraceae), *Hypochaeris maculata* L. (Asteraceae), *Calliandra eriophylla* Benth. (Fabaceae), *Cyperus pseudovegetus* Steud. (Cyperaceae), *Tillandsia aeranthos* Loisel. (Bromeliaceae), *Oxalis magnifica* Rose (Oxalidaceae); as well as some fungi of genera *Pycnoporus* (Polyporaceae) and Psilocybe (Strophariaceae), as well as cattle dung. But no drosophilid emerged. Even so, some species as *D. bromelioides* Pavan and da Cunha, *Zygothrica orbitalis* and *Z. ptilialis* Burla, which are commonly found in flowers and fungi (Schmitz and Hofmann 2005; Gottschalk et al. 2009), were present in our samples (banana baited traps).

New pampean species

The largest genus of the Drosophilidae, *Drosophila*, represented slightly more than 97% of the sampled specimens, but another genus also was common in our samples: *Rhinoleucophenga*, notable for its relatively high diversity in the Pampa, with ten collected species, when compared with similar studies in other biomes. Furthermore, except *R. gigantea* Thomson, all the species of this genus are here being recorded for the first time from Pampa biome, although *R. obesa* and *R. punctulata* have been recorded in the Atlantic rainforest of southern Brazil (De Toni et al. 2007; Hochmüller et al. 2010) and in central Brazilian Cerrado (Blauth and Gottschalk 2007; Chaves and Tidon 2008).

From the ten species of the genus found in our samples, six constitute undescribed species. Three of them are described here as new species, *Rhinoleucophenga pampeana* sp. nov., *Rhinoleucophenga missionera* sp. nov. and *Rhinoleucophenga sulina* sp. nov. This increases the total number of *Rhinoleucophenga* species by 15%. The genus is entirely New World in distribution, with most species from South and Central America; only one species, *R. obesa* is widespread throughout North America.

Genus *Rhinoleucophenga* Hendel

Rhinoleucophenga Hendel, 1917: 44. Type species: *R. pallida* Hendel 1917

Rhinoleucophenga pampeana sp. nov.

Examined material. 2♂ and 3♀, collected in traps with fermented banana, in a natural area of Pampa biome in the municipality of Bossoroca, RS, Brazil, in April of 2012.

Type series. Holotype: 1♂ labelled "*Rhinoleucophenga pampeana*; HOLOTYPE ♂; Brazil, Rio Grande do Sul, Bossoroca. S28° 44'89" W54° 56'64", 07.iv.2012 col.: JL Poppe; banana bait". Postabdomen of holotype dissected, stored in microvials with glycerin, stored on the same pin with the respective specimen. Paratypes: 1♂ and 3♀ labelled "*Rhinoleucophenga pampeana*; PARATYPE; Brazil, Rio Grande do Sul, Bossoroca. S28° 44'89" W54° 56'64", 07.iv.2012 col.: JL Poppe; banana bait".

Type locality. Bossoroca, Rio Grande do Sul, Brazil.

Diagnosis. Head covered with ca. 200 scattered interfrontal setulae, thorax brownish and abdomen brownish with brown stripes that make the terminal abdominal portion darker. Transversal veins and the end of R2+3, R4+5 and M veins strongly clouded; costal and subcostal cell clouded. The R2+3 vein presents four clouded supernumerary veins. Male terminalia as in Figure 8 a-b.

Description. ♂. Head (Fig 4A). Front yellow brownish, covered with ca. 200 scattered interfrontal setulae, frontal length 0.89mm (0.78-1); frontal index = 0.98 (0.83-1.14); top-to-bottom width ratio = 1.13 (1.07-1.19); ocellar triangle-to-front length ratio = 0.28 (0.23-0.33); or1/or3 ratio = 0.92 (0.75-1.10); or2/or1 ratio = 1.02 (0.92-1.13); vibrissal index = 0.33 (0.29-0.37). Carina prominent and sulcate. Cheek index = 9.95 (8.6-11.3). Eye index = 1.54 (1.52-1.56). Antennas with the scape and pedicel same color as frons, flagellomere grayish, arista plumose, with 10 dorsal branches and 8 ventral branches plus terminal fork. Palpus yellow with ca. 50 setae along lower margin.

Thorax (Fig 5A). Thorax length 3.15mm (2.91-3.4). Scutum and scutellum brownish; scutum with a median yellow brownish longitudinal stripe in the anterior portion; 14 rows of acrostichal setulae. 3 pairs of prescutellar setae, the central pair strongest, about 76.5% (66-87) of posterior dorsocentral setae. Only one pair of postpronotal setae. Transverse distance of dorsocentral setae 5.89x (5.53-6.25) of longitudinal distance. Basal scutellar setae divergent. Sterno index = 0.87 (0.84-0.9); median katepisternal setae absent. Halteres yellow whitish. Legs light yellow.

Wings (Fig 6A). Transverse veins and the end of R2+3, R4+5 and M veins strongly clouded; costal and subcostal cell clouded. In the holotype, the R2+3 vein with four clouded supernumerary veins that extend in direction to the costal vein, but not reaching it. In the ♂ paratype the posterior supernumerary vein is directed to the R4+5 vein. Length 5.91mm (5.52-6.3); width 2.57mm (2.34-2.8). Indices: C = 4.79 (4.73-4.85); Hb = 0.47 (0.42-0.53); Ac = 0.95 (0.9-1); 4c = 0.57 (0.57-0.58); 4v = 1.39 (1.37-1.42); 5x = 0.72 (0.66-0.79); M = 0.36 (0.33-0.40); prox.x = 2.71 (2.70-2.72).

Abdomen (Fig 7A). Brownish, with faint brown stripes covering 1/3 of tergite II and ½ of the tergite III, medially interrupted only on tergite II. Wider stripes posterior to tergite IV making the abdomen darker in the posterior portion.

Body length 5.85mm (5.7-6).

Terminalia ♂ (Fig 8 A-B). Aedeagus ring-like, dorsoventrally flattened, with short protrusions on the top. Apodeme long and bifurcated bifurcate in posterior portion. Surstyli fused to epandrium, on each side with ca. 28 black rod-shaped prensisetae slightly round at tip. About 8 inner setae and 4 outer setae in each side. About 4 superior setae and 25-30 lower setae in each side of epandrium. Small cerci with ca. 20-25 setae on each half.

♀. Head (Fig 4B). Same color pattern as male, front covered with ca. 200 scattered interfrontal setulae. Front length = 0.86mm (0.8-0.92); frontal index = 0.95 (0.75-1.15); top-to-bottom width ratio = 1.12 (1-1.24); ocellar triangle-to-front length ratio = 0.37 (0.29-0.45); or1/or3 ratio = 0.8 (0.63-0.96); or2/or1 ratio = 0.6 (0.5-0.69). Vibrissal index = 0.4 (0.36-0.44). Carina prominent and sulcate. Cheek index = 10.3 (9.08-11.6).

Eye index = 1.42 (1.38-1.47). Antennas with the scape and pedicel same color as frons, flagellomere grayish; arista plumose with 11 dorsal branches and 8 ventral branches plus terminal fork. Palpus in the same front color, with ca. 50 setae along lower margin.

Thorax (Fig 5B). Color pattern lighter than male. Thorax length 2.87mm (2.6-3.13). Scutum and scutellum brownish. 14 rows of acrostichal setulae. 3 pairs of prescutellar setae, the central pair strongest, about 71% (67-75) of posterior dorsocentral setae. Only one pair of postpronotal setae. Transverse distance of dorsocentral setae 5.6x (5.2-6) of longitudinal distance. Basal scutellar setae divergent. Sterno index = 1.05 (0.8-1.3); median katepisternal setae absent. Halteres yellow whitish. Legs light yellow.

Wings (Fig 6B). Transverse veins and the end of R2+3, R4+5 and M veins strongly clouded; costal and subcostal cell clouded. Vein R2+3 with two clouded supernumerary veins that extend in direction to the costal vein, but not reaching it. Length 5.8mm (5.6-6); width 2.4mm (2.3-2.5). Indices: C = 4.51 (4.28-4.75); Hb = 0.48 (0.37-0.6); Ac = 1.05 (1-1.11); 4c = 0.66 (0.63-0.7); 4v = 1.6 (1.51-1.7); 5x = 0.8 (0.7-0.9); M = 0.39 (0,3-0.49); prox.x = 2.85 (2.79-2.92).

Abdomen (Fig 7B). Same color pattern of male.

Body length: 5.73mm (5.17-6.3).

Terminalia ♀ (Fig 8 C-D). Cerci long with four apical setae on each half. Epiproct with ca. 20 setae and the hypoproct with ca. 40, being 8 apical setae longer than the others. Spermathecal capsule slightly rounded and without apparent friezes.

Etymology. The species name refers to the biome where it was found, the Pampa biome.

Distribution. Known only for the type locality.

Biology. Collected in traps with fermented banana.

Rhinoleucophenga missionera sp. nov.

Examined material. 1♂ and 4♀, collected in traps with fermented banana, in a natural area of Pampa biome in the municipality of Bossoroca, RS, Brazil, in December of 2011.

Type series. Holotype: ♂ labelled "*Rhinoleucophenga missionera*; HOLOTYPE ♂; Brazil, Rio Grande do Sul, Bossoroca. S28° 44'89" W54° 56'64", 23.xii.2011 col.: JL Poppe; banana bait". Postabdomen of holotype dissected, stored in microvials with glycerin, stored on the same pin with the respective specimen. Paratypes: 4♀ labelled "*Rhinoleucophenga missionera*; PARATYPE; Brazil, Rio Grande do Sul, Bossoroca. S28° 44'89" W54° 56'64", 23.xii.2011 col.: JL Poppe; banana bait".

Type locality. Bossoroca, Rio Grande do Sul, Brazil.

Diagnosis. Head covered with ca. 50 scattered interfrontal setulae, thorax brownish and abdomen brownish with brown stripes that make the terminal abdominal portion darker. Wings hyaline, IC= 3.57 in male and 3.51 in female. Male terminalia as in Figure 9 A-B.

Description. ♂. Head (Fig 4C). Front brown, covered with ca. 50 scattered interfrontal setulae, frontal length 0.73mm; frontal index = 1.17; top-to-bottom width ratio = 1.60; ocelar triangle-to-front length ratio = 0.33; or1/or3 ratio = 0.85; or2/or1 ratio = 0.77; vibrissal index = 0.3. Carina prominent and sulcate. Cheek index = 8.77. Eye index = 1.47. Antenna with flagellomeres same color as frons, arista plumose, with 6 dorsal branches and 5 ventral branches plus terminal fork. Palpus light brownish with ca. 20 setae along lower margin.

Thorax (Fig 5C). Thorax length 2.22mm. Scutum and scutellum brownish; scutum with three narrow longitudinal brown stripes between the dorsocentral setae. 12 rows of acrostichal setulae. 3 pairs of prescutellar setae, the central pair strongest, about 53% of posterior dorsocentral setae. Only one pair of postpronotal setae. Transverse distance of dorsocentral setae 4x of longitudinal distance. Basal scutellar setae divergent. Sterno index = 1; median katepisternal setae absent. Halteres yellow whitish. Legs yellow.

Wings (Fig 6C). Hyaline. Length 3.65mm; width 1.60mm. Indices: C = 3.57; Hb = 0.37; Ac = 1.21; 4c = 0.88; 4v = 2.09; 5x = 1.23; M = 0.62; prox.x = 3.46.

Abdomen (Fig 7C). Brownish with brown stripes slightly interrupted covering 1/2 of the tergite II, III and IV. Wider stripes posterior to tergite V making the abdomen brown darker in the posterior portion.

Body length: 4.67mm.

Terminalia ♂ (Fig 9 A-B). Aedeagus oval, elongate dorsoventrally; straight in apical portion. Paraphyses long and slightly twisted. Apodeme long and bifurcate in posterior portion.

♀. Head (Fig 4D). Same color pattern as male. Front covered with ca. 50 scattered interfrontal setulae. Front length = 0.62mm (0.52-0.72); frontal index = 1.01 (0.93-1.09); top-to-bottom width ratio = 1.16 (1.08-1.25); ocelar triangle-to-front length ratio = 0.32 (0.28-0.36); or1/or3 ratio = 0.98 (0.93-1.03); or2/or1 ratio = 0.61 (0.56-0.66). vibrissal index = 0.32 (0.29-0.36). Carina prominent and sulcate. Cheek index = 8.9 (7.9-9.9). Eye index = 1.45 (1.42-1.48). Antenna with scape and pedicel same color as frons, flagellomere grayish; arista plumose with 6 dorsal branches and 5 ventral branches plus terminal fork. Palpus brownish, with ca. 20 setae along lower margin.

Thorax (Fig 5D). Same color pattern as male. Thorax length 1.92mm (1.78-2.06). Scutum and scutellum brownish. 12 rows of acrostichal setulae. 3 pairs of prescutellar setae, the central pair strongest, about 53% (53-54) of posterior dorsocentral setae. Only one pair of postpronotal setae. Transverse distance of dorsocentral setae 4x (3.5-4.5) of longitudinal distance. Basal scutellar setae divergent. Sterno index = 0.99 (0.87-1.12); median katepisternal setae absent. Halteres yellow whitish. Legs brownish.

Wings (Fig 6D). Hyaline. Length 3.9mm (3.7-4.1); width 1.6mm (1.5-1.7). Indices: C = 3.51 (3.27-3.74); Hb = 0.42 (0.35-0.5); Ac = 1.39 (1.3-1.48); 4c = 0.91 (0.87-0.96); 4v = 2.09 (1.94-2.23); 5x = 1.53 (1.23-1.83); M = 0.65 (0.59-0.7); prox.x = 3.38 (3.20-3.56).

Abdomen (Fig 7D). Same color pattern of male.

Body length: 3.96mm (3.52-4.4).

Terminalia ♀ (Fig 9 C-D). Cerci long with ca. 30 setae, three longer apical setae in each one. Epiproct short with ca. 14 similar setae. Hypoproct larger than long, with ca. 30, being 3 apical setae longer. Spermathecal capsule slightly elongate and narrow medially.

Etymology. The species name refers to the region where it was found, in the state of Rio Grande do Sul State, region of Missões.

Distribution. Known only for the type locality.

Biology. Collected in traps with fermented banana.

Rhinoleucophenga sulina sp. nov.

Examined material. 2♂ and 2♀, collected in traps with fermented banana, in a natural area of Pampa biome in the municipality of Bossoroca, RS, Brazil, in December of 2011 and in April of 2012.

Type series. Holotype: ♂ labelled "*Rhinoleucophenga sulina*; HOLOTYPE ♂; Brazil, Rio Grande do Sul, Bossoroca. S28° 44'89" W54° 56'64", 23.xii.2011 col.: JL Poppe; banana bait". Postabdomen of holotype dissected, stored in microvials with glycerin, stored on the same pin with the respective specimen. Paratypes: 1♂ and 1♀ labelled "*Rhinoleucophenga sulina*; PARATYPE; Brazil, Rio Grande do Sul, Bossoroca. S28° 44'89" W54° 56'64", 23.xii.2011 col.: JL Poppe; banana bait".

Type locality. Bossoroca, Rio Grande do Sul, Brazil.

Diagnosis. Head covered with ca. 60 scattered interfrontal setulae, thorax brownish and abdomen brownish with interrupted brown stripes on tergites II, III and IV; tergites V, VI and VII darker in the males and lighter in the females. Wings hyaline, IC= 2.46 to male and 2.55 to female. Male terminalia as in Figure 12 A-C.

Description. ♂. Head (Fig 4E). Front brownish, covered with ca. 60 scattered interfrontal setulae, frontal length 0.61 mm (0.52-0.7); frontal index = 1.27 (1.11-1.44); top-to-bottom width ratio = 1.05 (1-1.11); ocellar triangle-to-front length ratio = 0.32 (0.30-0.34); or1/or3 ratio = 1.19 (1.14-1.25); or2/or1 ratio = 0.71 (0.66-0.76); vibrissal index = 0.24 (0.21-0.28). Carina prominent and sulcate. Cheek index = 8.33 (8.16-8.5). Eye index = 1.54 (1.5-1.58). Antenna same color as frons, arista plumose, with 7 dorsal branches and 6 ventral branches plus terminal fork. Palpus in the same color as front, with ca. 20 setae along lower margin.

Thorax (Fig 5E). Thorax length 2.03mm (1.82-2.25). Scutum and scutellum brownish; 16 rows of acrostichal setulae. 3 pairs of prescutellar setae, the central pair strongest, about 59% (58-60) of posterior dorsocentral setae. Only one pair of postpronotal setae. Transverse distance of dorsocentral setae 4.53x (4.3-4.77) of longitudinal distance. Basal scutellar setae divergent. Sterno index = 1.01 (1.01-1.02); median katepisternal setae absent. Halteres yellow whitish. Legs light yellow.

Wings (Fig 6E). Hyaline. Length 3.32mm (3.3-3.35); width 1.65mm (1.5-1.8). Indices: C = 2.46 (2.42-2.5); Hb = 0.37 (0.35-0.39); Ac = 1.50 (1.49-1.51); 4c = 1.28 (1.27-1.29); 4v = 2.5 (2.4-2.6); 5x = 2.1 (2.9-2.12); M = 1.10 (1.04-1.16); prox.x = 3.84 (3.8-3.88).

Abdomen (Fig 7E). Brownish with brown strips interrupted medially covering 1/3 of the tergites II, III, IV and 2/3 of the tergites V, VI and VII making terminal portion of abdomen brown.

Body length: 4.07mm (3.8-4.35).

Terminalia ♂ (Fig 10 A-C). Epandrium microtrichose with surstyli fused. Approximately 17 prensisetae and about 5 inner setae and 13 outer setae in each side. About 15 superior setae and 30 lower setae in each side. Small cerci with ca. 35 setae in each one, with 3-4 longer setae in the apical portion. Aedeagus oval, wider medially and the apical portion is narrower than the base. Apodeme long and bifurcate in the posterior region. Hypandrium wider than length.

♀. Head (Fig 4F). Same color pattern as male, front covered with ca. 60 scattered interfrontal setulae. Front length = 0.56mm (0.52-0.6); frontal index = 1.3 (1.16-1.44); top-to-bottom width ratio = 1.29 (1.15-1.43); ocellar triangle-to-front length ratio = 0.29 (0.24-0.34); or1/or3 ratio = 1.03 (0.95-1.10); or2/or1 ratio = 0.76 (0.76-0.77); vibrissal index = 0.35 (0.3-0.41). Carina prominent sulcate. Cheek index = 11.97 (11.75-12.2). Eye index = 1.73 (1.72-1.73). Antenna same color as frons; arista plumose with 8 dorsal branches and 6 ventral branches plus terminal fork. Palpus same color as frons, with ca. 20 setae along lower margin.

Thorax (Fig 5F). Color pattern same as male. Thorax length 1.9mm (1.9-2). 12 rows of acrostichal setulae. 3 pairs of prescutellar setae, the central pair strongest, about 54% (52-56) of posterior dorsocentral setae. Only one pair of postpronotal setae. Transverse distance of dorsocentral setae 4.29x (3.72-4.86) of longitudinal distance. Basal scutellar setae divergent. Sterno index = 0.94 (0.93-0.95); median katepisternal setae absent. Halteres yellow whitish. Legs light yellow.

Wings (Fig 6F). Hyaline. Length 3,5mm (3.4-3.6); width 1.6mm (1.5-1.7). Índices: C = 2.55 (2.5-2.6); Hb = 0.32 (0.28-0.37); Ac = 1.44 (1.44-1.45); 4c = 1.25 (1.25-1.26); 4v = 2.53 (2.48-2.58); 5x = 2.17 (2-2.35); M = 1.14 (1.12-1.17); prox.x = 3.75 (3.72-3.78).

Abdomen (Fig 7F). The pattern of stripes of the tergites II, III and IV is the same as the male, but color pattern is lighter than the male.

Body length: 3.7mm (3.5-3.8).

Terminalia ♀ (Fig 10 D-E). Cerci long with ca. 30 setae, 7 longer apical setae in each one. Epiproct with ca. 15 setae. Hypoproct with ca. 20, being 12 longer setae. Spermathecal capsule slightly rounded.

Etymology. The species name refers to the region where it was found in the Brazilian territory.

Distribution. Known only for the type locality.

Biology. Collected in traps with fermented banana.

Discussion: The species that are described here unquestionably belong to the genus *Rhinoleucophenga* based on the following features: strong prescutellar setae, only two katepisternal setae, only one pair of postpronotal setae, basal scutellar setae divergent, frons densely covered with scattered interfrontal setulae; surstyli fused to epandrium bearing small, peg-like prensisetae; aedeagus simple, reduced.

Rhinoleucophenga pampeana sp. nov. resembles *R. obesa*, *R. joaquina* Schmitz, Gottschalk & Valente, *R. lopesi* Malogolowkin and *R. gigantea* based on the annular structure of the aedeagus and an apodeme that is long and bifurcate posteriorly. It also resembles *R. obesa* for having wings with clouded cross veins, arista with 10 dorsal branches and legs yellowish. Resemblances to *R. matogrossensis* Malogolowkin include the coloration and body size, and 14 rows of acrostichal setulae. But it clearly differs of all known species of the genus by its distinctive pattern of clouding wings and by the clouded supernumerary veins in the R2+3 veins.

R. missionera sp. nov. resembles *R. matogrossensis* by the darker color pattern and by the transverse distance between dorsocentral setae (4x the longitudinal distance), however it differs of *R. matogrossensis* for presenting arista with 6 dorsal branches and 5 ventral branches. It also resembles to *R. lopesi* by the thorax color, for 12 rows of acrostichal setulae, and hyaline wings, but it differs of *R. lopesi* for presenting 3 pairs of prescutellar setae and the transverse distance of dorsocentral setae 4x (3.5-4.5) of longitudinal distance.

R. sulina sp. nov. resembles *R. subradiata* by the body color pattern, including the striped pattern on the tergites. It differs from *R. subradiata* by the arista with long branches, and by differences in the aedeagus structure, which is similar to other species of this genus, with an aspect slightly annular and by an apodeme that is long and bifurcated.

Unfortunately, like most members of the *Rhinoleucophenga*, very little is known of the habitats, distribution or biology of these new species. And further researches are necessary to fill these gaps.

Historical occurrence

The first record of a Drosophilidae in the Pampa biome was probably *Rhinoleucophenga gigantea*, by Thomson (1896) in Buenos Aires, Argentina. It was followed by other sparse records in the same country between the decades of 1920 and 1930 by general dipterologists (Duda 1929; Malloch 1934; Séguy 1934), followed by studies carried out by geneticists and taxonomists specialized in Drosophilidae in the decade of 1940 (Fernandez Gianotti 1944; Frota-Pessoa 1947). Since 1980’s with studies mainly of Fontdevila, Vilela, Hasson and Fernandez the records of drosophilids in the Argentinean portion of Pampa have increased, but they are normally sparse records focused mainly on systematic and evolutionary issues, with few broad inventories (rare exceptions are Fernandez and Lopez 1995, in Mar del Plata, and Montes et al. 2011, in Tandil). In Uruguay the first record of Drosophilidae was

Scaptomyza graminum Fallén and *S. nigripalpis* Malloch (Malloch 1934), but most of the records are more recent from the inventory researches developed by Goñi (Goñi et al. 1998, 2002, 2012). In the Brazilian Pampa, the first record of drosophilids is from the classical study of Dobzhansky and Pavan (1943) followed by few, but classical studies of Cordeiro, Pavan, Frota-Pessoa and Brncic between the decades of 50 and 70, motivated mainly by the fertile researches in Genetic and Evolution with Neotropical species of *Drosophila* as model organisms that had been proposed by Th. Dobzhansky in the country. Since 1990's the Drosophilidae records in the Brazilian Pampa has been increased by the studies developed mainly by the research group of UFRGS coordinated by one of us (VLS Valente), however most of these studies have been developed in the region of Porto Alegre city.

Currently, there are 92 described species recorded in the Pampa biome (if considered the three new species of *Rhinoleucophenga* genus described in the present research this number increases to 95 species) (Table 2), 10 of which are exotic species. *D. busckii* Coquillett, *D. immigrans*, *D. melanogaster* Meigen, *D. simulans*, *D. ananassae* Doleschall, *D. kikkawai* Burla and *D. virilis* Sturtevant are present in Neotropical region since early studies and is not clear when they invaded the Pampa. More recently was recorded the invasion of *Zaprionus indianus* in the Pampa (Castro and Valente 2001; Goñi et al. 2001; Lavagnino et al. 2008). This species probably invaded the Pampa coming from the north, after its first record in America done by Vilela (1999), in São Paulo, Brazil. Other more recent invasive species is *D. malerkotliana* Parshad & Paika that was recorded for the first time in the Neotropics by Sene in 1976 (Val and Sene 1980), in the Northeast region of Brazil, but there is only one record of this species in the Pampa done by Garcia et al. (2008). After that, *D. malerkotliana* has not been recorded in the Pampa, although it is common in the tropical environments of South America. Among the more recent invasive species *D. subobscura* Collin seems to be the unique that invaded the Pampa coming from Temperate region, since it was previously recorded in Chile (Brncic and Budnik 1979; Brncic et al. 1981) and was recorded for the first time in the Argentinean Pampa by Prevosti et al. (1983) and Lopez (1985), followed by Goñi and Martinez (1995) in Uruguay. Until today, it was never found in Brazil.

In the Brazilian Pampa, were recorded up to now 83 out of the 92 species recorded in Pampa biome (if considered the three new species of *Rhinoleucophenga* genus described in the present research these numbers increase to 86 and 95 species, respectively). In the Argentinean and Uruguayan Pampa were recorded 30 and 26 species, respectively. Although the Brazilian Pampa contains almost three times more species than the Argentinean Pampa, in the latter there are six species that only were recorded in this area of Pampa up to now: *Cladochaeta bomplandi* Malloch, *D. koepferae* Vilela, *D. serenensis* Brncic, *Scaptomyza pallida* Zetterstedt, *Scaptomyza spinipalpis* Séguy and *Scaptomyza striaticeps* Wheeler and Takada. However, in the Uruguayan Pampa there are no exclusive species, but there are three species: *D. subosbcura*, *Scaptomyza graminum* and *Scaptomyza nigripalpis* that only have been recorded in the Uruguayan and Argentinean Pampa. Also there are other seven species recorded between Uruguay and Brazil: *D. arassari* da Cunha and Frota-Pessoa, *D. denier* Blanchard, *D. hydei*, *D. maculifrons* Duda, *D. mediovittata* Frota-Pessoa, *D. ornatifrons* Duda and *D. virilis* that have not been recorded in the Argentinean portion of Pampa.

This superiority in the Brazilian richness could be related by two main factors, such as the proximity of the Brazilian Pampa with the Atlantic rainforest and the higher sample efforts employed in this area.

Thus an important aspect observed here is the number of sampled areas in each country and the proximity among these sites (Table 3). Although the Uruguayan Pampa seems to be better explored, based on a higher number of localities, 38 (Fig 2 and 11), only eight of them are not concentrated near the coast and the region of Montevideo, and at some of these localities only one collection was developed (Goñi et al. 1998). A huge, unsampled area occurs in the central region of Uruguay. The same problem exists for the Argentinean Pampa, where there are few diversity inventories focusing specifically on this biome, most of the studies are about genetic issues (Salzano 1955; Ruiz et al. 1984; Soto et al. 2005) or are only about a specific species or sporadic samples including Pampa and other environments (Frota-Pessoa 1947; Pavan 1959; Brncic 1978; Vilela 1990). Only two sampled points are not in the Buenos Aires and Mar del Plata neighborhoods and thus is possible to observe that most part of the Argentinean Pampa is not sampled (Fig. 2).

Despite Brazilian Pampa being the richest when compared with Uruguay and Argentina, the Brazilian researches present the same problem of the sampled localities being concentrated, in this case mainly near Porto Alegre (Table 3). There are only two sampled localities in the central region of Rio Grande do Sul State (Loreto et al. 1998; Tidon-Sklorz and Sene 2001), one locality in the northwest of the State (Poppe et al. 2012) and one sampled locality in the southwest region (Barros 1950) but this last point was only a reference to *D. paranaensis* Barros, therefore as can be observed in the figure 2 there are many gaps without sampling in the Brazilian Pampa. According to MMA (2007) there are some areas considered priority to conservation studies, and the most part of these areas are in the central and northwest of Rio Grande do Sul State and consequently were not explored yet, being important localities for future study.

