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Diversity of Drosophilidae (Insecta, Diptera) in the Restinga forest of southern Brazil

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ABSTRACT

Although members of Drosophilidae are frequently the topic of ecological studies in Brazil, few have explored Restinga or, until only recently, Pampa biome environments. This study proposes to describe the diversity and temporal variation of the Drosophilidae assemblage from a Restinga forest of Rio Grande do Sul, Brazil. We performed monthly collections from February 2013 to January 2014 using yeasted banana-baited traps. A total of 25,093 individuals of 46 species were sampled. *Drosophila simulans* and the *D. willistoni* subgroup were the dominant taxa; *D. polymorpha*, *D. immigrans*, *D. paraguayensis* and *Zygothrica orbitalis* were of intermediate abundance, and the other 40 species were rare. Based on sampling effort estimators, our collections were sufficient. Jaccard and Morisita indices evaluated using ANOSIM reveal little similarity in the composition of samples across months. Canonical correspondence analysis shows that the variables of maximum and minimum temperature are the main factors responsible for differentiation of the species composition of the assemblage throughout the year, whereby collections in the coldest periods (July, August and September) are those with a more differentiated composition. In these months, the dominance of *D. simulans* and the *D. willistoni* subgroup decreases while increased abundance of the *D. tripunctata* group (as *D. paraguayensis*) and *Z. orbitalis* occurs. In comparison to other studies carried out in environments in southernmost Brazil, we observed a similar pattern of fluctuation in abundance over the year, with a higher abundance of dominant species in warmer months and population sizes decreasing in colder months.

Introduction

Insects play an important role in the diversity of natural habitats and are essential for habitat maintenance (Didham et al., 1996; Samways, 2015). Studies on Drosophilidae, a well-studied Diptera family, have shed light on species coexistence and the relationship between insects and environments (Shorrocks and Rosewell, 1986; Sevenster and Van Alphen, 1993; Yamashita and Hijii, 2003; Mata et al., 2008), noting that drosophilids are sensitive to changes in habitat conditions, with excellent potential as bioindicators (Mata et al., 2008, 2010). Members of Drosophilidae have been studied throughout Brazil, and their assemblages are characteristic of different ecosystems (Martins, 1987; Val and Marques, 1996; Schmitz et al., 2007; Döge et al., 2008; Mata et al., 2008; Bizzo et al., 2010; Rohde et al., 2010; Poppe et al., 2014).

The Brazilian coast is characterized by Restinga forest, a particular ecosystem formed on sandy and nutrient-poor soils that is characterized by heterogeneous vegetation with a strong marine influence. This ecosystem is mainly associated with the Atlantic forest biome and is distributed along the entire Brazilian coast, occupying 80% of the coastal area. The Restinga forest macroclimate fluctuates according to its latitudinal location, being more stable than in field areas but varying more widely than forests with richer soils. The structure and distribution of Restinga forests are a result of depositional features determined by successive changes in sea

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level during the Quaternary period (Lacerda et al., 1982; Coutinho, 2006; Behling et al., 2009; Magnago et al., 2010).

In the state of Rio Grande do Sul, Brazil, the Restinga forest has a particular physiognomy and flora related to the Pampa biome. This ecosystem is conditioned by a temperate climate, which contrasts with the tropical influence that predominates on the northern coast of the state (Waechter, 1985; Bencke, 2009). Recently, areas of Restinga have been used for the development of commercial forests, mainly eucalyptus and pine, which is changing this environment and giving rise to a new structural configuration (Fonseca and Diehl, 2004). Overall, in addition to other negative effects, the loss of any natural habitat can cause a severe decrease in biodiversity, affecting the rate of population growth, reducing the length and diversity of the food chain and changing interactions among species (Forero-Medina and Vieira, 2007).

Different environments of southern Brazil have been characterized in terms of their Drosophilidae fauna. However, most research to date has been conducted in the Atlantic forest, forests and agricultural areas of the Pampa biome and urbanized areas (Valente and Araújo, 1991; Silva et al., 2005; Hochmüller et al., 2010; Garcia et al., 2012; Poppe et al., 2012, 2013). Furthermore, studies of Restinga were performed in Santa Catarina and São Paulo States (Bizzo and Sene, 1982; Bizzo et al., 2010) where the environmental conditions are different due to the Atlantic forest influence. Therefore, the present study sought to report the diversity and temporal variation in Drosophilidae species of Restinga forest in southernmost Brazil, with a goal of joining efforts in characterization of the environments of the Pampa biome. Moreover, investigation of the species richness of the fauna and flora of different environments is a priority measure for studies focused on species conservation and

