Eight new records of drosophilids (Insecta; Diptera) in the Brazilian savanna.

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Introduction

The Brazilian savanna, locally known as the Cerrado biome, is the second largest South American domain in size (ca 2.000.000 km^2) and one of the 25 biodiversity hotspots in the world (Myers *et al*., 2000). This savanna compared to all the savanna formations in the world has the highest plant species richness, composed by a mosaic of physiognomies ranging from open fields to gallery forests (Eiten, 1972; Oliveira and Marquis, 2002). However, the drosophilid fauna of this region, is still poorly studied. The first regular collections of these insects in the Brazilian savanna occurred at the end of the 1990's and were concentrated around the Federal District (Tidon *et al*., 2003; Ferreira and Tidon, 2005; Tidon, 2006). Currently, 112 drosophilid species are known in this biome (Blauth and Gottschalk, 2007; Mata *et al*., 2008; Chaves and Tidon, 2008), and 102 of them are endemic to the Neotropical Region. This diversity is probably a sub-estimation of the real richness, since several protected areas in this biome have not been adequately sampled.

This paper lists the drosophilid flies collected in five previously unsampled sites in the Brazilian savanna. In addition, it adds eight new occurrences (seven species and one genus) to the current drosophilid list for this biome.

Materials and Methods

The (five previously unsampled) sites surveyed were: A: Fazenda Trijunção, BA (14º49’ S; 45º58’ W), B: Parque Nacional Chapada dos Guimarães, MT (15º24’S; 55º49’W), C: Parque Estadual Serra Dourada, GO (15º57’S; 50º07’W), D: Parque Nacional das Emas, GO (18º15’S; 52º53’W), and E: Parque Estadual Serra de Caldas, GO (17º44’S; 48º39’W). The geographic locations were obtained with a Garmin II GPS, and additional details for each site sampled such as habitat types, collection dates, and methods are shown in Table 1.

To collect richness data accurately, different sampling strategies were utilized, mainly in the forest environments, which concentrate the highest number of drosophilid species (Tidon, 2006). Sampling was based on retention traps, fallen fruits of *Mauritia flexuosa*, *Campomanesia phaea*, *Byrsonima basiloba*, living flowers of *Hibiscus sabdariffa* and *Bombax cyathophorum*, and living fungus of *Pleurotus sp.*, *Tricholoma sp.,* and one unidentified genus (Table 1).

Fifteen retention traps (Tidon and Sene, 1988) were placed in the focal area for at least three days. These traps collected within its chambers all the drosophilids that entered them attracted by the bait, banana fermented by *Saccharomyces cerevisiae*. The drosophilids retained during the collection period were anesthetized with ether while inside the traps. All captured flies were transferred into small vials and transported to the laboratory at the *Universidade de Brasília* for identification. Resources evaluated as breeding sites were stored on moist sand in individual containers plugged with a fine mesh net. All drosophilids emerged throughout the 15 days were retrieved, counted and identified.

We identified the trapped and emerged individuals, whenever possible, to the species level using keys, descriptions and, in some cases, by analyzing the male terminalia (Freire-Maia and Pavan, 1949; Frota-Pessoa, 1954; Magalhães, 1962; Val, 1982; Vilela, 1983; Vilela and Bächli, 1990; Chassagnard and Tsacas, 1993). References to the taxonomic authorities can be found in the Drosophilidae taxonomy database compiled by Bächli (2008). We deposited voucher specimens of each species in the University of Brasilia Drosophilid Collection.

Table 1. Details of the five sites sampled in the Brazilian savanna. (A) Fazenda Trijunção-BA, (B) Parque Nacional Chapada dos Guimarães-MT, (C) Parque Estadual Serra Dourada-GO, (D) Parque Nacional das Emas-GO, and (E) Parque Estadual Serra de Caldas-GO.