CONCLUSION

The Brazilian portion of the Uruguayan savanna has revealed a surprising diversity of Drosophilidae. The presence of six species recorded for the first in the pampas and even in Brazil, along with three new species of *Rhinoleucophenga*, reflects the importance of studies in natural areas of this endangered biome.

A total of 95 Drosophilidae species known thus far from the Brazilian, Uruguyan and Argentinian pampas is probably still a gross underestimate because most of this biome is still not intensively sampled, despite the fact that it includes areas considered to be of extremely high biological importance (Olson et al. 2001; MMA 2007). Most of collections in the present study were made with banana baits, but since some species are scarcely attracted by this substrate their low frequency does not at all represent the actual size of the population in the community, as Magalhães and Bjornberg (1957) and Magalhães (1962) have cautioned. A good case in point is *Rhinoleucophenga*, which is diverse in pampas but not commonly attracted to the bait traps. If the larvae of these flies are predators of grass-feeding scale insects and other sternorrhynchans, as a few scattered reports indicate, then their abundance and species diversity has been significantly underestimated for pampas.

The combination of this information and the knowledge of the current state of preservation of pampas biome stress the necessity of preserving natural areas of pampas, such as our collecting site, since modified areas, such as rural and urban areas, have already presented low richness of species (Poppe et al. 2012), representing a loss of diversity. Thus, is extremely important the creation of new conservation areas to preserve the natural biodiversity of pampas.

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TABLES

Table 1: Absolute abundance of the collected drosophilids in the municipality of Bossoroca during the collecting periods.

Species	April/2011	July	October	December	April/2012	Total
Amiota sp01	0	1	0	0	1	2
A. sp02	0	0	0	17	0	17
Drosophila antonietae Tidon-Sklorz & Sene	1	0	5	9	6	21
D. arassari da Cunha & Frota-Pessoa	0	0	0	6	0	6
D. bandeirantorum Dobzhansky and Pavan	2	0	1	0	0	3
D. bocainensis Pavan & da Cunha	3	0	0	0	1	4
D. briegeri Pavan & Breuer	4	6	4	0	0	14
D. bromelioides Pavan & da Cunha	4	1	0	0	0	5
D. busckii Coquillett	1	2	2	0	0	5
D. buzzatii Patterson & Wheeler	190	0	23	30	143	386
D. capricorni Dobzhansky & Pavan	2	0	0	0	0	2
D. cardini Sturtevant	40	6	0	2	0	48
D. flexa Loew	0	1	0	0	0	1
D. fuscolineata Duda	2	0	3	0	0	5
D. hydei Sturtevant	66	0	15	1	0	82
D. immigrans Sturtevant	2	2	123	1	0	128
D. kikkawai Burla	0	1	0	0	0	1
D. maculifrons Duda	73	222	7	3	0	305
D. mediopicta Frota-Pessoa	1	5	5	0	0	11
D. mediopunctata Dobzhansky & Pavan	12	402	6	1	3	424
D. mediostriata Duda	2	0	0	0	0	2
D. melanogaster Meigen	0	0	36	0	0	36
D. mercatorum Patterson & Wheeler	120	3	125	19	48	315

D. nebulosa Sturtevant	0	3	3	0	0	6
D. nigricruria Patterson & Mainland	0	1	11	4	0	16
D. onca Dobzhansky & Pavan	0	10	12	2	0	24
D. ornatifrons Duda	0	22	21	5	1	49
D. pallidipennis Dobzhansky & Pavan	4	1	12	12	0	29
D.paraguayensis Duda	3	23	12	1	0	39
D. piratininga Ratcov & Vilela	0	1	0	0	0	1
D. polymorpha Dobzhansky & Pavan	33	4	14	15	0	66
D. prosaltans Duda	2	0	3	0	0	5
D. simulans Sturtevant	622	15	1832	449	312	3230
D. sp2	0	2	0	0	0	2
D. sp3	0	1	0	0	0	1
D. sp7	0	0	1	6	0	7
D. sturtevanti Duda	4	0	0	1	0	5
D. willistoni Sturtevant	107	5	84	336	18	550
Rhinoleucophenga gigantea Thomson	5	0	0	4	0	9
R. lp10	0	1	0	1	0	2
R. lp3	0	0	0	1	0	1
R. lp5	0	0	0	1	0	1
R. missionera sp. nov.	0	0	0	5	0	5
R. obesa Loew	0	0	0	0	4	4
R. pampeana sp. nov.	0	0	0	0	5	5
R. punctulata Duda	1	0	0	8	6	15
R. subradiata Duda	0	0	0	17	0	17
R. sulina sp. nov.	0	0	0	3	1	4
Zaprionus indianus Gupta	95	0	1	2	22	120
Zygothrica ptilialis Burla	0	6	0	0	0	6

Z. orbitalis Sturtevant	0	2	0	0	1	3
Gr. annulimana (females)	0	0	2	0	0	2
Gr. cardini (females)	105	12	32	4	3	156
Gr. repleta (females)	0	3	164	60	186	413
Gr. saltans (females)	1	0	6	0	1	8
Gr. tripunctata (females)	16	464	57	3	0	540
Total	**1523**	**1228**	**2622**	**1029**	**762**	**7164**

Table 2: List of collected drosophilids in the pampas biome, and the respective locality where they were record. To locality codes see Table 3.

Genus	Group	Species	Locality code
Cladochaeta	*bomplandi*	*C. bomplandi* Malloch	20
Drosophila	*annulimana*	*D. annulimana* Duda	8
		D. arassari da Cunha & Frota-Pessoa	3, 56
		D. schineri Pereira & Vilela	4, 6, 8, 11
	bromeliae	*D. bromelioides* Pavan & da Cunha	3, 8
	busckii	*D. busckii* Coquillett	3, 6, 8, 15, 17, 21, 27, 32, 46, 52, 53, 57, 61, 65, 68, 70
	calloptera	*D. quadrum* Wiedemann	6
	canalinea	*D. piratininga* Ratcov & Vilela	3, 8
	cardini	*D. cardini* Sturtevant	3, 6, 8, 11, 71
		D. cardinoides Dobzhansky & Pavan	4, 5, 6, 8, 9, 11, 15
		D. neocardini Streisinger	8, 9
		D. polymorpha Dobzhansky & Pavan	3, 4, 6, 8, 9, 11, 15, 21, 32, 46, 52, 65, 66
	coffeata	*D. fuscolineata* Duda	3
		D. pagliolii Cordeiro	2, 4
	dreyfusi	*D. briegeri* Pavan & Breuer	3
	flavopilosa	*D. cestri* Brncic	4, 6, 8, 11, 16
		D. cordeiroi Brncic	8, 16
		D. flavopilosa Frey	8, 16, 21, 32
		D. incompta Wheeler &Takada	4, 6, 8, 11, 12, 16
	guarani	*D. alexandrei* Cordeiro	6
		D. griseolineata Duda	4, 6, 8, 11, 15
		D. maculifrons Duda	3, 4, 6, 8, 11, 15, 33

	D. ornatifrons Duda	3, 5, 6, 8, 56, 71
immigrans	*D. immigrans* Sturtevant	3, 4, 5, 6, 8, 11, 15, 17, 27, 32, 50, 51, 54, 55, 57, 63, 64, 65, 66, 68, 69, 70, 71
melanogaster	*D. ananassae* Doleschall	8, 21
	D. kikkawai Burla	3, 4, 6, 8, 11, 21
	D. melanogaster Meigen	3, 4, 6, 8, 11, 15, 17, 21, 25, 26, 32, 47, 48, 49, 53, 54, 55, 56, 57, 61, 62, 66, 68, 69, 70
	D. malerkotliana Parshad & Paika	8
	D. simulans Sturtevant	3, 4, 5, 8, 11, 15, 17, 21, 27, 32, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 61, 62, 65, 68, 69, 70
mesophragmatica	*D. gaucha* Jaeger & Salzano	4, 6, 8, 11, 17, 21, 27, 32, 52, 53, 64, 65, 68, 70, 71
obscura	*D. subosbcura* Collin	17, 27, 52
pallidipennis	*D. pallidipennis* Dobzhansky & Pavan	3, 6, 8, 15
repleta	*D. aldrichi* Patterson	15
	D. antonietae Tidon-Sklorz & Sene	3, 8, 11, 14,15
	D. buzzatii Patterson & Wheeler	3, 5, 8, 15, 17, 18, 21, 29, 30, 32, 46, 52, 53, 65
	D. hydei Sturtevant	3, 4, 6, 8, 11, 15, 17, 32, 46, 52, 61, 62, 63, 65, 66, 68, 69, 71
	D. koepferae Fontdevila & Wasserman	18, 30
	D. mercatorum Patterson & Wheeler	3, 4, 6, 8, 11, 15, 18, 21, 27, 30, 52, 71
	D. meridionalis Wasserman	8, 18, 30, 54, 56, 71

	D. nigricruria Patterson & Mainland	3, 15, 17
	D. onca Dobzhansky & Pavan	1, 3, 8, 15
	D. paranaensis Barros	13
	D. repleta Wollaston	15, 21, 32, 70, 71
	D. serido Vilela & Sene	6
	D. zottii Vilela	8
saltans	*D. prosaltans* Duda	3, 4, 6, 8, 11
	D. pulchella Sturtevant	8
	D. sturtevanti Duda	3, 4, 6, 8, 11
sticta	*D. sticta* Wheeler	8
tripunctata	*D. angustibucca* Pavan	4, 6, 8, 11
	D. bandeirantorum Dobzhansky and Pavan	3, 6, 8, 15
	D. cuaso Bächli, Vilela & Ratcov	8
	D. mediopicta Frota-Pessoa	3, 4, 5, 6, 8, 11
	D. mediopunctata Dobzhansky & Pavan	3, 4, 5, 6, 8, 11, 15
	D. mediosignata Dobzhansky & Pavan	4, 6, 8, 11
	D. mediostriata Duda	3, 4, 6, 8, 11
	D. mediovittata Frota-Pessoa	8, 52, 53, 71
	D. nappae Vilela, Valente & Basso-da-Silva	8, 71
	D. neoguaramunu Frydenberg	8
	D.paraguayensis Duda	3, 5, 8, 15, 71
	D. paramediostriata Townsend & Wheeler	8
	D. roehrae Pipkin & Heed	8
	D. trifilum Frota-Pessoa	8
virilis	*D. virilis* Sturtevant	15, 32, 65

	willistoni	*D. bocainensis* Pavan & da Cunha	3, 5, 6, 8, 10, 23
		D. capricorni Dobzhansky & Pavan	3, 4, 6, 8, 11
		D. fumipennis Duda	8
		D. nebulosa Sturtevant	3, 6, 8, 17, 21, 46, 49, 52, 54, 57, 67, 68
		D. parabocainensis Carson	8, 10
		D. paulistorum Dobzhansky & Pavan	4, 6, 8, 11
		D. willistoni Sturtevant	3, 4, 6, 8, 11, 15, 21, 26, 32, 33, 37, 38, 41, 46, 48, 49, 50, 52, 56, 57, 59, 65, 66, 68, 69, 71
	Ungrouped	*D. caponei* Pavan & da Cunha	8
		D. denieri Blanchard	8, 46, 49, 60, 64
		D. flexa Loew	3, 8
		D. lutzii Sturtevant	8
		D. serenensis Brncic	18, 30
Leucophenga	*Ungrouped*	*L. maculosa* Coquillett	15, 20, 24
Rhinoleucophenga	*Ungrouped*	*R. gigantea* Thomson	3, 21
		R. obesa Loew	3
		R. Punctulata Duda	3
		R. pampeana sp. nov.	3
		R. missionera sp. nov.	3
		R. sulina sp. nov.	3
		R. subradiata Duda	3
Scaptomyza	*vittata*	*S. nigripalpis* Malloch	21, 32
	Ungrouped	*S. graminum* Fallen	19, 32
		S. pallida Zetterstedt	24
		S. spinipalpis Seguy	26
		S. striaticeps Wheeler & Takada	21

Zaprionus	*armatus*	*Z. indianus* Gupta	3, 8, 15, 22, 31, 32, 34, 35, 36, 39, 40, 42, 43, 44, 45
Zygothrica	*bilineata*	*Z. bilineata* Williston	7
	hypandriata	*Z. hypandriata* Burla	8
	orbitalis	*Z. orbitalis* Sturtevant	3
	vittimaculosa	*Z. vittimaculosa* Burla	4, 6, 8, 11, 15
	Ungrouped	*Z. ptilialis* Burla	3, 8

Table 3: Locality codes where drosophilids had been reported from pampas biome, with their respective coordinates, and the references where each site was mentioned. *: the present study.

Code	Site	Country	Coordenates	References
1	Arroio Teixeira	Brazil	30°41'0" S 51°23'0" W	Diniz & Sene (2004)
2	Bexiga (Santa Maria)	Brazil	29°59'24" S 52°22'40" W	Cordeiro (1963)
3	Bossoroca	Brazil	28°43'48" S 54°54'00" W	Poppe et al.*
4	Eldorado	Brazil	30°5'2" S 51°36'57" W	Magalhães (1962), Cordeiro (1963), Loreto et al. (1998)
5	Guaiba	Brazil	30°6'50" S 51°19'30" W	Saavedra et al. (1995a; 1995b)
6	Itapuã	Brazil	30°23'20" S 51°2'60" W	Cordeiro (1951), Burla & Pavan (1953), Grimaldi (1990), Valente & Araújo (1991), Loreto et al. (1998)
7	Morungava (Gravataí)	Brazil	29°51'3" S 50°55'3" W	Grimaldi (1990)
8	Porto Alegre	Brazil	30°1'59" S 51°13'48" W	Dobzhansky & Pavan (1943), Frota-Pessoa (1954), Brncic (1978), Brncic & Valente (1978), Val (1982), Santos & Valente (1990), Valiati & Valente (1996), Loreto et al. (1998), Castro & Valente (2001), Ananina et al. (2002), Costa et al. (2003), Vilela et al. (2004), Silva et al. (2005a; 2005b), Schmitz & Hofmann (2005), Garcia et al. (2008), Garcia et al. (2012),
9	Restinga (POA)	Brazil	30°1'59" S 51°13'48" W	Napp & Cordeiro (1981)
10	Ponta Grossa (POA)	Brazil		Carson (1954), Wheeler & Magalhães (1962)
11	Santa Maria	Brazil	29°41'0" S 53°48'0" W	Loreto et al. (1998)
12	Santa Vitoria do Palmar	Brazil	33°31'8" S 53°22'5" W	Hofmann & Napp (1984)
13	Santana do Livramento	Brazil	30°52'39" S 55°31'59" W	Barros (1950)
14	São Francisco de Assis	Brazil	29°33'0" S 55°7'51" W	Tidon-Sklorz & Sene (2001)
15	São Luiz Gonzaga	Brazil	28°22'51" S 55°00'62" W	Poppe et al. (2012)
16	Sapucaia	Brazil	29°50'20" S 51°8'38" W	Brncic (1978), Val (1982), Hofmann & Napp (1984)

17	Tandil	Argentina	37°19'0" S 59°09'0" W	Montes et al. (2011)
18	Arroyo Escobar	Argentina	34°20'18" S 58°43'27" W	Fontdevila et al. (1982), Hasson et al. (1991), Hasson et al. (1992), Rodriguez et al. (2000)
19	Bahia Blanca	Argentina	38°43'0" S 62°16' 0" W	Malloch (1934)
20	Bompland	Argentina	29°49'3" S 57°25'40" W	Malloch (1934), Frota-Pessoa (1947), Vilela & Bachli (1990)
21	Buenos Aires	Argentina	34°36'12" S 58°22'54" W	Brethes (1907), Knab (1912), Fernandez Gianotti (1944), Pavan (1959), Hackman (1959), Wheeler et al. (1962), Krivshenko (1963), Freire-Maia & Freire-Maia (1964), Wheeler & Takada (1966), Thomson (1869), Spassky et al. (1971), Ashburner & Leumeunier (1976), Brncic (1978), Vilela (1990), Godoy-Herrera & Silva (1997), Arriaza-Onel & Godoy-Herrera (1999), Soto et al. (2005)
22	Diamante	Argentina	32°4'0" S 60°39'0" W	Lavagnino et al. (2008)
23	El Destino (Buenos Aires)	Argentina	38°46'01" S 60°27'0" W	Salzano (1955)
24	Germania (Buenos Aires)	Argentina	34°34'13" S 62°3'4" W	Staatliches Museum für Naturkunde Stuttgart, Germany, Collection (1988)
25	San José (Buenos Aires)	Argentina	34°52'12"S 58°20'6" W	Duda (1929)
26	La Plata	Argentina	34°55'16" S 57°57'16" W	Seguy (1934), Cordeiro et al. (1958), Hackman (1959), da Cunha et al. (1959), Wheeler (1970), Spassky et al. (1971) Singh & Rhomberg (1987), Hale & Singh (1987, 1991)
27	Mar Del Plata	Argentina	38° 0'0" S 57°33'0" W	Lopez (1985), Brncic (1987), Fernandez & Lopez (1995), Fernandez et al. (2000)
28	Moreno (Buenos Aires)	Argentina	34°39'4" S 58°47'24" W	Hasson et al. (1992)
29	Otamendi	Argentina	34°14'3" S 58°53'10" W	Fernandez et al. (1999), Casals et al. (2003)
30	Quilmes	Argentina	34°43'0" S 58°16'0" W	Ruiz et al. (1984), Fontdevila et al. (1988), Hasson et al. (1992), Rossi et al. (1996), Fanara et al. (1999), Fanara & Hasson (2001), Casals et al. (2003), Sorensen et al. (2005)

31	San Pedro	Argentina	33°40'46" S 59°40'1" W	Lavagnino et al. (2008)
32	Montevideo	Uruguay	34°53'1" S 56°10'55" W	Malloch (1934), Hackman (1959), Wheeler et al. (1962), Fresia et al. (2001), Goñi et al. (2001), Goñi et al. (2002), Valente et al. (2003), Rohmer et al. (2004), Ayrinhac et al. (2004), David et al. (2006), Yassin et al. (2007)
33	Melo	Uruguay	32°21'58" S 54°10'16" W	Da Cunha & Dobzhansky (1954), Salzano (1995)
34	Arazati	Uruguay	34°54'30" S 56°9'17" W	Goñi et al. (2002)
35	Castilos	Uruguay	33°53'00" S 57°40'00" W	Goñi et al. (2002)
36	Colônia Valdense	Uruguay	34°24'36" S 57°14'48" W	Goñi et al. (2002)
37	La Coronilla	Uruguay	33°54'0" S 53°31'0" W	Garcia et al. (2006)
38	Laguna Negra	Uruguay	34°1'0" S 53°38'0" W	Garcia et al. (2006)
39	LasBrujas	Uruguay	34°38'0" S 56°20'0" W	Goñi et al. (2002)
40	Maldonado	Uruguay	34°54'0" S 54°57'0" W	Goñi et al. (2002)
41	Piriapolis	Uruguay	34°54'0" S 54°57'0" W	Sassi et al. (2005)
42	Rivera	Uruguay	30°54'9" S 55°33'2" W	Goñi et al. (2002)
43	Rocha	Uruguay	34°29'0" S 54°21'0" W	Goñi et al. (2002)
44	San Javier (Parque Nacional Esteros de Farrapos)	Uruguay	32°39'59" S 58°8'10" W	Goñi et al. (2002)
45	Santa Lucia	Uruguay	34°27'9" S 56°23'47" W	Goñi et al. (2002)
46	Arroyo Gajo del Lunarejo, Rivera	Uruguay	31°13'60" S 55°52'60" W	Goñi et al.(1998)
47	Salto, Salto	Uruguay	31°23'2.27" S 57°56'59" W	Goñi et al.(1998)
48	Tacuarembo, Tacuarembo	Uruguay	31°22'60" S 57°56'60" W	Goñi et al.(1998)
49	Mercedes, Soriano	Uruguay	33°15'38" S 58°1'43" W	Goñi et al.(1998)
50	Sauce del Cebollatí, Lavalleja	Uruguay	34°0'14" S 54°57'40" W	Goñi et al.(1998)
51	Potrero Grande, Rocha	Uruguay	34°29'0" S 54°20'58" W	Goñi et al.(1998)
52	Boca del Sarandi, Rocha	Uruguay	34°28'60" S 54°21'0" W	Goñi et al.(1998)
53	Sarandi del Consejo, Rocha	Uruguay	34°28'60" S 54°21'0" W	Goñi et al.(1998)

54	Castillos, Rocha	Uruguay	34°11'60" S 53°51'0" W	Goñi et al.(1998)
55	Costa Azul, Rocha	Uruguay	34°37'56" S 54°9'25" W	Goñi et al.(1998)
56	Cerro del Toro, Maldonado	Uruguay	34°53'0" S 54°58'30" W	Goñi et al.(1998)
57	Terrazas de Puerto, Piriápolis, Maldonado	Uruguay	34°51'53" S 55°16'10" W	Goñi et al.(1998)
58	Las Flores, Piriápolis, Maldonado	Uruguay	34°49'37" S 55°18'39" W	Goñi et al.(1998)
59	Santa Lucia del Este, Canelones	Uruguay	34°47'17" S 55°32'13" W	Goñi et al.(1998)
60	El Pinar, Canelones	Uruguay	34°47'7" S 55°54'37" W	Goñi et al.(1998)
61	Rincón de Melilla, Montevideo	Uruguay	34°50'2" S 56°3'25" W	Goñi et al.(1998)
62	Parque Lecocq, Montevideo	Uruguay	34°47'31" S 56°20'3" W	Goñi et al.(1998)
63	Punta Espinillo, Montevideo	Uruguay	34°49'45" S 56°21'59" W	Goñi et al.(1998)
64	Paso de la Arena, Montevideo	Uruguay	34°49'13" S 56°20'26" W	Goñi et al.(1998)
65	Parque Vaz-Ferreira, Montevideo	Uruguay	34°53'41" S 56°15'23" W	Goñi et al.(1998)
66	Facultad de Agronomia, Montevideo	Uruguay	34°50'11" S 56°13'11" W	Goñi et al.(1998)
67	Jardin Botanico, Montevideo	Uruguay	34°51'33" S 56°12'1" W	Goñi et al.(1998)
68	Plaza fabini, Montevideo	Uruguay	34°54'20" S 56°11'39" W	Goñi et al.(1998)
69	IIBCE, Montevideo	Uruguay	34°53'14" S 56°8'33" W	Goñi et al.(1998)
70	Facultad de Ciencias, Montevideo	Uruguay	34°52'55" S 56° 7'2" W	Goñi et al.(1998)
71	Sierra de Minas	Uruguay	34°30'59" S 55°20'07" W	Goñi et al. (2012)

FIGURE LEGENDS

Figure 1: South America indicating the pampas biome (light green) and the collecting point (the municipality of Bossoroca) in the south of Brazil (28° 45’024”S 54° 56’729”W).

Figure 2: Pampas biome map showing all of the sampled sites in the Brazilian (o), Uruguayan (Δ) and Argentinean Pampa (□). The black line represents the pampas biome boundaries. References for the numbers are in Table 3. Map source: Google Earth® 2012.

Figure 3: Relative abundance of the most common species in each sampled period: April of 2011 (April/2011); July of 2011 (July); October of 2011 (October); December of 2011 (December); April of 2012 (April/2012).

Figure 4: Head of *Rhinoleucophenga pampeana* sp. nov. (Male (a), Female (d)), *Rhinoleucophenga missionera* sp. nov. (Male (b), Female (e)) and *Rhinoleucophenga sulina* sp. nov. (Male (c), Female (f)). Scale bar = 0.5 mm.

Figure 5: Thorax of *Rhinoleucophenga pampeana* sp. nov. (Male (a), Female (d)), *Rhinoleucophenga missionera* sp. nov. (Male (b), Female (e)) and *Rhinoleucophenga sulina* sp. nov. (Male (c), Female (f)). Scale bar = 0.5 mm.

Figure 6: Wings of *Rhinoleucophenga pampeana* sp. nov. (Male (a), Female (d)), *Rhinoleucophenga missionera* sp. nov. (Male (b), Female (e)) and *Rhinoleucophenga sulina* sp. nov. (Male (c), Female (f)). Scale bar = 0.5 mm.

Figure 7: Abdomen of *Rhinoleucophenga pampeana* sp. nov. (Male (a), Female (d)), *Rhinoleucophenga missionera* sp. nov. (Male (b), Female (e)) and *Rhinoleucophenga sulina* sp. nov. (Male (c), Female (f)). Scale bar = 0.5 mm.

Figure 8: Terminalia of *Rhinoleucophenga pampeana* sp. nov. Epandrium (a), aedeagus (b), dorsal view of female terminalia (c), ventral view of female terminalia (d) and the spermatheca (aa).

Figure 9: Terminalia of *Rhinoleucophenga missionera* sp. nov. Ventral view of aedeagus (a), lateral view of aedeagus (b), dorsal view of female terminalia (c), ventral view of female terminalia (d) and the spermatheca (aa).

Figure 10: Terminalia of *Rhinoleucophenga sulina* sp. nov. Epandrium (a), ventral view of aedeagus (b), dorsal view of aedeagus (c), dorsal view of female terminalia (d), ventral view of female terminalia (e) and the spermatheca (aa).

Figure 11: Detailed view of the most intensively sampled areas of pampas in (A) Brazil, (B) Argentina and (C) Uruguay.

