



The diversity of Drosophilidae in the South American pampas: update of the species records in an environment historically neglected.

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Introduction

In the last decades many faunal surveys of Drosophilidae have been done in different Neotropical environments in Brazil, such as Atlantic rainforest (De Toni *et al.*, 2007), Cerrado (Mata *et al.*, 2008), mangrove swamps (Schmitz *et al.*, 2007), Caatinga (Tidon-Sklorz and Sene, 1995), Pantanal (Val and Marques, 1996), Amazonian rainforest (Martins, 1987), Araucarian forest (Saavedra *et al.*, 1995; Cavasini *et al.*, 2014), Restinga (Bizzo *et al.*, 2010). However, the Pampas biome, which is the southernmost environment, was neglected by the researchers mainly for being an open grassland environment and apparently lacking breeding and feeding resources to drosophilids.

The pampas covers southernmost Brazil, all of Uruguay, and the central region of eastern Argentina. It is a heterogeneous landscape, with a matrix of natural grasses and small patches of forest. The seasons are well defined, and the annual temperature range is extremely pronounced, ranging from negative values in the winter up to 40°C in the summer. This temperature range has been pointed as determinant to the presence and maintenance of Drosophilidae species in the region (Poppe *et al.*, 2013, 2015).

Only recently Poppe *et al.* (2014) highlighted the high diversity of drosophilids in this environment. The same is noticed in Uruguay (Goñi *et al.*, 1998, 2002, 2012), while in Argentina most of information comes from the studies focused predominantly on genetics and/or autecology (Wheeler and Magalhães, 1962; Hale and Singh, 1991). Thus, it is evident the poor knowledge of the Drosophilidae fauna in the South grasslands of South America.

Poppe *et al.* (2014) mentioned the record of 95 species in the grasslands of Brazil, Uruguay, and Argentina. After that, some studies have been performed in pampas pointing the record of more species, including the new invasion of *D. suzukii*. Thus, the present report is an update of the list of recorded Drosophilidae species in the South American pampas.

Material and Methods

A comprehensive literature search of species recorded in the pampas of Brazil, Uruguay, and Argentina was performed, including not only taxonomic studies, but also genetic, evolutionary, and ecological ones. Some species records are from unpublished samples performed by us in the Brazilian pampas (28°45'01"S 54°56'55"W; 30°20'44"S 54°19'32"W). These data updated the species list presented by Poppe *et al.* (2014) to that biome.

Results and Discussion

Thirteen species were included in the pampas species list proposed by Poppe *et al.* (2014): *Drosophila senei* Vilela, *D. suzukii* Matsumura, *D. trapeza* Heed and Wheeler, *Hirtodrosophila levigata* (Burla), *H. mendeli* (Mourão, Gallo and Bicudo), *H. morgani* (Mourão, Gallo and Bicudo), *Mycodrosophila projectans* (Sturtevant), *Paraliodrosophila antennata* (Wheeler), *Rhinoleucophenga joaquina* Schmitz,

Gottschalk and Valente, *R. punctuloides* Poppe, Schmitz and Valente, *Zygothrica poeyi* (Sturtevant), *Z. prodispar* Duda, and *Z. dispar* Wiedemann (Table 1). Except for the first two species, the pampas represents the southernmost record region to the other ones.

Table 1. List of Drosophilidae flies recorded in the Pampas. *: new species added in the list presented by Poppe *et al.* (2014). New record localities, 1: 30°20'44"S 54°19'32"W; 2: 29°11'09"S 54°53'50"W (Robe *et al.*, 2014); 3: 31°48'58"S 52°25'55"W (Robe *et al.*, 2014); 4: 32°32'25"S 52°32'34"W (Robe *et al.*, 2014); 5: 28°45'01"S 54°56'55"W.