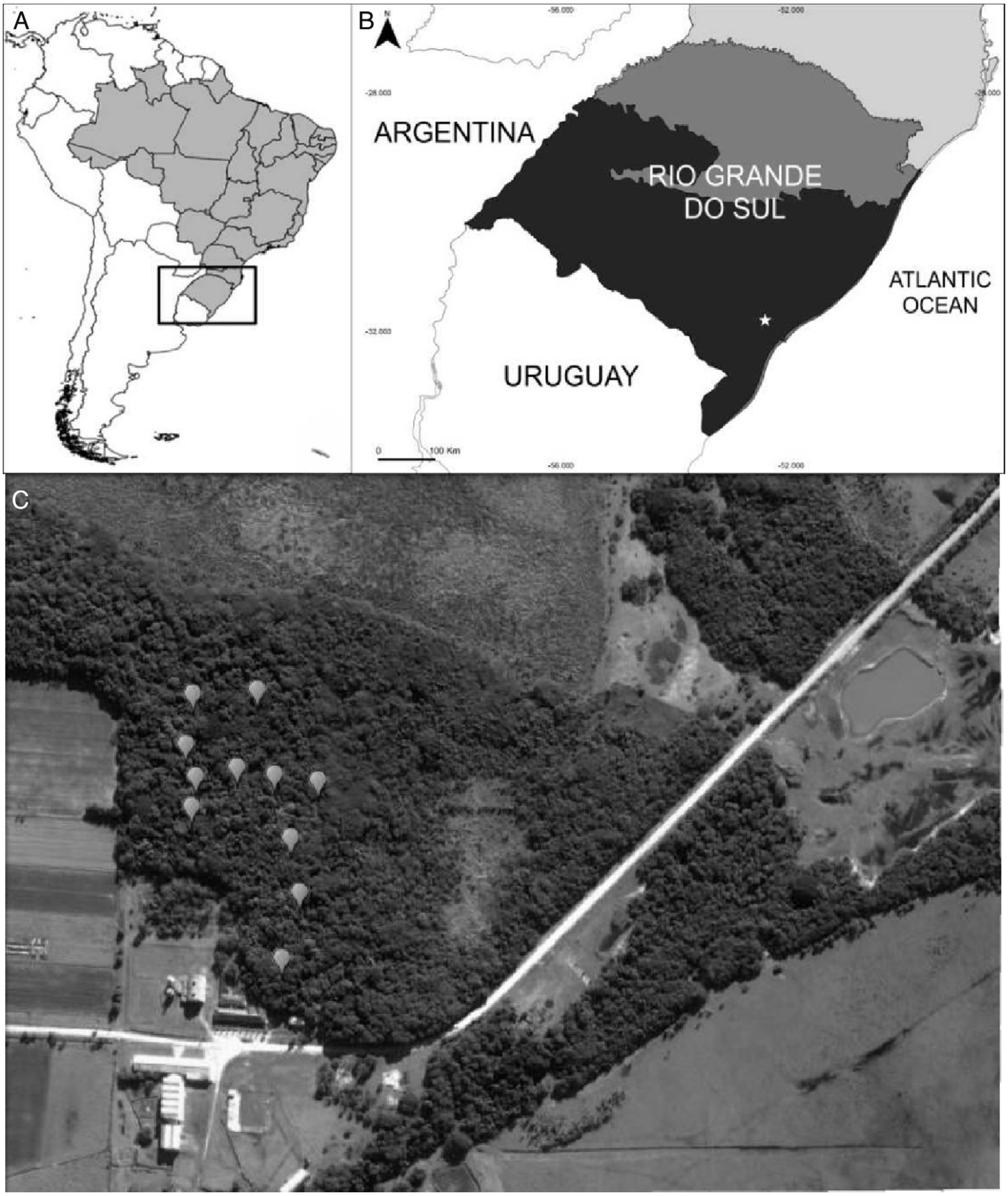

Fig. 1. Studied area. (A) Map of South America, with Brazil in gray and the state of Rio Grande do Sul highlighted. (B) Map of the state of Rio Grande do Sul, highlighting the Horto Botânico Irmão Teodoro Luís (HBITL) with a circle. (C) Satellite image of the Restinga forest of HBITL, with the positions of the traps shown (Google Earth).

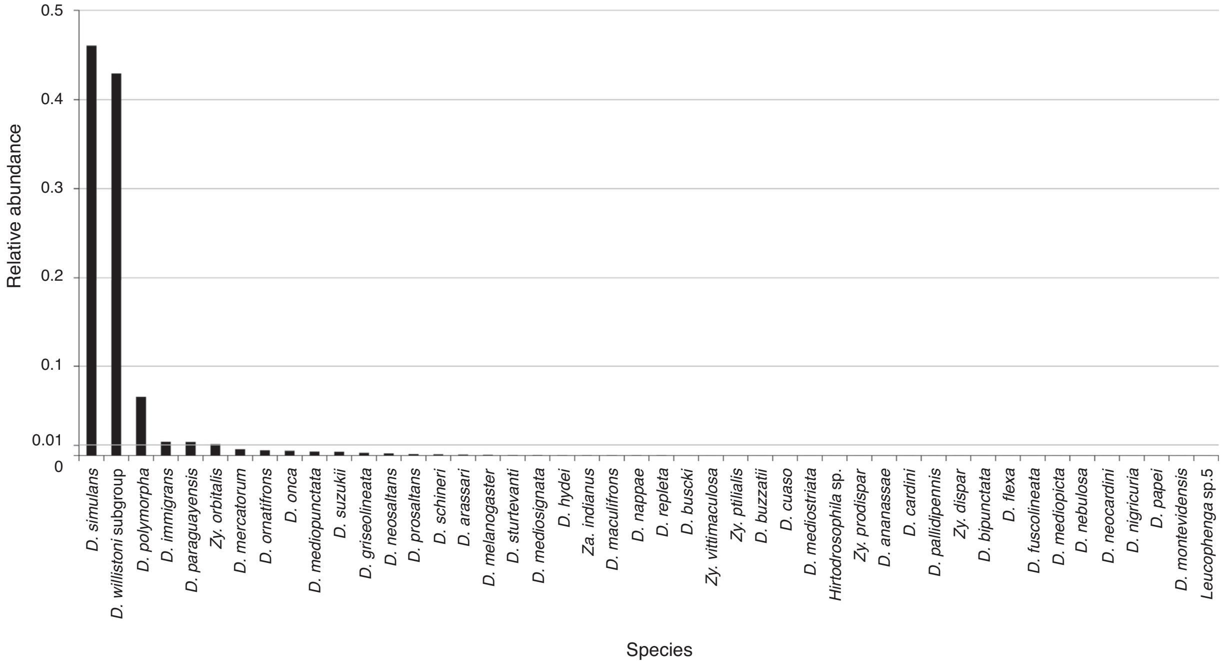

Fig. 2. Rank abundance plot of the relative abundances of Drosophilidae species sampled in the Horto Botânico Irmão Teodoro Luis (HBITL), Brazil, from February 2013 to January 2014.

understanding their temporal dynamics. Accordingly, this study in a Restinga forest with permanent conservation area status will assist in the maintenance of its environmental integrity and support future studies.