Figure 1:

Figure 2

Figure 3

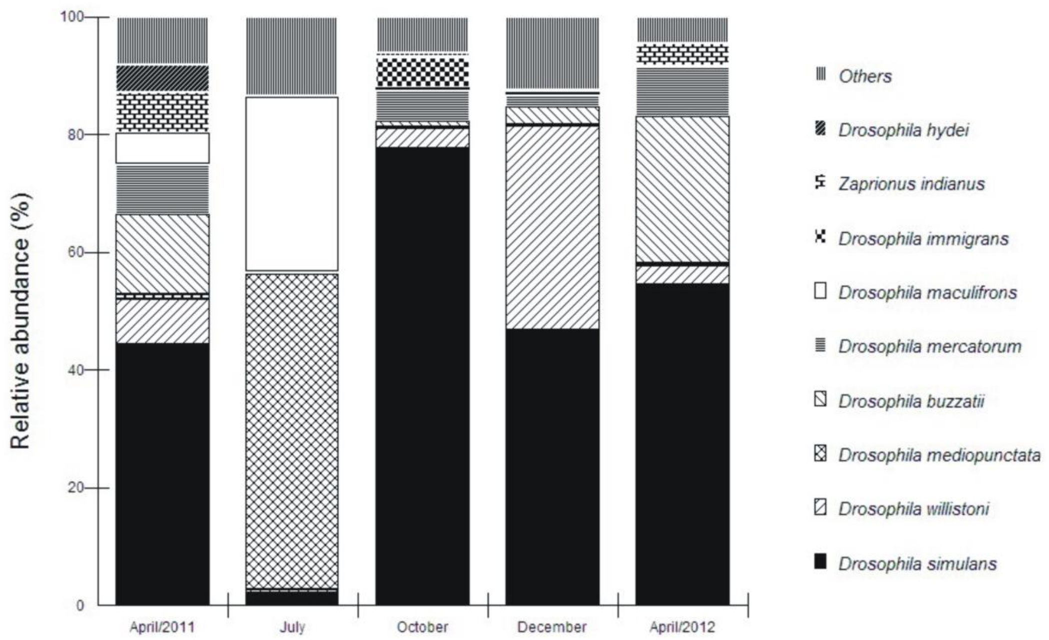
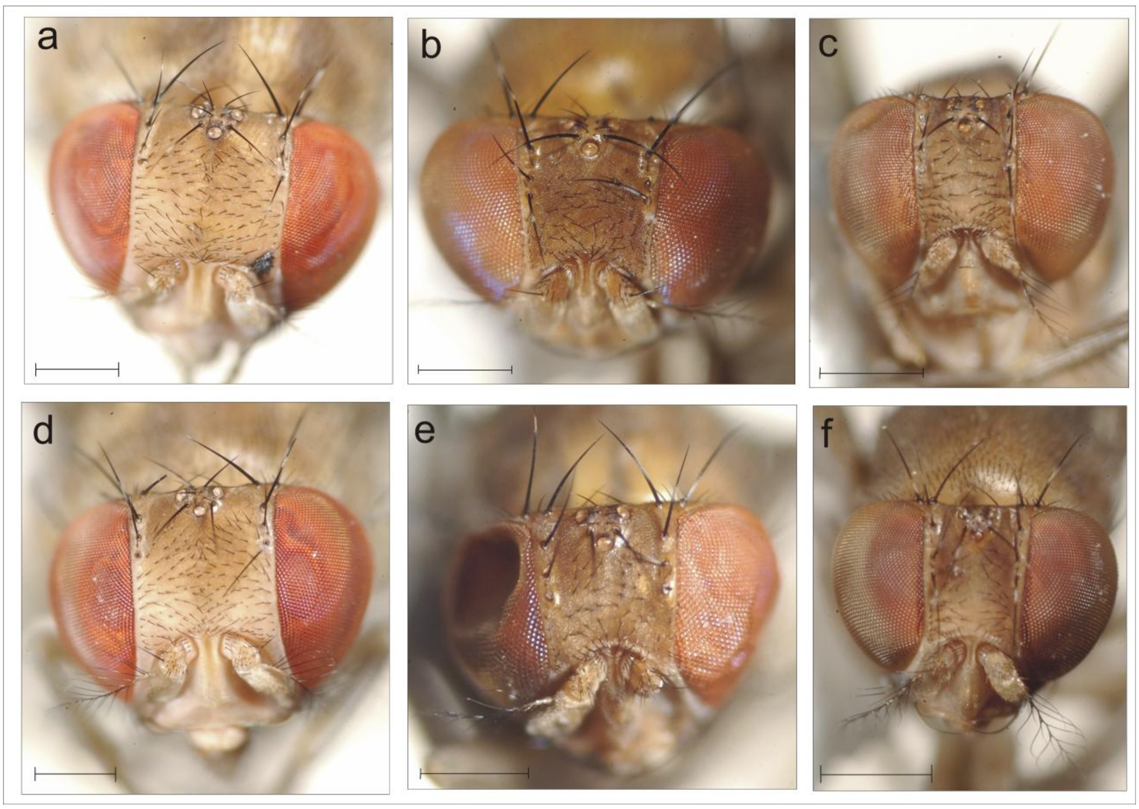


Figure 4



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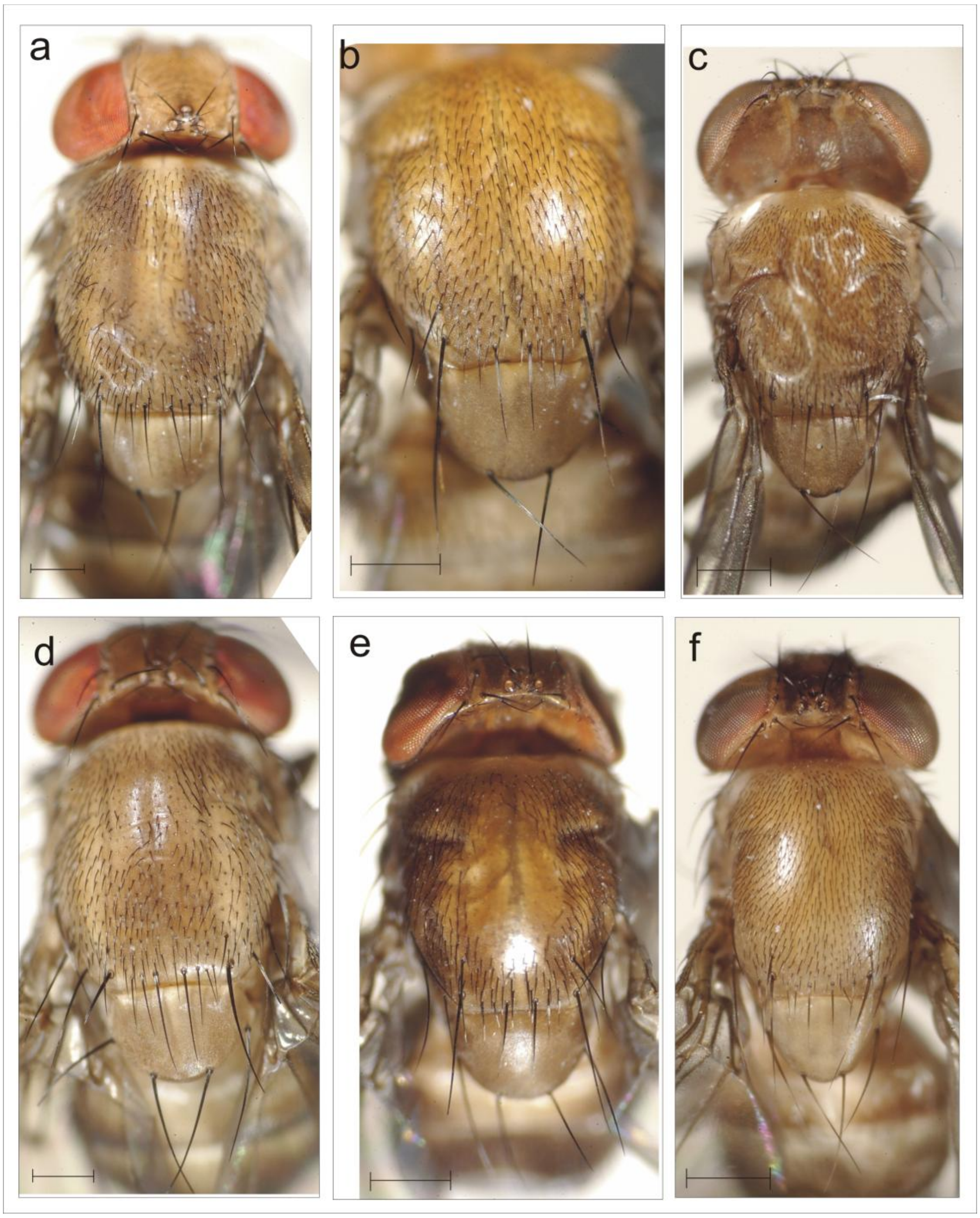

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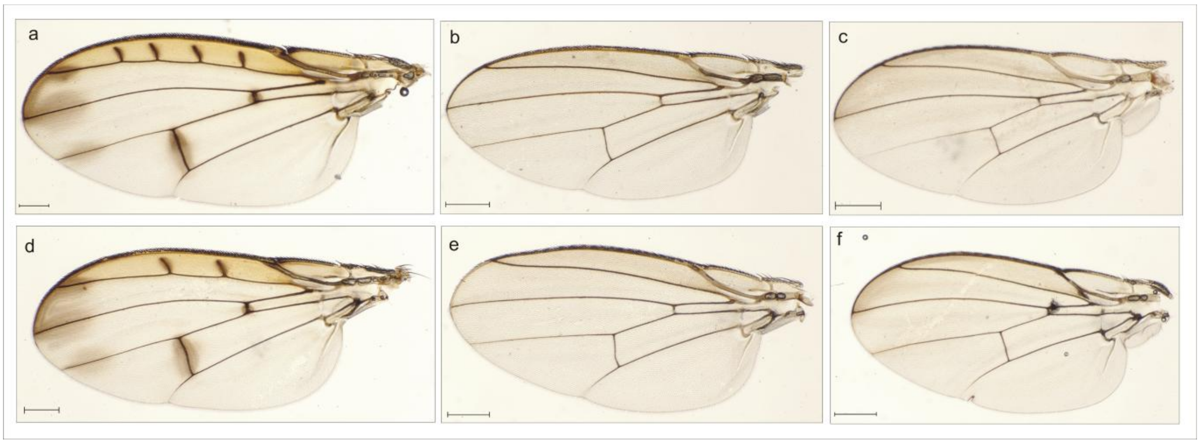

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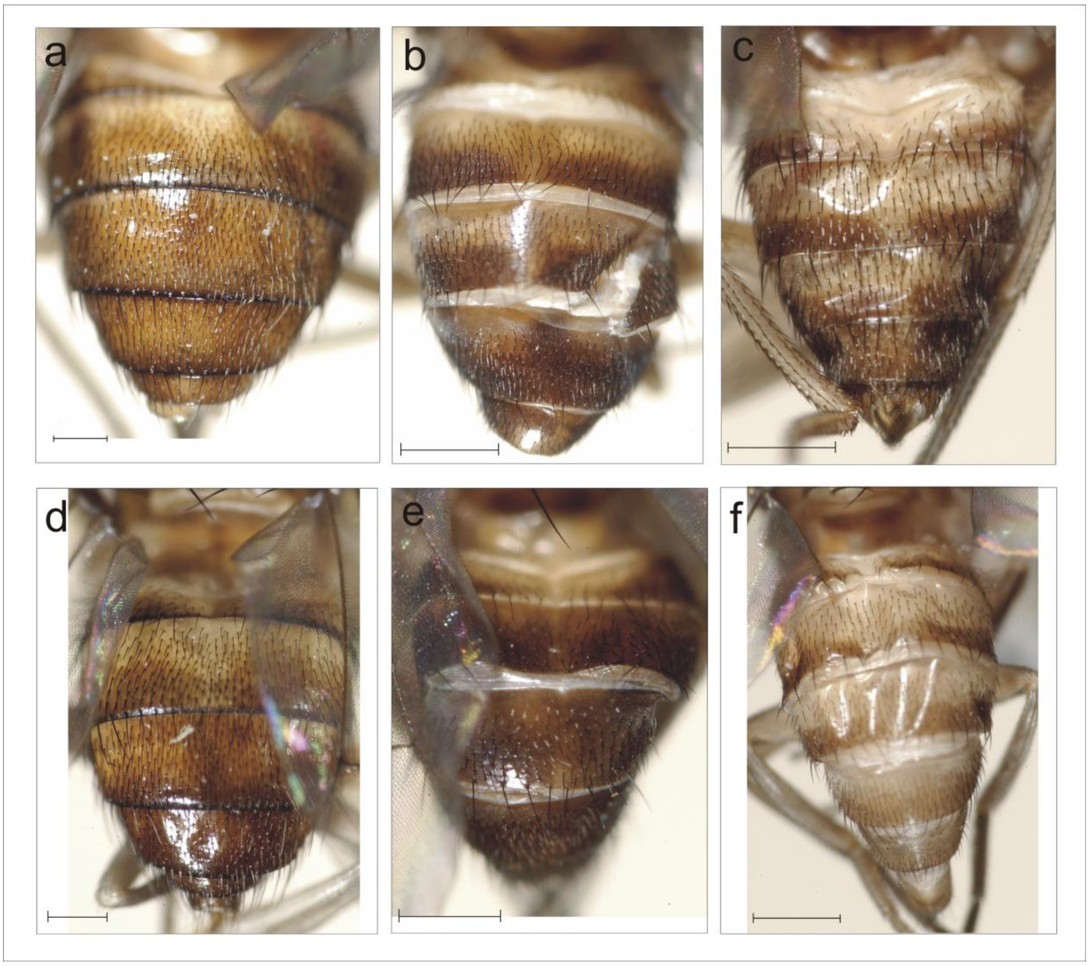

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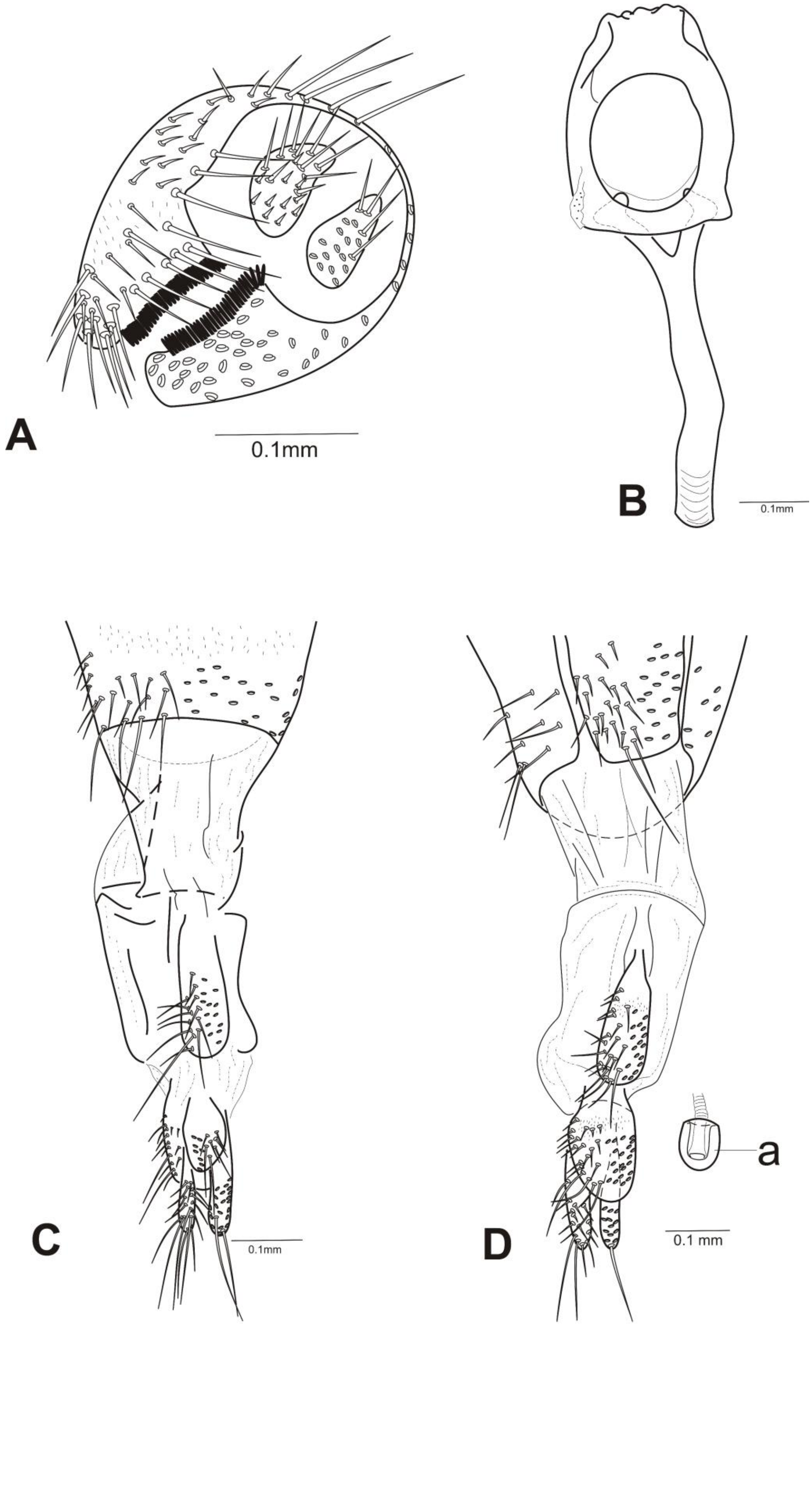

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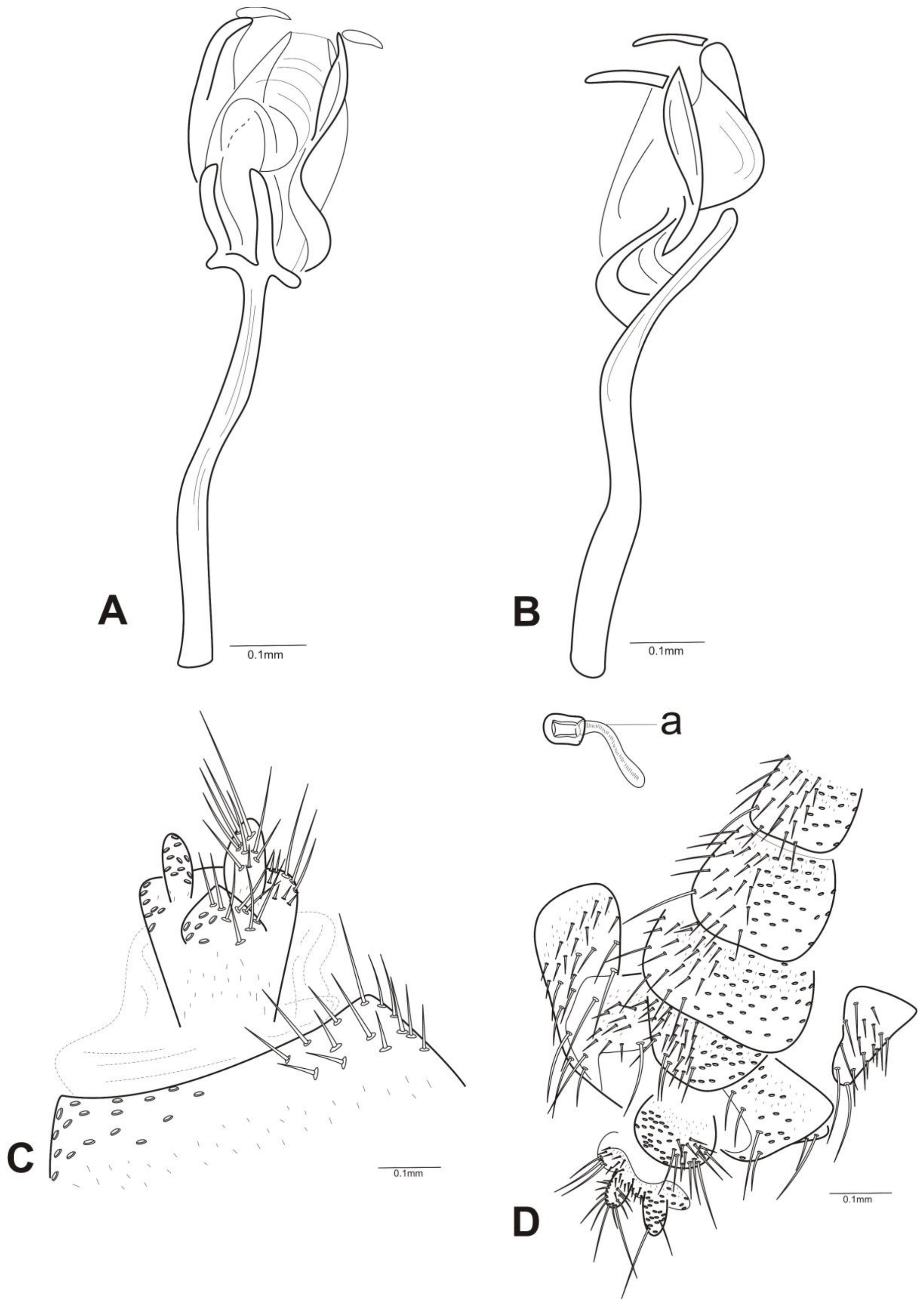
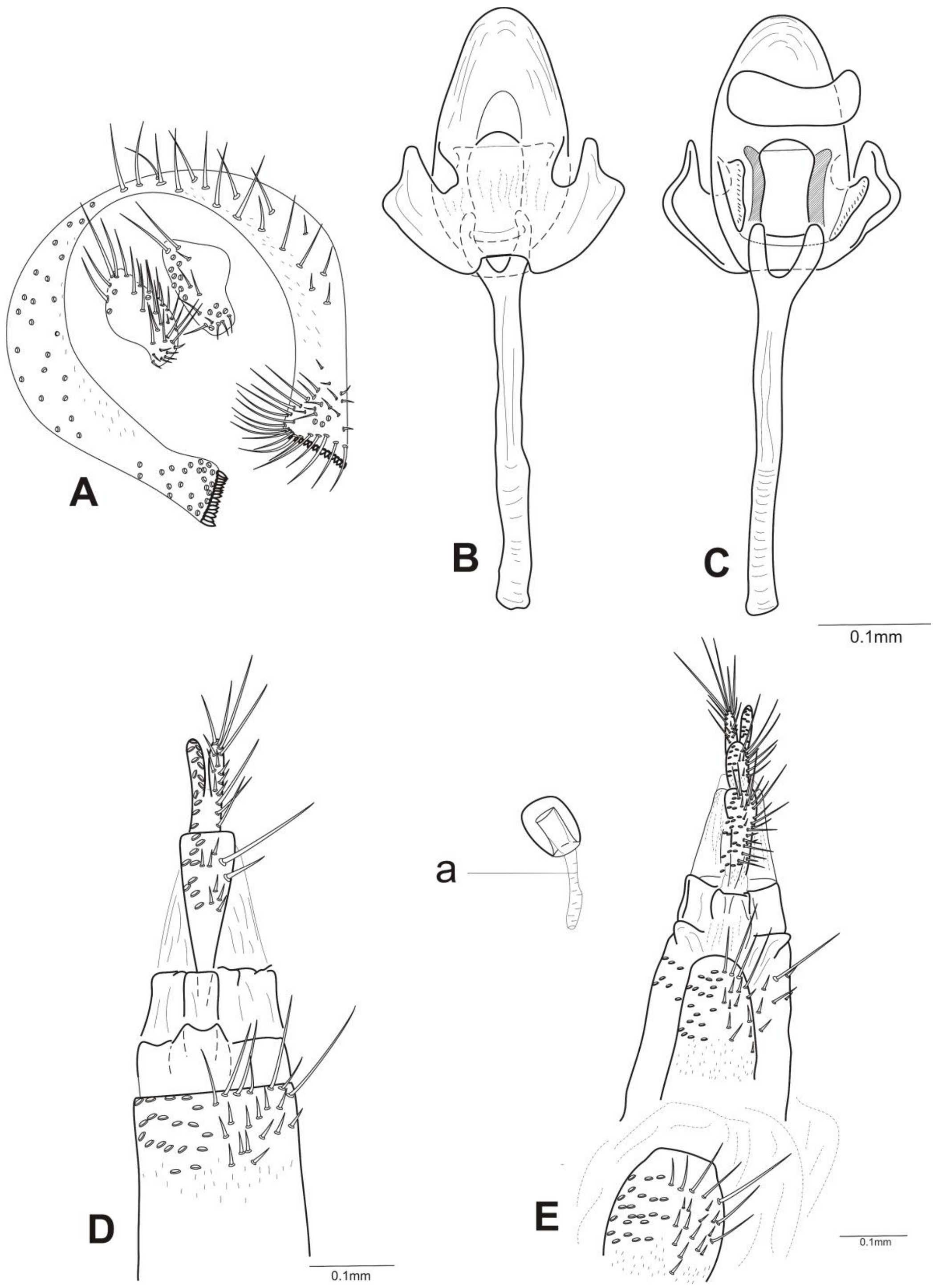


Figure 10



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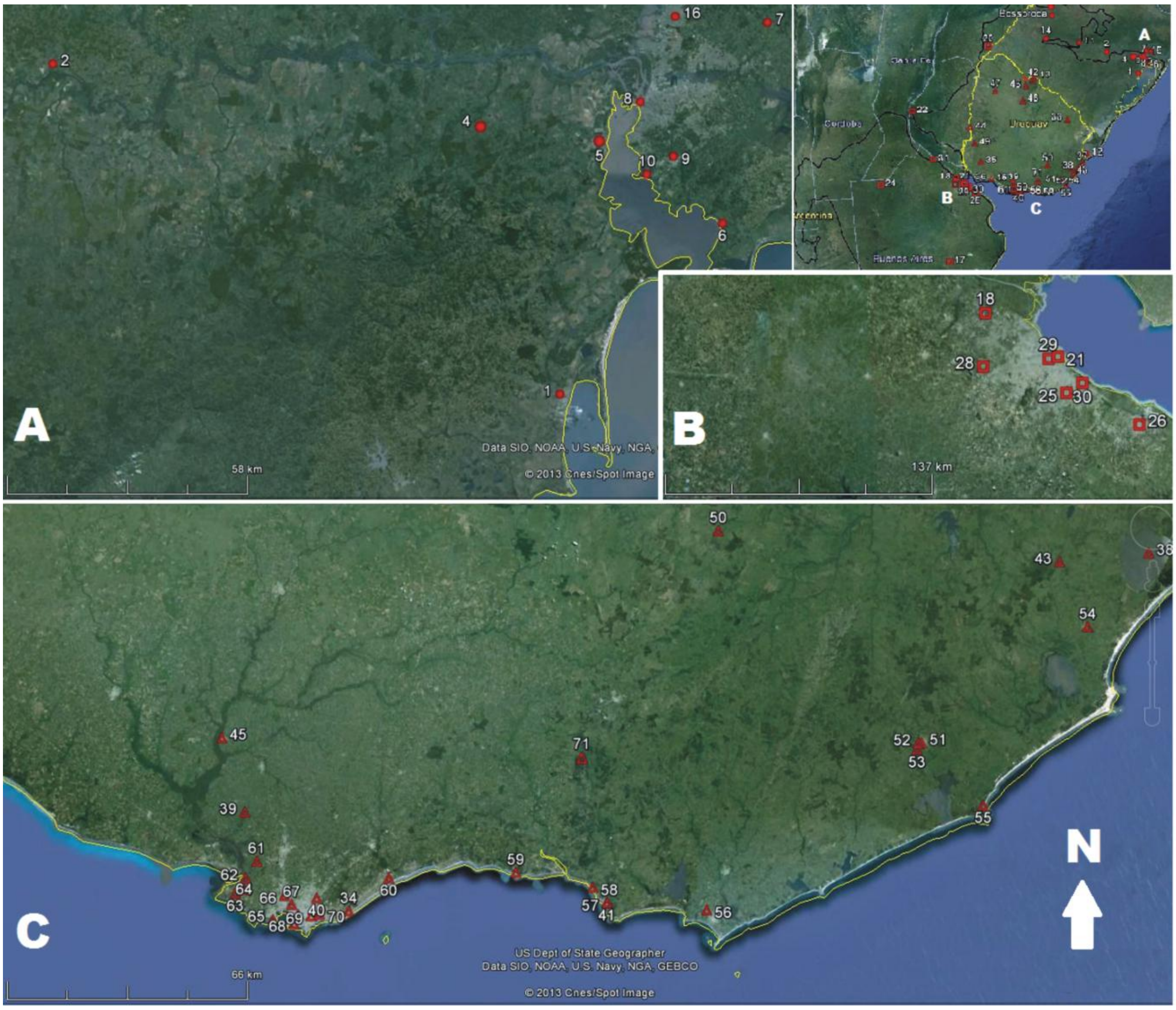
A
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58 km
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3. CAPÍTULO III

Manuscrito a ser submetido ao periódico Insect Conservation and Diversity

Title: Drosophilidae flies in the Pampas biome: Spatial and Temporal components.

Running title: Drosophilidae flies in the Pampa.

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Abstract: The species composition and the relative abundance of an insect community can vary through time and space due many reasons, including climatic variables and habitat preferences. Drosophilids are insects very sensible to environmental factors and the Pampa is a poorly studied biome that shows both spatial heterogeneity and striking climatic variations. Seasonal collections with banana baited traps were performed in a natural area of Pampa biome in Rio Grande do Sul, southern Brazil. Diversity was measured through the observed species richness (S_{obs}); species richness estimated by rarefaction method (S_{rar}); Shannon-Wiener heterogeneity index (H') and Smith-Wilson evenness index (Evar). The Kruskal-Wallis test was used to test the effects of season and environment on species abundance fluctuations and diversity measures. The potential interactions between spatial and temporal components were tested using the chi-square test, followed by residual analysis. Both exotic and Neotropical species were more concentrated in the edge and inner of forest patches, although this tendency was influenced by the seasonal climate variation. However, the open field cannot be slighted, once some species seem to prefer this more hostile part of the Pampa, such as some species of the *D. repleta* group. Variations in the diversity measures also was observed. The seasonality seems to have a stronger effect on the characterization of the Pampean Drosophilidae assemblage, although the type of environment and the interactions between the two components also act important roles.

INTRODUCTION

According to Wolda (1988), the species composition and the relative abundance of an insect community can vary through the time due many reasons, including climatic variables and the availability of resources in the environment.

To Brncic *et al*. (1985) the seasonal pattern of occurrence of each species is the product of a long and continuous process of adaptation to the environmental conditions in which the species live. Thus the community structure is reflection of the species behavior to the climatic variables (Da Cunha & Magalhães (1965). Thereby the tolerance of species to environmental variables is fundamental to their pattern of distribution through an area, being that the temperature influences directly the flight activity of insects as drosophilids (Pavan *et al*., 1950). Within big families as Drosophilidae and in regions of large range of temperature, as the Temperate region, different species normally answer differently to the environmental factors (Patterson, 1943; Dobzhansky & Epling, 1944; Pipkin, 1952).

Studies of these interactions between insects and the environmental variables are fundamentals since insects are able to answer quickly to the alterations in the environment and, thus they can play excellent roles as bioindicators (Kremen *et al*., 1993), highlighting the insects that present high capability of moving (Brown Jr., 1996), such as drosophilids.

