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Assemblage of drosophilids (Diptera, Drosophilidae) inhabiting flooded and nonflooded areas in the extreme South of Brazil

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ABSTRACT

Several studies on the potential use of drosophilid assemblages as bioindicator systems have been carried out in the last years. Nevertheless, the successful application of these organisms in these systems requires adequate filling of several knowledge gaps. In this sense, little is known about drosophilid assemblages in wetlands and flooded areas. The present study provides the first survey of drosophilid species inhabiting such environments in the extreme South of Brazil and compares general beta-diversity patterns between assemblages of flooded versus nonflooded areas. The specimens were collected with banana-baited traps, and the assemblages recovered in eight wetlands of the southernmost coast of Brazil were compared to those recovered from seven nonflooded areas of the Pampa and Atlantic Forest biomes. A total of 5028 and 2571 individuals encompassing 27 and 37 species were collected in the flooded and nonflooded areas, respectively. The differential species composition patterns presented between these areas was statistically supported, which seems to be related to the lower beta-diversity presented by swamps, especially in regard to dominance patterns. So, the open and climatically harsher environment provided by wetlands possibly constitutes a hostile environment for the entry and, mainly, for the persistence of several native Drosophilidae species, in contrast to some exotic and more plastic species (as *Drosophila simulans* and *Zaprionus indianus*). Since the diversity gradient of flooded areas does not seem to be related to the conservation status of the swamp, our results question the use of Drosophilidae species as bioindicators of environmental disturbance and antropic influence in wetlands.

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Introduction

According to Keddy (2010), wetlands are ecosystems of transition between aquatic and terrestrial environments, whose biota is particularly adapted to flooded conditions. These areas may support significant parts of current biodiversity, serving as habitat even for predominantly terrestrial groups, like Insects. For Diptera, for example, aquatic or semi-aquatic larval stages are encountered in at least 32 of the 155 families (Williams and Feltmate, 1992; Sundermann et al., 2007). In fact, dipteran species are important components of these ecosystems, where they act on the energy flow and nutrient cycling, serving also as bioindicators of water quality (Moulton, 1998; Sankarperumal and Pandian, 1992).

Drosophilidae is one of the most diverse and widely distributed dipteran families, encompassing more than 4300 species (Bächli, 2016), encountered throughout different environments above sea level, with the exception of Polar Regions (Throckmorton, 1975). Almost all species in this family present terrestrial life cycles, without any aquatic or semi-aquatic stage across development (Tsacas and Legrand, 1979). Nevertheless, different species present different ecological requirements, and some Drosophilidae species are specially adapted to wet conditions, as observed by Robe et al. (2014) and Freitas (2017) with correlational niche inference methods or by Kellermann et al. (2012a,b) with experimental assessments of physiological resistance. Moreover, according to Oldroyd (1964), even so-called terrestrial species require “damp” micro-habitats.

Although the occurrence of Drosophilidae has already been recorded in freshwater wetlands (Keiper et al., 2002), the composition of their assemblages in these habitats is still poorly understood. Until now, few species were registered in these environments,

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Table 1
List of sampling points, with their respective coordinates and sampling dates.

Code	Sampling cities	Sampling date	Latitude	Longitude
1	Taim (TA)	28/12/2015	−32.49	−52.58
2	APP Estrada Velha (EV)	20/04/2016	−32.12	−52.15
3	APA Lagoa Verde (LV)	25/11/2015	−32.16	−52.18
4	Lago dos Biguás – FURG (LB)	20/04/2016	−32.07	−52.16
5	Ilha dos Marinheiros (IM)	21/12/2015	−31.99	−52.15
6	Osório (OS)	22/11/2016	−29.97	−50.23
7	Três Cachoeiras – Poço (TCPA)	23/11/2016	−29.44	−50.05
8	Três Cachoeiras – Ponte do Poço (TCPPA)	23/11/2016	−29.44	−50.05
9	Santa Maria (SM)	24/08/2010	−29.68	−53.81
10	São Nicolau (SN)	08/09/2010	−28.21	−55.24
11	Pelotas (PEL)	26/08/2011	−31.76	−52.33
12	Canguçu (CAN)	26/08/2011	−31.39	−52.67
13	Horizontina (HOR)	13/10/2010	−27.62	−54.31
14	Cruz Alta (CA)	28/05/2011	−28.64	−53.6
15	Chapecó (CHAP)	30/08/2010	−27.09	−52.61

