



Diversity and associations between Drosophilidae (Diptera) species and Basidiomycetes in a Neotropical forest

FELIPE B. VALER¹, EDUARDO BERNARDI², MAYARA F. MENDES³,
MONICA L. BLAUTH³ and MARCO S. GOTTSCHALK³

¹Departamento de Biologia Celular e Molecular e Bioagentes Patogênicos, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (USP), Avenida Bandeirantes, 3900, 14049-900 Ribeirão Preto, SP, Brasil

²Departamento de Microbiologia e Parasitologia, Instituto de Biologia, Campus Capão do Leão, Universidade Federal de Pelotas (UFPel), Caixa Postal 354, 96010-000 Pelotas, RS, Brasil

³Departamento de Ecologia, Zoologia e Genética, Instituto de Biologia, Campus Capão do Leão, Universidade Federal de Pelotas (UFPel), Caixa Postal 354, 96010-000 Pelotas, RS, Brasil

Manuscript received on May 21, 2015; accepted for publication on July 27, 2015

ABSTRACT

Drosophilidae is one of the most representative families of insects that occurs in fungal fruiting bodies of Basidiomycetes; however, the diversity and community structure of mycophagous Drosophilidae in the Neotropical region is poorly known. The aims of the present study were to describe the diversity of mycophagous Drosophilidae and to investigate its colonization of fungal hosts in a forest of southern Brazil. From 120 fungal samples (patches of mushrooms) of 17 Basidiomycetes genera, flies were recorded emerging from 70 samples and collected in adult stages of 25 fungal samples, for a total of 4897 drosophilids belonging to 31 species and 5 genera. *Drosophila* Fallén was the most species-rich genus, whereas *Hirtodrosophila* Duda was the dominant genus. Studies performed in the Holarctic region indicate that mycophagous drosophilid have generalist habits; however, our results showed that most drosophilids use fewer than two fungal hosts, and most species of *Hirtodrosophila* and *Leucophenga* were restricted to abundant fungal species, suggesting a specialization for these resources. The most specialized fauna emerged from *Auricularia*, which was the most frequent fungal genus in our collection, and this result supports the assumption that specialization depends on the availability of fungal resources over time.

Key words: generalist species, mycophagy, mushrooms, Pampa biome, specialist species, trophic niche.

INTRODUCTION

A variety of insects colonize fruiting bodies of Basidiomycetes fungi; however, Drosophilidae is usually the most representative family because the flies use these bodies for feeding, mating and breeding sites (Bunyard 2003, Courtney et al.

1990, Shorrocks and Charlesworth 1980, Toda et al. 1999). Mycophagy in Drosophilidae is most likely derived from detritivorous feeding habits and may have arisen several times during their evolution (Throckmorton 1975). This habit is possibly synapomorphic in the genera *Hirtodrosophila* Duda, *Mycodrosophila* Oldenberg and *Zygothrica* Wiedemann (Grimaldi 1987) and

Correspondence to: Marco Silva Gottschalk
E-mail: marco.gottschalk@yahoo.com

homoplastic in *Drosophila* Fallén, *Leucophenga* Mik and *Scaptomyza* Hardy (Courtney et al. 1990). Mycophagy was also suggested in the genera *Paraliodrosophila* Duda and *Paramycodrosophila* Duda (Vilela and Bächli 2007), which are closely related to *Hirtodrosophila*, *Mycodrosophila* and *Zygothrica* (Grimaldi 1990).

In certain species of Drosophilidae, fungal fruiting bodies are the only resource required throughout the life cycle, and such behavior is observed in *Hirtodrosophila* (except for rare instances, see Grimaldi 1987) and *Mycodrosophila* species, which are primarily mycophagous because they depend on the fungi for breeding and feeding sites (Courtney et al. 1990). In other drosophilids, mycophagy is more labile, and fungi represent only a fraction of the resources used. Such behavior can be observed in species of *Zygothrica*, which can be found in fungi and flowers, or *Drosophila*, which may use a wide range of hosts, such as cacti, flowers, sap, fruits and fungi (Carson 1971, Markow and O'Grady 2008).

Despite a broad larval niche, a number of *Drosophila* species were dominant in the fungi collected from the Holarctic region, where most of the ecological studies of mycophagous drosophilids have been conducted (Lacy 1984, Takahashi et al. 2005, Wertheim et al. 2000). The main *Drosophila* species groups that emerged from this resource were *pinicola*, *quinaria*, *testacea* and *tripunctata*, which all belong to the *immigrans-tripunctata* radiation (Markow and O'Grady 2006, Remsen and O'Grady 2002). However, in certain mycophagous communities, other genera stand out in species richness and abundance, such as *Hirtodrosophila*, *Leucophenga* and *Mycodrosophila* (Bunyard 2003, Toda et al. 1999, Tuno 1999). For instance, *Leucophenga* was the dominant genus and *Hirtodrosophila* was the most species-rich genus among the flies emerging from fungi in a large-scale field survey in Australia (van Klinken and Walter 2001).

In the Neotropical region, few studies have addressed mycophagous Drosophilidae. From the 1940s to 1960s, taxonomic studies described a number of mycophagous *Hirtodrosophila* and *Zygothrica* species but did not investigate their ecology (Burla 1956, Cordeiro 1952, Frotapessoa 1945, 1951, Mourão et al. 1965). Despite the frequency and diversity of Drosophilidae, few ecological studies have reported their feeding, mating or breeding habits on mushrooms (Grimaldi 1987, Heed 1957). In Brazil, such collections were conducted in only two biomes: the Atlantic Rainforest biome (Gottschalk et al. 2009, Val and Kaneshiro 1988) and the Cerrado biome (Roque et al. 2006, Roque and Tidon 2008). These studies expanded the knowledge of drosophilid species with mycophagous habits.

Although studies of Holarctic fauna have provided important contributions to our understanding of the ecology and evolution of mycophagous drosophilids, the characteristics of fauna in this region may differ from those of the Neotropical region as suggested by Courtney et al. (1990). Thus, the aim of this study was to describe the diversity of the drosophilid assemblage associated with fungal species of Basidiomycetes in a forest area of the Pampa biome located in southern Brazil.

MATERIALS AND METHODS

STUDY AREA

We conducted this study in a restinga forest located in a grassland formation of 23 ha belonging to the Federal Conservation Unit Horto Botânico Irmão Teodoro Luís (HBITL, 31°48'54"S, 52°25'48"W), Rio Grande do Sul, Brazil (Fig. 1). HBITL is located in the Pampa biome and surrounded by wetlands, grassland areas and semideciduous forest remnants (Wolff et al. 2008).