Sites	Date	Collection	Habitats	Collection methods
A	18-21/04/2008	1	yard	retention traps
		2	palm swamp forest	retention traps
		3		fallen fruits of *Mauritia flexuosa*
		4	cerrado sensu strictu	retention traps
B	08/05-02/06/2008	5	gallery forest	retention traps
		6		fallen fruits of *Byrsonima basiloba*
		7		living flowers of *Hibiscus sabdariffa*
		8	mesophytic forest	retention traps
		9		living flowers of *Bombax cyathophorum*
		10		living fungus of *Tricholoma sp.*
		11	gallery forest	retention traps
		12		fallen fruits of *Campomanesia phaea*
		13		living fungus of *Pleurotus sp.*
		14		living fungus of an unidentified genus
		15	gallery forest	retention traps
		16		living flowers of *B. cyathophorum*
C	21-23/07/2008	17	gallery forest	retention traps
		18	yard	retention traps
		19	gallery forest	retention traps
D	24-26/07/2008	20	gallery forest	retention traps
		21	cerrado sensu strictu	retention traps
		22	gallery forest	retention traps
E	27-30/07/2008	23	cerrado sensu strictu	retention traps

Results and Discussion

A total of 3,610 drosophilids were collected and classified into 45 species from eight genera (*Drosophila*, *Hirtodrosophila*, *Mycodrosophila*, *Neotanygrastrella*, *Rhinoleucophenga*, *Scaptodrosophila*, *Zaprionus* and *Zygothryca*). *Drosophila* was the most representative genus, with 34 recognized and two undetermined species, the latter probably belonging to the *Drosophila repleta species* group (*Drosophila* FT1) and *D. willistoni* group (*Drosophila* FT2) (Table 2). This richness

Table 2. Species collected in five previously unsampled sites in the Brazilian savanna. Numbers indicate collections mentioned in Table 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Total
Drosophila																								
D. ananassae	-	1	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	3
D. arauna	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
D. austrosaltans	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
D. bromelioides	3	11	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22
D. busckii	1	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	-	7
D. buzzatii	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
D. calloptera	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
D. canalinea	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
D. caponei	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
D. cardini	19	68	1	60	1	-	2	-	-	-	-	-	-	-	1	-	1	-	1	35	54	39	-	282
D. cardinoides	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
D. cuaso	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
D. fumipennis	1	3	2	-	-	2	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	10
D. impudica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
*D. lutzii**	-	-	-	-	-	-	41	-	5	-	-	-	-	-	-	51	-	-	-	-	-	1	-	98
D. malerkotliana	-	1	-	5	-	-	-	-	-	-	2	5	-	-	4	-	-	-	-	-	-	-	-	17
D. mediopunctata	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
D. melanogaster	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	8
D. mediostriata	1	14	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	17
D. mercatorum	-	3	-	-	2	-	-	-	-	-	-	-	-	-	1	-	-	-	-	3	10	3	-	22
D. nebulosa	14	325	21	191	-	2	-	-	-	-	-	-	-	-	1	-	1	1	4	-	-	-	-	560
D. nigricuria	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4	3	-	10
D. pagliolii	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
D. paraguayensis	-	2	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	6
D. paranaensis	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-	4
D. polymorpha	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2
D. repleta	1	2	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17
D. saltans	1	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	2	-	1	-	1	-	-	7
D. schildi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
D. simulans	27	182	16	169	-	-	1	-	-	-	4	1	-	-	3	-	1	9	2	19	66	36	-	536
D. sturtevanti	1	2	-	7	11	-	-	10	-	-	-	-	-	-	7	-	-	3	1	-	-	-	2	44
D. willstoni	-	20	9	5	-	-	1	-	-	-	-	34	-	-	-	-	-	-	-	-	-	-	-	69
Drosophila FT1	-	2	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
Drosophila FT2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Hirtodrosophila																								
*H. subflavohalterata**	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	7
Mycodrosophila																								
*M. projetans**	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	8
Neotanygastrella																								
N. FT1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	2

represents more than 40% of all drosophilid species currently known in the Brazilian savanna (Blauth and Gottschalk, 2007; Chaves and Tidon, 2008; Mata *et al*., 2008). This study presents seven new drosophilid species occurrences for the biome (*Drosophila lutzii*, *Hirtodrosophila subflavohalterata*, *Mycodrosophila projetans*, *Rhinoleucophenga angustifrons*, *R. fluminensis*, *R. punctulata*, *Zygothrica apopoeyi*) and the first record of the genus *Neotanygastrella*, represented by one unidentified species.