Notes: Points 1–8 represent flooded swamps, whereas points 9–15 represent non-flooded woods.

among which we may highlight: *Drosophila quinaria* Loew (1866) and *D. tripunctata* Loew (1862), which emerged from larvae eating *Symplocarpus foetidus* (L.) Salisb. ex W.P.C. Barton (1817) in decomposition (Grimaldi and Jaenike, 1983); *D. deflecta* Malloch (1924) in *Nuphar lutea* (L.) Sm. (1809); and *D. palustris* Spencer (1942) and *D. subpalustris* Spencer (1942), which feed on decaying leaves and twigs (Keiper et al., 2002). Another species discovered in wetlands is *D. aguape* Val and Marques (1996), collected from *Eichhornia azurea* (Sw.) Kunth (1843) flowers collected along the Paraguay River, in the Brazilian Pantanal Biome, state of Mato Grosso (Val and Marques, 1996). This apparent low diversity in limnic wetlands contrasts with that found in mangroves (Schmitz et al., 2010), where at least 69 species have been recorded with the use of banana baited traps. However, most species observed by Schmitz et al. (2010) are probably transient in mangroves, since the authors did not find rearing sites in this environment. So, it is possible that this wide disagreement in putative richness patterns is an outcome of the use of different sampling strategies.

Considering the increasing degradation of wetlands and flooded areas (Finlayson, 2007), the search for an understanding of the ecological patterns and processes operating in these areas persists. So, this study aims to provide a first step toward a better knowledge of the presence and ecological influence of Drosophilidae species in wetlands and flooded areas, supplying an inventory of the assemblages of drosophilids inhabiting such environments in the extreme South of Brazil and comparing beta-diversity patterns between flooded versus nonflooded areas.

Materials and methods

Study area

Drosophilids were collected between 2010 and 2016 along different flooded or nonflooded areas located in the extreme South of Brazil. This region is especially profitable for such studies since it presents almost 39% of its surface covered by lakes and lagoons (Schwarzbold and Schäfer, 1984), whose water regime depends exclusively on the pluviometric regime (Vieira, 1983). Sampling in wetlands were performed in eight flooded areas distributed along the Rio Grande do Sul (RS) Coastal Plain, in the municipalities of Rio Grande, Osório and Três Cachoeiras (Table 1, Supplementary Material – Fig. S1). These areas were characterized as swamps in virtue of the dominance of trees that are rooted in hydric soils, but not in peat (Keddy, 2010), including tropical mangroves swamps and bottomland forests in floodplains. Nevertheless, the eight swamp sampling points covered a heterogeneous set of environments, with different kinds or levels of anthropic influences:

(1) Ecological Station of Taim (TA), in Rio Grande – RS, a flooded area of more than 34,000 hectares within the Pampa Biome that encompasses a large area of protected swamps (Gomes et al., 1987), sheltering several animal and plant taxa, including some endangered species. Nevertheless, the ecological station is still impacted by livestock farming and forestry, which is related to the use of agrochemicals. The sampled area was near a forestry with *Pinus* plantations, used for the extraction of wood.
(2) Environmental Protection Area of Estrada Velha (EV), in Rio Grande – RS, encompassing 18 hectares of woods within the Pampa Biome that are periodically flooded. This area is impacted by atmospheric pollution related to the vehicle flow of BR392 and to nearby industries and also by cattle rearing and agricultural practices.
(3) Environmental Protection Area of Lagoa Verde (LV), in Rio Grande – RS, that covers 510 hectares of protected area inside the urban portion of Rio Grande, in the Pampa Biome. This area is impacted by housing anthropic presence and by the atmospheric pollution from the highway located in the vicinity.
(4) The Biguás Lake (LB), located inside the *Campus* of Universidade Federal do Rio Grande (FURG), in an open area of the Pampa Biome within Rio Grande – RS. The lake has an extension of approximately 1.5 ha and a maximum of 2 m of depth, being periodically enriched by organic matter from domestic effluents that contributes to the outcrop of aquatic macrophytes and phytoplankton, alternating the trophic state of the lake between eutrophy and hypereutrophy.
(5) Ilha dos Marinheiros (IM), in Rio Grande – RS, an island of 39 km^2, located at the Southern margin of the Patos Lagoon, in the Pampa Biome, which embraces a swamp with less than 1 m depth. It is mostly explored by agricultural practices, and, as a consequence, is impacted by the use of agrochemicals.
(6) A particular property, in Osório (OS) – RS, located between two lakes (Emboaba and Emboabinha), in the transition between the Atlantic Forest and the Pampa Biomes. It is mostly impacted by the breeding of domestic animals, and by the inadequate disposal of materials near the lakes.
(7) Waterfall Poço das Andorinhas (TCPA), in Três Cachoeiras – RS, characterized by a natural and locally conserved landscape of the Atlantic Forest, with low anthropic interference, mainly related to touristic practices.
(8) Bridge of the Well (TCPPA), in Três Cachoeiras – RS, located approximately 7 km of the waterfall Poço das Andorinhas (point 7), in the Atlantic Forest Biome. It is crossed by a local road, with low vehicle flow.