The forest structure of HBITL shows at least three vegetation strata: arboreal, shrub and

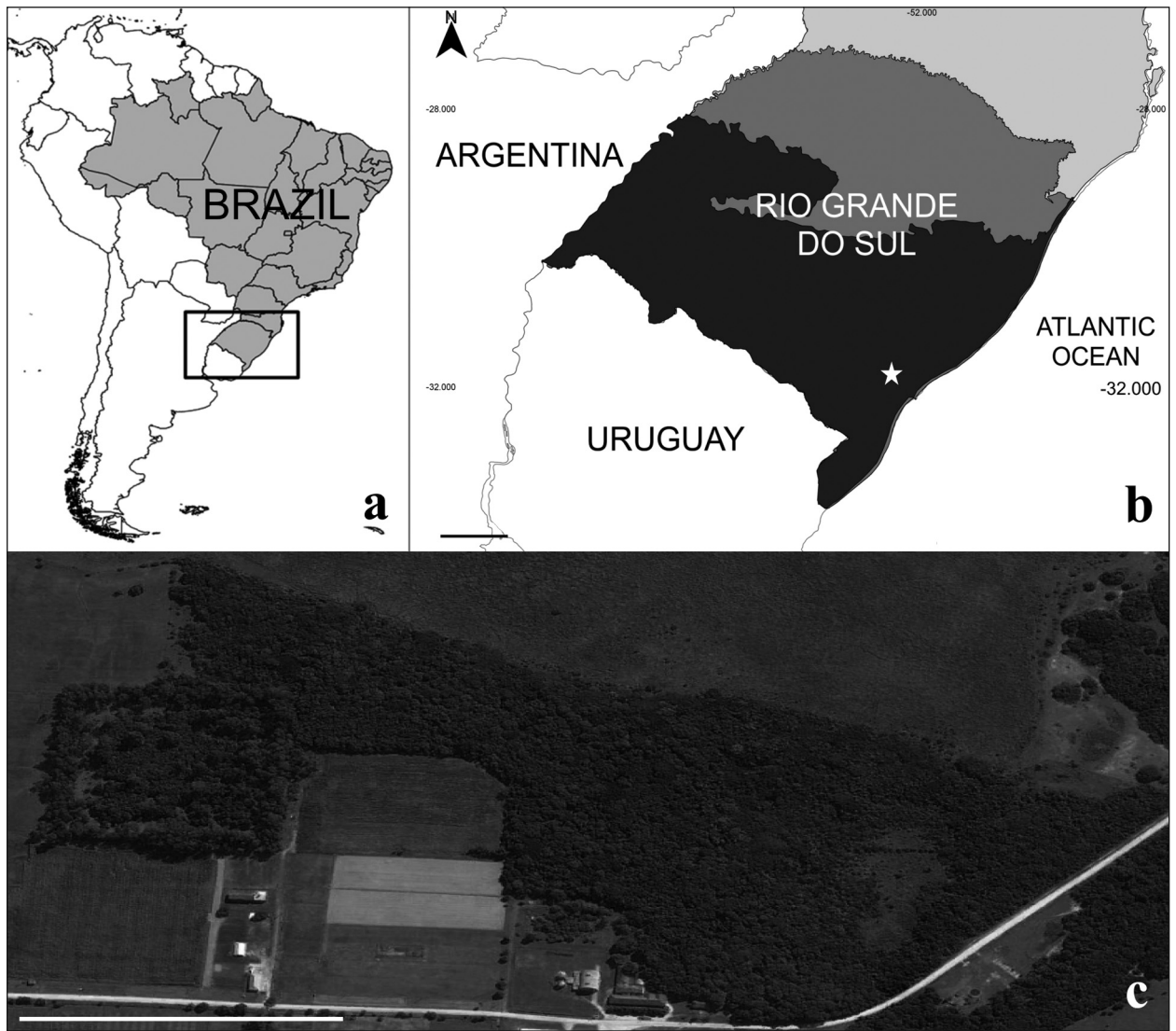


Figure 1 - Location of the Horto Botânico Irmão Teodoro Luís (HBITL). **a.** Map of South America with Brazil in gray and state of Rio Grande do Sul highlighted. **b.** Map of Rio Grande do Sul indicating the location of HBITL with a star, Pampa biome in black and Atlantic Rainforest biome in dark gray. **c.** Satellite photograph of the surveyed restinga forest area of HBITL (Source: Google Earth®). Scale bars: 100 km in **b**; 300 m in **c**.

herbaceous. *Ficus luschnathiana* (Miq.) Miq. is a frequent canopy tree, but other native species also occur, such as *Trichilia clausenii* C.DC., *Cordia americana* (L.) Gottshling & J.E.Mill., *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn., *Nectandra megapotamica* (Spreng.) Mez, *Cupania vernalis* Cambess, *Luehea divaricata* Mart. & Zucc. and *Sorocea bonplandii* (Baill.) W.C. Burger, Lanjouw & Boer. The exotic species *Asparagus setaceus* (Kunth) Jessop is widely distributed from

the forest edge of the HBITL, and a large number of trees at different ages provide continuous contributions to the abundance of coarse woody debris (J.N. Schlee Jr, unpublished data).

According to the Köppen classification system, the climate is Cfa (Kottek et al. 2006). Meteorological measurements performed between 1971 and 2000 reveal that the average annual temperature is 17.8°C and maximum and minimum monthly mean temperatures are 28.2°C and 8.6°C,

respectively (Agrometeorological Station of Pelotas 2014). The rainfall is 1367 mm/year, there are approximately 120 rainy days, and the annual relative humidity is 80%.

DATA COLLECTION

We searched for Basidiomycetes fungi along a 200 m trail, and we extended our search 10 m on either side of the trail. Fruiting bodies were found on the roots of plants, in leaf litter or on decaying wood. The collections were performed monthly between February and May 2011 and between February and June 2013, and each collection was conducted over a period of three hours in the morning. The sampling units of this study are patches of fungal bodies. We also captured adults on fruiting bodies with a net or entomological aspirator and fixed them in 70% ethanol in the field.

All of the fruiting bodies found in the field were collected, and when a large number of mushrooms was observed in the same location, only a fraction were collected. The collected fruiting bodies were transported in plastic bags to the laboratory, where they were then weighed to quantify the fungal mass, stored in glass vials with autoclaved sand and covered with synthetic mesh. To prevent dehydration, distilled water was added to the sand. Samples were maintained at $25 \pm 1^\circ\text{C}$ for four or five weeks, and during this period, the emergent insects were aspirated daily and fixed in 70% ethanol.

IDENTIFICATION OF BIOLOGICAL MATERIAL

We identified drosophilid males based on their external morphology and genitalia according to Frota-Pessoa (1945), Burla (1956), Wheeler and Takada (1971), Grimaldi (1987, 1990) and Vilela and Bächli (1990, 2004, 2007). We prepared the genitalia in accordance with Wheeler and Kambyssellis (1966) and using Kaneshiro's (1969) modifications. The females were identified based

on the external morphology to the lowest taxonomic level possible. For analyses, we considered the abundance of females of cryptic species to be proportional to the number of emerged males from the same sample.

The fungus genera and/or species were identified via photos of the fruiting bodies taken in the field according to Lincoff (1981, 2010), Putzke and Putzke (1998), Polese (2005) and Laessle and Lincoff (2010). Because of the advanced decay stage of certain fruiting bodies, we could not identify a number of samples, which were excluded from the analyses conducted at the species level.

ASSEMBLAGE CHARACTERIZATION

We assessed species dominance within the assemblage via a Whittaker plot that considered the relative abundance of species (pi , ratio between the absolute abundance of species i and total abundance) (Krebs 1999). Sampling adequacy was evaluated using emergence data from Basidiomycetes samples in a randomized species accumulation curve. The bootstrap method was used to support the sampling sufficiency (Epps and Arnold 2010, Smith and van Belle 1984) and performed with EstimateS v. 8.2.0 software with 500 randomizations (Colwell 2006).