In the last decades the studies about the ecology of Drosophilidae have achieved significant advances specially in the Neotropical region (Araújo & Valente, 1981; Saavedra *et al*., 1995b; Tidon *et al*., 2003; Tidon, 2006; Torres & Madi-Ravazzi, 2006; De Toni *et al*., 2007; Mata *et al*., 2008, 2010; Gottschalk *et al.*, 2009; Schmitz *et al*., 2010), however in many environments still there is a lack of knowledge about the relation between species and the environmental variables. One of these environments is the pampas biome, where there are few studies and the most of them are concentrated within or next to urban areas (Valente & Araújo, 1991; Saavedra *et al*., 1995b; Garcia *et al*., 2012; Poppe *et al*., 2012), being poorly known the behavior of drosophilids in natural areas.

In previous papers about the Drosophilidae diversity in pampas, was verified a reduction of diversity when comparing a forest patch and an urban area (Poppe *et al.*, 2012) and the influence of climatic variables on the fluctuations of populations of several species (Poppe *et al.*, 2013). In the present study, we aim to investigate the distribution pattern of the Drosophilidae species and diversity measures in different types of natural environments in a relatively well-conserved area of pampas and the influence of the season in the abundance and habitat choice of each species. So, we tested the null hypothesis that seasons and types of environments, do not affect the abundances of drosophilids in pampas biome.

MATERIAL AND METHODS

Seasonal collections were performed in a natural area of pampas biome, within the limits of the municipality of Bossoroca in the Rio Grande do Sul State, southern Brazil (28° 45’024”S 54° 56’729”W) (Fig 1), in the period of April 2011 to April 2012. This environment is classified as Uruguayan Savanna (WWF, 2013), which is compound mainly by C4 grasses, consisting a double structure of vegetation; the superior layer is characterized by *Aristida jubata* Herter (Poaceae) and the inferior layer by rhizomatous grasses of Poaceae family (*Axonopus jesuiticus* Araujo, *Paspalum notatum* Herter, *Paspalum lepton* Schult and *Axonopus affinis* Chase) being possible occur an alteration in the species composition according to the soil conditions (Boldrini *et al*., 2010). The climate in the region is the Cfa according to Köppen classification, presenting hot and dry summers and cold and wet winters.

In each collection, thirty banana-baited traps (Tidon & Sene, 1988) were left in the field during three days, in a distance of around forty meters one from others. Trying a higher comprisement of each sampling, the traps were equally distributed among open field, edge of forest and inner of forest patches.

Collected flies were maintained in ethanol 96% until identification. The identification was made using external morphology and the male terminalia, consulting specialized literature. Analysis of male terminalia was conducted according to Bächli *et al*. (2004). Some individuals belonging to *Drosophila repleta* Sturtevant, *D. tripunctata* Sturtevant, *D. cardini* Sturtevant, *D. saltans* Sturtevant and *D. annulimana* Pavan & da Cunha species groups that remained unidentified at species level were not scored for statistical analysis of species abundance and diversity measures. However, they were considered in the total number of individuals (N).

The climatic data of each sampled period were obtained at COOPATRIGO (Cooperativa Tritícola Regional) in the municipality of Bossoroca. Principal component analyses (PCA) were employed to derive principal component scores for temperature and humidity, using the software Past 1.34 (Hammer *et al*., 2001). Figure 2 shows the PCA scores of the sampled periods. The temperature score was calculated based in the maximum, minimum and medium temperature during the period of collections (four days before the beginning of the collections more during the three days while the traps were in the field), and the humidity score was based in the daily medium humidity and the rainfall level accumulated during the period of collections.

Diversity data were measured as follows: (1) observed species richness (S_{obs}); (2) species richness estimated by rarefaction method (S_{rar}); (3) Shannon-Wiener heterogeneity index (H'); and (4) Smith-Wilson evenness index (E_{var}). Of these, H' and E_{var} were calculated using the software Ecological Methodology (Krebs, 1999). Natural logarithm (ln) was used to calculate H'. For S_{rar}, all samples were standardized to 31 specimens, to nullify the effect of N (number of individuals) in species richness, using Biodiversity-Pro version 2 (McAleece *et al.*, 1997).

The Kruskal-Wallis test was used to test the effects of season and environment on species fluctuation, and to compare ecological indices through seasons in each environment (open field, edge of forest and inner of forest patches). For populations of Drosophilidae with at least 1% of abundance during the sampled period, potential interactions between spatial and temporal components on the species abundance determination were tested using the chi-square test, followed by residual analysis. These analyses were performed using the PASW Statistics software (http://www.spss.com.hk/statistics/).

Specificity and Fidelity indices were measured for each species in the Open field, Edge of forest and Inner of forest, according Dufrene & Legendre (1998). $Specificity_{ij} = Nindividuals_{ij} / Nindividuals_{i}$, where $Nindividuals_{ij}$ is the mean number of species *i* across sites of group *j*, and $Nindividuals_{i}$ is the sum of the mean numbers of individuals of species *i* over all groups. $Fidelity_{ij} = Nsites_{ij} / Nsites_{j}$, where $Nsites_{ij}$ is the number of sites in cluster *j* where species *i* is present, and $Nsites_{j}$ is the total number of sites in that cluster.

The influence of space and time on assemblage diversity was estimated by the following calculation: H’between = H’total - (Nj H’j)/ Nt; where H’between is the value of H’ for a given component; H’total is the value of H’ considering all the samples together; Nt is the total number of individuals in all samples, Nj is the number of individuals in category j, H’j is H’ within category j. Spatial (open field, edge of forest and inner of forest) and temporal (seasons) components were considered.

RESULTS AND DISCUSSION

The Collected Species and the Distribution Pattern

A total of 7,164 drosophilids of fifty-one species were collected, including members of the genera *Drosophila*, *Rhinoleucophenga*, *Amiota*, *Zaprionus* and *Zygothrica* (Table 1). The genus *Drosophila* was responsible for 97% of the total drosophilids collected. The most abundant species in the sampled period were *Drosophila simulans* Sturtevant (45.13%), followed by *D. willistoni* Sturtevant (7.69%), *D. mediopunctata* Dobzanhsky & Pavan (5.93%), *D. buzzatii* Patterson & Wheeler (5.40%), *D. mercatorum* Patterson & Wheeler (4.40%), *D. maculifrons* Duda (4.13%), *D. immigrans* Sturtevant (1.80%) and *D. hydei* Sturtevant (1.14%). Genus *Zaprionus* was also abundant, being represented by *Z. indianus* Gupta (1.68%). All these species are easily found in pampas biome (Silva *et al*., 2005; Garcia *et al*., 2012; Poppe *et al*., 2012).

Although the community had been composed mainly by Neotropical species (45 species), the dominance of *D. simulans* was high enough to cause a high proportion of exotic specimens collected (Fig 3). *D. simulans* is an exotic cosmopolitan species (Patterson & Stone, 1952) and its high abundance is commonly observed in many environments through the Neotropical Region (Brncic *et al.*, 1985; Saavedra *et al*., 1995b; Goñi *et al.*, 1998; Tidon, 2006; De Toni *et al*., 2007; Garcia *et al*., 2012). Other exotic species, *Zaprionus indianus* is an interesting case of invasion. It was recorded in the American continent for the first time by Vilela in 1999, and from then it was observed as dominant species in many South American environments (De Toni *et al*., 2001; Castro & Valente, 2001; Goñi *et al*., 2001; Tidon *et al*., 2003). However in pampas it does not seem to be a common species.

Exotic species are more common in disturbed environments (Ferreira & Tidon, 2005; Tidon, 2006; Gottschalk *et al.*, 2007), although they have been also found in environments relatively preserved. Here, in a general way, both exotic and native species were more concentrated in the edge and inner of forest patches, but this tendency was influenced by the seasonal climate variation. Table 2 presents data on the behavior of those species more abundant in the sample (at least 1% of relative abundance). Data is presented as the number of individuals observed in a specific site and season of sampling, and the total number of individuals in that site considering all the seasons. Thus among the exotic species, *D. simulans* was more abundant in open field during the samples of October (spring), but in the next periods, December and April (summer and autumn, respectively), it was more abundant in the inner and edge of forest (Fig 4a). *D. immigrans* and *Zaprionus indianus* were present in all the environments, but *D. immigrans* was more abundant in October (spring) in the edge and inner of forest (Fig 4b) and *Zaprionus indianus* was abundant mainly in April (autumn) in all the environments (Fig 4c).

Among the Neotropical species *D. willistoni* was never present in the open field, being abundant in the edge of forest during the samples of April (autumn) but always more abundant in the inner of forest patches, mainly in the December samples (summer) (Fig 5a). *D. mediopunctata* was also never present in the open field and only one specimen was collected in the edge of forest in April/2011, thereby it was much more abundant in the forest and mainly in July (winter) (Fig 5b). *D. maculifrons* as *D. willistoni* and *D. mediopunctata* was never present in the open field, being mainly found in the inner of forest patches in July (winter) (Fig 5c).

On the other hand, the Neotropical species of *D. repleta* group as *D. buzzatii*, *D. mercatorum* and *D. hydei* were predominantly more abundant in the open field mainly in the April samples (autumn), and in the edge of forest in the October samples (spring). But *D. buzzatii* in December (summer) was more frequent in the inner of forest patches (Fig 6a), *D. mercatorum* was frequent in the inner of forest in October (spring) (Fig 6b) and *D. hydei* was almost absent in this environment (Fig 6c).

Other species, such as *D. flexa* Loew, *D. kikkawai* Burla, *D. mediostriata* Duda and *R.* lp10 although less abundant in the samples, showed high specificity to the edge of forest, being present only in this environment. In the inner of forest also there are some specific species, such as *D. briegeri* Pavan & Breuer, *D. capricorni* Dobzhansky & Pavan, *D. nebulosa* Sturtevant, *D. piratininga* Ratcov & Vilela, *D.* sp2, *D.* sp3, *R. sulina* sp. nov., *R.* lp3, *R.* lp5 and *Zygothrica ptilialis* Burla. This specificity and fidelity of the species for one of the environments is reflected in the characterization of each community (Mata *et al*., 2008b) as can be observed in figure 7.

This preference pattern of species for a specific environment has been observed by others as Sene *et al*. (1980), Val *et al*. (1981), Vilela *et al*. (1983), Martins (1987), Chassagnard *et al*. (1997), Tidon (2006), Mata *et al*. (2008) and Schmitz *et al*. (2010) that highlighted the preference of species of *D. repleta* group for open environments and the low relation with cold temperatures (Wasserman *et al*., 1973; Vilela, 1983). Despite this tendency of higher abundance in open environments of some species of *D. repleta* group, *D. mercatorum* has been abundantly found in all the Brazilian biomes, including forest areas (Araújo & Valente, 1981; Mateus *et al*., 2006; Tidon, 2006; Mata *et al*., 2008; Hochmüller *et al*., 2010; Garcia *et al*., 2012).

The preference for forest and less urbanized areas of *D. willistoni*, *D. guarani* species group, *D. tripunctata* species group and the exotic *D. immigrans* has already been stressed by other authors in other environments and also in pampas (Toda 1973;

Araújo & Valente, 1981; Saavedra *et al*., 1995b; Döge *et al*., 2004; Ferreira & Tidon, 2005; Silva *et al*., 2005; Tidon, 2006; Garcia *et al*., 2012; Poppe *et al*., 2012)

Furthermore, the environmental preference of these species also is related with their climatic preference. Previous studies as Dobzhansky & Pavan (1950) Valente & Araújo (1986) and Saavedra *et al*. (1995b) stressed the preference of the species of *D. tripunctata* group for low temperatures. The same preference for cold periods is observed to *D. maculifrons* (Dobzhansky & Pavan, 1950; Salzano, 1955, Poppe *et al*., 2013) and according to Franck & Valente (1985) and Saavedra *et al*. (1995b) *D. maculifrons* is also well related with humid environments, what is consistent with the forest characteristics in the pampas. On the other hand, species of short life cycle as *D. willistoni* are favored for higher temperatures that improving the availability of resources as fruits and consequently increase the abundance of these species in the community during hot periods (Dobzhansky & Pavan, 1950; Franck & Valente, 1985; Sevenster & van Alphen, 1993; Martins, 1995; Garcia *et al*., 2012).

To the exotic species *D. simulans* and *Z. indianus*, the cold periods seem to be an important limiting factor, decreasing drastically the abundance of those species in all the environments where they occurred. However, in a disturbed area of pampas Poppe *et al*. (2012) noticed a decrease in the abundance of those species in hot periods (summer), it probably happened because in our samples, during the summer period, the specimens migrate to the forest patches, what do not happen in altered areas of pampas.

Diversity measures

Analyzing the distribution pattern of the species among the studied environments during the sampled period, it is observed that along with the species abundance fluctuation, variations in the diversity measures such as Shannon-Wiener heterogeneity index (H'), the total abundance (N), observed species richness (S_{obs}), species richness estimated by rarefaction method (S_{rar}) and Smith-Wilson evenness index (E_{var}) (table 3) also occur.

In the Open field the highest heterogeneity index (H') was observed in April/2011, period of autumn (Kruskal-Wallis test: $p<0.01$) (Fig 8a). This may be explained by the high humidity and intermediate temperature in this period, since in July and December the diversity index values decreased drastically. This reduction in the periods of July and December also was followed by decreases in the total abundance (N), observed species richness (S_{obs}) and species richness estimated by rarefaction method (S_{rar}) (Kruskal-Wallis test: $p<0.001$) (Fig 8b, c and d, respectively). On the other hand, the Smith-Wilson evenness index (E_{var}) was higher in July and December (Fig 8e), but it did not change in the other sampled periods (Kruskal-Wallis test: $p>0.05$), probably due to the influence of the low abundance of collected specimens (N= 3 and 6, respectively) with the relative high richness (S_{obs}= 3 and 5, respectively). Together these values caused the statistical increase of the E_{var} index in the open field, as observed by Tidon (2006) in the Cerrado and by De Toni *et al*. (2007) in the Atlantic rainforest.

As noticed to open field samples, the H' index in the edge of forest was also lowest in the coldest period (July) (Kruskal-Wallis test: $p<0.05$) (Fig 8a), not showing differences among the other sampled periods. This decrease in the H' index was followed by the S_{obs} in the coldest and in the second hottest periods (July and April/2012, respectively) (Kruskal-Wallis test: $p<0.01$) and by the S_{rar} in the periods of July, December and April/2012 (Kruskal-Wallis test: $p<0.05$) as noticed in the figures 8c and 8d. However, the E_{var} index was highest in the warmer periods (December and April/2012) (Kruskal-Wallis test: $p<0.01$) (Fig 8e). According again to the data noticed in the open field, the highest N was in the October samples (spring) (Kruskal-Wallis test: $p<0.001$) (Fig 8b).

Differently of the open field and edge of forest, the H' index did not vary significantly among the forest samples (Kruskal-Wallis test: $p>0.05$) (Fig 8a). On the other hand, the E_{var} index was lowest in the July samples (Kruskal-Wallis test: $p<0.05$) (Fig 8e) and the N was highest in the October samples (Kruskal-Wallis test: $p<0.05$) (Fig 8b), as they were in the open field and in the edge of forest. As the richness indices, S_{obs} was lowest in both autumn periods (Kruskal-Wallis test: $p<0.01$) (Fig 8c) and the S_{rar} was lowest in the autumn period of 2012 (Kruskal-Wallis test: $p<0.05$) (Fig 8d).

Therefore, the general tendency was the edge and inner of forest patches presenting higher values to the diversity measures than the open field. This tendency occurs mainly in stressing periods (table 3) as July and December (temperatures around 2 and 36°C, respectively) when some drosophilids, such as the species of *D. repleta* group, seem to move from the open field to the inner of forest patches. This climatic influence on the drosophilids and other dipterans fauna, causing migratory activity and alterations in the diversity measures was already observed in other environments (Parsons, 1989; Ravenscroft, 1994; Fonseca *et al*., 2006; Marinoni *et al*., 2006; Tidon, 2006; Kivinen *et al*., 2007; Mata *et al*. 2008; Costa *et al*., 2008; Hilário *et al*., 2012) and in altered areas of pampas (Poppe *et al*., 2013).

In Tropical regions the humidity has higher influence on the diversity measures than in the Temperate regions, as can be noted in the Cerrado (Silva *et al*., 2011), but in pampas there is not a defined dry period and the humidity presents low influence on the drosophilids community (Poppe *et al*., 2013). Thus regulator action of the temperature over the drosophilids was clear in the open field community, which was limited for periods of high and low temperatures. On the other hand, in July the E_{var} index was higher in the open field due the decrease of the total abundance of collected drosophilids in this environment (table 3 and Fig 8e), as noted in dry periods in the Tropical region (Tidon, 2006).

Other relevant aspect in the forest is the availability of resources, mainly during the spring, what probably caused an increase in the forest richness, and consequently in the number of collected specimens (Sevenster & VanAlphen, 1993). Thus, this highest availability of resources in the forest patches would be sufficient to make this environment naturally richest. Also in the spring was detected the highest abundance of *D. simulans*, whose abundance may have influenced negatively the H' index in the sample of this period. This negative influence on the diversity measures by dominant species has already been observed in ecological analyzes with drosophilids (Saavedra *et al*., 1995b; Silva *et al*., 2005; De Toni *et al*. 2007).

Therefore, despite the interaction between sites and seasons, the seasonality seems to be the main environmental component on the characterization of the Pampean Drosophilidae community (Table 4). This importance of the climatic variables on the fluctuation of diversity of drosophilids community has been stressed by many authors worldwide, as Shorrocks (1975) and Brncic *et al*. (1985), in England and Chile, where the seasonality explained 82.4% and 63.3% of the diversity index, respectively, and also comparing contrasting areas in pampas (Silva *et al*., 2005; Poppe *et al*., 2012). The part of the diversity index that was not explained by the seasonal and spatial components could be explained by other aspects not analyzed here, such as intra and interespecific interactions, microclimatic components, vertical distribution, resource availability and genetic aspects.

Concluding remarks

The results showed in the present study suggest that there is a strong interaction between climatic factors and the habitat choice for several species of drosophilids in a natural area of pampas biome. In general, the forest patch showed a higher diversity and richness, with several species being absent in the open field and also presenting high specific to this environment. However, the open field cannot be slighted, once some species show preference for this site, especially in seasons with mild temperatures, such as some species of the *D. repleta* group. But in hard conditions of cold or heat, when the diversity and abundance of drosophilds in the open field are drastically reduced, the forest patch seems to be used as a refuge by the drosophilids, acting as a center of recolonization, reinforcing its importance to the maintenance of the biodiversity in pampas. Furthermore, this function must be still more important in the future, when the temperatures probably will be higher, as highlighted by the IPCC (Intergovernmental Panel on Climate Change) as a consequence of the global warming (Orsini, 2007).

Therefore, the natural fields and mainly the forest patches of pampas must be preserved in conservation units and the supervision procedures must be developed to avoid the strong reduction of the forest patches in the rural areas, where the biodiversity has been reduced, considering the Drosophilidae fauna (Poppe *et al*., 2012).

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TABLES

Table 1: Absolute abundance of the collected drosophilids in each season and site, in alphabetic order. Op. Field: Open Field; Ed. Forest: Edge of Forest; Forest: Inner of Forest patches.

Species	Autumn/2011			Winter			Spring			Summer			Autumn/2012			Total
	Op. Field	Ed. Forest	Forest	Op. Field	Ed. Forest	Forest	Op. Field	Ed. Forest	Forest	Op. Field	Ed. Forest	Forest	Op. Field	Ed. Forest	Forest	
Amiota sp02											7	10				17
A. sp01						1							1			2
Drosophila antonietae		1						1	4	1	2	6	1		5	21
D. arassari											2	4				6
D. bandeirantorum		1	1						1							3
D. bocainensis	2		1										1			4
D. briegeri			4			6			4							14
D. bromelioides		4				1										5
D. busckii		1		1	1		2									5
D. buzzatii	187	3					12	7	4	1	6	23	124	6	13	386
D. capricorni			2													2
D. cardini	26	8	6	2	3	1						2				48
D. flexa					1											1
D. fuscolineata			2					3								5
D. hydei	62	3	1				4	9	2			1				82
D. immigrans		2			1	1	2	44	77	1						128
D. kikkawai					1											1
D. maculifrons		9	64		10	212		4	3		1	2				305
D. mediopicta			1		4	1		1	4							11
D. mediopunctata		1	11			402			6			1			3	424
D. mediostriata		2														2
D. melanogaster							7	12	17							36
D. mercatorum	55	35	30		2	1	24	62	39		10	9	10	11	27	315
D. nebulosa						3			3							6
D. nigricruria					1		9	2			1	3				16

D. onca						10		2	10			2				24
D. ornatifrons						22		4	17			5			1	49
D. pallidipennis		4			1			6	6		6	6				29
D. paraguayensis		2	1			23			12			1				39
D. piratininga						1										1
D. polymorpha		16	17			4		4	10			15				66
D. prosaltans		1	1					1	2							5
D. simulans	85	335	202	2	7	6	398	639	795	3	147	299	85	70	157	3230
D. sp2						2										2
D. sp3						1										1
D. sp7							1				3	3				7
D. sturtevanti		3	1									1				5
D. willistoni		31	76		1	4		8	76		25	311		1	17	550
Rhinoleucophenga gigantea	5										3	1				9
R. lp3												1				1
R. lp5												1				1
R. lp10					1						1					2
R. missionera sp. nov.											2	3				5
R. obesa													2	1	1	4
R. pampeana sp. nov.														2	3	5
R. punctulata	1										7	1	2	2	2	15
R. subradiata											11	6				17
R. sulina sp. nov.												3			1	4
Zaprionus indianus	28	53	14					1				2	9	7	6	120
Zygothrica ptilialis						6										6
Zy. orbitalis						2								1		3
Gr. *annulimana* (females)									2							2

Gr. *cardini* (females)	32	40	33		3	9	1	12	19			4			3	156
Gr. *repleta* (females)					1	2	46	57	61		14	46	122	13	51	413
Gr. *saltans* (females)		1						1	5						1	8
Gr. *tripunctata* (females)		4	12		11	453		13	44			3				540
Total	483	560	480	5	49	1174	506	893	1223	6	248	775	357	114	291	7164

Table 2: Chi-squared (X^2) and Kruskal-Wallis tests to evaluate the preference of the drosophilids for one of the sites and the influence of the season on this preference during the sampled period. Only the species with at least 1% of relative abundance in the total sample were considered in the analyzis. -: absence of data; empty cell: not significant residue; na: not analyzed due to sparse data; n/N: species total abundance as encountered in the indicated site and season / species total abundance in that site considering all the seasons. Only statistically significant chi-squared adjusted residuals are presented.

	X^2					Kruskal-Wallis	
species	site	season	n/N	adjusted residual	p-value	p-value (site)	p-value (season)
Drosophila simulans	Open field	spring	398/573	7.1	<0.001	<0.05	<0.001
	Edge of forest	autumn	335/1198	9.2	<0.001	<0.05	<0.001
	Forest	summer	299/1459	10.2	<0.001	<0.05	<0.001
Drosophila immigrans	Open Field	summer	1/3	na	na	<0.05	<0.001
	Edge of forest	spring	44/47		>0.05	<0.05	<0.001
	Forest	spring	77/78		>0.05	<0.05	<0.001
Zaprionus indianus	Open field	autumn	28/37		>0.05	>0.05	<0.001
	Edge of forest	autumn	53/61		>0.05	>0.05	<0.001
	Forest	autumn	14/22		>0.05	>0.05	<0.001
Drosophila willistoni	Open field	-	-	-	-	-	-
	Edge of forest	autumn	31/66	6.0	<0.001	<0.001	<0.01
	Forest	summer	311/484	4.1	<0.001	<0.001	<0.01
Drosophila mediopunctata	Open Field	-	-	-	-	-	<0.05
	Edge of forest	autumn	1/1	na	na	<0.001	<0.05
	Forest	winter	398/422		>0.05	<0.001	<0.05
Drosophila maculifrons	Open Field	-	-	-	-	-	-
	Edge of forest	spring	4/24	4.8	<0.001	<0.001	<0.05
	Forest	winter	212/281	3.4	<0.001	<0.001	<0.05
Drosophila buzzatii	Open field	autumn	187/324	8.4	<0.001	<0.01	<0.01
	Edge of forest	spring	7/22	4.7	<0.001	<0.01	<0.01
	Forest	summer	23/40	13.6	<0.001	<0.01	<0.01
Drosophila mercatorum	Open field	autumn	55/84	5.3	<0.001	>0.05	<0.001
	Edge of forest	spring	62/120	2.7	<0.01	>0.05	<0.001
	Forest	spring	39/106		>0.05	>0.05	<0.001
Drosophila hydei	Open Field	autumn	62/66	6.2	<0.001	>0.05	<0.001
	Edge of forest	spring	9/12	5.5	<0.001	>0.05	<0.001
	Forest	summer	1/4	na	na	>0.05	<0.001

Table 3: Diversity measures in each site and season during all the sampled period. The traps were grouped two by two to the analyzis. APR-11: April of 2011; JUL-11: July of 2011; OCT-11: October of 2011, DEC-11: December of 2011; APR-12: April of 2012. H': Shannon-Wiener heterogeneity index; N: total abundance of collected species; S_{obs}: observed species richness; S_{rar}: species richness estimated by rarefaction method; E_{var}: Smith-Wilson evenness index.

		Open field					Edge of Forest					Inner of Forest				
	Traps	APR-11	JUL-11	OCT-11	DEC-11	APR-12	APR-11	JUL-11	OCT-11	DEC-11	APR-12	APR-11	JUL-11	OCT-11	DEC-11	APR-12
H'	01.02	1.584	0	0.862	0	1.371	1,430	0	1.32	1.657	0.503	2.149	1.403	1.877	1.638	0.906
	03.04	2.115	0.918	0.497	0	1.226	2,066	0	1.105	1.684	0.918	1.601	1.791	1.397	1.937	0.918
	05.06	2.308	0	0.631	0	1.322	2,245	0	0.717	2.365	1.98	1.779	0	1.343	1.74	1.611
	07.08	2.437	0	1.035	1.5	1.135	1,800	0	2.18	1.357	1.585	2.328	2.009	1.881	2.043	1.204
	09.10	2.465	0	1.284	0	2.295	1,824	2.852	1.477	2.309	1.201	2.458	1.742	1.624	2.667	1.963
N	01.02	94	0	79	0	48	123	5	212	20	9	137	208	204	235	15
	03.04	71	3	91	1	47	138	6	218	32	3	70	235	188	296	9
	05.06	131	0	168	1	11	85	4	167	40	37	94	4	252	85	18
	07.08	103	0	86	4	18	63	3	75	64	3	86	202	160	74	57
	09.10	52	0	35	0	26	106	16	138	78	29	48	47	285	31	21
Sobs	01.02	7	1	5	0	5	7	4	9	7	2	8	9	15	12	3
	03.04	6	2	4	1	4	13	3	11	8	2	7	16	13	15	2
	05.06	9	0	7	1	3	11	1	7	9	7	7	4	12	10	5
	07.08	7	0	6	3	3	7	3	11	7	3	11	13	13	11	6
	09.10	7	0	4	0	7	12	9	14	11	3	8	6	14	11	7
Srar	01.02	5.35	0	3.61	0	2.9	4.86	0	4.51	0	0	5.97	3.98	6.77	4.65	2.68
	03.04	5.46	0	2.73	0	2.47	7.59	0	4.51	7.81	0	5.81	5.44	5.47	5.56	0
	05.06	5.93	0	3.16	0	3	7.55	0	3.36	8.18	3.6	4.89	0	5.24	5.69	3.82
	07.08	6.27	0	4.38	0	2.61	5.48	0	7.97	5.57	0	7.2	6.19	6.48	6.9	2.81
	09.10	6.56	0	3.88	0	4.85	6.86	0	5.47	8.48	2.8	7.05	4.98	5.66	11	4.58
Evar	01.02	0.329	0	0.253	0	0.259	0.297	0	0.235	0.55	0.475	0.301	0.199	0.319	0.236	0.471
	03.04	0.515	0.924	0.214	1	0.25	0.383	0	0.251	0.464	0.924	0.425	0.27	0.307	0.249	0.924
	05.06	0.26	0	0.218	1	0.661	0.39	0	0.216	0.516	0.407	0.278	0	0.282	0.309	0.512
	07.08	0.556	0	0.319	0.932	0.482	0.35	0	0.44	0.404	1	0.361	0.286	0.305	0.374	0.293
	09.10	0.602	0	0.371	0	0.56	0.363	0.796	0.314	0.512	0.701	0.479	0.325	0.249	0.625	0.551

Table 4: Contribution of temporal and spatial components to the Shannon-Wiener heterogeneity index (H') in the assemblages of drosophilids in a natural area of Pampa biome.