Otherwise, sampling in nonflooded environments encompassed mainly wooded areas, distributed along the interior of the state of Rio Grande do Sul and the South of Santa Catarina (SC), in the municipalities of Santa Maria, São Nicolau, Pelotas, Canguçu, Horizontina, Cruz Alta and Chapecó (Table 1, Supplementary Material – Fig. S1):

(9) A fragment of Atlantic Forest, located in Santa Maria – RS (SM), distant about 6 km from the urban area of the municipality, near the transition to the Pampa Biome. The edge of this fragment is located near a highway with intense vehicle flow and there are housing units in the vicinity.
(10) A wood in São Nicolau – RS (SN), characterized as a transitional area between the Atlantic Forest and Pampa Biomes, whose edges are bathed by a small stream, distant about 1 km from the urban area of the municipality. In the immediate vicinity

of the collection point, the environment is visibly degraded by anthropic action, mostly by agricultural practices.

(11) A restinga forest of the Pampa Biome, located in Pelotas – RS (PEL), within the Horto Botânico Irmão Teodoro Luis of the Universidade Federal de Pelotas (UFPEL), harboring a dense vegetation of medium to large sized trees, within a humid and well preserved environment.

(12) A closed forest fragment of the Pampa Biome, within Canguçu – RS (CAN), located about 15 km from the municipality. It is a well preserved area, used mainly for fruticulture and tourism purposes.

(13) A small Atlantic Forest fragment within the rural area of the municipality of Horizontina – RS (HOR), distant about 5 km of the city, whose vicinities are used for agricultural exploration and livestock farming.

(14) A fragment of Atlantic Forest in the rural area of Cruz Alta – RS (CA), presenting medium to large sized trees that help to maintain the microclimate of the forest. Even so, the region is surrounded by degraded areas, used mainly for agricultural exploration.

(15) A wood inserted within a rural property of the municipality of Chapecó – SC (CHAP), encompassing a fragment of Atlantic Forest that is enriched by organic matter of both animal and plant origin. The sampling region is close to a housing area that is explored mainly for fruticulture.

Collections

The flies were collected with banana-baited traps sprinkled with baker's yeast (*Saccharomyces cerevisae* Meyen ex E.C. Hansen (1883)), constructed according to Tidon and Sene (1988). Ten baits were distributed in each of the sampling points, where they were left for 2–3 days. In the flooded areas, the baits were tied in trees or in riparian vegetation close to the water surface, with a distance of 1–4 m from each other, according to the size of the flooded area, whereas in nonflooded areas, baits were randomly suspended at a height of approximately 1.5 m within more or less dense forest areas, with a distance of about 10 m from each other. After collections, the flies were preserved in ethanol 100%. As the distance between traps was not enough to consider them independent samples, traps were grouped in each sampling point.

Identification

First, the specimens were screened and identified based on their external morphology, and after, the terminalia of the males was dissected and prepared to proceed the identification (Bachli et al., 2004). So, with the help of identification keys and aedeagus illustrations available in the literature (Freire-Maia and Pavan, 1949; Grimaldi, 1990; Vilela and Bächli, 1990), males could be identified to the species level, whereas females of cryptic groups were only identified at the group level, and later proportionally distributed according to the identity of the males of the same group collected at the same point for analysis purposes. After determination, each slide was fixed with Canada balsam. All specimens were labeled and stored in 100% alcohol and deposited in the Drosophilidae Collection of Museu de História Natural Carlos Ritter (MHNCR).