Genus	Group	Species
<i>Cladochaeta</i>	<i>bomplandi</i>	<i>C. bomplandi</i> (Malloch)
<i>Drosophila</i>	<i>annulimana</i>	<i>D. annulimana</i> Duda
		<i>D. arassari</i> da Cunha & Frota-Pessoa
		<i>D. schineri</i> Pereira & Vilela
	<i>bromeliae</i>	<i>D. bromelioides</i> Pavan & da Cunha
	<i>busckii</i>	¹ <i>D. busckii</i> Coquillett
	<i>calloptera</i>	<i>D. quadrum</i> (Wiedemann)
	<i>canalineae</i>	<i>D. piratininga</i> Ratcov & Vilela
	<i>cardini</i>	<i>D. cardini</i> Sturtevant
		<i>D. cardinoides</i> Dobzhansky & Pavan
		<i>D. neocardini</i> Streisinger
		¹ <i>D. polymorpha</i> Dobzhansky & Pavan
	<i>coffeata</i>	<i>D. fuscolineata</i> Duda
		<i>D. pagliolii</i> Cordeiro
	<i>dreyfusi</i>	<i>D. briegei</i> Pavan & Breuer
	<i>flavopilosa</i>	<i>D. cestri</i> Brncic
		<i>D. cordeiroi</i> Brncic
		<i>D. flavopilosa</i> Frey
		<i>D. incompta</i> Wheeler & Takada
	<i>guarani</i>	<i>D. alexandrei</i> Cordeiro
		<i>D. griseolineata</i> Duda
		¹ <i>D. maculifrons</i> Duda
		<i>D. ornatifrons</i> Duda
	<i>immigrans</i>	<i>D. immigrans</i> Sturtevant
	<i>melanogaster</i>	<i>D. ananassae</i> Doleschall
		<i>D. kikkawai</i> Burla
		<i>D. malerkotiana</i> Parshad & Paika
		<i>D. melanogaster</i> Meigen
		¹ <i>D. simulans</i> Sturtevant
		^{1,5} <i>D. suzukii</i> Matsumura
	<i>mesophragmatica</i>	<i>D. gaucha</i> Jaeger & Salzano
	<i>obscura</i>	<i>D. subobscura</i> Collin in Gordon
	<i>pallidipennis</i>	<i>D. pallidipennis</i> Dobzhansky & Pavan
	<i>repleta</i>	<i>D. aldrichi</i> Patterson
		<i>D. antonietae</i> Tidon-Sklorz & Sene
		<i>D. buzzatii</i> Patterson & Wheeler
		<i>D. hydei</i> Sturtevant

		<i>D. koepferae</i> Fontdevila & Wasserman
		¹ <i>D. mercatorum</i> Patterson & Wheeler
		<i>D. meridionalis</i> Wasserman
		<i>D. nigricruria</i> Patterson & Mainland
		<i>D. onca</i> Dobzhansky & Pavan
		<i>D. paranaensis</i> Barros
		<i>D. repleta</i> Wollaston
		^{5*} <i>D. senei</i> Vilela
		<i>D. serido</i> Vilela & Sene
		<i>D. zottii</i> Vilela
	<i>saltans</i>	<i>D. prosaltans</i> Duda
		<i>D. pulchella</i> Sturtevant
		<i>D. sturtevanti</i> Duda
	<i>sticta</i>	<i>D. sticta</i> Wheeler
	<i>tripunctata</i>	<i>D. angustibucca</i> Pavan
		<i>D. bandeirantorum</i> Dobzhansky & Pavan
		<i>D. cuaso</i> Bächli, Vilela & Ratcov
		<i>D. mediopicta</i> Frota-Pessoa
		¹ <i>D. mediopunctata</i> Dobzhansky & Pavan
		<i>D. mediosignata</i> Dobzhansky & Pavan
		<i>D. mediostriata</i> Duda
		<i>D. mediovittata</i> Frota-Pessoa
		<i>D. nappae</i> Vilela, Valente & Basso-da-Silva
		<i>D. neoguaramunu</i> Frydenberg
		<i>D. paraguayensis</i> Duda
		<i>D. paramediostriata</i> Townsend & Wheeler
		<i>D. roehrae</i> Pipkin & Heed
		^{5*} <i>D. trapeza</i> Heed & Wheeler
		<i>D. trifulum</i> Frota-Pessoa
	<i>virilis</i>	<i>D. virilis</i> Sturtevant
	<i>willistoni</i>	<i>D. bocainensis</i> Pavan & da Cunha
		<i>D. capricorni</i> Dobzhansky & Pavan
		<i>D. fumipennis</i> Duda
		<i>D. nebulosa</i> Sturtevant
		<i>D. parabocainensis</i> Carson
		<i>D. paulistorum</i> Dobzhansky & Pavan
		<i>D. willistoni</i> Sturtevant
	<i>Ungrouped</i>	<i>D. caponei</i> Pavan & da Cunha
		<i>D. denieri</i> Blanchard
		<i>D. flexa</i> Loew
		<i>D. lutzii</i> Sturtevant
		<i>D. serenensis</i> Brncic
<i>Hirtodrosophila</i>	<i>glabrifrons</i>	^{3*} <i>H. levigata</i> (Burla)
	<i>hirticornis</i>	^{2,3*} <i>H. mendeli</i> (Mourão, Gallo and Bicudo)