The use of each fungal species by drosophilid species was evaluated according to the ratio between the number of colonized samples and total number of samples of each fungal species. To assess the species' temporality, the relative frequency of fungal species was calculated as the ratio between the number of collections in which the species were observed and total number of collections (eight collections).

We conducted Spearman's correlations between the total number of samples, total mass of fungal samples, relative frequency of each fungal species and total abundance and species richness of emerged drosophilid species.

To visualize the association between host fungal species and drosophilid species, we performed a correspondence analysis (CA) in the program Past v.2.17b (Hammer et al. 2001). The fungal species were considered as the independent variable, and drosophilid species abundance (with Log transformation) was considered as the dependent variable. To avoid the inclusion of subsampled species of drosophilid and fungus, three criteria were adopted for inclusion in the CA: (1) drosophilid species must have an absolute abundance ≥ 10 individuals; (2) drosophilid species must have been collected in at least three samples; and (3) fungal samples had to be determined to the species level. To test the significance of the groups obtained with the CA, an analysis of similarity (ANOSIM) using the Bray-Curtis index was conducted using the program Past v.2.17b. The Bonferroni correction for multiple comparisons was performed to adjust the significance.

RESULTS

We collected 4897 drosophilids in 5 genera and 31 species. Of these, 4620 individuals in 26 species emerged from 70 samples, and 277 adult flies in 17 species were collected on 25 samples (Tables I and II, respectively). Although the drosophilid assemblage was not sampled to saturation, our sampling approach was sufficiency (Fig. 2) because we recovered 90% of the 29 drosophilid species predicted by the bootstrap analysis.

To characterize our collection, the number of samples, mass (g) and relative frequency for each collected fungal species are shown in Table I. The number of fungus samples was positive correlated with the total abundance ($r_s = 0.683$; $p = 0.0003$; $df = 22$) and species richness of the emerged flies ($r_s = 0.719$; $p = 0.0001$; $df = 22$). The abundance of emerged flies was also correlated with the total mass of fungal samples ($r_s = 0.497$; $p = 0.015$; $df = 22$), although species richness was not ($r_s =$

0.349 ; $p = 0.102$; $df = 22$). In addition, the relative frequency of fungal species was positive correlated with drosophilid abundance ($r_s = 0.702$; $p = 0.0002$; $df = 22$) and species richness ($r_s = 0.744$; $p = 0.00005$; $df = 22$).

Out of 120 Basidiomycetes samples identified at the genus level, 57 were positive for drosophilid emergence or adult collection events, whereas 63 were negative (see Appendix S1 in Supporting Information – Supplementary Material). Positive samples of *Coprinus* Persoon, *Lycoperdon* Persoon, *Cantharellus* Jussieu, *Cortinarius* (Persoon) Gray, *Panus* Fries and *Clitocybe* (Fries) Stauder were not observed, although *Lycoperdon* was sampled on five occasions. For most of the observed fungal species, less than 50% were colonized by drosophilids, and *Agaricus* Linnaeus, *Auricularia* Bulliard ex Jussieu, *Polyporus* P. Micheli ex Adanson and *Melanoleuca* Patouillard were the only genera that had colonized more than 50% of the samples. Of these last four genera, only *Melanoleuca* was sampled in a single collection, whereas *Agaricus* and *Auricularia* were the second- and third-most sampled genera (19 and 18 samples, respectively), indicating that they are important resources for the drosophilid assemblage. Furthermore, considering the relative frequency of fungal species as a measure of temporal availability, *Auricularia auricula-judae* (Bulliard) J. Schröter was the most common species at a relative frequency of 0.75, and it was followed by *A. polytricha* (Montagne) Saccardo and *Polyporus* sp.1, which both had a relative frequency of 0.40.

The dominant drosophilid species in our samples ($\pi_i \geq 0.10$) were *Hirtodrosophila morgani* aff., which had the highest relative abundance, *H. mendeli* (Mourão, Gallo and Bicudo), *Zygothrica bilineata* (Williston) and *H. levigata* (Burla) (Fig. 3). The intermediary species ($0.10 > \pi_i \geq 0.01$) were *Z. ptialis* Burla, *H. morgani* (Mourão, Gallo and Bicudo), *Leucophenga* sp.L002, *Drosophila paraguayensis* Duda and *Leucophenga* sp.L001.

TABLE I
Absolute abundance of drosophilid species that emerged from Basidiomycetes fungal species and sample number, relative frequency and mass of the fungal species sampled in Horto Botânicoirmão Teodoro Luis, southern Brazil.

Drosophilidae Species	Auricularia auricularia-judae	Marasmius sp.7	Auricularia polytricha	Agaricus sp.4	Polyporus sp.1	Leptota sp.	Agaricus sp.	Agaricus sp.7	Pleurotus sp.2	Marasmius sp.2	Melanolenca sp.	Tricholoma spp.	Marasmius spp.	Gastrum sp.3	Pycnoporus sp.1	Marasmius sp.4	Agaricus sp.6	Leptota sp.1	Marasmius sp.5	Agaricus sp.2	Ganoderma sp.2	Trametes sp.2	Marasmius sp.8	Not Identified	Total (ni)	
<i>Hirtodrosophila morgani</i> aff.	1348			2																					1350	
<i>H. mendeli</i>	510		118																						628	
<i>Zygothrica bilineata</i>		459				100			1		23	1												28	612	
<i>H. levigata</i>	11	5		10		68			35	31								1	1				1	415	578	
<i>Z. pitalalis</i>	2	10		1	27	30			18	11	6	6		13									1	211	330	
<i>H. morgani</i>	304		19																						323	
<i>Leucophenga</i> sp.2				88				32		30	30	5					2	7		2				46	164	
<i>Drosophila paraguayensis</i>	7			30				7	7	3		13	1	14			8		2	2					133	
<i>Leucophenga</i> sp.1		65		1				27		10	3		9											44	67	
<i>Drosophila</i> sp.Z2									4	10														1	63	
<i>H. subflavohalterata</i> aff.1	13		49																						59	
<i>Mycodrosophila projectans</i>	1			1	30				1							2					3	3		18	59	
<i>D. ornatifrons</i>					2						4				10				3					35	54	
<i>H. subflavohalterata</i> aff.2			37																						37	
<i>L. maculosa</i> cf.				16				18																	34	
<i>Leucophenga</i> sp.5				25				1																	26	
<i>D. nappae</i>				2		7			5																14	
<i>Mycodrosophila</i> sp.M001																								11	11	
<i>H. subflavohalterata</i>			10																						10	
<i>Z. laevifrons</i>																									10	
<i>D. griseolineata</i>																					2				8	
<i>D. simulans</i>																								5	5	
<i>H. pleurostrigata</i>			5																						5	
<i>D. cardini</i>																								1	1	
<i>D. melanogaster</i>																									1	
<i>D. willistoni</i> cf.																									1	
Total (ni)	2196	539	238	134	108	107	98	78	71	55	34	32	28	14	14	12	10	8	7	4	3	3	2	825	4620	
Number of samples	11	4	4	5	7	2	1	1	2	2	1	2	2	1	1	1	2	3	1	1	1	1	1	13	70	
Mass (g)	702	135	249	141	427	36	34	16	204	14	19	46	29	22	53	10	35	17	17	31	54	48	18	695	3052	
Frequency	0.75	0.25	0.38	0.25	0.38	0.25	0.13	0.13	0.25	0.13	0.13	0.25	0.25	0.13	0.13	0.13	0.13	0.25	0.13	0.13	0.13	0.13	0.13	0.13	0.75	-

TABLE II
Absolute abundance of drosophilid species sampled as adults on Basidiomycetes fungal species in Horto Botânico Irmão Teodoro Luis, southern Brazil.