Data analysis

Abundance and richness were measured for each of the sampling points, as well as for flooded and nonflooded areas taken as a whole. These last were also evaluated in regard to dominance patterns (dominance index = 1 – Simpson index, which ranges from 0, when all taxa are equally present, to 1, when one taxon dominates the community completely), as measured in PAST 2.17c (Hammer et al., 2001). Sample rarefaction curves were constructed for each studied environments, with confidence intervals measured through 1000 randomizations, as implemented in PAST 2.17c. The differences between assemblages encountered in flooded and

Table 2
List of species collected in flooded areas (points 1–8), with abundance and richness values recorded for each and for the total set of points.

Group	Species	1. TA	2. EV	3. LV	4. LB	5. IM	6. OS	7. TCPA	8. TCPPA	Total
annulimana	*Drosophila schineri*	0	0	2	0	0	0	0	0	2
busckii	*D. busckii*	0	0	0	5	1	0	0	0	6
bromeliae	*D. bromelioides*	0	35	0	0	0	0	0	0	35
calloptera	*D. atrata*	0	8	0	0	0	0	0	0	8
cardini	*D. cardini*	0	25	0	0	0	0	0	0	25
	D. polymorpha	0	4	12	0	14	0	0	0	30
dreyfusi	g.*dreyfusi* (NI)*	0	0	0	0	2	0	1	0	3
guarani	*D. griseolineata*	0	5	0	0	0	1	0	0	6
immigrans	*D. immigrans*	0	0	0	23	8	0	3	0	34
melanogaster	*D. simulans*	6	283	1814	630	848	42	87	30	3740
	D. suzukii	0	44	13	9	6	12	23	6	113
repleta	*D. hydei*	0	4	14	73	6	3	0	0	100
	D. mercatorum	0	49	25	12	17	3	0	1	107
	D. onca	0	0	0	0	0	0	21	4	25
	D. repleta	0	0	0	0	3	0	0	0	3
	D. serido	0	0	2	0	0	15	0	0	17
	D.buzzatii	0	12	118	2	3	62	0	0	197
saltans	*D. sturtevanti*	0	0	0	0	0	0	1	0	1
tripunctata	*D. bandeirantorum*	0	2	0	0	0	0	0	0	2
	D. mediosignata	0	52	0	0	0	0	0	0	52
	D. paraguayensis	0	7	0	0	0	0	0	0	7
	D. roehrae	0	2	0	0	0	0	0	0	2
willistoni	sg. *willistoni*	0	2	0	0	39	0	0	0	41
	Leucophenga sp.	0	24	0	0	0	1	0	0	25
	Rhinoleucophenga punctuloides	0	0	11	0	0	0	0	0	11
	R. obesa	0	0	0	2	0	0	0	0	2
armatus	*Zaprionus indianus*	0	204	156	72	2	0	0	0	434
Abundance		6	762	2167	828	949	139	136	41	5028
Richness		1	17	10	9	12	7	6	4	27
Dominance										0.56

Notes: NI, non-identified.

Table 3
List of species collected in nonflooded areas (points 9–15), with abundance and richness values recorded for each and for the total set of points.