All species considered new records for the Brazilian savanna have already been registered in the Neotropical Region, mainly in the south and southern Brazil. *Drosophila lutzii* is well distributed in South America, and its distribution seems to be associated with *Ipomoea* flowers (Schmitz and Hofmann, 2005). *Mycodrosophila projetans* is considered a widespread fly in the Neotropical Region (Wheeler and Takada, 1963), but the localities where it occurs in Brazil were not published; therefore, this is its first documented record of *M. projetans* in this biome. *Hirtodrosophila subflavohalterata* and *Zygothrica apopoeyi* were registered by Burla (1956) only in southern Brazil, in the states of Rio de Janeiro and São Paulo, respectively. Among the species from the genus *Rhinoleucophenga* recorded in this paper, *R. punctulata* has never been registered in Brazil before; it was known only in Bolivia (Malogolowkin, 1946). R. *angustifrons* and *R. fluminensis*, in contrast, were already registered in Brazil, in the Atlantic Forest biome (Malogolowkin, 1946; Lima, 1950). There are eight species of the genus *Neotanygastrella* known in the neotropics, and two of them (*N. chymomyzoides* and *N. tricoloripes)* are considered widespread in this region (Val *et al*., 1981). However, these two species have never been recorded in the Brazilian savanna before. Thus, this is the first record of the genus *Neotanygastrella* in this biome.

Nominal species not commented on here have already been recorded in the Brazilian savanna, and additional details about their distributions are available at Chaves and Tidon (2008). The two undetermined species of the genus *Drosophila* are possibly undescribed species: *Drosophila* FT1 probably belongs to the *D. fasciola* subgroup of the *D. repleta* group, and *Drosophila* FT2 was represented by only one male, whose external morphology and terminalia are similar to those of the *D. willistoni* group.

In short, this work adds eight new records (seven species and one genus) to the list of drosophilids compiled by Chaves and Tidon (2008), confirming that the drosophilid community of the Brazilian savanna is actually understudied. This region deserves more inventories, particularly using different collection methods, and in areas distant from urban centers.

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Rapidly evolving genes show well-resolved but conflicting phylogenies: Evidence from *Drosophila simulans* complex.

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Introduction

The *D. simulans complex,* which includes *D. simulans, D. mauritiana,* and *D. sechellia*, is a model system for the experimental study of speciation, sexual selection, and adaptation. Remarkably, the phylogenetic relationship of these species to each other has not been adequately resolved (summarized in Kliman *et al*., 2000; Harr *et al*., 1998; Ting *et al*., 2000). Specifically, a clear molecular phylogeny has not emerged, despite obvious behavioral, morphological, and physiological differences among these species. For example, the male genitalia of the three species are readily distinguishable from each other (Ashburner, 1989). Furthermore, these are good biological species as F_1 males between these species are completely sterile. Two issues make resolution of the relationships among these species problematic. First, a number of genes do not resolve the species – alleles of one species are often nested within alleles of another. Second, even when monophylly is observed, the branching order of the species is often inconsistent among genes.

Ting *et al*. (2000) advocated "speciation" genes, those loci that contribute to divergence between species (*e.g*., those involved in gametogenesis, behavior, morphology), as ideal genes for constructing a phylogeny of closely related species. To demonstrate their point, Ting *et al.* (2000) used the rapidly evolving *OdsH* to try to resolve the *D. simulans* clade phylogeny. Their analysis of the gene tree of *OdsH* suggested that *D. sechellia* split from *D. simulans* first, followed shortly by *D. mauritiana*. This result was congruent with the overall result of Harr *et al*. (1998), but not with analyses of several other genes (Kliman *et al*., 2000). A possible explanation for the observed discrepancy between Ting *et al.* and Kliman *et al.* is that any rapidly evolving gene will resolve a gene tree, but that these gene trees may not correspond to the actual species tree.

In this study, we test the idea that the resolved tree produced by Ting *et al*. is typical of that produced by any rapidly evolving gene. We also show that the trichotomy of the *simulans* clade is unlikely to be resolved.

Materials and Methods

We queried the NCBI sequence repository for all nucleotide sequences from *D. mauritiana*, and combined with sequence data produced in our lab for a set to 114 unique genes. Whole genome