Drosophilidae Species	<i>Auricularia auricula-judae</i>	<i>Marasmius sp.7</i>	<i>Polyporus sp.1</i>	<i>Marasmius sp.</i>	<i>Marasmius sp.5</i>	<i>Pleurotus sp.2</i>	<i>Agaricus sp.</i>	<i>Marasmius sp.2</i>	<i>Tricholoma sp.</i>	Not Identified	Total (ni)
<i>Zygothrica ptilialis</i>	50	13	8		3	3	2			6	85
<i>Zygothrica sp.Z002</i>	36										36
<i>Hirtodrosophila levigata</i>	14	1	5					1		7	28
<i>H. morgani</i> aff.	24										24
<i>H. mendeli</i>	19		3								22
<i>Z. bilineata</i>		16							1	4	21
<i>H. morgani</i>	18		2								20
<i>Drosophila paraguayensis</i>				3						16	19
<i>D. nappae</i>			5								5
<i>Z. dispar</i>		4									4
<i>Z. prodispar</i>		4									4
<i>D. willistoni</i> cf.			3								3
<i>Z. vittimaculosa</i>	2										2
<i>Drosophila sp.Z2</i>								1			1
<i>H. subflavohalterata</i> aff.1	1										1
<i>Mycodrosophila projectans</i>										1	1
<i>Z. orbitalis</i>	1										1
Total (ni)	165	38	26	3	3	3	2	2	1	34	277
Number of samples	6	2	5	1	1	1	1	1	1	6	25

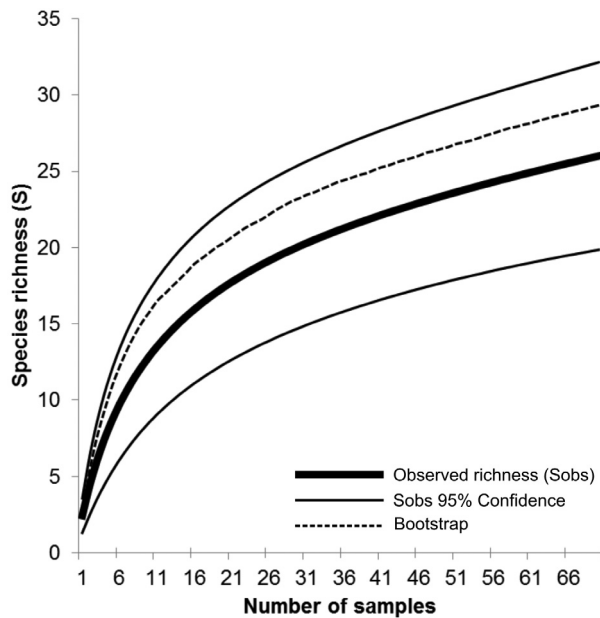


Figure 2 - Species accumulation curve for the drosophilid assemblage and species richness estimation by the bootstrap method.

These nine species accounted for approximately 90% of emerging individuals. The remaining 17 species were rare in our sample ($p_i < 0.01$).

Fig. 4 shows the number of fungal species from which the drosophilid species emerged, and it provides an overview of the resource range that these species can use, suggesting varying degrees of specialization. The drosophilid species that use other resources in addition fungi, such as *D. paraguayensis* and *Z. ptilialis* (Garcia et al. 2012), utilize a larger number of fungal species for larva rearing. Similarly, predominantly mycophagous species, such as *H. levigata* and *Mycodrosophila projectans* (Sturtevant) (Courtney et al. 1990), were also common and found in a large number of fungal host species. Five species of *Zygothrica* [*Z. dispar* (Wiedemann), *Z. orbitalis* (Sturtevant), *Z. prodispar* Duda, *Z. vittimaculosa* Burla and

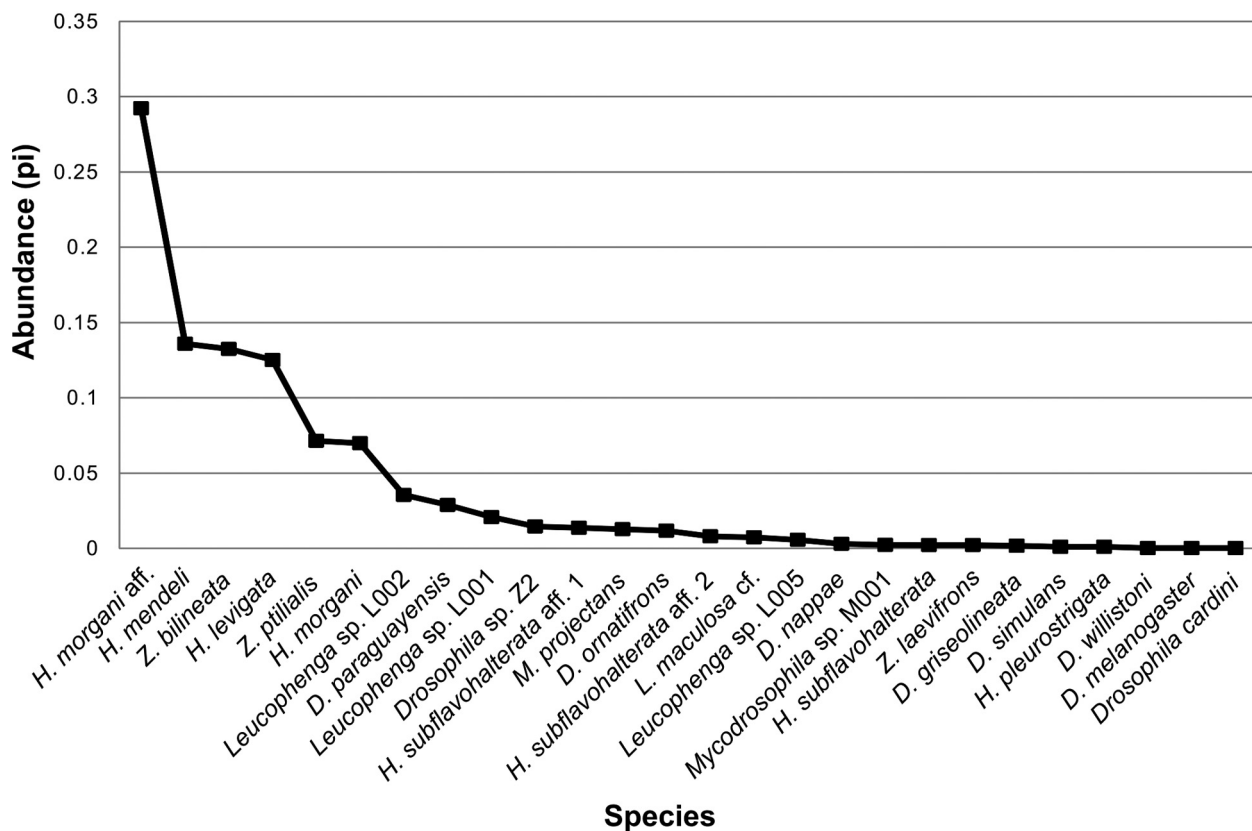


Figure 3 - Whittaker plot of relative abundance (π) of emerged drosophilid from Basidiomycetes fungal species in Horto Botânico Irmão Teodoro Luís.