Material and methods

Studied area

Horto Botânico Irmão Teodoro Luis (HBITL) is located in the municipality of Capão do Leão, Rio Grande do Sul, Brazil, at 31°47′48″S, 52°15′45″W (Fig. 1A and B). HBITL has been a protected area since 1964, with approximately 25 ha located in the Pampa biome (Guerra et al., 2015). It is composed of a mosaic of Restinga forest surrounded by wetlands and anthropogenic habitats, such as pasture and a few low buildings. The forest consists of different strata: trees, shrubs, and herbaceous plants, with xeromorphic, succulent and thorny vegetation (Rodrigues, 2005). Large fig trees are common, and they stand in the forest canopy. HBITL presents a long history of anthropic interference. Intended for research and academic purposes, it has been under the responsibility of the Universidade Federal de Pelotas since the 1960s.

According to Kottek et al. (2006), the climatic classification of this region is Cfa, presenting a mesothermal and super humid climate with no distinct dry season. The climatological normals recorded in 2013 and 2014 included an average annual temperature of 17.2 °C (the lowest and highest average temperatures were 4.2 °C and 24.8 °C, respectively) and 81.6% relative humidity (the lowest and highest average relative humidities were 75.3% and 85.0%, respectively). The environmental data were collected from *Estação Agroclimatológica de Pelotas* (2015) at 31°52′00″S, 52°21′24″W.

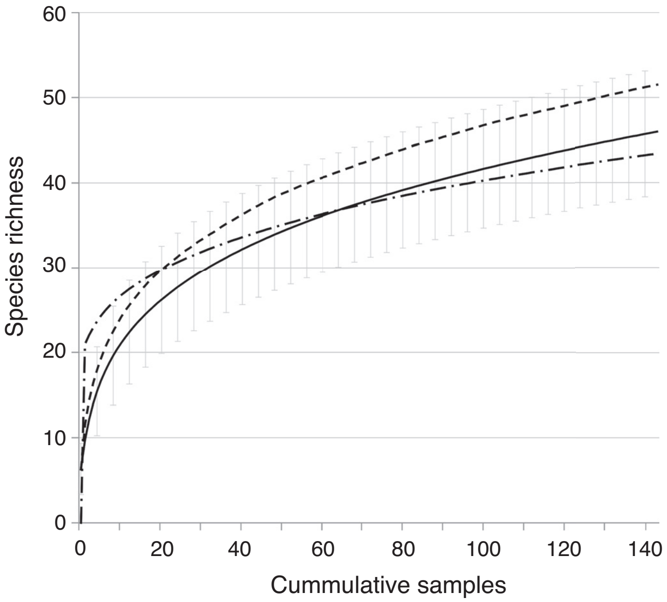

Fig. 3. Randomized accumulated curve of observed species (solid lines, bars are 95% confidence intervals) and bootstrap (dashed line) and Michaelis–Menten (dash-dotted lines) richness estimators constructed with Drosophilidae species sampled in the Horto Botânico Irmão Teodoro Luis (HBITL), Brazil, from February 2013 to January 2014.

Specimen sampling and identification

From February 2013 to January 2014, specimens were collected monthly using 12 traps constructed according to Tidon and Sene (1988) and baited with approximately 150 g of banana and 1.5 g of dry yeast. The traps were tied to trees at a height of approximately 1.5 m and distanced at 60 m from one another (Fig. 1C). The traps were kept in the field for 3 days.

The collected specimens were preserved in 70% ethanol and identified based on their external morphology according to the current literature. Male terminalia of sibling species were dissected according Bächli et al. (2004) for species-level identification. Females of sibling species were identified by external morphology and, when possible, the species level was determined according to the quantities of males in each trap for analysis purposes. Vouchers were deposited in the Drosophilidae collection of the *Museu de Ciências Naturais Carlos Ritter* (MCNCR).

Data analysis

To characterize the assemblage, we used the absolute and relative abundances of each species (n_i and p_i, respectively) and the species richness (number of species in the sample, S). We verified the spatial autocorrelation of the species composition among the traps in each sampling event using Mantel tests with Jaccard and Morisita indices. The significance level was calculated with 999 permutations. To perform the tests, geographic coordinates were determined for the position of each trap. The distances between traps were calculated based on the Euclidean distance. These analyses were conducted in the R program version 3.1.2 (R Core Team, 2013) with the ade4 package (Ecological Data Analysis, 2015). As we did not observe statistically significant correlations between the distances and assemblage compositions in any of the months studied (Supplementary material 1), we used the composition of individual traps as the statistical unit for subsequent analyses (unless otherwise stated).

We generated a rank-abundance curve to evaluate dominance in the assemblage based on the total relative abundance of species in all samples, and we constructed a randomized accumulation curve of the observed species with 95% confidence intervals using EstimateS v.8.0 software (Colwell, 2006). Bootstrap and Michaelis–Menten species richness estimators were used to verify the sampling effort.