Component	**H**	**%**
Temporal	1.336	48.57
Spatial	1.068	38.82
Not explained	0.347	12.61
Total	2.751	100

FIGURE LEGENDS

Figure 1: South America Map pointing out the collecting area (the municipality of Bossoroca) in the south of Brazil (28° 45’024”S 54° 56’729”W) in the Uruguayan Savanna (delimited by the broken line).

Figure 2: Results of the PCA scores to the temperature and humidity values during the sampled period in a natural area of Pampa biome.

Figure 3: The relative abundance of Exotic species (excluding *Drosophila simulans*), Neotropical species and *D. simulans* in the sampled period.

Figure 4: Fluctuations of absolute abundance in the Open field, Edge of forest and Inner of forest during the seasons. Only the exotic species with at least 1% of abundance in the total sampled are shown. a: *D. simulans*; b: *D. immigrans*; c: *Zaprionus indianus*. Apr-11: April of 2011; Jul-11: July of 2011; Oct-11: October of 2011; Dec-11: December of 2011; Apr-12: April of 2012.

Figure 5: Fluctuations of absolute abundance in the Open field, Edge of forest and Inner of forest during the seasons. Only the Neotropical species with at least 1% of abundance in the total sampled are shown. a: *D. willistoni*; b: *D. mediopunctata*; c: *D. maculifrons*. Apr-11: April of 2011; Jul-11: July of 2011; Oct-11: October of 2011; Dec-11: December of 2011; Apr-12: April of 2012.

Figure 6: Fluctuations of absolute abundance in the Open field, Edge of forest and Inner of forest during the seasons. Only the Neotropical species of *Drosophila repleta* group, with at least 1% of abundance in the total sampled are shown. a: *D. buzzatii*; b: *D. mercatorum*; c: *D. hydei*. Apr-11: April of 2011; Jul-11: July of 2011; Oct-11: October of 2011; Dec-11: December of 2011; Apr-12: April of 2012.

Figure 7: Specificity and fidelity values to the most abundant species (at least 1% of the total sampled) and the species that only occur in one type of environment through the seasons during all the sampled period. A: Open field; B: Edge of forest; C: Inner of forest; sim: *Drosophila simulans*; imm: *D. immigrans*; ind: *Zaprionus indianus*; wil: *D. willistoni*; mac: *D. maculifrons*; med: *D. mediopunctata*; buz: *D. buzzatii*; hyd: *D. hydei*; mer: *D. mercatorum*; fle: *D. flexa*; med: *D. mediostriata*; kik: *D. kikkawaii*; bri: *D. briegeri*; neb: *D. nebulosa*; cap: *D. capricorni*; pir: *D. piratininga*; pit: *Zygothrica ptilialis*; sul: *Rhinoleucophenga sulina* sp. nov. Apr-11: April of 2011; Jul-11: July of 2011; Oct-11: October of 2011; Dec-11: December of 2011; Apr-12: April of 2012.

Figure 8: Variation of the diversity measures in the Open field, Edge of forest and Inner of forest during the seasons. The bars of Standard error also are showed. a: Shannon-Wiener heterogeneity index (H'); b: total abundance of collected species (N); c: observed species richness (S_{obs}); d: species richness estimated by rarefaction method (S_{rar}); e: Smith-Wilson evenness index (E_{var}).

Figure 1

Figure 2

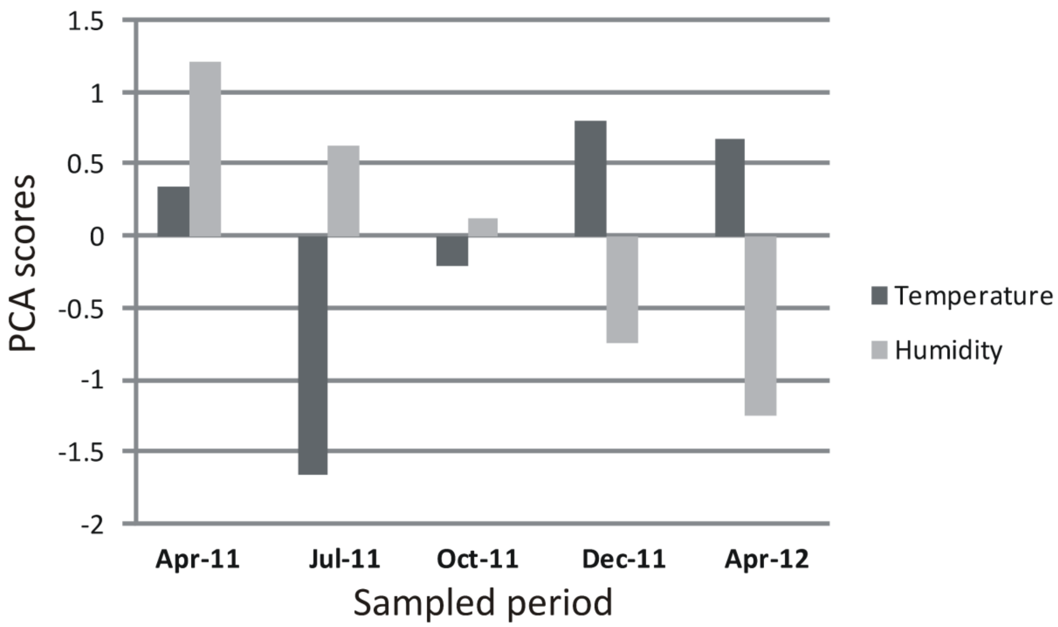


Figure 3

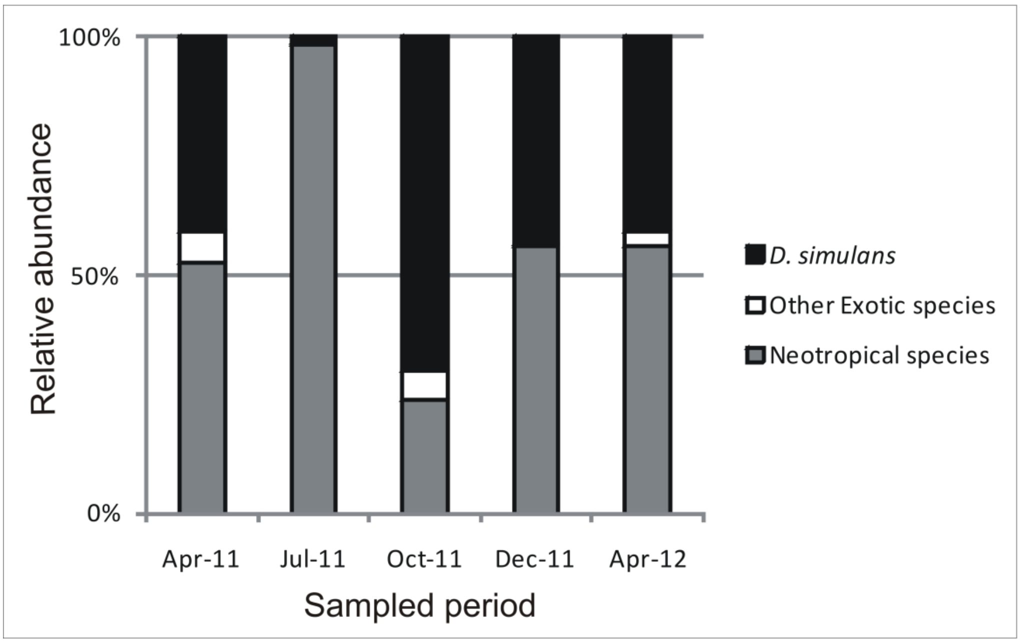


Figure 4

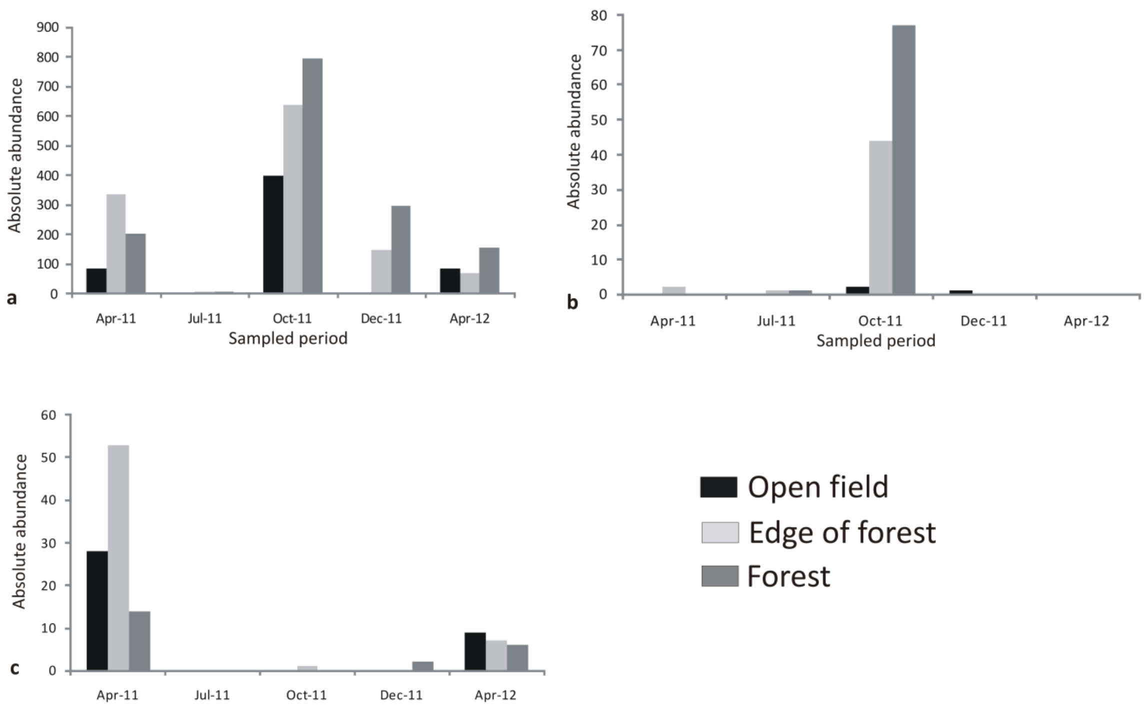


Figure 5

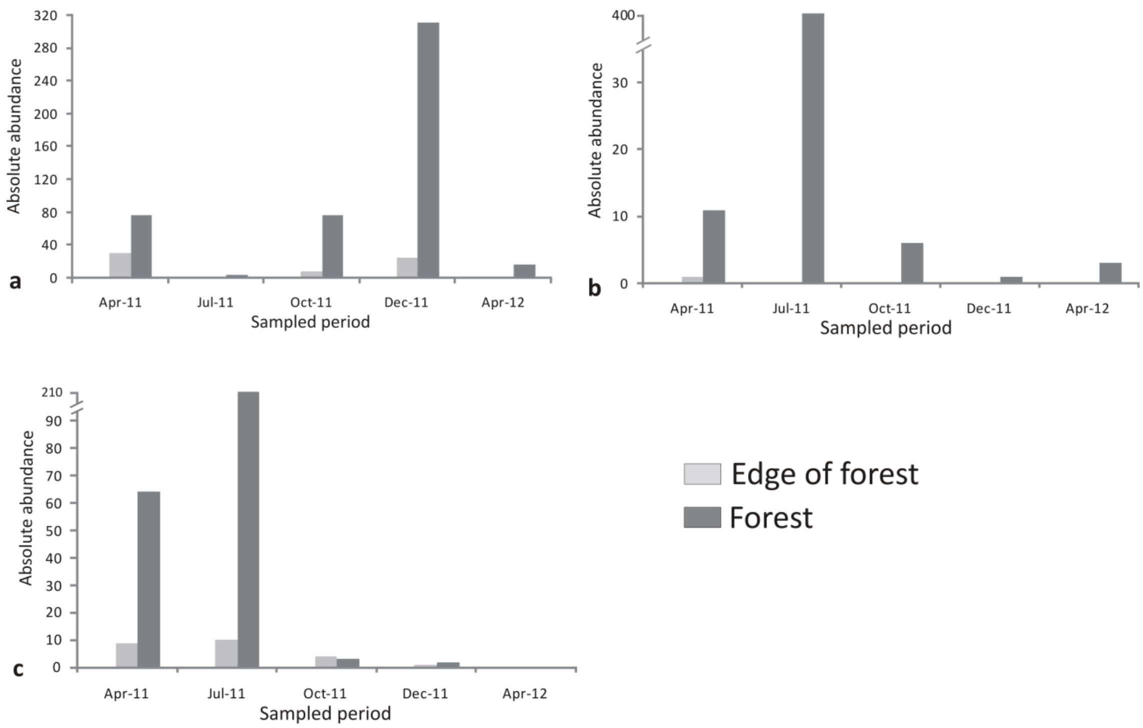


Figure 6

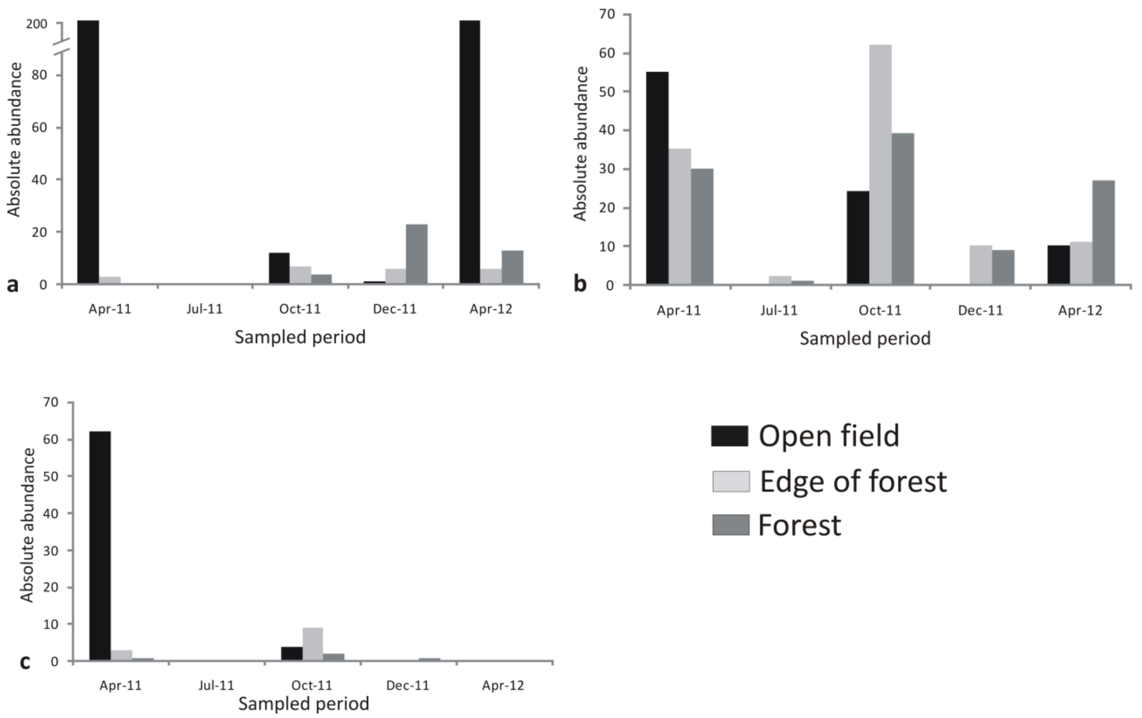


Figure 7

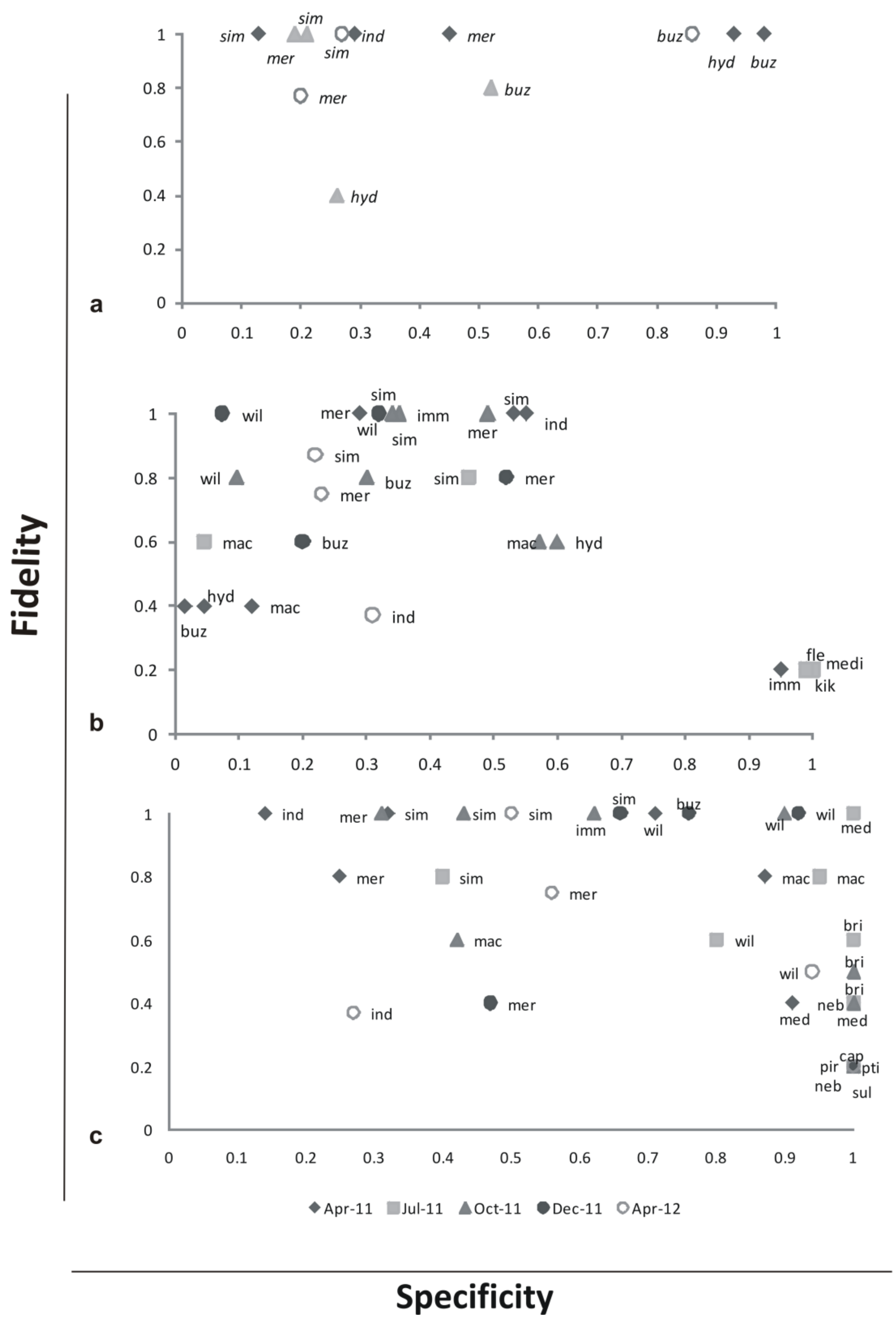
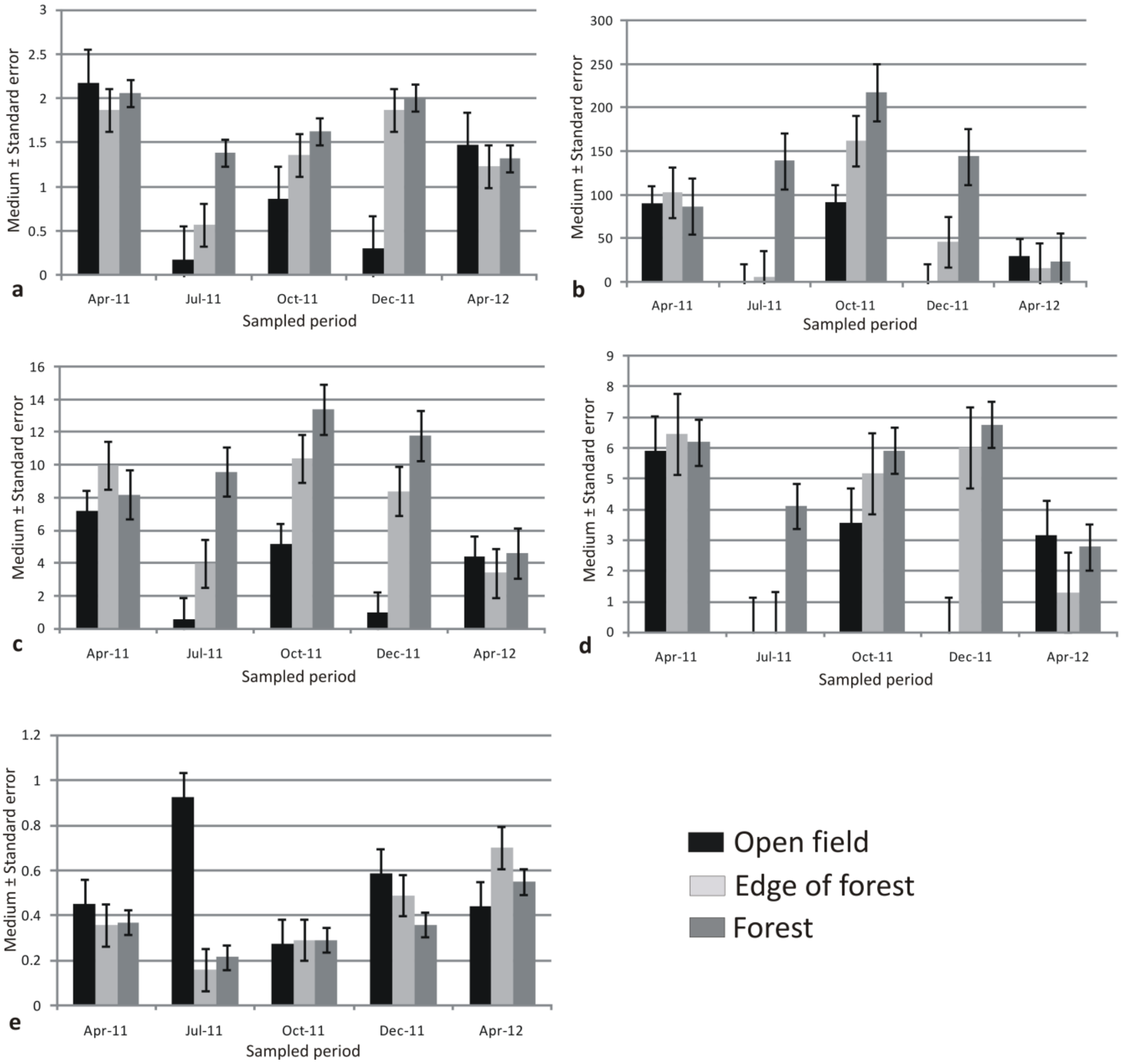


Figure 8


a
b
c
d
e
Medium ± Standard error
Sampled period
Apr-11
Jul-11
Oct-11
Dec-11
Apr-12
Open field
Edge of forest
Forest

4. CAPÍTULO IV

4.1.CONSIDERASÕES GERAIS

4.1.1. Principais Conclusões

O bioma Pampa revelou uma surpreendente diversidade de Drosophilidae, principalmente por ser um bioma que vinha sendo menosprezado e considerado de baixa biodiversidade.

A importância de estudos em áreas naturais do Pampa foi destacada com a grande diversidade do gênero *Rhinoleucophenga*, nesse que é o primeiro levantamento de Drosophilidae em uma área natural de bioma Pampa na região noroeste do estado do Rio Grande do Sul.

Apesar do destaque do gênero *Rhinoleucophenga*, a maior riqueza do gênero *Drosophila* já era esperada, uma vez que este é o principal gênero da família Drosophilidae, e também o gênero mais estudado e mais atraído por isca de banana, como já percebido em outras pesquisas no bioma Pampa e também em outros ambientes. Mesmo assim, ocorreram primeiros registros para espécies desse gênero. O que, mais uma vez, destaca a grande diversidade deste que é o bioma mais meridional do Brasil.

O papel das manchas de mata como refúgio para os drosofilídeos em períodos de *stress* térmico afirma a intima relação entre sazonalidade e ambiente na determinação do padrão de abundância e distribuição das espécies pelo ambiente. Essa característica tende a ganhar ainda mais importância na manutenção da diversidade de Drosophilidae do Pampa no futuro, com a elevação das temperaturas previstas pelo IPCC (*Intergovernmental Panel on Climate Change*) como conseqüência do aquecimento global.

Mas o ambiente de campo aberto não pode ser negligenciado, uma vez que este também é fundamental na manutenção das espécies de Drosophilidae no Pampa, ou seja, algumas espécies, como as do grupo da *Drosophila repleta*, mostraram-se altamente específicas para este ambiente. Em outras palavras, apesar de menos diverso, este ambiente também está contribuindo para a manutenção das assembléias de Drosophilidae do Pampa.

A forte influência da sazonalidade na determinação da flutuação das assembléias de Drosophilidae ficou clara, mas ainda há uma pequena parcela da diversidade que não teve sua variação explicada e pode ter ocorrido em função de aspectos como a interação intra e entre populações, condições microclimáticas, distribuição vertical das populações, aspectos genéticos ou talvez a influência dos métodos de coleta, porém, estes fatores não foram analisados nesta pesquisa.

Na comparação entre Brasil, Uruguai e Argentina, a superioridade de riqueza de espécies do Pampa brasileiro pode ser relacionada com alguns fatores não analisados na presente pesquisa, como a proximidade do Pampa com a Mata Atlântica, o esforço amostral empregado em cada pesquisa, incluindo a freqüência de coletas, o tempo das armadilhas em campo, o número de recursos e armadilhas utilizadas, entre outros fatores que podem influenciar na variação de riqueza de espécies em cada região amostrada do Pampa.

Porém, os três países apresentam a mesma problemática quanto ao estudo da diversidade de Drosophilidae no bioma Pampa, sendo os pontos amostrados aglomerados em pequenas regiões, deixando grandes lacunas geográficas totalmente desconhecidas até o momento. E no caso do Brasil muitas dessas lacunas são áreas consideradas como prioritárias pelo Ministério do Meio Ambiente para o desenvolvimento de estudos voltados a preservação ambiental, como os estudos de levantamento de fauna, onde muito provavelmente os pesquisadores ainda podem encontrar muitas espécies novas.

De maneira geral, confirmamos que o Pampa, assim como outros biomas melhor estudados, como a Mata Atlântica, por exemplo, apresenta uma grande diversidade de drosofilídeos, os quais expõem uma acentuada relação com as variáveis climáticas. A combinação dessas informações e o conhecimento do atual estado de preservação do bioma Pampa ressaltam a necessidade de um contínuo estudo para desvendar a diversidade de Drosophilidae neste bioma ainda tão pouco conhecido. Também confirmamos a importância das manchas de mata como refúgio para as espécies de animais do Pampa, o que torna fundamental a preservação destes ambientes.

5. ANEXOS

5.1. Normas de publicação no Journal of Zoological Systematics and Evolutionary Research

Aims and Scope

The *Journal of Zoological Systematics and Evolutionary Research* (JZSER) is a peer-reviewed, international forum for publication of high-quality research on systematic zoology and evolutionary biology. The aim of the journal is to provoke a synthesis of results from morphology, physiology, animal geography, ecology, ethology, genetics, population genetics, developmental biology and molecular biology. Purely taxonomic and predominantly cytogenetic manuscripts will not be accepted except in rare cases, and then only at the Editors-in-Chief's discretion. Only papers in English language are accepted.

Pre-submission English-language editing. Authors for whom English is a second language may choose to have their manuscript professionally edited before submission to improve the English. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

General Information

Types of contribution: JZSER publishes Original Articles, Reviews, Short Communications, Letters to the Editors and Book Reviews. To encourage scientific exchange and discussions, authors are invited to send critical comments on previously published articles.

Content: The contents of manuscripts submitted to JZSER must not have been submitted to any other journal in parallel or published previously. Authors are solely responsible for the contents of their contribution and are assumed to have the necessary authority for publication.