Groups	Species	9. SM	10. SN	11. PEL	12. CAN	13. HOR	14. CA	15. CHAP	Abund.
busckii	*D. busckii*	0	0	2	0	1	0	0	3
canalinea	*D. piratininga*	0	0	1	0	0	0	0	1
cardini	*D. cardini*	0	0	0	0	0	4	0	4
	D. neocardini	0	0	0	0	0	0	4	4
	D. polymorpha	6	9	5	1	6	2	28	57
coffeata	*D. fuscolineata*	0	1	0	0	0	2	1	4
guarani	*D. maculifrons*	0	0	0	0	1	1	0	2
	D. ornatifrons	1	2	4	40	2	22	8	79
immigrans	*D. immigrans*	0	5	8	0	71	1	147	232
melanogaster	*D. kikkawai*	0	0	0	0	1	0	0	1
	D. simulans	0	1138	13	1	49	0	541	1742
mesophragmatica	*D. mesophragmatica*	1	1	0	0	0	0	0	2
pallidipenis	*D. pallidipennis*	0	0	0	0	1	0	3	4
repleta	*D. hydei*	2	0	0	0	0	0	3	5
	sg. *fasciola* sp.1	0	0	0	2	0	0	0	2
	D. mercatorum	0	24	0	0	5	0	57	86
	D. onca	21	8	10	0	10	4	6	59
	D. papei	0	0	0	0	3	0	0	3
	D.buzzatii	2	0	0	3	0	0	0	5
saltans	*D. neosaltans*	0	0	0	0	0	0	9	9
	D. prosaltans	8	0	0	0	0	0	0	8
tripunctata	*D. bandeirantorum*	0	0	0	0	0	0	3	3
	D. cuaso	0	0	0	0	0	0	2	2
	D. fragilis	0	0	0	0	0	0	2	2
	D. mediopicta	0	2	0	0	0	3	0	5
	D. mediopunctata	0	5	14	25	0	24	2	70
	D. montevidensis	0	0	0	2	0	0	0	2
	D. paraguayensis	0	5	63	0	0	5	22	95
	D. sp.M4	0	0	0	0	0	0	2	2
	D. trifilum	0	0	0	0	0	0	2	2
	D. roehrae aff.	0	0	0	0	0	3	0	3
	D. bocainensis	0	0	5	0	0	0	0	5
willistoni	sg. *willistoni*	0	0	4	0	0	0	28	32
	Leucophenga sp.	0	0	0	2	0	0	0	2
armatus	*Zaprionus indianus*	0	0	0	0	0	2	7	9
orbitalis	*Zygothrica orbitalis*	0	0	1	0	0	0	0	1
vittimaculosa	*Zygothrica vittimaculosa*	0	0	0	0	0	6	18	24
Abundance		41	1200	130	76	150	79	895	2571
Richness		7	11	12	8	11	13	21	37
Dominance									0.47

nonflooded areas were evaluated through a Non-metric multidimensional scaling (NMS) (Melo and Hepp, 2008), in PAST 2.17c, using Bray–Curtis dissimilarity measures. Raw abundance data was also employed in a one-way Analysis of Similarities (ANOSIM) (Clarke, 1993) performed in PAST 2.17c, with the same dissimilarity measures. A Similarity Percentage analysis (SIMPER) (Clarke, 1993) was then performed with Bray–Curtis, in order to assess the contribution of each species to the observed patterns. The indicator value (*IndVal*) (Dufrene and Legendre, 1997) of each species to flooded or nonflooded environments was finally measured with the use Labdsv 1.8-0 package (Roberts, 2016) in R version 3.3.3 (R Core Team, 2013). Group-wise partitioning of the species composition datasets was performed with the package hierDiversity (Marion et al., 2015) in R version 3.3.3 (R Core Team, 2013), which allowed to evaluate within-group (alpha), among-group (beta), and pooled-total (gamma) diversity components in different hierarchical levels: (L2) for flooded and nonflooded environments; (L3) for the total set of collection points. This analysis was performed with Shannon's entropy diversity orders, with confidence evaluated through 999 bootstrap replicates.

After realizing the strong association of *Drosophila suzuki* Matsumura (1931) to swamps, we decided to withdrawn this species in all the statistical analyzes. In fact, since the first record of this species in south Brazil in 2013, it rapidly spread across the area (Deprá et al., 2014), which suggests the use of this species could add noise to the analyses, in face of the temporal differences between samplings performed in flooded (2015–2016) and nonflooded (2010–2011) environments.

Results

We collected 5028 specimens along the eight flooded sampling points, and these encompassed 27 species of four Drosophilidae genera and, in *Drosophila*, 13 species groups (Table 2). Among these samples, abundance varied from 6 to 2167 (in TA and LV, respectively), whereas richness ranged from 1 to 17 (in TA and EV, respectively).

These values contrast with those found for nonflooded areas, where a total of 2571 specimens comprising 37 species of four genera and 13 *Drosophila* groups were sampled (Table 3). In these localities, abundance varied from 41 to 1200 (in SM and SN, respectively), whereas richness ranged from 7 to 21 (in SM and CHAP, respectively).