		³ * <i>H. morgani</i> (Mourão, Gallo and Bicudo)
<i>Leucophenga</i>	Ungrouped	<i>L. maculosa</i> Coquillett
<i>Mycodrosophila</i>	Ungrouped	^{3,4} * <i>M. projectans</i> (Sturtevant)
<i>Paraliodrosophila</i>	Ungrouped	² * <i>P. antennata</i> Wheeler
<i>Rhinoleucophenga</i>	Ungrouped	<i>R. gigantea</i> (Thomson)
		⁵ * <i>R. joaquina</i> Schmitz, Gottschalk & Valente
		<i>R. missionera</i> Poppe <i>et al.</i>
		<i>R. obesa</i> (Loew)
		<i>R. pampeana</i> Poppe <i>et al.</i>
		<i>R. punctulata</i> Duda
		⁵ * <i>R. punctuloides</i> Poppe, Schmitz & Valente
		<i>R. subradiata</i> Duda
		<i>R. sulina</i> Poppe <i>et al.</i>
<i>Scaptomyza</i>	<i>mesoscapto</i> myza	<i>S. striaticeps</i> Wheeler & Takada
		<i>S. nigripalpis</i> Malloch
	<i>parascapto</i> myza	<i>S. pallida</i> (Zetterstedt)
	<i>scapto</i> myza s. str.	<i>S. graminum</i> (Fallén)
	Ungrouped	<i>S. spinipalpis</i> Seguy
<i>Zaprionus</i>	<i>armatus</i>	<i>Z. indianus</i> Gupta
<i>Zygothrica</i>	<i>atriangula</i>	³ * <i>Z. poeyi</i> (Sturtevant)
	<i>bilineata</i>	³ <i>Z. bilineata</i> (Williston)
	<i>dispar</i>	^{3,5} * <i>Z. dispar</i> (Wiedemann)
		³ * <i>Z. prodispar</i> Duda
	<i>hypandriata</i>	<i>Z. hypandriata</i> Burla
	<i>orbitalis</i>	<i>Z. orbitalis</i> (Sturtevant)
	<i>vittimaculosa</i>	<i>Z. vittimaculosa</i> Burla
	Ungrouped	³ <i>Z. ptialis</i> Burla

Rhinoleucophenga was the genus presenting most new records of species in the pampas, beyond the two species previously mentioned other four species are under description process by J.L. Poppe (data not shown).

Poppe *et al.* (2014) highlighted the presence of 10 exotic species in the pampas. Deprá *et al.* (2014) pointed the first record of *D. suzukii* in the South America continent, after that, the respective species was recorded in many localities in the pampas increasing to 11 the number of exotic species in this environment.

New areas were included as sampled sites to the Brazilian pampas: São Gabriel (30°20'44"S, 54°19'32"W), Santiago (Robe *et al.*, 2014) (29°10'9"S, 54°53'50"W), Pelotas (Robe *et al.*, 2014) (31°48'58"S, 52°25'55"W), and Rio Grande (Robe *et al.*, 2014) (32°32'25"S, 52°32'34"W). A total of 108 Drosophilidae species are now known from the Brazilian, Uruguyan, and Argentinian pampas (Table 1). Twelve of 13 new recorded species were found only in the Brazilian pampas; only *D. suzukii* is widespread by the Brazilian and Uruguayan pampas (B. Goñi, pers. comm.). Despite it is probably still a gross underestimate of pampas diversity, since most of this biome is still not intensively sampled, the presented data indicate the high diversity of Drosophilidae in the South America grasslands, an environment historically neglected by the researchers due its "poor diversity" appearance.

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A description of the adult *Drosophila* miRNome.

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Introduction

MicroRNAs (miRNAs, miRs) are short (18–24 nucleotides, nt) non-coding RNAs present in all eukaryotes, which play crucial roles during the post-transcriptional gene expression regulation (Ambros, 2004).

According to genomic localization, miRNAs can be classified as intergenic or intragenic. Most of the human miRNAs described up to date (deposited in miRBase) are intergenic (68%). Among the intragenic miRNAs, 12% are located in intronic regions and the others are located in coding exons, repetitive regions, long non-coding RNAs or non-coding regions. miRNA genes are often located near to other miRNA being part of clusters. Clusters of close miRNAs tend to be intergenic and are expressed as polycistronic, co-regulated units that contain their own promoters (Saini, Griffiths-Jones, and Enright, 2007). In contrast, intragenic miRNAs are typically co-expressed with their host gene, although instances of independent transcriptional regulation have also been reported in intronic miRNA. Additionally differences in the expression of polycistronic mRNA located in introns have been associated to regulated alternative splicing (Bell, Buvoli, and Leinwand, 2010).

miRNA biogenesis is a multi-step process requiring several enzymes. In the canonical biogenesis miRNAs are usually transcribed by RNA polymerase II as long primary transcripts (pri-miRNA) (Y. Lee *et al.*, 2004), although, it has also been described pri-miRNA transcription by RNA polymerase III (Babiarz, Ruby, Wang, Bartel, and Blelloch, 2008). Pri-miRNA presents 7-methylguanosine cap (m⁷G) in 5' end and they are polyadenylated in 3' end (Cai, Hagedorn, and Cullen, 2004). First, the maturation process begins in the nucleus, where pri-miRNA is folded into a characteristic hairpin structure that is asymmetrically and specifically cut near the stem-loop by RNase III Drosha, generating one or more hairpin structures known as pre-miRNA (approximately 65 nt) (Y. Lee *et al.*, 2003). Drosha needs the binding of RNA protein DGCR8 (Pasha in *Drosophila*) as co-factor, which together form the microprocessor complex (Denli, Tops, Plasterk, Ketting, and Hannon, 2004). Second, pre-miRNAs are then transported to the cytoplasm by exportin 5