To evaluate the influence of temporal variation on the assemblage over the sampling period, we conducted canonical correspondence analysis (CCA) using the absolute abundance of species collected in three or more samples or $n_i > 10$ and the climatic variables maximum temperature (TM), minimum temperature (Tm), relative humidity (RH) and precipitation (PR) as independent variables (Supplementary material 2). This analysis was conducted using PAST 3.0 software (Hammer et al., 2001). For the statistical analysis, we used the average values of each environmental variable over a period of 5 days, 3 days with the traps in the field and 2 days prior to the collections. The environmental data were standardized ($Z_i = (X_i - \bar{X})/S$, where X_i is the value of the environmental variable for sample i, S is the standard deviation of the samples, and $\bar{X}$ is the average of the samples).

Finally, we conducted Spearman correlation analysis between the total abundance and species richness in each month and the environmental variables. We used Bonferroni correction to minimize type I error. The tests were conducted using PAST 3.0 software.

Results

A total of 25,093 individuals, belonging to 46 species in five genera of Drosophilidae, were collected and identified, including the first record of *D. neosaltans* for the state of Rio Grande do Sul. Twelve females were identified as species groups or subgroups and were not included in the statistical analyses. Table 1 presents the absolute and relative abundances of the specimens identified.

Table 1
Abundances of Drosophilidae species sampled from February 2013 to January 2014 in the Horto Botânico Irmão Teodoro Luís (HBITL), Brazil.

Species	n_i	p_i
Drosophila genus		
Dorsilopha subgenus		
D. busckii group		
D. busckii Coquillett, 1901	5	[a]
Drosophila subgenus		
D. annulimana group		
D. arassari Cunha and Pavan, 1947	27	0.001
D. schineri Pereira and Vilela, 1987	32	0.001
D. cardini group		
D. cardini Sturtevant, 1916	2	[a]
D. neocardini Streisinger, 1946	1	[a]
D. polymorpha Dobzhansky and Pavan, 1943	1587	0.063
D. coffeata group		
D. fuscolineata Duda, 1925	1	[a]
D. guarani group		
D. griseolineata Duda, 1927	74	0.003
D. maculifrons Duda, 1927	9	[a]
D. ornatifrons Duda, 1927	142	0.005
D. immigrans group		
D. immigrans Sturtevant, 1921	374	0.015
D. pallidipennis group		
D. pallidipennis Dobzhansky and Pavan, 1943	2	[a]
D. repleta group		
D. buzzatii Patterson and Wheeler, 1942	3	[a]
D. hydei Sturtevant, 1921	10	[a]
D. mercatorum Patterson and Wheeler, 1942	170	0.007
D. nigricuria Patterson and Mainland, 1943	1	[a]
D. onca Dobzhansky and Pavan, 1943	129	0.005
D. papei Bächli and Vilela, 2002	1	[a]
D. repleta Wollaston, 1958	7	[a]
D. tripunctata group		
D. bipunctata Patterson and Mainland, 1943	1	[a]
D. cuaso Bächli, Vilela and Ratcov, 2000	3	[a]
D. mediopicta Frota-Pessoa, 1954	1	[a]
D. mediopunctata Dobzhansky and Pavan, 1943	107	0.004
D. mediosignata Dobzhansky and Pavan, 1943	13	[a]
D. mediostriata Duda, 1925	3	[a]
D. nappae Vilela, Valente and Basso-da-Silva, 2004	8	[a]
D. paraguayensis Duda, 1927	370	0.015
D. montevidensis Goñi and Vilela, 2016	1	[a]
Siphlodora subgenus		
D. flexa Loew, 1866	1	[a]
Sophophora subgenus		
D. melanogaster group		
D. ananassae Doleschall, 1858	2	[a]
D. melanogaster Meigen, 1830	23	0.001
D. simulans Sturtevant, 1919	11,090	0.442
D. suzukii Matsumura, 1931	100	0.004
D. saltans group		
D. neosaltans Pavan and Magalhães, 1950	57	0.002
D. prosaltans Duda, 1927	36	0.002
D. sturtevanti Duda, 1927	16	[a]
D. willistoni group		
D. nebulosa Sturtevant, 1916	1	[a]
D. willistoni subgroup	10,336	0.413
Hirtodrosophila genus		
Hirtodrosophila sp.	3	[a]
Leucophenga genus		
Leucophenga sp.5	1	[a]
Zaprionus genus		
Za. vittiger group		
Za. indianus Gupta, 1970	10	[a]
Zygothrica genus		
Zy. ptilialis Burla, 1956	4	[a]
Zy. dispar group		
Zy. dispar (Wiedemann, 1830)	2	[a]
Zy. prodispar Duda, 1925	3	[a]
Zy. orbitalis group		
Zy. orbitalis (Sturtevant, 1916)	307	0.012
Zy. vittimaculosa group		
Zy. vittimaculosa Burla, 1956	5	[a]
Total	25,081	

n_i, absolute abundance; p_i, relative abundances.

[a] $p_i < 0.001$.