Zygothrica sp.Z002], were only collected as adult flies and did not emerge from the fruiting bodies (Table II).

With the exception of *H. levigata* and *H. morgani* aff., all of the *Hirtodrosophila* species emerged from two species of *Auricularia*: *A. auricula-judae* and *A. polytricha*. *Auricularia auricula-judae* was the main resource of *H. morgani* aff., the dominant species in the assemblage, although only two flies of this species emerged from *Polyporus* sp.1. Other species that displayed a narrow range of fungal trophic resources were *L. maculosa* cf. (Coquillett in Johnson) and *Leucophenga* sp. L005, which were sampled emerging from *Agaricus* sp.4 and *Agaricus* sp.7. Certain species were commonly sampled in banana-baited traps, such as the exotic *Drosophila melanogaster* Meigen and *D. simulans* Sturtevant, and the Neotropical species *D. cardini*

Sturtevant and *D. willistoni* cf. Sturtevant were collected emerging from a single species of fungi.

Fig. 5 shows the two first axes obtained with the CA. Axis 1 explains 15.54% of the total variation of the sample, whereas axis 2 explains 13.18%. Three independent clusters of drosophilid species were established and corroborated by ANOSIM ($p = 0.0003$ for all comparisons): (1) four species of *Hirtodrosophila* (*H. mendeli*, *H. morgani*, *H. morgani* aff. and *H. subflavohalterata* aff.1) were associated with *A. auricula-judae* and *A. polytricha*; (2) *Leucophenga* sp.L001, *Leucophenga* sp.L002, *Leucophenga* sp.L005 and *Z. bilineata* were associated with *Agaricus* sp.4, *Agaricus* sp.6, *Agaricus* sp.7, *Lepiota* sp.1 and *Marasmius* sp.7; and (3) *D. ornatifrons*, *D. paraguayensis*, *H. levigata*, *M. projectans* and *Z. ptitialis* were associated with a wider range of fungal species

and genera, including *Agaricus* sp.2, *Agaricus* sp.6, *Fomitopsis* sp.1, *Geastrum* sp.3, *Lepiota* sp.1, *Marasmius* sp.2, *Marasmius* sp.4, *Marasmius* sp.5,

Marasmius sp.7, *Pleurotus* sp.2, *Polyporus* sp.1, *Pycnoporus* sp.1 and *Trametes* sp.2. The last group appears to be composed of more generalist species.

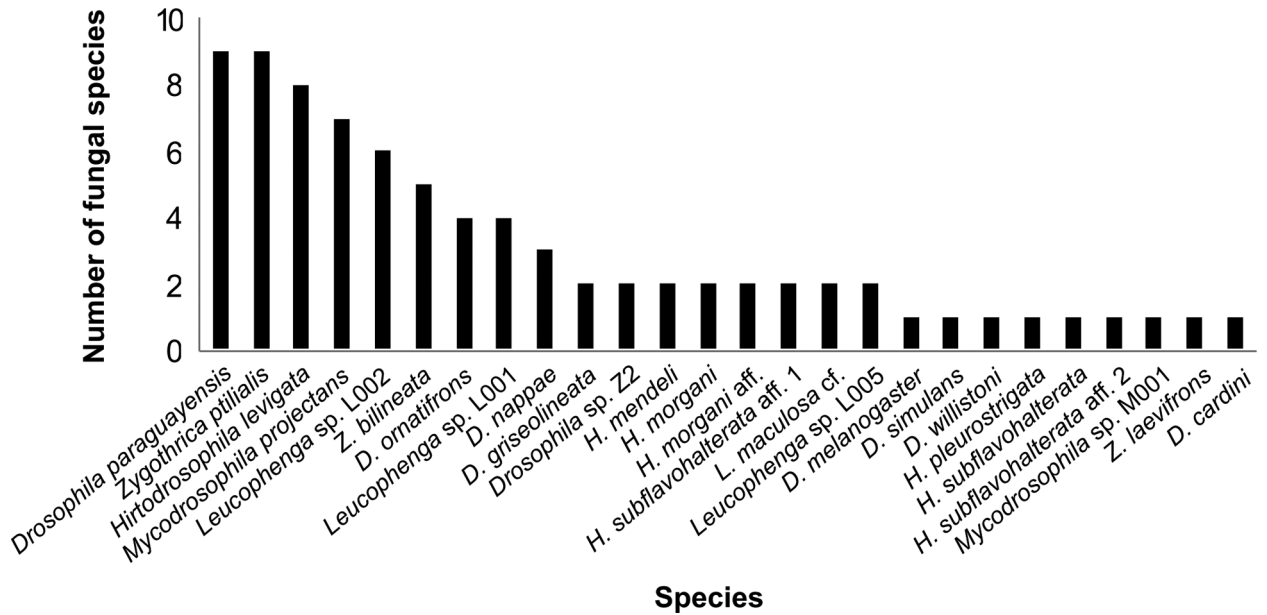


Figure 4 - Number of Basidiomycetes fungal species used by each species of drosophilid.