Submission and reviewing process: Manuscripts are submitted to JZSER online, i.e. electronically, from the corresponding author's JZSER ScholarOne Manuscript (formerly known as Manuscript Central) account. You will need your files in an electronic format, an Internet connection, and a user ID and password for the site. Except for Book Reviews, online submission is mandatory.

After submission, each paper enters the pre-review stage. This policy reduces effort of authors, editors and reviewers. In the pre-review stage manuscripts that do not fit into the scope of JZSER or have only low relevance to a broader readership, or do not meet the formal standards and thus have a low probability of being accepted, are identified and can be rejected by the Editors without consulting referees. Manuscripts that are suitable for consideration go into the peer-review process which will be handled by the editor in charge of the particular field (authors may suggest a particular editor during the submission procedure). Authors may suggest suitable referees (full names and affiliations). However, the referees nominated by the authors must not necessarily be contacted. Papers may be returned for modification or revision. Authors resubmitting revised manuscripts should follow the same procedure as for submission of new manuscripts. The Editor-in-Chief makes the final decision on the acceptance for publication. After acceptance the final manuscript and figure files must be uploaded. The figures must be high-resolution scans (JPEG, TIFF or EPS files).

Agreements: Authors must send a completed and signed Copyright Transfer Agreement (CTA) to the Production Editor after manuscript acceptance.

OnlineOpen: OnlineOpen is a pay-to-publish service from Wiley that offers authors whose papers are accepted for publication the opportunity to pay up-front for their manuscript to become open access. Each OnlineOpen article will be subject to a one-off fee to be met by or on behalf of the Author in advance of publication. Upon online publication, the article (both full-text and PDF versions) will be available to all for viewing and download free of charge (for details see 5.4)

Early View: *Journal of Zoological Systematics and Evolutionary Research* is covered by Wiley-Blackwell's Early View service. Early View articles are complete full-text articles published online in advance of their publication in a printed issue. Articles are therefore available as soon as they are ready, rather than having to wait for the next scheduled print issue. Early View articles are complete and final. They have been fully reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after online publication. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the traditional way. They are therefore given a Digital Object Identifier (DOI), which allows the article to be cited and tracked before it is allocated to an issue. After print publication, the DOI remains valid and can continue to be used to cite and access the article. For more information on DOI, click here.

Preparation of Manuscripts

The following remarks may assist you in preparing your manuscript for submission to JZSER. We strongly encourage our authors to follow these guidelines as it facilitates both the peer-review and the editorial process.

Structure of manuscripts

The first page of the manuscript should contain the following information: title, name(s) of author(s), affiliation(s) of authors (including full address, phone and fax numbers, and email addresses), indication of corresponding author and up to five keywords.

The text should be divided into the following ten sections: Abstract, Introduction, Material and Methods, Results, Discussion, Acknowledgements, Second Summary (if required, in German, French, Italian or Spanish language and headed by an appropriate translation of the title of the paper), References, Tables (consecutively numbered), and Figure Legends. Figures and Supporting Information are uploaded as separate files and should not be included into the Main Document. The system generates a single

PDF file with all submitted files, excluding any supporting information.

Formats

JZS has no strict length limits, but manuscripts should be as concise as possible (e.g., recommended length for Original Articles: 10,000 words including references; Short Communications: 5,000 words including references). Manuscripts have to be submitted on DIN A 4 page format, 1,5 spacing, with a wide margin. When preparing your file, please use only standard fonts such as Times, Times New Roman or Arial for text, and Symbol font for Greek letters, to avoid inadvertent character substitutions. In particular, please do not use Japanese or other Asian fonts. Do not use automated or manual hyphenation. Please use page and line numbers to facilitate identifying the reviewer's comments.

Units, abbreviations and nomenclature

The S.I. System is relevant to measures. All biological and chemical names, or other technical terms, should be given according to the most recent international nomenclature. Authorities of scientific name should be cited, but only once when the name appears in the text for the first time. Alternatively, if taxon names are listed in tables (e.g., specimen list) authorities may also included there. It is not necessary to mention authorities in the title or summaries, unless the article is specifically taxonomic. Where commercially available substances, reagents, or equipment are used, the manufacturer's name and address (city and country is sufficient) should be provided in the 'Materials and Methods' section, along with the generally accepted common name.

Scientific botanical and zoological species names, as well as Latin terminology such as *in vitro*, *in vivo*, *de novo* etc., will be printed in italics. They should either be typed as such, or underlined with a single straight line. Unless there is risk of ambiguity, names of species can be abbreviated on being mentioned a second time and thereafter throughout the text, thus, *Heterobasidion annosum* would become *H. annosum*.

Tables and Figures

Tables should not be created as graphics files, but by using the table function in MS Word or the tabulator in other programmes. Tables must have a brief title and may have footnotes.

Each figure and scheme should have a legend. These should be listed together at the end of the reference section of the text file rather than being included with the drawings in the graphics files. Good quality graphics should be submitted for referees and editors. For high quality reproduction, high resolution graphics must be supplied.

Each figure and table must have a reference in the text. It is essential that text and image files be kept separate. Please mark the position of tables/figures in the text.

To facilitate production of quality artwork, we recommend that authors generate their artwork according to our digital illustration standards. Information on which can be found here: http://authorservices.wiley.com/bauthor/illustration.asp

*Software and format***.** The recommended format for all illustration files is TIFF or TIF. JPG and JPEG format will not be accepted. EPS (with preview) is acceptable but not recommended. Do not submit native application formats.
Resolution. Journal quality reproduction will require greyscale and color files at resolutions yielding approximately 300 ppi. Bitmapped line art should be submitted at resolutions yielding 600-1200 ppi. These resolutions refer to the output size of the file; if you anticipate that your images will be enlarged or reduced, resolutions should be adjusted accordingly.
File names. Illustration files should be given the 2- or 3-letter extension that identifies the file format used (i.e., .tif, .eps).

References

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. EndNote reference styles can be searched for here: http://www.endnote.com/support/enstyles.asp

Reference Manager reference styles can be searched for here: http://www.refman.com/support/rmstyles.asp

Literature will be quoted in the text with the year of publication, e.g. Mayr and Bock (2002) or (Mayr and Bock 2002). If more than one reference is quoted in the text, separate them by a semicolon, e.g. (Borkin 1999; Mayr and Bock 2000). If the same author is quoted with more than one source of the same year, mark them with a, b, etc. (e.g. Bock 2000a). If the cited literature has more than two authors, it should be quoted by the first author followed by et al. (e.g. Clark et al. 1999)

The list of references should only include publications cited in the text. The references have to be entered alphabetically, and must include the name(s) and initials of the author(s), year of publication in parentheses, title of paper, name of journal (abbreviated according to international rules, without dots), volume number (bold), page numbers. References to books must include surname(s) and initials of author(s), year of publication in parentheses, editor(s), complete title of the book edition, publisher, place of publication, page numbers.

Examples:
Journals: Larsen K, Wilson GDF (2002) Tanaidacean phylogeny, the first step: the superfamily Paratanaidoidea. J Zool Syst Evol Res **40**:205-222.

Books: Borkin LJ (1999) Distribution of amphibians in North Africa, Europe, Western Asia, and the former Soviet Union. In: Dulleman WE (ed), Pattern of Distribution of Amphibians: Global Perspective. John Hopkins University Press, Baltimore and London, pp 329-420.

Special Note: References and related citations which belong to the Supporting Information only should be clearly identified in the main text and Reference List with the insertion of an asterisk. For example, when citing a reference within the main text: '(*Larsen and Wilson 2002)'; when included in the Reference list: '*Larsen K, Wilson

GDF (2002) Tanaidacean phylogeny, the first step: the superfamily Paratanaidoidea. J Zool Syst Evol Res **40**:205-222'.

Supporting Information

Supporting Information can be a useful way for an author to include important but ancillary information with the online version of an article. Examples of Supporting Information include additional tables, data sets, figures, movie files, audio clips, 3D structures, and other related nonessential multimedia files.

Supporting Information should be cited within the article text, and a descriptive legend should be included. It is published as supplied by the author, and a proof is not made available prior to publication; for these reasons, authors should provide any Supporting Information in the desired final format.

For further information on recommended file types and requirements for submission, please visit: http://authorservices.wiley.com/bauthor/suppinfo.asp

It should be clearly stated at the time of submission that the Supporting Information is intended to be made available through the online edition. The Supporting Information is an integral part of the article and will be reviewed accordingly.

The availability of Supporting Information should be indicated in the main manuscript by a paragraph, to appear after the References, headed 'Supporting Information' and providing titles of figures, tables, etc. References and related citations which belong to the Supporting Information only should be clearly identified in the main text and Reference List with the insertion of an asterisk (see 3.3.3. References).

Electronic Submission of Manuscripts

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Structure of Drosophilidae Assemblage (Insecta, Diptera) in Pampa Biome (São Luiz Gonzaga/RS)

Estrutura da Assembléia de Drosophilidae (Insecta, Diptera) no Bioma Pampa (São Luiz Gonzaga/RS)

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ABSTRACT

The Brazilian Pampa (the southernmost end of the country) is currently a highly modified environment because of increasing agricultural activities. In many places, only small parts of grasslands remain inside an agricultural landscape. Drosophilidae (Diptera) have been widely used as a potential bioindicators to monitor the effects of anthropogenic changes in natural environments. However, the fauna of Drosophilidae in the Pampa Biome from natural and disturbed environments, still remains largely unknown. The present study represents one of the first attempts to fill this gap, showing results from monthly collections in the municipality of São Luiz Gonzaga (28°24'28"S, 54°57'39"W), in the Brazilian Pampa. A species inventory was carried out in two contrasting environments, an urban zone and a forest remnant (rural zone). In both areas banana-baited traps were used to capture adult drosophilids. The identification was made using external morphology and male terminalia. In total, 13,379 drosophilids were analyzed (rural zone: N= 8,812 and S_{obs}= 25; urban zone: N= 4,567 and S_{obs}= 16). In the present study, 16 (60%) out of 26 species were found exclusively or preferentially in the forest. The period of highest richness was between the months of June to November (roughly winter and spring), and the period of lowest richness was from December to May (roughly summer and autumn). An analysis of cluster by the Coefficient of Jaccard showed that species composition slightly changes when the period of the year with higher temperatures (from January to May) is compared with the period with lower temperatures (from June to October). The species abundances were also highly affected by seasonality, as revealed by the Morisita Index, since the samples clustered into similar groups in consecutive periods and in the same season, showing the seasonal preference of some species. The time component was a determinant in the diversity of the assemblage, surpassing the spatial effect. The strong reduction in diversity in the urban area when compared to a small forest patch is evidence of the importance of the natural environments in maintaining the diversity in the Pampa biome, currently a highly disturbed landscape.

Key-words: Pampa Biome; Drosophilidae; diversity; bioindicator.

RESUMO

O Pampa brasileiro (extremo sul do país) está, atualmente, vastamente modificado devido ao aumento das atividades agrícolas. Em muitos lugares, apenas pequenos fragmentos de campo permanecem em uma paisagem agrícola. Drosophilidae (Diptera) tem sido amplamente utilizadas como bioindicadores para monitorar os efeitos das mudanças antropogênicas em ambientes naturais. Porém, a fauna de Drosophilidae no Bioma Pampa de ambientes naturais ou perturbados, ainda permanece amplamente desconhecida. O presente estudo é uma das primeiras tentativas de preencher esta lacuna, apresentando resultados de coletas mensais no município de São Luiz Gonzaga (28°24'28''S, 54°57'39''W), no Pampa brasileiro. Um inventário de espécies foi conduzido em dois ambientes contrastantes, uma zona urbana e um remanescente de floresta (zona rural). Em ambos os locais, armadilhas com banana fermentada foram usadas para capturar drosofilideos adultos. A identificação foi feita através da morfologia externa e da terminália dos machos. No total, 13,379 drosofilideos foram analisados (zona rural: N= 8,812 and S_{obs}= 25; zona urbana: N= 4,567 and S_{obs}= 16). No presente estudo, 16 (60%) das 26 espécies coletadas foram encontradas exclusivamente ou preferencialmente no fragmento de mata. O período de maior riqueza foi entre os meses de junho a novembro (inverno-primavera), e o período de menor riqueza foi de dezembro a maio (verão-outono). Uma análise de cluster pelo Coeficiente de Jaccard mostrou que a composição da assembléia muda ligeiramente quando o período do ano com temperaturas mais elevadas (janeiro-maio) é comparado com o período de temperaturas menos elevadas (junho-outubro). A abundância das espécies foi também altamente afetada pela sazonalidade, como revelou o

Índice de Morisita, onde as amostras foram agrupadas em períodos consecutivos dentro de uma mesma estação, mostrando a preferência sazonal de algumas espécies. O componente tempo foi determinante na diversidade da assembléia, superando o efeito espacial. A forte redução na diversidade na área urbana quando comparada com o pequeno fragmento de floresta, torna evidente a importância do ambiente natural para a preservação da diversidade no bioma Pampa, atualmente com sua paisagem altamente alterada.

Palavras-chave: Bioma Pampa; Drosophilidae; diversidade; bioindicador.

INTRODUCTION

The loss of biodiversity has become a central issue, with the recognition that the increasing human pressure on landscapes and natural habitats results in population or species extinction at unprecedented rates. Populations are entities in a continuous process of change. Even when the community and the ecosystem do not seem to be changing, intrinsic factors like the density, mortality, birth rate, food availability, among others, are in constant fluctuation, and the species that compose this community keep in constant adjustment for these changes (Odum, 1988). The environment change affects the occurrence and the abundance of some resources used by species that in turn may respond differently to these alterations. In this sense, it is possible to distinguish two kinds of time-based change: the predictable change (like the daily variation and the seasonal variation), which can increase or decrease the diversity of local species, and the unpredictable change (stochastic events, and catastrophic sometimes), which can cause a decrease in specific diversity (Begon *et al*., 1996). Changes in land use, including urbanization and agricultural expansion, concomitant with the fragmentation and disturbance of the natural environments, may substantially alter species distributions and diversity. The existence of large gaps in species occurrence data and comprehensive monitoring schemes are, therefore, strong impediments to the detection of these processes (Kivinen, 2007).

The Brazilian Pampa is currently a highly modified environment. The Pampa Biome is a landscape mostly neglected by biodiversity studies, despite its high diversity and characteristic wildlife and flora. This ecosystem extends over an area of approximately 700,000 km^2 of mainly plain lowlands, shared between Argentina, Brazil and Uruguay (Bilenca & Miñarro, 2004). In Brazil, it covers the southernmost end of the country, in the state of Rio Grande do Sul. This portion represents about 176,000 km^2, approximately 63% of the area of the state and 2.1% of the Brazilian territory (Collares, 2006). The original landscape is predominantly covered by grasslands, although these are sometimes naturally invaded by arboreal formations of deciduous seasonal forest and ombrophilous dense forest, remarkably in northern and eastern parts of the State of Rio Grande do Sul (IBGE, 2004), where the biome is bordered by the Atlantic Forest biome. Unfortunately, the Pampa has been suffering a wide loss of diversity and habitat due to the fast agricultural expansion started in the 1970's, aggravated recently by plans to convert wide areas of grasslands to monoculture of trees, according to the Agricultural Census (IBGE, 2006). In many places, only small parts of grasslands remain inside an agricultural landscape (Risser, 1997; Porto, 2002; Bencke, 2003). Currently, just 11.7% of the Pampa Biome has been spared human influence in Rio Grande do Sul (PROBIO, 2007).

Flies of family Drosophilidae (Diptera) have been widely used in scientific research as a paradigmatic model and more recently have become a target taxon of biodiversity inventories and suggested as a potential bioindicator to monitor the effects of anthropogenic changes in natural environments (Avondet *et al*., 2003; Ferreira & Tidon, 2005; Gottschalk *et al*., 2007). They are a diverse and relatively well-known taxon, easily sampled with a low cost, and very sensitive to environmental changes. However, the fauna of Drosophilidae in the Pampa Biome, both from natural and disturbed environments, still remains largely unknown. Although the state of Rio Grande do Sul has been one of the most targeted study areas in Brazil concerning Drosophilidae diversity, most of the studies have been conducted in localities belonging to the Atlantic Forest Biome (Petersen, 1960; Franck & Valente, 1985; and others), while the Pampa Biome has been largely neglected, being one of the most unexplored in Brazil, as noted by Gottschalk *et al*. (2008). In fact, the only Drosophilidae diversity inventories performed in this Biome in Brazil sampled localities in or nearby the city of Porto Alegre: a forested area at Itapuã State Park (Valente & Araújo, 1991), a rural grassland area in Guaíba (Saavedra *et al*., 1995) and the urban region of the city of Porto

Alegre (Silva *et al*., 2005; Garcia *et al*., 2008; Garcia *et al.*, 2012; Silva *et al*., 2008). Recently, Hochmüller *et al*. (2010) conducted a survey in a transition area between Pampa Biome and Atlantic Forest Biome in the municipality of Cruz Alta. Similarly, outside Brazil, only a few Drosophilidae inventories have been conducted in the biome, a study carried out in Argentina (Fernández Iriarte & Lopez, 1995) and one in Uruguay (Goñi *et al.*, 1997, 1998), besides sparse records.

The present study represents one of the first attempts to fill this gap, showing results from monthly collections in the municipality of São Luiz Gonzaga, Rio Grande do Sul, in the Brazilian Pampa. A biodiversity inventory was carried out in two contrasting environments, an urban zone and a forest remnant.

MATERIALS AND METHODS

Study area

The collections were carried out in two areas in the municipality of São Luiz Gonzaga (28°24'28"S, 54°57'39"W), northwest of the state of Rio Grande do Sul, southern Brazil, a region of subtropical climate characterized by rainy weather and well defined seasons, with negative temperatures during the winter and a hot summer. The region has been heavily degraded, consisting nowadays of medium and small-sized cities in a predominantly agricultural landscape, with the natural grasslands highly disturbed and the forested areas reduced to just small patches of secondary forests. It is located near the northern border of the Pampa Biome, as defined by IBGE (2004).

Two contrasting localities were surveyed. The urban zone (UZ), in downtown (28°24’39’’S, 54°57’371”W), is situated in the main urban and commercial area of the municipality (Fig 1a). According to the criteria described by Ruszczyk (1986/1987), based on percentage of vegetal cover like was done by Gottschalk *et al.* (2007) in Florianópolis, this area can be considered as having a medium urbanization level. The rural zone (RZ), located about 10 km from downtown collection point (28°22'51.2"S, 55°00'8.62''W), is a small native fragment of deciduous seasonal forest inside a region originally with predominance of steppe savanna, today largely replaced with agricultural areas (Fig 1b).

Collections and identification

In both areas banana-baited traps (Tidon & Sene, 1988) were used to capture adult drosophilids. For each sample, one kilogram of banana were mashed, sprinkled with baker's yeast and distributed in 5 traps hung in the trees at about 1.5 m above the ground, where they were kept for five days. Samples were taken monthly from September 2007 to September 2008, and in November 2008 and January 2009.

Flies were maintained in ethanol 70% until identification. The identification was made using external morphology and male terminalia, consulting specialized literature. Analysis of male terminalia was conducted according to Bächli *et al.* (2004).

Some individuals belonging to *Drosophila repleta*, *D. tripunctata* and *D. guarani* species groups that remained unidentified at species level were not scored for statistical analysis of species abundance and diversity measures (just ~ 8% of total sample). However, they were considered in the total number of individuals (N) and the number of individuals of Neotropical species (N_{nat}).

Voucher specimens of the material collected were deposited in the Laboratory of Zoology of Universidade Regional Integrada do Alto Uruguai e das Missões (URI) in Santo Ângelo, RS, Brazil.

Data analysis

Diversity data were measured as follows: (1) observed species richness (S_{obs}); (2) species richness estimated by rarefaction method (S_{rar}); (3) Shannon-Wiener heterogeneity index (*H'*); and (4) Smith-Wilson evenness index (E_{var}). Of these, *H'* and E_{var} were calculated using the software Ecological Methodology (Krebs, 1999). Natural logarithm (ln) was used to calculate *H'*. For S_{rar}, all samples were standardized to 11 specimens, to nullify the effect of *N* (number of individuals) in species richness, using BiodiversityPro version 2 (McAleece *et al*., 1997). The correlation among S_{obs}, S_{rar}, *H'*, E_{var} and *N* was tested by Linear correlation *r* in Past 1.34 (Hammer *et al*., 2001).

Statistically significant differences in values of *H'*, E_{var}, S_{obs}, *N*, N_{exot} and N_{nat} between collection points were analyzed using the T test, in Past 1.34 (Hammer *et al*., 2001). The preference of some species for a specific environment was tested with Wilcoxon tests based on their absolute abundances, using the same software.

The influence of space and time on assemblage diversity was estimated by the following calculation: $H'_{between} = H'_{total} - (Nj\ H'j)/Nt$; where $H'_{between}$ is the value of *H'* for a given component; H'_{total} is the value of *H'* considering all the samples together; *Nt* is the total number of individuals in all samples, *Nj* is the number of individuals in category *j*, *H'j* is *H'* within category *j*. Spatial (urban and rural zones) and temporal (monthly collections) components were considered.

The similarity between samples was investigated by cluster analysis using UPGMA method, in Past 1.34 (Hammer et al., 2001). Similarity measures were Coefficient of Jaccard and Morisita index of similarity. Coefficient of Jaccard is a binary coefficient (deals with presence/absence data), so it was used to compare the similarities in species composition among samples. On the other hand, Morisita index deals with quantitative data, so it was used to compare samples in terms of relative abundance of each species. As the original Morisita index showed little differences between our samples, we used it after a logarithmic transformation [ln(x+1)], as recommended by Wolda (1981) and Krebs (1999) for communities with few species in common and many rare species, as the present sample.

RESULTS AND DISCUSSION

Species occurrence and abundances

In total, 13,379 drosophilids were analyzed (RZ: N= 8,812 and S_{obs}= 25; UZ: N= 4,567 and S_{obs}= 16), distributed as 26 species, 23 of which belonging to genus *Drosophila*. One species probably has not been described yet, and was called here *Drosophila* sp.Q2. This is the same species referred to by the same name by Gottschalk *et al.* (2007), in a study that reported its occurrence it in Morro da Lagoa da Conceição and Morro da Cruz, in Florianópolis, SC, and found abundantly by Sabrina C. F. de Oliveira in the Unidade de Conservação Ambiental Desterro (UCAD), also in Florianópolis (pers. comm.). The genera *Zygothrica*, *Zaprionus* and *Leucophenga* were represented by just one species each (Tables 1 and 2).

Two species of *Drosophila*, *D. aldrichi* and *D. repleta*, were recorded in the State of Rio Grande do Sul for the first time. For *D. aldrichi* this is the new southernmost record. With these new records, the number of described drosophilid species known for Rio Grande do Sul rises to 86. Also were found *D. nigricruria*, *D. virilis* and *Leucophenga maculosa*, which were just recently found for the first time in Rio Grande do Sul by Hochmüller *et al*. (2010).

From the 26 species found, six are exotic. Except for one collection in RZ, during the January of 2009, in summer, *D. simulans* was always the most abundant species in our study, showing expressive dominance in UZ (68% of the total of individuals) and being also the most abundant species in RZ (48% of the individuals). This species frequently is the most abundant exotic species in natural environments in Brazil (Sene *et al.*, 1980; Torres & Madi-Ravazzi, 2006; Schmitz *et al.*, 2007; Bizzo *et al.*, 2010; Hochmüller *et al.*, 2010). Its sibling species, *D. melanogaster*, is also commonly found in synanthropic environments, although with lower abundances, as in the present study. *Zaprionus indianus* is a recent invader (Vilela, 1999) and became a very abundant species in urbanized environments. Therefore, the abundance of *Z. indianus* in São Luiz Gonzaga seems to be comparatively lower than in other locations (Castro & Valente, 2001; De Toni *et al*., 2001; Ferreira & Tidon, 2005; Silva *et al.*, 2005; Gottschalk *et al.*, 2007), where it represents, in some situations, more than half of collected individuals. In São Luiz Gonzaga, this species achieved a total relative abundance of about 5% in the urban zone and of about 1% in the forest fragment, similar to the results found by Hochmüller *et al.* (2010) in Cruz Alta, in the region of transition between the Atlantic Forest and Pampa Biome in the countryside of Rio Grande do Sul. The present study adds new evidence that the populations of this species are relatively small in this region. As this region is next to the southern limit of its distribution (Uruguay and northern Argentina), it is possible that this species is represented by marginal populations living in suboptimal conditions, limited by weather conditions like lower temperatures.

Another similarity between the drosophilids assemblages from São Luiz Gonzaga and Cruz Alta is the relatively higher representativeness of *D. immigrans* and *D. busckii,* when compared to other studies in Brazil. Contrasting *Z. indianus,* these species seem to be related to more temperate weather, becoming markedly rarer in northernmost localities (Ferreira & Tidon, 2005; Torres & Madi-Ravazzi, 2006; Gottschalk *et* al., 2007; Schmitz *et al.*, 2007; Bizzo *et al.*, 2010). The other exotic species, *D. virilis*, is not commonly attracted to banana-baited traps and was represented by one individual only.

Between the Neotropical species, the most common were *D. mercatorum*, *D. hydei* and *D. buzzatii* in UZ (all belonging to *D. repleta* group) and *D. mercatorum*, *D. polymorpha* and *D. willistoni* in RZ. The abundances of the species of the *D. repleta* group are underestimated, since discrimination of females is difficult and just the males were identified. However, assuming that the relative abundances of the females were the same as of the males, *D. mercatorum* is the most common Neotropical species in São Luiz Gonzaga. This differentiates the assemblages of drosophilids collected in São Luiz Gonzaga from the assemblages found in Porto Alegre and in localities of Atlantic Forest and Amazon Biomes, where *D. willistoni* is almost always the most abundant Neotropical species (Martins, 1987; Silva *et al.*, 2005; Gottschalk *et al.*, 2007). Again, the results of the present study are similar to the findings by Hochmüller *et al.* (2010) in Cruz Alta, where a lower representativeness of *D. willistoni* was observed. On the other hand, in Cruz Alta *D. mercatorum* did not achieve expressive abundances (*D. maculifrons* was the most abundant Neotropical species). A high representativeness of *D. mercatorum* was found by Ferreira & Tidon (2005), in Brasília, Cerrado Biome, where it also was the most abundant Neotropical species.

Some important absences can be noticed in assemblages of drosophilids in São Luiz Gonzaga, like *D. malerkotliana*, *D. paulistorum* and *D. saltans* species group, taxa that are quite common in most part of Brazil. *Drosophila malerkotliana* (an introduced species) and *D. saltans* species group also seem to be absent in Cruz Alta, while *D. paulistorum* is present at low abundance in that locality (Hochmüller *et al.*, 2010).

The preference of some species for a given environment has been reported by many authors (Dobzhansky & Pavan, 1950; Sene *et al.*, 1980; Ferreira & Tidon, 2005; Tidon, 2006). In the present study, 16 (60%) out of the 26 species were found exclusively or preferentially in the forest, while nine did not express any preference and just one was exclusive of the city (Table 3). This last case was *D. repleta*, which in spite of being a

Neotropical species, was introduced in many regions around the world, being currently a cosmopolitan species, normally associated to anthropic presence. The preference for the forest patch was higher among the Neotropical species, 70% of which occurring exclusively or preferentially in this environment; however, when only the introduced species are considered, this proportion decreased to one third.

Diversity measures

The highest diversity was found in RZ, considering either heterogeneity (H') or species richness (S_{obs} or S_{rar}) (Table 4). Avondet *et al.* (2003), Gottschalk *et al.* (2007) and Garcia *et al.* (2012), in studies performed in the cities of Oxford, OH, USA, Florianópolis, SC, Brazil and Porto Alegre, RS, Brazil, respectively, found some differences in the abundance of species along an urban gradient, but did not find any decrease in diversity. On the other hand, other studies like those of Goñi *et al.* (1997), Ferreira & Tidon (2005) and Hochmüller *et al.* (2010), respectively, in Montevideo, Uruguay, Brasília, DF, Brazil and Cruz Alta, RS, Brazil, found some evidence of decrease in diversity in urbanized regions, when compared with natural environments. The factors that cause the decrease in diversity in some localities and not in others remain to be elucidated. Gottschalk *et al.* (2007) suggested that the existence of green areas nearby the urban areas could support the survival of native drosophilid species in the city. Considerable portions of natural environment remnants still persist in Florianópolis and, to a lesser extent, in Porto Alegre. On the other hand, the region where Cruz Alta and São Luiz Gonzaga are located is characterized by a highly human-modified landscape, with few and small patches of natural vegetation. Some authors point out that local biodiversity may be affected by the regional amount of remnant vegetation, with a fragmentation threshold below which diversity becomes dependent of patch size (Pardini *et al.*, 2010).