After ranking species based on their relative abundances, we observed two dominant taxa in the assemblage, with $p_i > 0.10$:

exotic *D. simulans* and the Neotropical *D. willistoni* subgroup (Fig. 2). The intermediary species, at $0.10 \geq p_i > 0.01$, were *D. polymorpha*, *D. immigrans*, *D. paraguayensis* and *Zygothrica orbitalis*. The remaining 40 species, at $p_i \leq 0.01$, were rare. Among the 46 species sampled, 12 were singletons (just one specimen collected) and four doubletons (only two specimens collected). Thus, 40% of the species were occasionally or poorly sampled. Fig. 3 shows the randomized accumulation curve of the observed species (S_{obs}) with 95% confidence intervals and the richness curves based on bootstrap and Michaelis–Menten estimators. The bootstrap estimator predicted 51 species, close to the species richness values observed. This estimator curve is entirely within the range of the 95% confidence intervals of the S_{obs} curve, exhibiting high similarity between them. Furthermore, the Michaelis–Menten estimator showed a lower richness than S_{obs}, and the meeting point of the curves indicated the inflection point of the S_{obs} curve (i.e., the point where the species accumulation rate decreases). These results suggest that we collected the majority of the species in this location with this type of baited trap.

Among the four climatic variables considered in our analysis, i.e., the maximum (TM) and minimum temperatures (Tm), relative humidity (RH) and precipitation (PR), TM and Tm had the most influence on Drosophilidae species over the studied year. These variables and PR were highly related to axis 1 of CCA, which explains 52.7% of the variation in the data (Fig. 4). Axis 2, corresponding to 43.5% of the variation in the data, was equally related to TM, Tm and RH. In the CCA plot, February, March, April, May, June, October, November and December 2013 and January 2014 occurred close to the intersection of the axes, where *D. simulans*, the *D. willistoni* subgroup and *D. polymorpha* were the dominant species and the abundance of *D. neosaltans*, *D. prosaltans* and *D. suzukii* increased (Fig. 4). The first three species were present in all months, but their abundances also decreased in July, August and September, when the temperatures decreased (Fig. 5). In months with lower temperatures, i.e., July and August, *Zy. orbitalis* was the dominant species, and the abundance of *D. mediosignata*, *D. mediopunctata*, *D. melanogaster*, *D. paraguayensis*, *D. griseolineata*, *D. arassari* and *D. hydei* increased. Other species of *Zygothrica* were also sampled in August, totaling 226 individuals. In contrast, *D. immigrans*, *D. mercatorum*, *D. repleta*, *D. nappae*, *D. sturtevanti*, *D. ornatifrons*, *D. schineri*, *D. onca*, *D. mediostriata* and *D. maculifrons* did not show marked variation in abundance during the sampled year. Precipitation (PR) had little influence on species composition in this study.

When we analyzed the monthly variation in terms of the absolute abundance (Fig. 6A) and species richness (Fig. 6B) of Drosophilidae, we observed a high absolute abundance in warmer months. The same pattern was not found for species richness. Using the Spearman test, we correlated absolute abundance with climatic variables and observed abundance to be positively correlated with TM ($R = 0.58$, $p = 0.048$) and Tm ($R = 0.71$, $p = 0.01$) (Fig. 7), whereas absolute abundance was not correlated with the other two variables (RH: $R = -0.15$, $p = 0.63$; and PR: $R = -0.46$, $p = 0.14$). Additionally, species richness showed no correlation with any climatic variable (TM: $R = -0.03$, $p = 0.92$; Tm: $R = -0.12$, $p = 0.70$; RH: $R = -0.38$, $p = 0.22$; and PR: $R = -0.57$, $p = 0.053$), though the pattern of species dominance changed in the coldest months, as observed in the CCA plot.

Discussion

Southern expansion of the distribution of collected species

The new record of *D. neosaltans* expands its geographic distribution limit to the latitude of 31°47′48″S (Silva et al., 2005; Gottschalk et al., 2008, 2009; Garcia et al., 2012; Poppe et al., 2012, 2014; Valer et al., 2013; Deprá et al., 2014; Hochmüller et al., 2010). Before our survey, the southernmost record of *D. neosaltans* was in Florianópolis, SC (27°35′49″S, 48°32′58″W), and the species was recorded more often in forested areas of the Atlantic forest biome (De Toni et al., 2007; Gottschalk et al., 2007; Döge et al., 2008). In addition, the limits of the distributions of *D. cuaso*, *D. fuscolineata*, *D. flexa*, *D. maculifrons*, *D. nigricuria* and *D. papei* have been expanded further south. These species, which are rare in samples from different studies, were previously recorded in the northern region of the state of Rio Grande do Sul (Gottschalk et al., 2009; Hochmüller et al., 2010; Garcia et al., 2012; Poppe et al., 2012, 2014; Valer et al., 2013, 2016; Deprá et al., 2014).

The Drosophilidae assemblage of HBITL

Of the five genera sampled in our study, *Drosophila* was the richest. Because this genus frequently uses decaying fruits, a large number of species is expected when sampling is conducted using yeast and banana baits (reviewed in Gottschalk et al., 2008). We also sampled one species each of the genera *Hirtodrosophila* and *Leucophenga*, in addition to five species of *Zygothrica*, which are commonly found in mushrooms, where they feed, lay eggs, rear larvae and/or carry out sexual courtship (Grimaldi, 1987; Val and Kaneshiro, 1988; Courtney et al., 1990; Valer et al., 2016). The capture of species of these genera with banana baits typically occurs sporadically. Finally, the Drosophilidae assemblage also included the genus *Zaprionus*, represented by one exotic species, *Za. indianus*, which is frequently caught by banana traps.