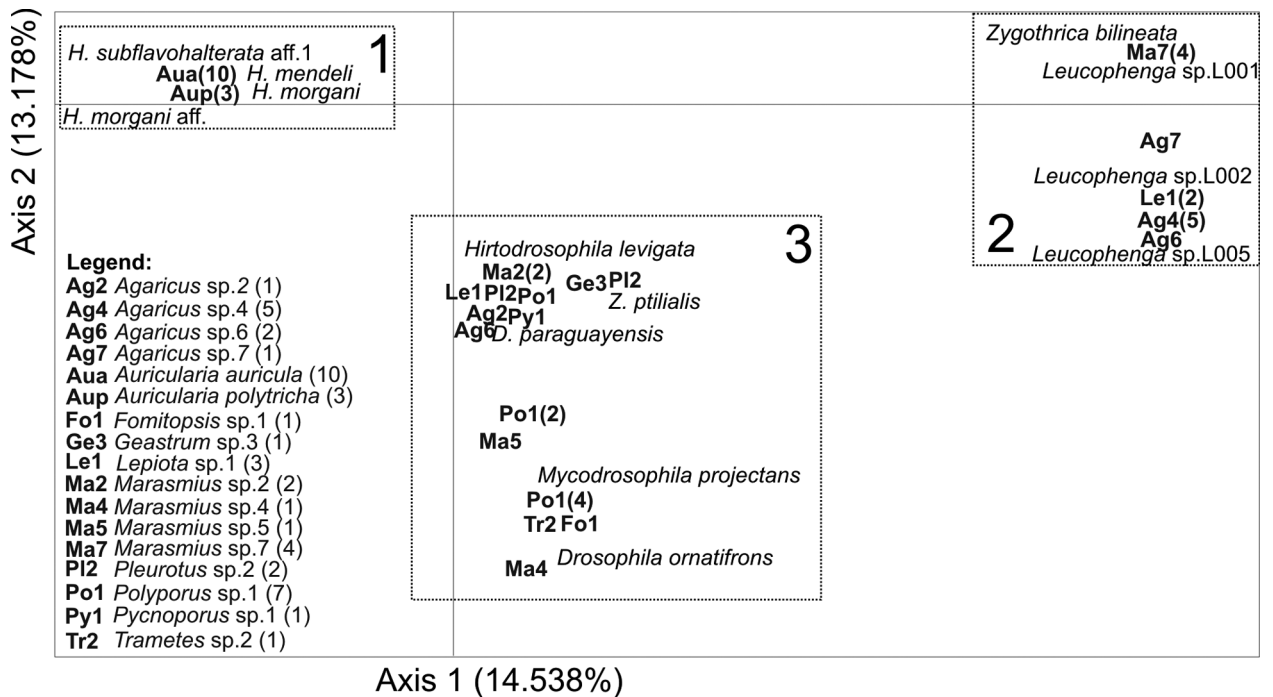


Figure 5 - Axis 1 and 2 of the correspondence analysis (CA) relating the species of drosophilid and host fungal species from which they emerged. Samples of fungi are shown in bold. The number of samples used in the CA is shown in brackets in the legend. The numbered squares represent independent groups of drosophilid species according the analysis of similarity (ANOSIM).

DISCUSSION

In this study, we provided valuable information on the diversity of Basidiomycetes resources used by drosophilid species in the Neotropical region. We provided emergence data for 26 species, including *Hirtodrosophila levigata*, *H. mendeli*, *H. pleurostrigata* (Burla), *Zygothrica bilineata* and *Z. ptilialis*, for which no fungal host has previously been reported (Gottschalk et al. 2009, Grimaldi 1987, Roque et al. 2006, Roque and Tidon 2008, Val and Kaneshiro 1988). Nine collected species could not be identified (*Drosophila* sp.Z2, *H. morgani* aff., *H. subflavohalterata* aff.1, *H. subflavohalterata* aff.2, *Mycodrosophila* sp.M001, *Zygothrica* sp.Z002, *Leucophenga* sp.L001, *Leucophenga* sp.L002 and *Leucophenga* sp.L005) and may represent undescribed species. This unexplored diversity highlights the importance of the sampled area for additional taxonomic surveys, particularly because the dominant species of the assemblage (*H. morgani* aff.) is an undescribed species.

The increased availability of fungal species (measured by number of samples and relative frequency) led to increased abundance and species richness of the associated drosophilid. Döge et al. (2015) observed that the availability of resources is the main factor affecting the size of the populations of two species of drosophilids. We observed a positive correlation between for the mass of fungal species and drosophilid abundance, although a correlation was not observed between mass of fungal species and drosophilid species richness. Apparently, the drosophilid species were restricted to ovipositing or breeding on fungal species and could only colonize a limited number of species despite the high availability.

Regarding the diversity of the assemblage, our results are consistent with previous studies that assessed the emergence of drosophilid from Basidiomycetes fungi in the Neotropics, and we showed that the most species-rich genus was *Drosophila* and most abundant genus was

Hirtodrosophila (Gottschalk et al. 2009, Roque et al. 2006). The dominance of *Hirtodrosophila* is most likely associated with the high colonization rates of *Auricularia* (Appendix S1) and high sample number of this fungus, which is the main trophic resource for *Hirtodrosophila* larvae in our study.

Drosophilids associated with fungi have been subject to extensive sampling in Japan (Toda et al. 1999), the United States (Lacy 1984) and Europe (Shorrocks and Charlesworth 1980), and these studies reported that a few species were highly dominant in relation to others, which is consistent with our results. The highest number of *Drosophila* species groups observed in our study belonged to the *immigrans-tripunctata* radiation, which is notable for its mycophagous habits (Courtney et al. 1990, Gottschalk et al. 2009, Lacy 1984, Morales-Hojas and Vieira 2012). *Drosophila paraguayensis*, which belongs to *tripunctata* species group, are frequently found on fruit but was the most abundant *Drosophila* species in our samples, suggesting that fungi are important breeding sites for this species, especially in summer and autumn. Saavedra et al. (1995) recorded a low emergence of *D. paraguayensis* from fruit in summer and autumn, although this species was observed breeding on fruit in spring and winter. In addition, Garcia et al. (2012), Hochmüller et al. (2010) and Poppe et al. (2012) sampled *D. paraguayensis* and observed low abundance in summer and autumn with banana-baited traps. Most likely, this versatile species changes its larval resources (i.e., fruit and fungi) seasonally, and this behavior is supported by the results of our study because *D. paraguayensis* was sampled in nine fungal species.

Drosophila melanogaster, *D. simulans* and *D. willistoni* cf., species, which breed on fruit, were collected occasionally in low abundance on fungus. The colonization of fungi by *D. willistoni* corroborates Roque et al. (2006), who collected this species emerging from Agaricales and Boletales.

van Klinken and Walter (2001) sampled 37 species of drosophilids emerging from fungi

belonging to Agaricales, Aphyllophorales, Auriculariales and Boletales in Australia and suggested that their niches overlap. Although certain species of *Hirtodrosophila*, *Leucophenga*, *Lissocephala* Malloch, *Mycodrosophila*, *Paramycodrosophila*, *Scaptodrosophila* Duda and *Zygothrica* emerged exclusively from fungi, species of *Drosophila*, *Leucophenga* and *Scaptodrosophila* also emerged from flowers and fruits. The trophic niche of *Hirtodrosophila*, *Mycodrosophila* and *Drosophila* species are similar in the Neotropical region, with the former two genera restricted to fungi (Grimaldi 1987, Heed 1957) and *Drosophila* using other substrates as breeding resources (Carson 1971). However, for *Leucophenga* species in the Neotropics, fungal fruiting bodies are the only substrate in which they are known to rear their larvae (Gottschalk et al. 2009, Heed 1957, Roque et al. 2006).

Of the species collected on fruiting bodies, a subset of *Zygothrica* species did not emerge, which is consistent with Grimaldi (1987), who reported the use of fungal fruiting bodies as a courtship arena for *Zygothrica*. However, adult *Leucophenga* were not collected on fruiting bodies and were only collected as they emerged. Lachaise and Tsacas (1983) suggest that the feeding and breeding sites of *Leucophenga* species are distinct, which could explain our results.

Data in the literature suggest that most mycophagous drosophilid species have generalist habits because fruiting bodies are considered to be nutritionally homogeneous (Courtney et al. 1990, Hanski 1989). Thus, oligophagous or specialist species would be uncommon, and records reporting such habits would most likely be artefacts of insufficient sampling effort (Hanski 1989). Our results provide evidence that is inconsistent with this hypothesis because only 9 of the 26 drosophilid species were observed colonizing three or more fungal species, most of the recorded species were collected in fewer than two fungal species, and most species of *Hirtodrosophila* and *Leucophenga*

were restricted to abundant fungal species (in collected sample mass), indicating specialization for these resources. In addition, we found species of drosophilid that were closely associated with *Auricularia*, *Agaricus*, *Lepiota* and *Melanoleuca*. The emerged fauna of *Auricularia* were the most particular and primarily represented by species of the *hirticornis* group of *Hirtodrosophila*. *Auricularia* was the most frequent and abundant in HBITL, and according to Courtney et al. (1990), these features are necessary to host a specialized fauna. The generalist habit described by Courtney et al. (1990) and Hanski (1989) in the Holarctic region may be a result of the absence of specialist species of *Hirtodrosophila*, *Leucophenga* and even *Zygothrica* because *Drosophila* is the dominant genus of mycophagous drosophilid assemblages in the Holarctic.