Here, no significant difference between UZ and RZ in the abundance of exotic species (N_{exot}) was observed, but the number of specimens of endemic species from Neotropics (N_{nat}) was significantly lower in UZ (Table 4). This suggests that the forest patch in RZ can be easily invaded by at least some exotic species, probably because it is a small fragment of forest, in a region quite fragmented by agricultural cultures. On the other hand, for most Neotropical species, it is difficult to survive in face of the expansion of urban environments over natural ones.

In general, the observed species richness (S_{obs}) was higher in the period between June and November (roughly winter and spring), varying from 5 to 11 in UZ and from 10 to 18 in RZ, while it was lower from December to May (roughly summer and autumn), with 3 to 7 species in UZ and 3 to 9 in RZ (Table 4). A higher richness in drosophilid assemblages during winter was also found by Torres & Madi-Ravazzi (2006) in the state of São Paulo. In São Luiz Gonzaga, a severe dry and hot period during summer may have caused a negative effect on drosophilidae diversity.

The expressive abundance of *D. simulans* seemed to affect the indexes of heterogeneity (*H'*) and evenness (E_{var}) of the assemblage. The highest value of E_{var} was found in May 2008 (E_{var} RZ = 0.963 and E_{var} UZ = 0.525), period of autumn (Table 4), when the species richness and the relative abundance of *D. simulans* were lower in comparison to other months, which was observed again in summer periods. In RZ, the highest heterogeneity was observed during June 2008 (*H'*= 1.921), autumn, as opposed to the findings by Benado & Brncic (1994), in Chile, in a study that reported the lowest diversity in the same period. In UZ, the highest heterogeneity was found in October 2007 (*H'*= 1.569), spring, as found by De Toni *et al.* (2007) in Santa Catarina. The lowest diversity was found in both sites in September of 2008, winter, with *H'*= 0.252 in RZ and *H'*= 0.832 in UZ. This low diversity is due to the high dominance of *D. simulans*. The dominance of one species acting negatively on community diversity was noticed by De Toni *et al.* (2007) and Brncic *et al.* (1985), with a large dominance of *D. willistoni* and *D. simulans* in their collections, respectively.

Table 5 shows the correlations between the diversity measures in each site. In both sites, S_{obs} showed positive and significant correlation with N, while S_{rar} was correlated with H'. In UZ, H', E_{var} and S_{rar} showed a statistically significant positive correlation, while in ZR, E_{var} was negatively correlated with S_{obs} and N.

In spite of the interference caused by the dominance of *D. simulans*, the time component was a determinant in the diversity of the assemblage, although more than half of the diversity could not be explained by the analyzed components (Table 6). The time component in the present study showed a relatively high contribution to the diversity in comparison with other similar studies (Silva *et al.*, 2005; Gottschalk *et al.*, 2007; Schmitz *et al.*, 2010). These studies, however, performed just seasonal collections, and studies based on monthly collections, like the present one, are not common. These results stress the great effect of the temporal changes in drosophilid assemblages that, especially in areas with a marked seasonal regime, as the Pampas, surpass the spatial effects, even when contrasting environments are compared.

Similarity analysis

In general, the present results show that species composition, as measured by the Jaccard coefficient (Fig. 2), is markedly affected by seasonality, being more similar in the colder months of the year. All samples taken between June and October (late autumn, winter and early spring) clustered together within a group sharing at least 40% of the species, while all the samples from January to May (summer and early autumn) lied outside this group. The months of November and December (late spring) seem to be a transition period, as some samples clustered in the coldest period and other samples in the hottest period. The environment (urban or forested) was also shown to be an important factor, since some clustering between sites can be observed, but to a lesser extent than temporal factors.

The Morisita index showed that when the structure of the assemblage is considered, the effect of seasonality is evident, since samples clustered into similar groups in consecutive periods and in the same season, showing the seasonal preference of some species again (Fig. 3). Almost all samples taken showed a high abundance of *D. simulans*, so the clustering was more influenced by secondary species. The first group of samples to split off the cluster is composed roughly by samples collected in hot months in the urban zone, and can be characterized by a relatively high abundance of *Z. indianus*. The second group to split is constituted exclusively by samples from the forest patch, marked by a higher relative abundance of *D. polymorpha*. Some summer samples within this group formed a subcluster with, besides *D. polymorpha*, a higher representativeness of *D. willistoni*. The remaining samples, mainly urban samples, but also several samples from the forest patch, are those that showed the higher dominance of *D. simulans*. Among them, a group of samples collected in spring clustered together and have in common a relatively high abundance of *D. busckii* and *D. mercatorum*.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Studies that compared drosophilids assemblages in forested areas with urban ones have consistently found marked differences in relative species abundances, but not always in diversity (Avondet *et al*., 2003, Gottschalk *et al*., 2007). However, the present study is the second recent survey to strongly suggest a marked biodiversity loss with the expansion of urban landscapes in detriment of natural ones in the countryside of the state of Rio Grande do Sul. Hochmüller *et al.* (2010) found lower species richness in the urban area of Cruz Alta, when compared to a forest remnant nearby. The present study found the same pattern in São Luiz Gonzaga. Additionally, we could detect a reduction in diversity also when it was measured by Shannon-Wiener index and species richness by rarefaction. It is notable too that 70% of the Neotropical species showed a preference for the forest fragment, with a significant reduction in abundance of native species in the city, reinforcing the importance of natural environments to maintain the regional biodiversity. Similar results were relatively well documented in the Cerrado biome (Ferreira & Tidon, 2005, Tidon, 2006, Mata *et. al*., 2010), where it was also verified that many Neotropical species that occur in natural environments were absent in the city, while others decreased in abundance as the degree of urbanization increased. The Cerrado and the Pampa biomes are similar in being constituted by natural formations of forests inserted in a landscape dominated predominantly by savanna-like environments. Future studies, especially in the Pampa, could indicate if the patterns of response of the biodiversity to landscape modification are similar in the two biomes.

The forest remnant surveyed in the present study is a very small and disturbed fragment, inside an agricultural landscape, and, as we noticed, although still a refuge for Neotropical species absent in the city, is also highly invaded by some introduced species, bioindicators of disturbed environments. Considering that the assemblages of drosophilids in undisturbed natural environments of the Pampa are still completely unknown, future studies are needed to assess the portion of the biodiversity that can have already been lost in a landscape widely converted to agricultural fields. Although the impact of the urbanization on the natural assemblages is relatively well studied, the effects of the change of land use to agriculture and cattle raising are still little known.

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TABLES

TABLE 1: Monthly absolute abundance of drosophilid species collected in a forest patch in the rural zone (RZ) of São Luiz Gonzaga, RS, Brazil.

	SEP 07	OUC 07	NOV 07	DEC 07	JAN 08	FEBB 08	APR 08	MAY 08	JUN 08	JUL 08	AUG 08	SEP 08	NOV 08	JAN 09
D. aldrichi							2							
D. antonietae			2						5					
D. bandeirantorum										1				
D. busckii	171	288	169							3		46		
D. buzzatii	4	8	2	2					4	2	2	2		
D. cardinoides									9	11	2	5		
D. griseolineata										5		3		
D. hydei	11	12	3	1					1		1	1		
D. immigrans	21	11	17		1		1		4		8	11	50	
D. maculifrons			1						6	5	6	4		
D. mediopunctata	3								3	2	1	2		
D. melanogaster	22	11				3	12		44		29	19		
D. mercatorum	139	98	97	3	8	2			27	60	83	58	44	1
D. nigricuria	2	5				2	2	3	9	6	8	10	2	1
D. onca									2		2	5	5	
D. pallidipennis			2		9	4			3		13	7	17	
D. paraguayensis											1			
D. polymorpha	41	12	18	8	70	34	8	3	118	182	335	101	24	100
D. simulans	187	290	207	20	45	52	52	5	204	347	1340	1275	56	146
D. sp. Q2			3										6	
D. virilis	2													
D. willistoni					10	31	6		32	101	8	6	1	626
Gr. guarani (females)									1					
Gr. repleta (females)	196	77	71	11	3	3	5	2	37	95	78	52	73	2
Gr. tripunctata (females)	7		1				1		2	1		3		
Leucophenga maculosa									33					
Zaprionus indianus							44		7	3	3	3	23	9
Zygothrica vittimaculosa		3							4	23	9			

TABLE 2: Monthly absolute abundance of drosophilid species collected in the urban zone (UZ) of São Luiz Gonzaga, RS, Brazil.

	SEP 07	OUC 07	NOV 07	DEC 07	JAN 08	FEB 08	APR 08	MAY 08	JUN 08	JUL 08	AUG 08	SEP 08	NOV 08	JAN 09
D. bandeirantorum											13			
D. busckii	123	90	8							7	8			
D. buzzatii	3	78	3	2		2			1					
D. cardinoides				1							1			
D. hydei	8	53	42							2	10		2	
D. immigrans	4		8						1		13	1	17	
D. maculifrons									6					
D. melanogaster	6	10	7		2		2	5	22	8	59	16		
D. mercatorum	61	36	16		6		3	3	10	10	45	3	5	2
D. nigricruria	1			2					2					
D. pallidipennis											1		1	
D. polymorpha	6	10	1	2	1	1			2	2	6	1		
D. repleta					2									
D. simulans	179	205	87	25	20	134	13	33	178	190	1401	367	86	171
D. willistoni						6								
Gr. repleta (females)	80	143	17	3	1			1	17	34	15	4	3	1

TABLE 3: Drosophilidae species collected in São Luiz Gonzaga, RS, classified according to environment preference. Species without preference were abundantly present in both areas.

Only in forest	**Without preference**
D. aldrichi	*D. bandeirantorum*
D. antonietae	*D. busckii*
D. griseolineata	*D. buzzatii*
D. mediopunctata	*D. cardinoides*
D. onca	*D. hydei*
D. paraguayensis	*D. maculifrons*
D. sp.Q2	*D. melanogaster*
D. virilis	*D. simulans*
Leucophenga maculosa	*Zaprionus indianus*
Zygothrica vittimaculosa	**Only in city**
Preferentially in forest	*D. repleta*
D. immigrans *	
D. mercatorum **	
D. nigricruria **	
D. pallidipennis *	
D. polymorpha ***	
D. willistoni **	

$p<0.05$, ** $p<0.01$, *** $p<0.001$

TABLE 4: Monthly variation in Shannon-Wiener heterogeneity index (*H'*), Smith and Wilson's index of evenness (E_{var}), observed species richness (S_{obs}), species richness estimated by rarefaction (S_{rar}, for n=11), number of individuals (N), number of individuals of exotic species (N_{exot}) and number of individuals of Neotropical species (N_{nat}), of the assemblages of drosophilids in urban (UZ) and rural (RZ) zones of São Luiz Gonzaga, RS, Brazil.

		SEP 07	OUC 07	NOV 07	DEC 07	JAN 08	FEB 08	APR 08	MAY 08	JUN 08	JUL 08	AUG 08	SEP 08	NOV 08	JAN 09
E_{var}	ZU	0.214	0.471	0.332	0.433	0.461	0.218	0.337	0.525	0.243	0.28	0.163	0.127	0.263	0.148
	ZR	0.218	0.248	0.175	0.467	0.311	0.32	0.318	0.963	0.3	0.193	0.158	0.208	0.327	0.102
H' *	ZU	1.318	1.569	1.426	0.82	1.17	0.445	0.749	1.048	0.911	0.644	0.504	0.252	1.066	0.722
	ZR	1.649	1.366	1.408	1.137	1.269	1.387	1.443	1.066	1.921	1.53	0.941	0.832	1.902	0.85
S_{obs} **	ZU	9	7	8	5	7	5	5	4	9	7	11	5	6	3
	ZR	11	10	12	5	6	7	9	3	18	14	17	17	10	6
S_{rar} **	ZU	3.57	4.48	4.08	3.07	3.78	1.96	2.58	3.11	2.97	2.38	2	1.51	3.26	2.07
	ZR	4.49	3.65	3.78	3.55	3.63	3.8	4.04	3	5.21	4.18	2.82	2.68	5.43	2.73
N **	ZU	391	482	172	32	32	149	73	59	231	222	1560	388	133	214
	ZR	603	738	521	34	143	128	127	11	515	751	1851	1558	228	883
N_{exot}	ZU	312	305	110	25	23	140	70	56	210	208	1484	384	125	212
	ZR	403	600	393	20	46	55	109	5	259	353	1380	1354	129	155
N_{nat} *	ZU	79	177	62	7	9	9	3	3	21	14	76	4	8	2
	ZR	200	138	128	14	97	73	18	6	256	398	371	204	99	728

* $p<0.05$, ** $p<0.01$; all measures with significant differences are higher in RZ in comparison with UZ.

TABLE 5: Linear correlation *r* between *H'*, *Evar*, S_{obs}, S_{rar} and *N* in the drosophilid assemblages of urban (bottom left) and rural (top right) zones of São Luiz Gonzaga, RS, Brazil. *: $p<0.05$; **: $p<0.01$; ***: $p<<0.01$

	H'	E_{var}	S_{rar}	S_{obs}	*N*
H'		-0.055726	0.98521***	0.20562	-0.44076
E_{var}	0.59474*		-0.067533	-0.55521*	-0.55744*
S_{rar}	0.97521***	0.68576**		0.19631	-0.43809
S_{obs}	0.23405	-0.1764	0.25631		0.71515**
N	-0.22535	-0.417	-0.25384	0.64962*	

TABLE 6: Contribution of temporal and spatial components to the diversity in the assemblages of drosophilids in São Luiz Gonzaga, RS, Brazil.

	H'	%
Temporal	0,3991	25,4
Espacial	0,0906	5,77
Not explained	1.0815	68.83
Total	1.5713	100

FIGURE LEGENDS

FIGURE 1: Rio Grande do Sul Map showing the municipality of São Luiz Gonzaga and the sampling zones: Urban Zone (A); Rural Zone (B). Source: http://maps.google.com.br/maps?hl=pt-BR&tab=wl&q=sao%20luiz%20gonzaga

FIGURE 2: UPGMA dendrogram showing the similarity in species composition of monthly samples of drosophilids in urban (U) and rural (R) zone in São Luiz Gonzaga, RS, Brazil, according to Jaccard similarity index.

FIGURE 3: UPGMA dendrogram showing the similarity in species abundances of monthly samples of drosophilids in urban (U) and rural (R) zone in São Luiz Gonzaga, RS, Brazil, according to Morisita index.

FIGURE 1:

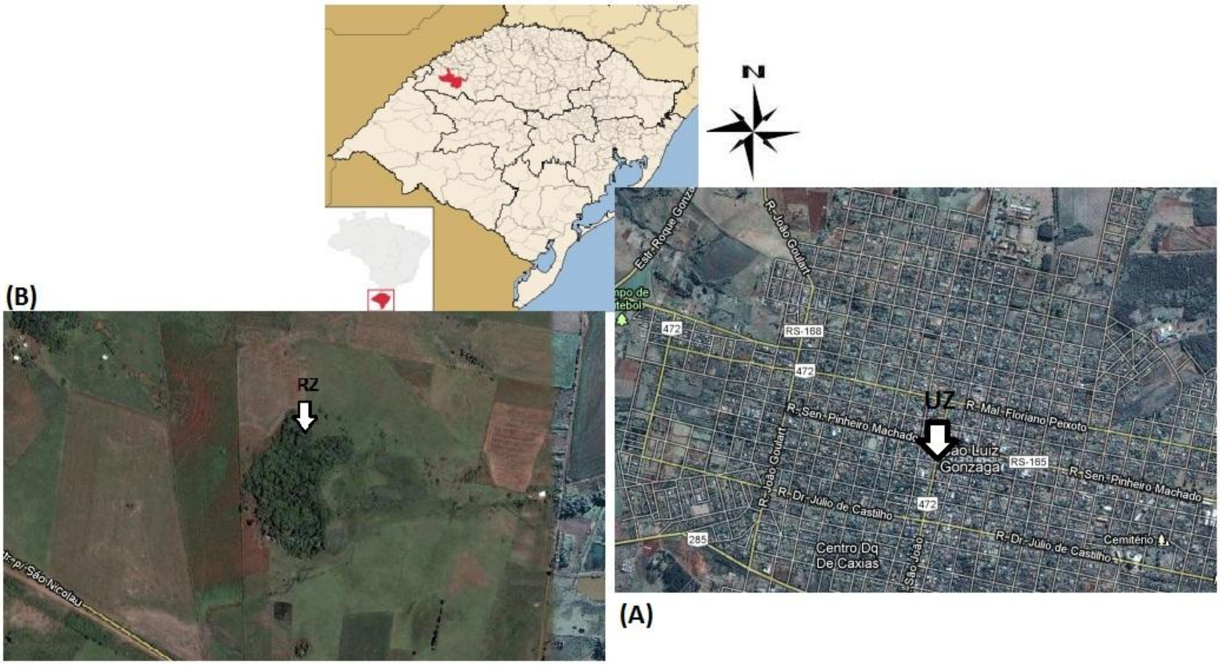

N
(B)
RZ
Estr. p/ São Nicolau
(A)
UZ
R. João Goulart
RS-168
472
R. Sen. Pinheiro Machado
R. Mal. Floriano Peixoto
São Luiz
Gonzaga
RS-165
R. Dr. Júlio de Castilho
285
Centro Dq
De Caxias
Cemitério

FIGURE 2:

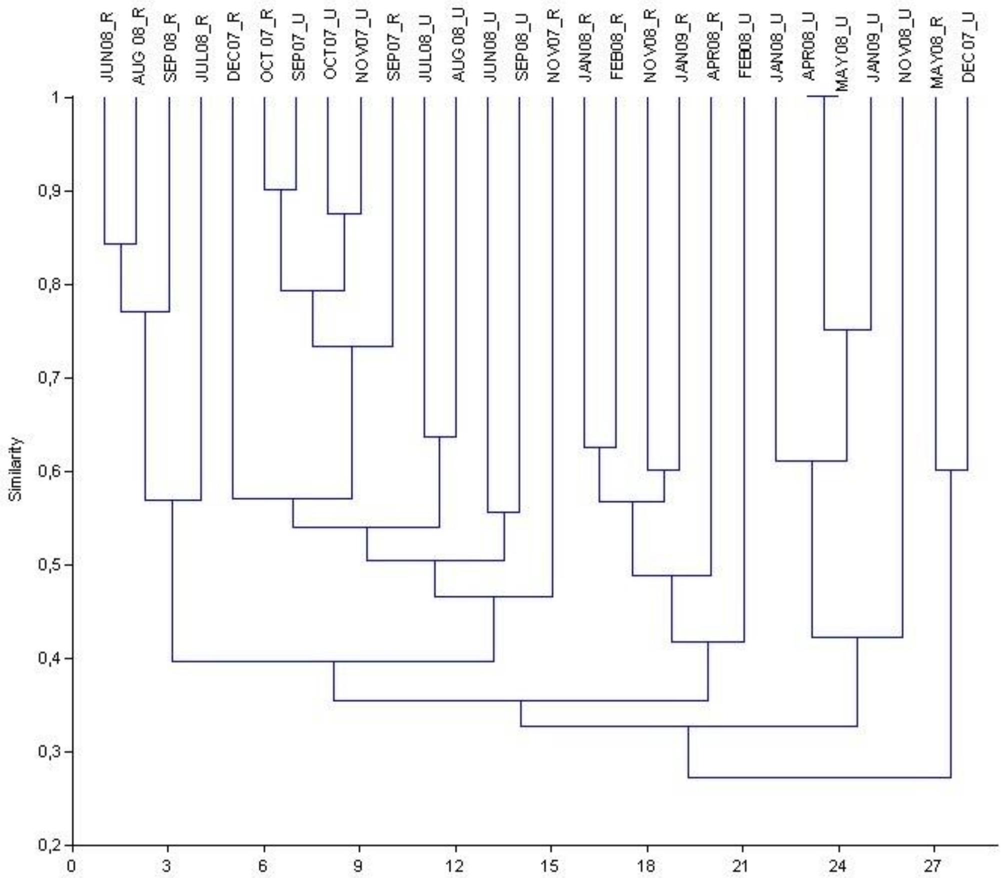
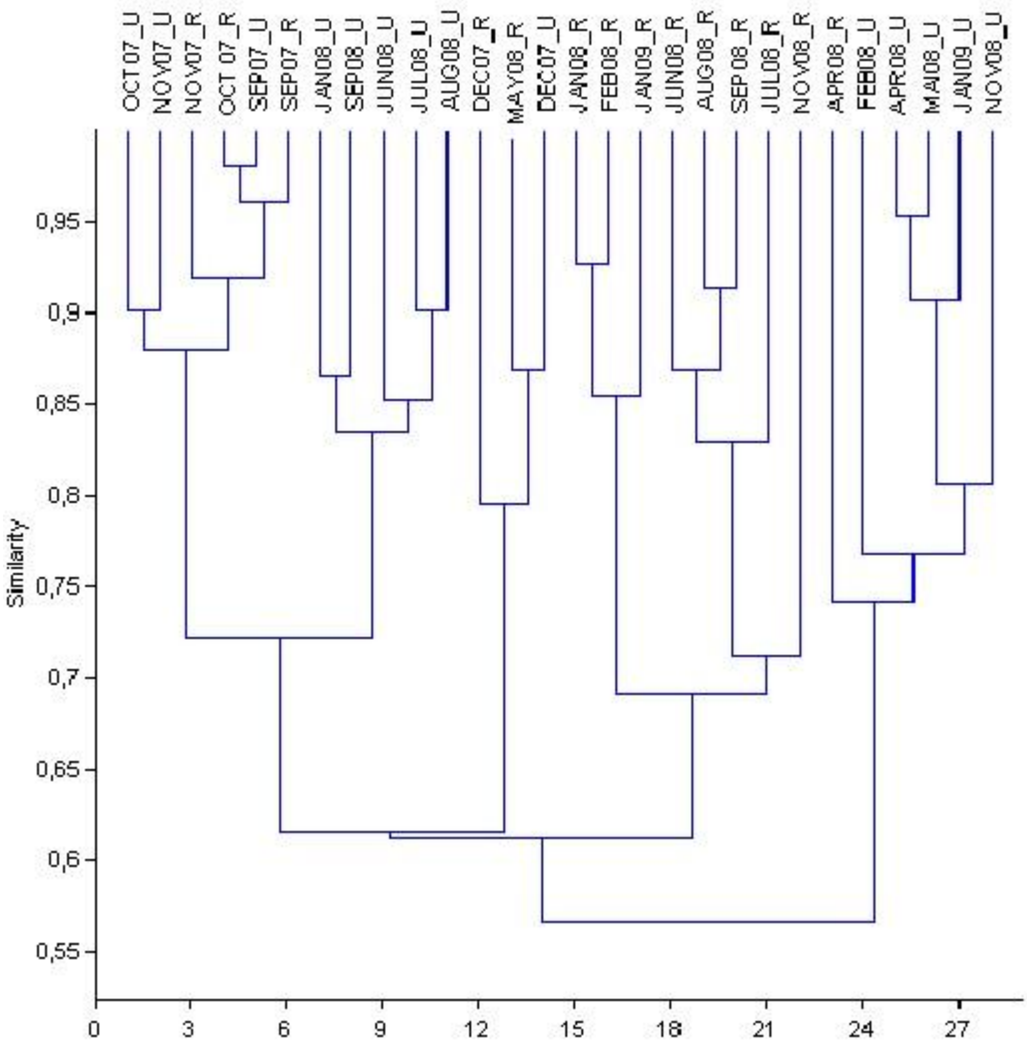

JUN08_R
AUG 08_R
SEP08_R
JUL08_R
DEC07_R
OCT 07_R
SEP07_U
OCT07_U
NOV07_U
SEP07_R
JUL08_U
AUG08_U
JUN08_U
SEP08_U
NOV07_R
JAN08_R
FEB08_R
NOV08_R
JAN09_R
APR08_R
FEB08_U
JAN08_U
APR08_U
MAY08_U
JAN09_U
NOV08_U
MAY08_R
DEC07_U
Similarity
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0
3
6
9
12
15
18
21
24
27

FIGURE 3:


OCT07_U
NOV07_U
NOV07_R
OCT07_R
SEP07_U
SEP07_R
JAN08_U
SEP08_U
JUN08_U
JUL08_U
AUG08_U
DEC07_R
MAY08_R
DEC07_U
JAN08_R
FEB08_R
JAN09_R
JUN08_R
AUG08_R
SEP08_R
JUL08_R
NOV08_R
APR08_R
FEB08_U
APR08_U
MAI08_U
JAN09_U
NOV08_U
Similarity
0,95
0,9
0,85
0,8
0,75
0,7
0,65
0,6
0,55
0
3
6
9
12
15
18
21
24
27

5.4. Manuscrito aceito para publicação no periódico Neotropical Entomology

Ecology, Behavior and Bionomics

Title: Population Dynamics of Drosophilids in the Pampa Biome in Response to Temperature.

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Running Title: Fluctuations of Drosophilidae in the Pampa Biome.

Abstract: Environmental variables such as temperature and rainfall can directly affect the community structure of dipterans; seasonal oscillations in the abundance of species of Drosophilidae reflect differences in how tolerant populations are to climatic conditions. Over a period of fourteen months, we collected samples in two habitat types in the Pampa biome in the municipality of São Luiz Gonzaga, RS, Brazil (28°24'28"S, 54°57'39"W). Through correlation analysis, the influence of environmental variables on Drosophilidae populations was evaluated for both collecting sites. The results suggested a negative correlation between the abundances of *Drosophila cardinoides* Dobzhansky & Pavan, *D. maculifrons* Duda, *D. melanogaster* Meigen, *D. nigricruria* Patterson & Mainland and *Zygothrica vittimaculosa* Burla with temperature, which is reflected in the distributions of those species within Brazil. Our findings are important for characterizing and preserving biodiversity in this almost-unknown biome in southern Brazil given the current climate change scenario.

Key-words: Drosophilidae; Pampa; seasonal oscillations; biodiversity.

Introduction

Environmental variables such as temperature, humidity and rainfall can directly affect the community structure of dipterans, including Drosophilidae (Patterson 1943, Dobzhansky & Epling 1944, Dobzhansky & Pavan 1950, Pipkin 1952, Pavan 1959, Valiati *et al* 2005). Thus, oscillations in the abundance of a species among seasons reflect differences in the tolerance of populations from a specific site to climatic conditions (Da Cunha & Magalhães 1965, Bélo & Filho 1976, Begon *et al* 1996, Sorte *et al* 2011).

Community assembly and disassembly processes may be largely cyclical (Toshihide *et al* 2005), and temperature may thus be an ecological variable of great significance (Wolda 1988). Alterations in seasonal cycles are expected to become even more common over the course of time, which could be attributed to global warming (Rodriguez-Trelles & Rodriguez 2009), and these alterations are easily observed in insects' behavior (Stork & Eggleton 1992, Marinoni *et al* 2006, Costa *et al* 2008, Medeiros *et al* 2012).

Studies analyzing the relationships between species and environmental variables become even more relevant when variations in the composition of the arthropod fauna occur as a consequence of climatic variations (Hoffmann & Harshman 1999, Balanyá *et al* 2009, Brisson *et al* 2006, Van Heerwaarden & Hoffmann 2007, Rodriguez-Trelles & Rodriguez 2009, Zivanovic & Mestres 2011, Silva *et al* 2011).

Knowing the thermal tolerance of a species can explain much about its origin and capacity for dispersal in the environment; many authors have highlighted the tolerance for higher temperatures in tropical species and the capacity of temperate species to survive higher variations in temperature, which makes temperate species more able to migrate to other environments (Janzen 1967, Cohet *et al* 1980, Goto & Kimura 1998, Rodriguez-Trelles & Rodriguez 2009, Sorte *et al* 2011, Silva *et al* 2011). Tolerance of high temperatures is fundamental in urban environments, which are known to be hotter than less developed and non-urbanized areas; this difference is reflected in the composition of the Drosophilidae fauna (Costa *et al* 2003, Gottschalk *et al* 2007). Additionally, populations in which individuals have a low temperature tolerance and low acclimatization capacity are more prone to extinction (Deutsch *et al* 2008).