We found seven exotic Drosophilidae species in HBITL, *D. ananassae*, *D. busckii*, *D. immigrans*, *D. melanogaster*, *D. simulans*, *D. suzukii* and *Za. indianus*, amounting to 11,604 individuals, which is slightly lower than the abundance of native species (13,488 individuals). The presence of exotic species has been observed in areas with different anthropization levels (Ferreira and Tidon, 2005; De Toni et al., 2007; Gottschalk et al., 2007; Garcia et al., 2008). Although HBITL is currently a protected area and therefore limited to research, the presence of exotic species can nonetheless be associated with anthropic action in HBITL and its surroundings in recent decades. In addition, we sampled a large number of *D. suzukii*, an invasive species recently introduced to South America; this species was first recorded in Brazil in February 2013 in the southern region (Deprá et al., 2014). This species was sampled in October 2013 at HBITL, located approximately 550 km from the site of its first record.

The species sampled in our study represent approximately 40% of the Drosophilidae species recorded in the Pampa biome (Poppe et al., 2016). The species richness observed in HBITL corroborates other studies of Drosophilidae diversity using the same sampling method in different areas of the Pampa and Atlantic forest biomes of the state of RS. These studies recorded 26–53 species, with *Drosophila* being the most abundant and specious genus (Hochmüller et al., 2010; Garcia et al., 2012; Poppe et al., 2012, 2014).

Our sampled assemblage was characterized by the dominance of *D. simulans* and the *D. willistoni* subgroup. Despite the fact that it is an exotic species, *D. simulans* is frequently found in different habitats in the Neotropical region (Sene et al., 1980; Torres and Madi-Ravazzi, 2006; Bizzo et al., 2010; Schmitz et al., 2010; Garcia et al., 2012), and a high abundance of this species has also been verified in areas of the Pampa biome (Hochmüller et al., 2010; Poppe et al., 2012), as well as in urbanized areas (Gottschalk et al., 2007; Garcia et al., 2012). The occurrence of the *D. willistoni* subgroup as the second most abundant taxon disagrees with the results of other studies from the Pampa biome (Hochmüller et al., 2010; Poppe et al., 2012), where it was uncommon or rare, most likely because other studies in the Pampa biome were conducted in areas of grassland vegetation or more anthropized environments. In contrast, the

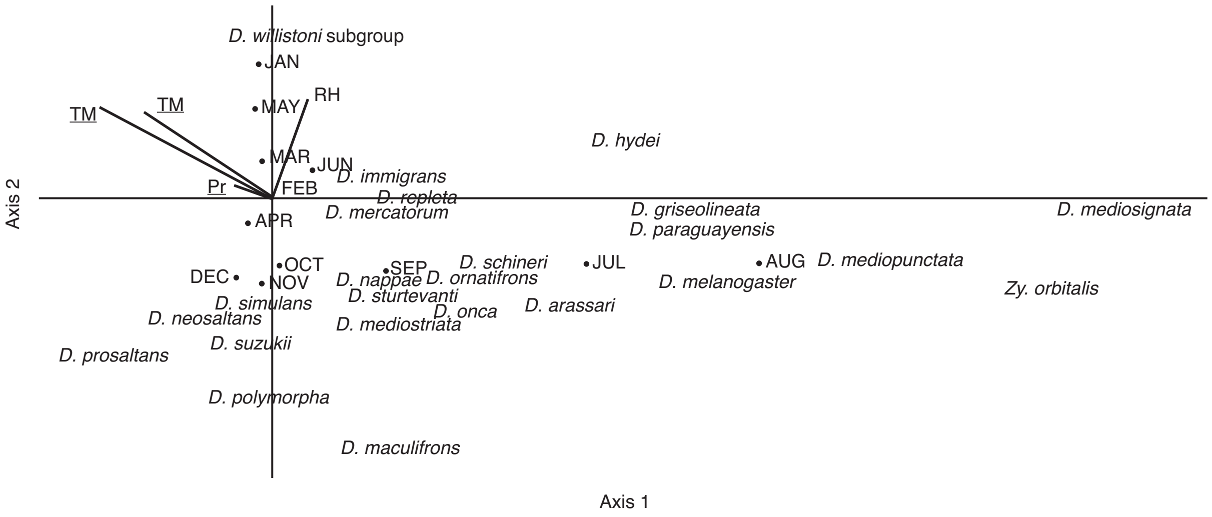

Fig. 4. Canonical correspondence analysis axes 1 and 2, showing the organization of species according to environmental variables (TM, maximum temperature; Tm, minimum temperature; RH, relative humidity; PR, precipitation) from February 2013 to January 2014.