In conclusion, the studied drosophilid assemblage was dominated by *Hirtodrosophila*, and *Drosophila* was the most species-rich genus. The dominance of *Hirtodrosophila* should reflect a specialist habit, whereas the higher species richness of *Drosophila* may reflect a generalist habit. The presence of drosophilids with specialist habits was most likely not a sampling artifact because the species richness did not increase with the mass of the fungal samples, suggesting a restriction imposed by fungal species on colonization by generalist drosophilids. The differences between the assemblage observed in our results and assemblages studied in the Holarctic region may have been caused by the lower species richness of specialized genera.

ACKNOWLEDGMENTS

We would like to thank Prof. Lizandra Jaqueline Robe, Prof. Cristiano Agra Iserhard and the anonymous reviewers for their helpful comments and improve the manuscript and Liliane Nachtigall Martins for their practical assistance in the field and fly identification. This work was supported

by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) under grant n° 472973/2013-4 and 471174/2013-0. The collection was authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), under permanent license for collecting biological material n° 25454-1.

RESUMO

Drosophilidae é uma das mais representativas famílias de insetos presentes em corpos de frutificação de fungos Basidiomicetes; no entanto, a diversidade e a estrutura da comunidade de Drosophilidae micófagos na região Neotropical é pouco conhecida. O objetivo deste estudo foi descrever a diversidade de Drosophilidae micófagos e investigar sua colonização em fungos hospedeiros em uma mata no sul do Brasil. De 120 amostras de fungos (agrupamentos de cogumelos), de 17 gêneros de Basidiomycetes, foram registradas moscas emergentes de 70 amostras e coletadas em estágio adulto de 25 amostras de fungos, totalizando 4.897 drosofilídeos pertencentes a 31 espécies e 5 gêneros. *Drosophila* Fallén foi o gênero com maior riqueza, enquanto *Hirtodrosophila* Duda foi o gênero dominante. Estudos realizados na região Holártica sugerem que drosofilídeos micófagos apresentam hábitos generalistas; no entanto, nossos resultados mostraram que a maioria dos drosofilídeos usam menos do que dois fungos hospedeiros, e que a maioria das espécies de *Hirtodrosophila* e *Leucophenga* foram restritas às duas espécies mais abundantes de fungos, indicando uma especialização por estes recursos. A fauna mais especializada emergiu de *Auricularia*, o qual foi o gênero fúngico mais frequente em nossa amostragem, e este resultado corrobora a hipótese de que a especialização depende da disponibilidade dos recursos fúngicos ao longo do tempo.

Palavras-chave: espécies generalistas, micofagia, cogumelos, bioma Pampa, espécies especialistas, nicho trófico.

REFERENCES

- AGROMETEOROLOGICAL STATION OF PELOTAS. 2015. [Cited on April 20, 2015]. Available from URL: <http://www.cpect.embrapa.br/agromet/estacao>.
- BUNYARD BA. 2003. Biodiversity and ecology of mycophilous Diptera in northeastern Ohio. *P Entomol Soc Wash* 105: 847-858.
- BURLA H. 1956. Die Drosophilidengattung *Zygothrica* und ihre beziehung zur *Drosophila*-untergattung *Hirtodrosophila*. *Mitt Zool Mus Berl* 2: 189-321.
- CARSON HL. 1971. The ecology of *Drosophila* breeding sites. Harold L. Lyon Arboretum Lecture, n. 2. Honolulu: The University of Hawaii Press, 28 p.
- COLWELL RK. 2006. EstimateS ver. 8.2.0: Statistical estimation of species richness and shared species from samples. Available from: <http://purl.oclc.org/estimates>.
- CORDEIRO AR. 1952. *Drosophila (Hirtodrosophila) caxiensis*, a new species of fungus-feeding fly from Brasil. *Dusenia* 3: 303-308.
- COURTNEY SP, KIBOTA TT AND SINGLETON TA. 1990. Ecology of mushroom-feeding Drosophilidae. In: Begon B, Fitter AH and Macfadyen A (Eds), *Advances in Ecological Research*, London: Academic Press, p. 225-274.
- DÖGE JS, DE OLIVEIRA HV AND TIDON R. 2015. Rapid response to abiotic and biotic factors controls population growth of two invasive drosophilids (Diptera) in the Brazilian Savanna. *Biol Invasions* 17: 2461-2474.
- EPPS MJ AND ARNOLD AE. 2010. Diversity, abundance and community network structure in sporocarp-associated beetle communities of the central Appalachian Mountains. *Mycologia* 102: 785-802.
- FROTA-PESSOA O. 1945. Sobre o subgênero *Hirtodrosophila*, com descrição de uma nova espécie (Diptera, Drosophilidae, *Drosophila*). *Rev Bras Entomol* 5: 469-483.
- FROTA-PESSOA O. 1951. *Drosophila (Hirtodrosophila) magnarcus* n. sp. (Diptera, Drosophilidae). *Rev Bras Entomol* 11: 407-411.
- GARCIA CF, HOCHMÜLLER CJC, VALENTE VLS AND SCHMITZ HJ. 2012. Drosophilid assemblages at different urbanization levels in the city of Porto Alegre, state of Rio Grande do Sul, Southern Brazil. *Neotrop Entomol* 41: 32-41.
- GOTTSCHALK MS, BIZZO LEM, DÖGE JS, PROFES MS, HOFMANN PRP AND VALENTE VLS. 2009. Drosophilidae (Diptera) associated to fungi: differential use of resources in anthropic and Atlantic Rain Forest areas. *Iheringia Ser Zool* 99: 442-448.
- GRIMALDI D. 1987. Phylogenetics and taxonomy of *Zygothrica* (Diptera: Drosophilidae). *B Am Mus Nat Hist* 186: 103-268.
- GRIMALDI D. 1990. A phylogenetic, revised classification of the genera in the Drosophilidae (Diptera). *B Am Mus Nat Hist* 197: 1-139.
- HAMMER Ø, HARPER DAT AND RYAN PD. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol Electron* 4: 1-9.
- HANSKI I. 1989. Fungivory: fungi, insects and ecology. In: Wilding N, Collins NM, Hammond PM and Webber JB (Eds), *Insect-fungus interaction*. London: Academic Press, p. 25-68.