In this context, the Pampa biome appears as a biome where the dynamics of biodiversity are poorly known and the seasonal patterns of structural variation in the insect assemblages, in this case Drosophilidae, are unexplored.

This biome, which is shared between subtropical and temperate regions in Brazil, Uruguay and Argentina (Bilenca & Miñarro 2004), is predominantly composed of grasslands, although these are sometimes naturally invaded by deciduous seasonal forest and ombrophilous dense forest in the northern and eastern parts of the State of Rio Grande do Sul, Brazil (IBGE 2004, Boldrini *et al* 2010). The biome is characterized by cool temperatures during the winter, approximately 10°C, and temperatures of approximately 38°C in the hot and dry summer, and it is considered an environment of extreme priority for conservation activities (MMA 2000).

Through the relationships between climatic variables and the abundance of drosophilids collected in two contrasting environments in the Pampa biome, the present study aims to contribute to a better understanding of the fluctuations and the structure of Drosophilidae assemblages that occur in the Pampa. Our hypothesis is based on the prediction that populations of exotic and native species behave differently with respect to oscillations in temperature and humidity.

Material and Methods

Study area

Samples of Drosophilidae were collected in two areas in the municipality of São Luiz Gonzaga (28°24'28"S, 54°57'39"W), northwest of the state of Rio Grande do Sul in southern Brazil (Fig 1) at an altitude of 231 m. This region has a subtropical climate characterized by rainy weather and well-defined seasons with negative temperatures in the humid winter and temperatures of approximately 38°C in the hot and dry summer. According to the Köppen classification system (Köppen 1931), it is a Cfa region. The region as a whole has been heavily degraded and consists of medium- and small-sized cities in a predominantly agricultural landscape. The natural grasslands are highly disturbed, and the forested areas have been reduced to just small patches of secondary forest. The region is located near the northern border of the Pampa Biome as defined by IBGE (2004).

Two contrasting localities were surveyed: an urban area (28°24’39’’S, 54°57’371”W) situated downtown in the main urban and commercial center of the municipality and a rural area located approximately 10 km from the downtown collection point (28°22’51.2”S, 55°00’8.62’’W). The rural area was a small native fragment of deciduous seasonal forest inside a region that was originally dominated by steppe savanna but has largely been replaced with agriculture. According to Powell (1997), the magnitude of dispersal among drosophilid species is highly variable, but the availability of feeding and breeding sites is determinant of the traveled distance, as some species are specialists and restricted to specific types of resources, and if necessary, they can travel for long distances to find those resources (Markow & Castrezana 2000). However, we exclusively used banana-baited traps as the collecting resource to minimize the influence of the use of different resources in our samples. Furthermore, between our sampled areas, there is a large availability of microhabitats, and it might be enough to avoid that species from an area migrate for long distances such as 10 km influenced by the availability of resources.

Collection and identification

In both areas, banana-baited traps (Tidon & Sene 1988) were used to capture adult drosophilids. For each sample, one kilogram of banana was mashed, sprinkled with baker's yeast and distributed among five traps. The traps were hung in trees approximately 1.5 m above the ground, where they were kept for five days. Samples were collected monthly from September 2007 to September 2008 and in November 2008 and January 2009.

During the period that the collections were made, environmental data including maximum temperature, minimum temperature, humidity and rainfall were recorded by the Meteorological Center of the Municipality of São Luiz Gonzaga. To estimate the differences between the temperatures in the urban and rural zones, simultaneous measurements were obtained at both of the sites. In the hottest periods, we registered temperatures that were up to 3ºC lower at the rural site compared to the urban zone, although the average difference was 2ºC.

The flies were preserved in 70% ethanol until identification. Identifications were made based on external morphology and male terminalia and by consulting specialized literature. The analysis of male terminalia was conducted according to Bächli *et al* (2004).

Some individuals belonging to the *Drosophila repleta*, *tripunctata* and *guarani* species groups that were unidentified at the species level were not scored for the statistical analysis of species abundance and diversity measures (~8% of the total sample). However, they were considered in the total number of individuals (*N*) and the number of individuals of Neotropical species (N_{nat}).

Voucher specimens of the collected material were deposited at Laboratory of Drosophila of the Universidade Federal do Rio Grande do Sul (UFRGS) in Porto Alegre, Rio Grande do Sul, Brazil.

Data analysis

The relationships between species abundance and the environmental variables—maximum and minimum temperatures, average maximum and minimum temperatures, average rainfall and average humidity during the collection week—were analyzed by means of Spearman's correlation coefficient (r_s) at a significance level of 5% (Zar 1999). This test is based on ranks and not the measures of the parameters of abundance, correcting for the zero-inflation of the dataset. Even so, we also employed a t-test to compare the average values of the environmental variables with the months in which the *Drosophila* species were present or absent. When the presuppositions were not satisfied (humidity and rainfall), the Wilcoxon-Mann-Whitney test was used.

To analyze the frequency of collection for each species during the sampled period, the Occurrence Constancy index of Dajoz (1983) was obtained by dividing the number of collections in which a given species occurred by the total number of collections and then multiplying that result by 100. Species with an index of $c \geq 50$ were considered constants, accessory species were those where $25 \leq c < 50$, and accidental species were those for which $c < 25$.

Results and discussion

During the 14 months of sampling, the temperature ranged from 2°C to 36°C with the lowest values in the period from May to August (late autumn and winter) and the highest temperatures during the period from October to February (late spring and summer). The humidity values were highest in periods of low temperatures, being approximately 76% in the period from May to July and approximately 44% in December and January. Although dry periods were common during the summer, periods of rain still occurred, so there was not a well-defined dry season as occurs in the Brazilian Cerrado, which is a similar environment to the Pampa but classified as Aw, tropical with a dry winter, according to the Köppen classification system (Tidon 2006). Under these conditions, we captured 13,379 drosophilids that represented four genera; 8,812 were collected in the rural site belonging to the genera *Drosophila*, *Leucophenga*, *Zaprionus* and *Zygothrica*, and 4,567 were collected in the urban site representing the genera *Drosophila* and *Zaprionus* (tables of abundance are available in Poppe *et al* 2012). The rural area always had a higher number of species, with 25 and 16 species collected from the rural and urban sites, respectively. Martins (1987), working in the Brazilian Amazon forest, stressed that some species of drosophilids are faithful to their habitat; this is noticeable primarily in tropical regions (Cohet *et al* 1980). In this context, the tolerance levels of these species to environmental variables determine their distribution and habitat fidelity (Janzen 1967).

Eleven of the species collected in the rural area (*Drosophila buzzatii* Patterson & Wheeler, *D. hydei* Sturtevant, *D. immigrans* Sturtevant, *D. melanogaster* Meigen, *D. mercatorum* Patterson & Wheeler, *D. nigricruria* Patterson & Mainland, *D. pallidipennis* Dobzhansky & Pavan, *D. polymorpha* Dobzanhsky & Pavan, *D. simulans* Sturtevant, *D. willistoni* Sturtevant and *Zaprionus indianus* Gupta) were constant (C≥50), six (*D. busckii* Coquillet, *D. cardinoides* Dobzanhsky & Pavan, *D. maculifrons* Duda, *D. mediopunctata*

Dobzhansky & Pavan, *D. onca* Dobzanhsky & Pavan and *Zygothrica vittimaculosa* Burla) were accessory (25≤C<50) and eight (*D. aldrichi* Patterson, *D. antonietae* Tidon-Sklorz & Sene, *D. bandeirantorum* Dobzhansky & Pavan, *D. griseolineata* Duda, *D. paraguayensis* Duda, *D. virilis* Sturtevant, *Leucophenga maculosa* Coquillett and *D*. sp.Q2) were accidental (C<25). In the urban area, only five species were constant (*D. mercatorum*, *D. melanogaster*, *D. polymorpha*, *D. simulans* and *Zaprionus indianus*), four were accessory (*D. busckii*, *D. buzzatii*, *D. hydei* and *D. immigrans*) and seven were accidental (*D. bandeirantorum*, *D. cardinoides*, *D. maculifrons*, *D. nigricruria*, *D. pallidipennis*, *D. repleta* Wollaston and *D. willistoni*).

As can be observed above, most of the exotic species in our samples were constant at both the rural and urban sites except for *D. busckii*, which was an accessory species at both the rural and urban sites, and *D. virilis*, which was present only at the rural site, being a rare species. However, only *D. melanogaster* among the exotic species presented a significant correlation with the environmental variables, and among the native species, only *D. cardinoides*, *D. maculifrons*, *D. nigricruria* and *Z. vittimaculosa* were significantly correlated with the analyzed climatic variables.

Therefore, only five of the 26 collected species exhibited some relationship with the measured environmental variables (Table 1). The abundance of *D. cardinoides* had a negative correlation with the average maximum and minimum temperatures (approximately 27 and 15°C, respectively) and the minimum temperature in the rural area (approximately 12°C), reinforcing its preference for colder periods (Fig 2a); in other words, the abundance of this species is increased in periods where the temperature decreases, and it is very reduced in periods of heat (Table 2). Although the abundance of this species increased in July 2008, when there was an elevation in the temperature, that event was not enough to affect the negative correlation between this species and low temperatures, as that increase in temperature was not sufficient to characterize a period of heat. The abundance of *D. maculifrons* exhibited a negative correlation with the average maximum temperature (approximately 27°C), thus also demonstrating a preference for colder weather. As can be seen in Figure 2b, the abundance of this species was higher during the colder months when the average of temperature was near 20°C, avoiding warmer periods (Table 2). The abundance of *D. nigricruria* was also negatively correlated with the maximum and minimum temperatures (approximately 29°C and 12°C, respectively) and with the average minimum and maximum temperatures (approximately 15 and 27°C, respectively) (Fig 2c), thus also presenting a good affinity to lower temperatures (Table 2).

The preferences of Neotropical species, primarily *D. maculifrons*, *D. nigricruria* and *D. cardinoides,* for cool regions can be demonstrated by their patterns of abundance and distribution through a comparison of the many inventory studies of Drosophilidae. Dobzhansky & Pavan (1950) found a dominance by *D. maculifrons* of between 40 and 52% in flowers of the genus *Bombax* in April 1949 in São Paulo, southeast of Brazil. The same authors also found this species to be common in their collections in the southern state of Paraná; however, in the same period in collections in Belém do Pará north of Brazil, *D. maculifrons* did not appear. This lack was also noted by Martins (2001) for that region. The absence of this species at low latitudes could confirm the indication from our correlations that *D. maculifrons* prefers low temperatures (Table 1), which is a common thermal characteristic of high latitudes. Mateus *et al* (2006), also in the states of São Paulo and Paraná, found *D. maculifrons* to be a constant species. The presence of *D. maculifrons* in Mato Grosso, Central Brazil was noted by Dobzhansky & Pavan (1950) and Pavan (1959), but more recently, this species was not found by Blauth & Gottschalk (2007), who just observed *D. cardinoides* as a common species in that region.

In the central region of Brazil, which is relatively warmer compared to the southern region, Ferreira & Tidon (2005), Tidon *et al* (2005), Tidon (2006) and Mata *et al* (2008) have also found *D. cardinoides*, *D. maculifrons* and *D. nigricruria* but always in low abundance.

In the state of Rio Grande do Sul in southernmost Brazil, Hochmüller *et al* (2010) recorded *D. nigricruria* and a high abundance of *D. maculifrons* in a period of low temperatures. Also in the southernmost region of Brazil, Araújo & Valente (1981), Saavedra *et al* (1995a, 1995b), Silva *et al* (2005), and Garcia *et al* (2012) noted *D. cardinoides* and *D. maculifrons* at relatively higher abundances, primarily in periods of lower temperatures.

Zygothrica vittimaculosa was negatively correlated with the highest temperatures (approximately 29°C), being more abundant in cold periods (Tables 1 and 2) from June through August (Fig 2d). Döge *et al* (2007) in Santa Catarina State also found a high number of species of *Zygothrica,* primarily in the cold months.

Among the exotic species, *Drosophila melanogaster* was negatively correlated with the lowest temperatures in both areas (approximately 12°C) and negatively correlated with the maximum temperatures in the urban area (approximately 29°C), being more abundant in periods of cold weather (June to September), as illustrated in Figures 2e (rural area) and 2f (urban area). David & Clavel (1969), McKenzie (1975) and Parsons (1978) stressed that *D. melanogaster* exhibits its highest breeding activity at temperatures between 12 and 20°C. Delpuech *et al* (1995), analyzing the variation in the ovarian size of different populations of *D. melanogaster*, noticed that individuals from temperate climates laid more eggs than did tropical individuals; aware of the tropical origin of *D. melanogaster*, the authors stressed that altitudinal variation and, consequently, temperature variation were responsible for this pattern.

Benado & Brncic (1994) and Poppe *et al* (2012) showed that even in comparisons between contrasting environments, the seasonal component is capable of explaining more of the diversity index than geographic location. Silva *et al* (2011) observed the seasonal influence over insect populations in the Cerrado, where extreme climatic conditions were able to decrease the abundance of Hymenoptera, Coleoptera, Diptera, Lepidoptera, Isoptera, Hemiptera and Trichoptera during the dry season, with these taxa becoming abundant again only in the subsequent wet period, when there was a high availability of flowers for use as feeding and breeding sites. Kimura *et al* (1977) reported that some species of drosophilids exhibit seasonal behavior due to the seasonality of the resources they utilize. Bizzo *et al* (2010) and Schmitz *et al* (2010) also stressed the hypothesis that the availability of resources for feeding and breeding are influenced by climatic conditions and are a determining factor for the distribution pattern of drosophilids in the mangroves and restingas of Santa Catarina, Brazil. Furthermore, Silva *et al* (2005) explained that the community diversity of drosophilids in the city parks of Porto Alegre, which is also in the southernmost Brazilian State, had a seasonal component. Thus, more specialized species would be more dependent on environmental conditions.

Other collected species such as *D. hydei*, *D. mercatorum*, *D. polymorpha*, and *D. willistoni* of Neotropical origin and *D. simulans* and *Z. indianus* of Afrotropical origin, although they were abundant and constant, did not exhibit correlations with the analyzed environmental variables; consequently, these species are little affected by the climatic variables analyzed here, and thus, they can also be easily found in the central and northern regions of Brazil, a tropical portion of the Neotropical region.

According to Brncic & Budnik (1987), species of drosophilids (and other animals) that are generalists are also good candidates for invading new territories, which are frequently far from their places of origin. Despite this characteristic of generalists and the invasive capacity of exotic species, the proportion of exotic species influenced by the environmental variables (17%) was similar to that of the native species (20%). Martins (2001), in an inventory of

Drosophilidae in the Amazon forest in northern Brazil, also noticed the influence of climatic variables in the behavior of an invasive species, *D. malerkotliana* Parshad & Paika, that was invasive due to its short life cycle and use of resources (fruits) at an initial decomposition level, which was regulated by variations in temperature and humidity. Many other researchers have found that the behavior of many drosophilids is influenced by environmental variables and shows a pronounced seasonality (Dobzhansky 1950, Benado & Brncic 1994, Tidon *et al* 2003, De Toni *et al* 2007, Bizzo *et al* 2010, Schmitz *et al* 2010, Garcia *et al* 2012).

Seasonality can explain a large part of the diversity of Drosophilidae and other insect families such as Culicidae (Costa *et al* 2008), Chrysomelidae (Linzmeier & Ribeiro-Costa 2008), Syrphidae (Souza-Silva *et al* 2001, Marinoni *et al* 2006), Formicidae (Medeiros *et al* 2012), Pentatomidae (Bortolotto *et al* 2012), Calliphoridae (Gião & Godoy 2006), Apidae (Dos Santos *et al* 2009), and Cicadellidae (Ott & Carvalho 2001); however, there are other elements that were not analyzed in this work, such as an urbanization gradient (Gottschalk *et al* 2007), vertical distribution (Kratz *et al* 1982), inter and intra-specific interactions between species and resource availability (Silva *et al* 2011, Medeiros *et al* 2012), all of which may also contribute to the fluctuation of this index.

Forest areas have the potential to act as microclimatic refuges to fauna in stressful situations, especially in highly degraded areas, as urbanized and open areas are normally hotter and drier than forest. Lucchese *et al* (2003) noted this function of parks in big cities, in this case the city of Porto Alegre in the south of Brazil. Thus, the conservation of forest patches in the Pampa biome is of high importance, as such patches contribute to the maintenance of species that are more sensitive to arid conditions. Greater aridity may be one reason for the reduced richness in the urban area compared to the rural area.

Species that are not able to migrate to other environments to avoid climatic stress are likely to suffer the most consequences from global warming. Therefore, knowledge of the behavior of species in relation to biotic and abiotic conditions in the field is fundamental for determining bioindicator species, which are very important in the current context of global warming and the devastation of natural areas.

In summary, the expectation that Drosophilidae populations in the Pampa biome fluctuate in response to the analyzed environmental variables was confirmed, but exotic and native species appear to be similarly affected. This study also detected some species that are negatively affected by higher temperatures. These species have the potential to be relatively more affected by climatic changes than others, possibly serving as bioindicators of global warming.

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Table Legends

Table 1: Spearman's correlation (r_s) between environmental variables (humidity, maximum temperature (max. temp.), minimum temperature (min. temp.), average maximum temperature, average minimum temperature and average rainfall) and the species of Drosophilidae that were represented by at least 20 collected individuals during the sampled period in the rural area (RA) and the urban area (UA) in São Luiz Gonzaga. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

species	N/N°col.		Humidity		Max. Temp.		Min. Temp.		Av. Max. Temp.		Av. Min. Temp.		Rainfall	
	RA	UA	RA	UA	RA	UA	RA	UA	RA	UA	RA	UA	RA	UA
Drosophila busckii	677/5	236/5	-0.048	0.12	-0.061	-0.103	0.082	0.042	0.017	-0.041	0.243	0.112	0.144	0.227
Drosophila buzzatii	26/8	89/6	-0.14	-0.18	-0.315	0.279	-0.278	0.297	-0.274	0.37	-0.168	0.383	0.218	0.135
Drosophila cardinoides	27/4	-	0.231	-	-0.472	-	-0.577*	-	-0.575*	-	-0.547*	-	0.155	-
Drosophila hydei	30/7	117/6	-0.313	0.153	-0.126	-0.086	-0.118	0.09	-0.073	-0.063	0.009	0.109	0.175	0.26
Drosophila immigrans	124/9	44/6	-0.353	0.004	-0.076	-0.113	-0.25	-0.357	-0.11	-0.253	-0.123	-0.299	-0.103	0.01
Drosophila maculifrons	22/5	-	0.271	-	-0.454	-	-0.485	-	-0.551*	-	-0.477	-	0.353	-
Drosophila melanogaster	140/7	137/10	-0.232	0.453	-0.48	-0.577*	-0.704**	-0.614*	-0.476	-0.623*	-0.556*	-0.531*	-0.127	0.375
Drosophila mercatorum	620/12	200/12	-0.209	0.19	-0.034	-0.345	-0.061	-0.264	-0.028	-0.32	0.061	-0.207	0.07	0.264
Drosophila nigricruria	50/11	-	0.189	-	-0.693**	-	-0.790***	-	-0.759**	-	-0.712**	-	0.135	-
Drosophila pallidipennis	55/7	-	-0.335	-	0.238	-	-0.2	-	0.068	-	-0.176	-	-0.278	-
Drosophila polymorpha	1054/14	32/10	0.063	-0.101	-0.055	-0.044	-0.265	-0.172	-0.165	-0.01	-0.167	-0.083	0.011	0.007
Drosophila simulans	4226/14	3089/14	0.132	0.279	-0.288	-0.248	-0.288	-0.301	-0.345	-0.264	-0.165	-0.173	0.209	0.185
Drosophila willistoni	821/9	-	0.344	-	0.064	-	0.009	-	-0.011	-	0.018	-	0.012	-
Leucophenga maculosa	33/1	-	0.172	-	-0.414	-	-0.449	-	-0.447	-	-0.447	-	0.33	-
Zaprionus indianus	92/7	258/9	0.252	0.335	-0.336	-0.365	-0.376	-0.064	-0.426	-0.301	-0.398	-0.216	-0.073	0.12
Zygothrica vittimaculosa	39/4	-	0.388	-	-0.523*	-	-0.371	-	-0.55	-	-0.407	-	0.349	-

Table 2: T-test comparison of the average values of the measured environmental variables (maximum and minimum temperatures, max. temp. and min. temp., respectively) ± standard error (SD) with the months in which the *Drosophila* species were present or absent in the rural area (RA) and in the urban area (UA). The Wilcoxon-Mann-Whitney test was applied to humidity and rainfall. Only the drosophilids for which Spearman's correlation coefficient was significant were included. *: p value to Wilcoxon-Mann-Whitney test

Species/Area	Humidity			Max. Temp.			Min. Temp.			Rainfall		
	p value*	Av. ± SD present	Av. ± SD absent	p value	Av. ± SD present	Av. ± SD absent	p value	Av. ± SD present	Av. ± SD absent	p value*	Av. ± SD present	Av. ± SD absent
Drosophila cardinoides/Rural	0.635	75.77± 12.95	70.03 ± 12.97	0.1	23 ± 4.7	28.9 ± 5.85	0.016	6.75 ± 4.6	14.10 ± 4.5	0.713	3.85 ± 4.8	6.5 ± 10.6
Drosophila maculifrons/Rural	0.364	77.92 ± 12.2	68.2 ± 12.31	0.206	24.4 ± 5.12	28.8 ± 6.2	0.198	9.40 ± 7.13	13.5 ± 4.12	0.203	9.5 ± 13.3	3.6 ± 6.0
Drosophila melanogaster/Rural	0.383	68.00 ± 9.4	75.5 ± 15.3	0.328	25.6 ± 6.10	28.9 ± 5.95	0.06	9.3 ± 5.4	14.7 ± 4.30	0.417	2.8 ± 4.40	8.6 ± 12.00
Drosophila nigricruria/Rural	0.368	72.8 ± 12.22	67.6 ± 16.7	0.013	25.65 ± 5.8	33.00 ± 2.7	0.041	10.8 ± 5.4	16.7 ± 2.9	1	4.4 ± 5.6	10.7 ± 18.5
Zygothrica vittimaculosa/Rural	0.24	78.25 ± 9.5	69.04 ± 13.3	0.05	22.5 ±3.9	29.1 ± 5.9	0.17	8.75 ± 5.8	13.3 ± 5.1	0.216	5.7 ± 4.2	5.7 ± 10.8
Drosophila melanogaster/Urban	0.24	62.8 ± 12.6	55.05 ± 12.75	0.013	27.1 ± 5.8	33.75 ± 2.65	0.08	10.7 ± 5.52	16.5 ± 3.9	0.35	7.10 ± 10.4	2.15 ± 4.3

Figure legends

Figure 1: Map of the State of Rio Grande do Sul showing the municipality of São Luiz Gonzaga and the sampling areas: the urban area (UA) and rural area (RA). Source: http://maps.google.com.br/ maps?hl=pt-BR&tab=wl&q=sao%20luiz%20gonzaga.

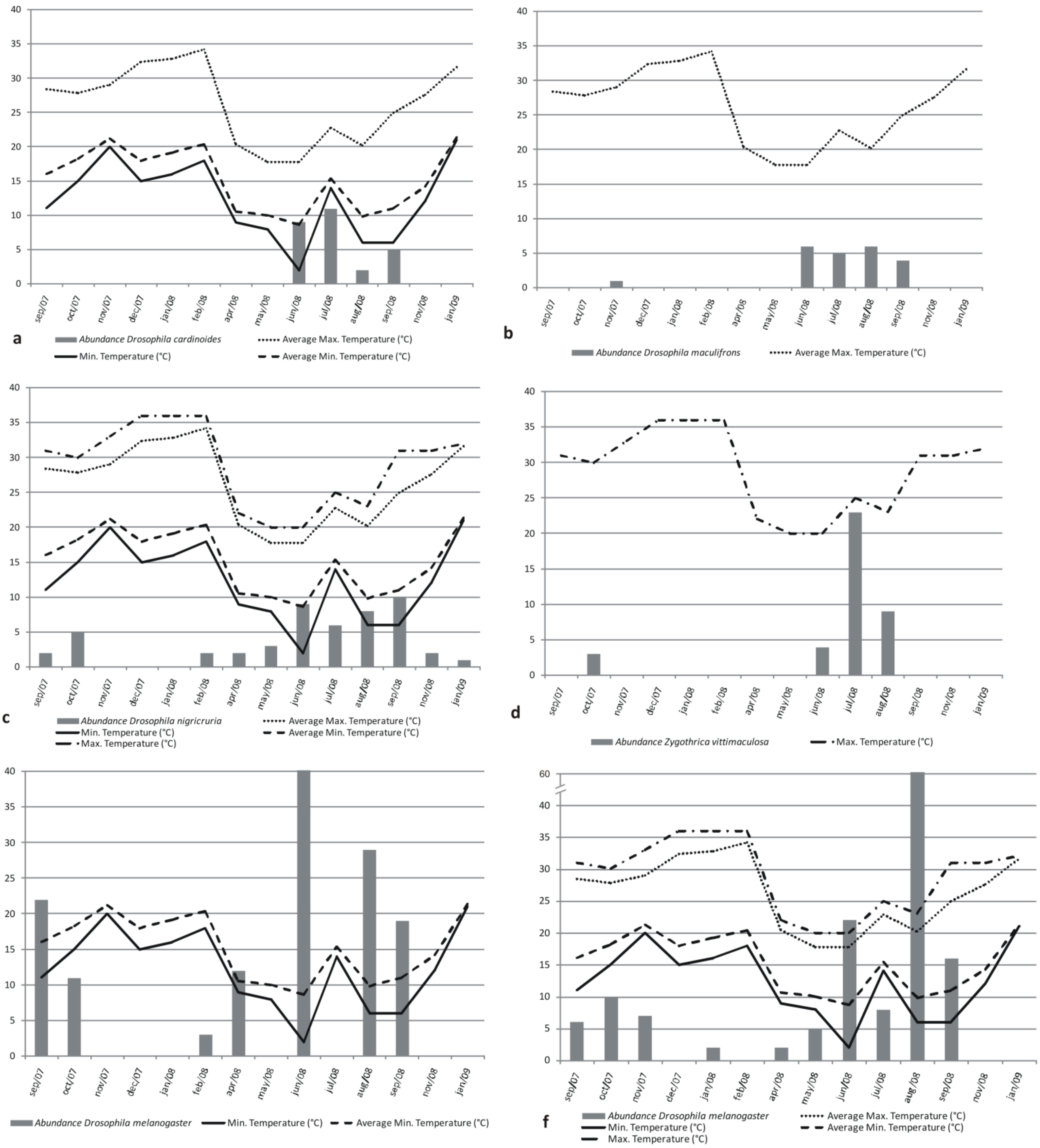
Figure 2: Influence of environmental variables (maximum temperature (Max. Temperature), minimum temperature (Min. Temperature), average maximum and minimum temperatures (Average Max. Temperature and Average Min. Temperature, respectively) on *Drosophila cardinoides* (a), *D. maculifrons* (b), *D. nigricruria* (c), *Zygothrica vittimaculosa* (d), *D. melanogaster* (rural area) (e), and *D. melanogaster* (urban area) (f).

All of the figures were prepared using the software program Corel Draw 12.

Figure 1


Atlantic rainforest biome
Pampa biome
Fonte: Mapa de Biomas do Brasil - IBGE, 2004
Rio de Janeiro - esc. 1:5.000.000
N
RA
UA
1992 m
Image © 2012 DigitalGlobe
Image © 2012 GeoEye
Google earth

Figure 2


a
Abundance Drosophila cardinoides
Average Max. Temperature (°C)
Min. Temperature (°C)
Average Min. Temperature (°C)
b
Abundance Drosophila maculifrons
Average Max. Temperature (°C)
c
Abundance Drosophila nigricruria
Average Max. Temperature (°C)
Min. Temperature (°C)
Average Min. Temperature (°C)
Max. Temperature (°C)
d
Abundance Zygothrica vittimaculosa
Max. Temperature (°C)
e
Abundance Drosophila melanogaster
Min. Temperature (°C)
Average Min. Temperature (°C)
f
Abundance Drosophila melanogaster
Average Max. Temperature (°C)
Min. Temperature (°C)
Average Min. Temperature (°C)
Max. Temperature (°C)
sep/07
oct/07
nov/07
dec/07
jan/08
feb/08
apr/08
may/08
jun/08
jul/08
aug/08
sep/08
nov/08
jan/09