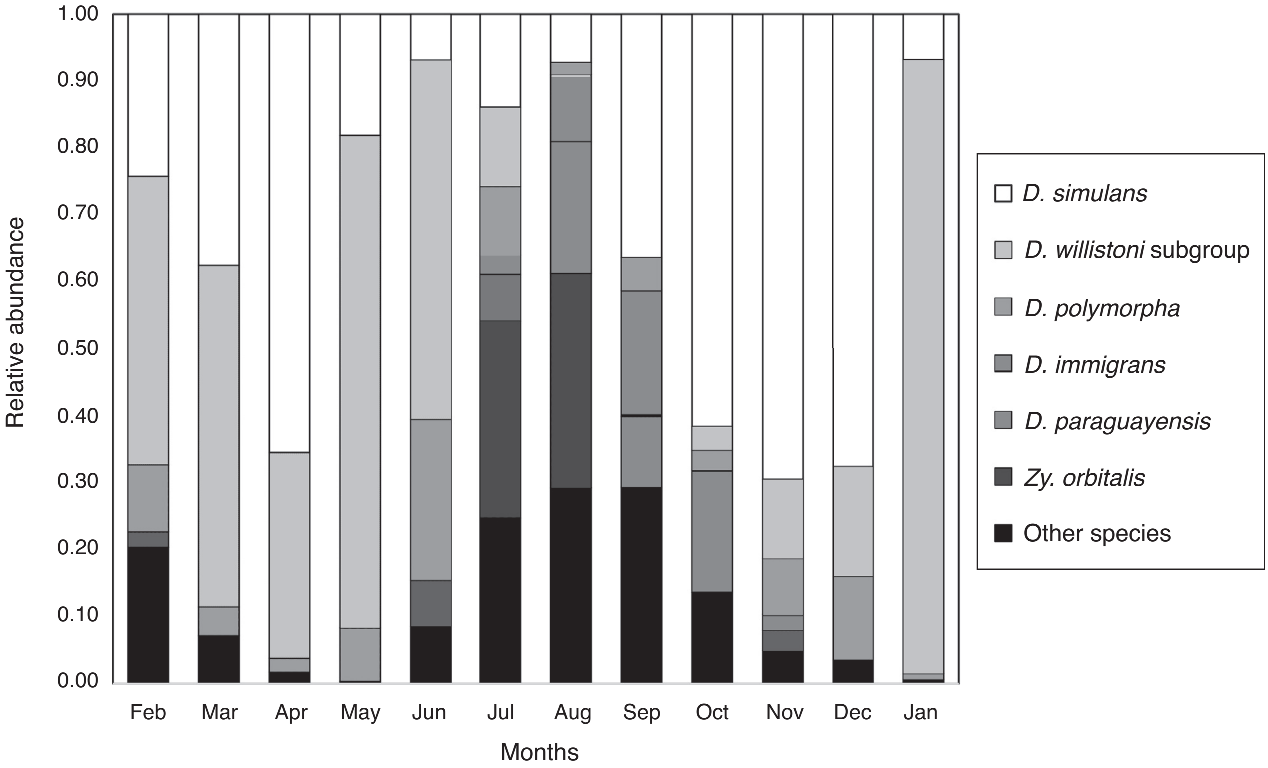

Fig. 5. Monthly relative abundance of species from February 2013 to January 2014 in the Horto Botânico Irmão Teodoro Luís (HBITL), Brazil.

D. willistoni subgroup has been well recorded in natural environments (Martins, 1987; Saavedra et al., 1995), though its abundance decreases in urbanized areas (Gottschalk et al., 2007; Hochmüller et al., 2010; Poppe et al., 2012).

Abiotic variables

Corroborating the literature to date, the studied Drosophilidae assemblage responds to climatic variation (Martins, 1987; Mata et al., 2008; Rohde et al., 2010). In temperate regions with four well-marked seasons, abundance of specimens is high in warm months but with low diversity, whereas abundance is low in cold months but with high richness (Petersen, 1960; Araújo and Valente, 1981; Franck and Valente, 1985; Saavedra et al., 1995; De Toni et al., 2007; Gottschalk et al., 2007; Poppe et al., 2013). Of the climatic variables, the maximum and minimum temperatures were those that most influenced the assemblage, with the minimum temperature being the most determinant in terms of species composition, as described in other studies conducted in different environments (Torres and Madi-Ravazzi, 2006; Bizzo et al., 2010; Poppe et al., 2013). In our study, 14 species exhibited well-marked seasonality, with abundance variation largely related to temperature changes (as evidenced by CCA). However, in the area studied by Torres and Madi-Ravazzi (2006), a positive correlation between species richness and rainfall was described. Associations between Drosophilidae assemblages and rainfall were also

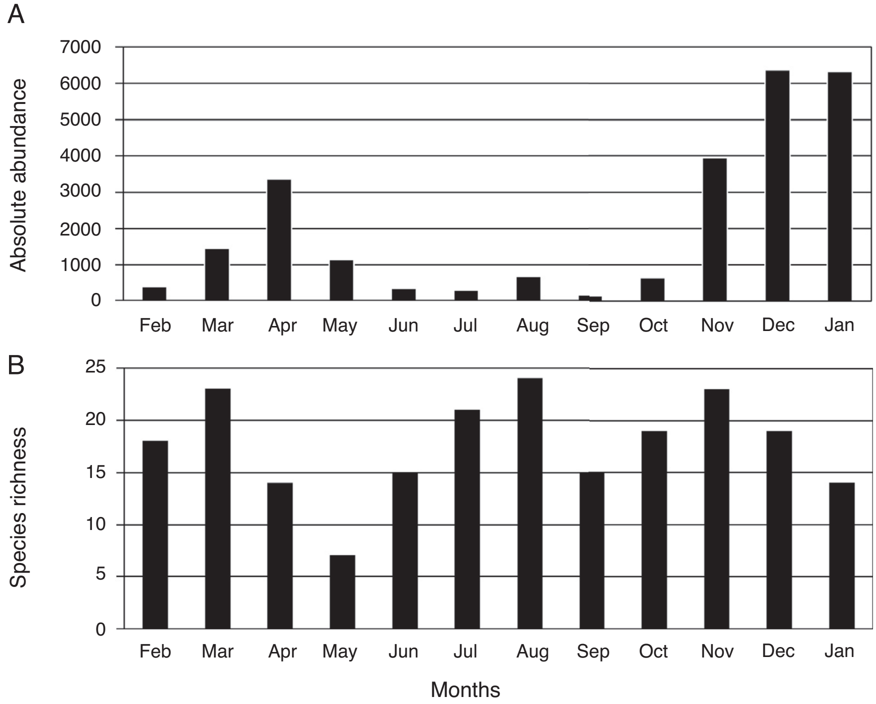

Fig. 6. Absolute abundance (A) and absolute richness of species (B) from February 2013 to January 2014 in the Horto Botânico Irmão Teodoro Luís (HBITL), Brazil.