- HEED WB. 1957. Ecological and distributional notes on the Drosophilidae (Diptera) of El Salvador. In: Wheeler MR (Ed), *Studies in the Genetics of Drosophila*, Texas: University of Texas Publication, p. 62-78.
- HOCHMÜLLER CJC, LOPES-DA-SILVA M, VALENTE VLS AND SCHMITZ HJ. 2010. The drosophilid fauna (Diptera, Drosophilidae) of the transition between the Pampa and Atlantic Forest Biomes in the state of Rio Grande do Sul, southern Brazil: first records. *Pap Avulsos Zool* 50: 285-295.
- KANESHIRO K. 1969. A study of the relationships of Hawaiian *Drosophila* species based on the external male genitalia. The University of Texas Publication 6918: 55-69.
- KOTTEK M, GRIESER J, BECK C, RUDOLF B AND RUBEL F. 2006. World Map of the Köppen-Geiger climate classification updated. *Meteorol Z* 15: 259-263.
- KREBS C. 1999. *Ecological Methodology*. San Francisco: Benjamin Cummings, 624 p.
- LACHAISE D AND TSACAS L. 1983. Breeding-sites in tropical African drosophilids. In: Ashburner M, Carson HL and Thompson Jr JN (Eds), *The Genetics and Biology of Drosophila*, London: Academic Press, p. 221-332.
- LACY R. 1984. Predictability, toxicity, and trophic niche breadth in fungus-feeding Drosophilidae (Diptera). *Ecol Entomol* 9: 43-54.
- LAESOE T AND LINCOFF G. 2010. *Mushrooms. The clearest recognition guides available*. London: Dorling Kindersley, 304 p.
- LINCOFF G. 1981. *Simon and Schuster's guide to mushrooms*. New York: A Fireside Book, 511 p.
- LINCOFF G. 2010. *The complete mushroom hunter: an illustrated guide to finding, harvesting and enjoying wild mushrooms*. Beverly: Quarry Books, 192 p.
- MARKOW TA AND O'GRADY PM. 2006. *Drosophila: A guide to species identification and use*. London: Academic Press, 259 p.
- MARKOW TA AND O'GRADY PM. 2008. Reproductive ecology of *Drosophila*. *Funct Ecol* 22: 747-759.
- MORALES-HOJAS R AND VIEIRA J. 2012. Phylogenetic patterns of geographical and ecological diversification in the subgenus *Drosophila*. *PLoS ONE* 7: 1-15.
- MOURÃO CA, GALLO AJ AND BICUDO HEMC. 1965. Sobre a sistemática de *Drosophila* no Brasil, com descrição de *D. mendeli* sp.n. e "Relação de espécies brasileiras do gênero *Drosophila*". *Cienc Cult* 17: 577-586.
- POLESE JM. 2005. *The pocket guide to mushrooms*. Slovakia: Könemann, 381 p.
- POPPE JL, VALENTE VLS AND SCHMITZ HJ. 2012. Structure of Drosophilidae assemblage (Insecta, Diptera) in Pampa Biome (São Luiz Gonzaga, RS). *Pap Avulsos Zool* 52: 185-195.
- PUTZKE J AND PUTZKE MTL. 1998. *O Reino dos Fungos*. Santa Cruz do Sul: EDUNISC, 606 p.
- REMSEN J AND O'GRADY P. 2002. Phylogeny of Drosophilinae (Diptera: Drosophilidae), with comments on combined analysis and character support. *Mol Phylogenet Evol* 24: 249-264.
- ROQUE F, FIGUEIREDO R AND TIDON R. 2006. Nine new records of drosophilids in the Brazilian savanna. *Drosoph Inf Serv* 89: 14-17.
- ROQUE F AND TIDON R. 2008. Eight new records of drosophilids (Insecta; Diptera) in the Brazilian savanna. *Drosoph Inf Serv* 91: 94-98.
- SAAVEDRA CCR, CALLEGARI-JACQUES SM, NAPP M AND VALENTE VLS. 1995. A descriptive and analytical study of four Neotropical drosophilid communities. *J Zool Syst Evol* 33: 62-74.
- SHORROCKS B AND CHARLESWORTH P. 1980. The distribution and abundances of the British fungal-feeding *Drosophila*. *Ecol Entomol* 5: 61-78.
- SMITH EP AND VAN BELLE G. 1984. Nonparametric estimation of species richness. *Biometrics* 40: 119-129.
- TAKAHASHI KH, TUNO N AND KAGAYA T. 2005. The relative importance of spatial aggregation and resource partitioning on the coexistence of mycophagous insects. *Oikos* 109: 125-134.
- THROCKMORTON LH. 1975. The phylogeny, ecology and geography of *Drosophila*. In: King RC (Ed), *Handbook of Genetics*, New York: Plenum Press, p. 421-469.
- TODA MJ, KIMURA MT AND TUNO N. 1999. Coexistence mechanisms of mycophagous drosophilids on multispecies fungal hosts: aggregation and resource partitioning. *J Anim Ecol* 68: 794-803.
- TUNO N. 1999. Insect feeding on spores of a bracket fungus, *Elfvigia applanata* (Pers.) Karst. (Ganodermataceae, Aphyllophorales). *Ecol Res* 14: 97-103.
- VAL FC AND KANESHIRO KY. 1988. Drosophilidae (Diptera) from the Estação Biológica de Boracéia, on the coastal range of the state of São Paulo, Brazil: geographical distribution. In: Vanzolini PE and Heyer WR (Eds), *Proceedings of a Workshop on Neotropical Distribution Patterns*, Rio de Janeiro: Acad Bras Cienc, p. 189-203.
- VAN KLINKEN RD AND WALTER GH. 2001. Larval hosts of Australia Drosophilidae (Diptera): A field survey in subtropical and tropical Australia. *Aust J Entomol* 40: 163-179.
- VILELA CR AND BÄCHLI G. 1990. Taxonomic studies on Neotropical species of seven genera of Drosophilidae (Diptera). *Mitt Schweiz Entomol Ges* 63(Suppl.): 1-332.
- VILELA CR AND BÄCHLI G. 2004. On the identities of nine Neotropical species of *Hirtodrosophila* (Diptera, Drosophilidae). *Mitt Schweiz Entomol Ges* 77: 161-195.
- VILELA CR AND BÄCHLI G. 2007. Revision of the Neotropical genus *Paraliodrosophila* (Diptera, Drosophilidae). *Mitt Schweiz Entomol Ges* 80: 291-317.
- WERTHEIM B, SEVENSTER JG, EIJS IEM AND VAN ALPHEN JJM. 2000. Species diversity in a mycophagous insect

- community: the case of spatial aggregation vs. resource partitioning. *J Anim Ecol* 69: 335-351.
- WHEELER MR AND KAMBYSELLIS MP. 1966. Notes on the Drosophilidae (Diptera) of Samoa. The University of Texas Publication 6615: 533-565.
- WHEELER MR AND TAKADA H. 1971. Male genitalia of some representative genera of American Drosophilidae. The University of Texas Publication 7103: 225-240.
- WOLFF LF, GOMES GC, RODRIGUES WF, BARBIERI RL, MEDEIROS CAB AND CARDOSO JH. 2008. Flora apícola arbórea nativa na região serrana de Pelotas para a apicultura sustentável na Metade Sul do Rio Grande do Sul, Documentos n. 242. Pelotas: Embrapa Clima Temperado, 37 p. Available from URL: <https://www.embbrapa.br/busca-de-publicacoes/-/publicacao/746892/flora-apicola-arborea-nativa-na-regiao-serrana-de-pelotas-para-a-apicultura-sustentavel-na-metade-sul-do-rio-grande-do-sul>.

embbrapa.br/busca-de-publicacoes/-/publicacao/746892/flora-apicola-arborea-nativa-na-regiao-serrana-de-pelotas-para-a-apicultura-sustentavel-na-metade-sul-do-rio-grande-do-sul.

SUPPLEMENTARY MATERIAL

Appendix S1 (Supporting Information) - Basidiomycetes samples that were positive (gray) and negative (black) for the emergence of Drosophilidae. The numbers under the charts represent the total number of samples collected from each Basidiomycetes genus.