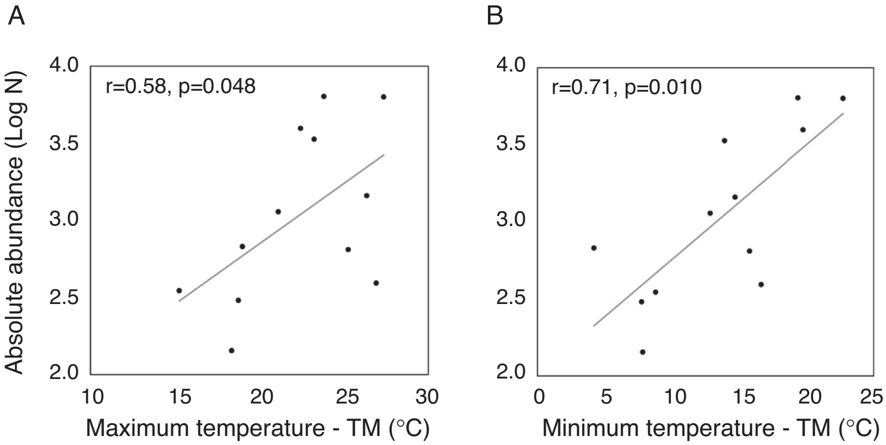

Fig. 7. Correlation analyses between the absolute abundance of species and the maximum and minimum temperatures.

stronger in studies performed in the Brazilian Cerrado, where the annual fluctuation in temperature year is smaller and the years are characterized by two seasons, one wet and one dry (Mata et al., 2008). According to Dobzhansky and Pavan (1950), precipitation should be more important for *Drosophila* species abundance because precipitation is essential for the flowering and fruiting of plants used as a trophic resource for Drosophilidae. In the HBITL area, precipitation has a secondary influence on the assemblage composition of Drosophilidae species (Fig. 4), most likely because fluctuation in this abiotic variable is less marked in southern Brazil.

Only three taxa were sampled during all 12 months of collection: *D. simulans*, *D. polymorpha* and the *D. willistoni* subgroup. These taxa show fluctuation in their abundance according to climatic variation. *Drosophila simulans* was dominant in April, November and December 2013, whereas the *D. willistoni* subgroup and *D. polymorpha* were dominant in January 2014, with the *D. willistoni* subgroup reaching an abundance that was greater than 90% of the total in that month. This pattern demonstrated that the dominant species were most abundant during warmer months, with an average temperature of 25 °C in April, November, and December of 2013 and January of 2014.

Zygothrica orbitalis was the dominant species in July and August 2013, when the maximum and minimum temperatures were low; for this species specifically, it is assumed that a lack of trophic resources for imagoes (i.e., mushrooms, Valer et al., 2016) causes them to visit the bait used. This fact, associated with the low abundance of the dominant *Drosophila* species, makes *Zy. orbitalis* the dominant species in August. In a recent study by our group, we sampled *Zy. orbitalis* emerging profusely from the fruits of *Psychotria* sp. (Rubiaceae, Magnoliophyta), a common bush present in HBITL (Mayara Ferreira Mendes, unpublished data), which may indicate that this fly species is attracted to these fruits for oviposition. When sampling in an area with a similar climate, Garcia et al. (2012) verified the same pattern in winter, with substitution of the dominant species in warmer times.

Drosophilidae of the Restinga forest

In Brazil, two studies focusing on Drosophilidae from Restinga environments have been performed (Bizzo and Sene, 1982; Bizzo et al., 2010). The areas sampled by Bizzo and Sene (1982) and Bizzo et al. (2010) have comparatively sparser vegetation and a greater marine influence (Falkenberg, 1999; Martins et al., 2008). Moreover, due to latitudinal differences, HBITL has a lower average temperature in the coldest month (approximately 4 °C) than the areas studied by Bizzo and Sene (1982) in São Paulo (46°56′W; 24°14′S) and by Bizzo et al. (2010) in Santa Catarina (48°27′49″W; 27°38′21″S), with averages in the coldest month of approximately 18 °C. Nonetheless, the average temperature in the warmest month is the same in the areas covered by these three studies (approximately 25 °C). Therefore, few similarities in species composition were observed when compared with our data, whereby the dominant species, with the exception of *D. simulans* and the *D. willistoni* subgroup, were different from Bizzo and Sene (1982) and Bizzo et al. (2010). In the Restinga forest of São Paulo, the *D. cardini* group (non-*D. polymorpha*) and *D. sturtevanti* were common, and *D. malerkotliana* was uncommon; in Restinga of Santa Catarina, *Za. indianus* and *D. fumipennis* were common and *D. nebulosa* was uncommon.

Conclusion

Foremost, our study increases knowledge about Drosophilidae fauna in the Restinga forest of the Pampa biome. The assemblage species richness observed and the record of *D. neosaltans* for the state of Rio Grande do Sul suggest that HBITL is an important area for the maintenance of the species of this family in this ecosystem. Temperature was found to be the predominant influence on the assemblage; *D. simulans* and the *D. willistoni* subgroup display positive relationships with this climatic variable, whereas this relationship is negative for other species. Due to differences in latitude and physiognomy, the Drosophilidae taxa observed were distinct from those of Restinga forests in northern areas.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rbe.2017.05.002.

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