So, although general abundance was higher for the swamps, richness appeared to be higher for nonflooded areas, which also presented lower dominance values (0.56 and 0.47 for flooded and nonflooded areas, respectively; $p = 0.001$) (Tables 2 and 3). The smaller richness for flooded in comparison to nonflooded environments was also suggested by the sample rarefaction curves, although this analysis suggests equilibrium in species representativeness was not reached in any of the cases (Fig. 1). The NMS also supported the existence of somehow differential composition patterns, and both environments presented non-overlapping

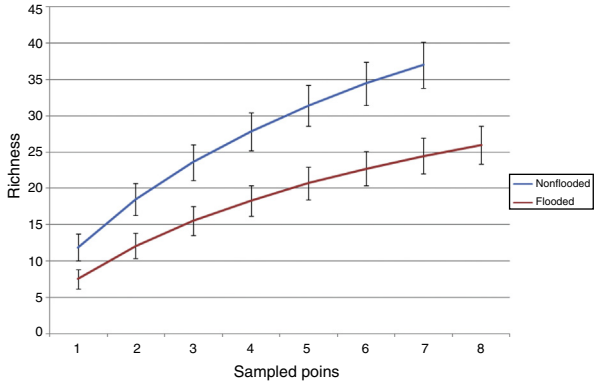

Fig. 1. Rarefaction curves of richness based on samples for flooded and nonflooded areas.

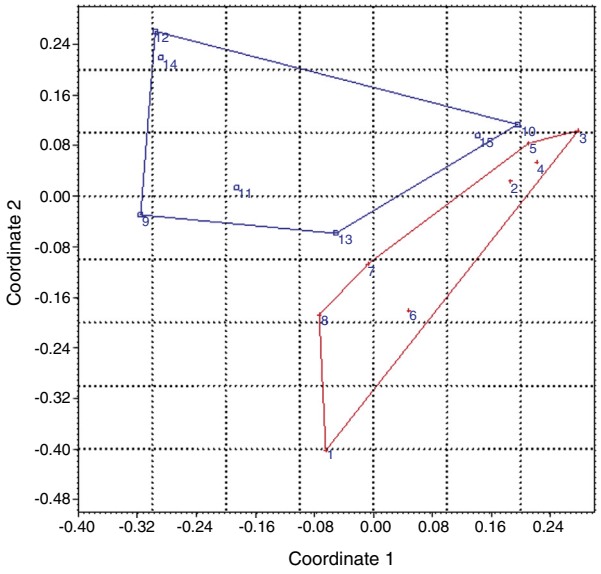

Fig. 2. Non-metric multi-dimensional scaling (NMS) ordination of the set of sampling points, according to Bray–Curtis dissimilarity measures. The plot represents the minimum convex polygon. Numbers refer to the different collection points, according to Table 1: 1–8 (in red), flooded areas; 9–15 (in blue), nonflooded areas.

minimum convex polygons when the two coordinates were considered (Fig. 2). The ANOSIM also supported a significant difference in species composition patterns between flooded and nonflooded areas, with values of 45.61 for mean rank within groups and 59.46 for mean rank between groups ($R = 0.2638$ and $p = 0.02$).

Hierarchical partitioning of diversity confirmed the higher alpha, beta and gamma-diversities presented in nonflooded environments when Shannon's entropy is taken into account (Table 4). In fact, the turnover of species observed for nonflooded areas was more than three times higher than the 0.975 quantile for flooded areas (Table 4). Moreover, beta-diversities for both kinds of environments (L2) were higher than those measured for the set of sampling points taken as a whole (L3) (Table 4), even with the higher turnover of species in the last case. Interestingly, this clear beta-diversity pattern fell apart when diversity was evaluated through simple richness measures, in which case L2 beta-diversity estimates were quite similar between environments (3.224 and 3.121 for flooded and nonflooded areas, respectively), but again higher than those obtained for the total set of points (1.562) (Supplementary Material – Table 1S).

SIMPER analysis revealed that the greater abundance of *D. simulans* Sturtevant (1919) in flooded areas explained almost 53% of the dissimilarity between the two set of areas (Table 5). Summed to this, the greater abundance of *Z. indianus* Gupta (1960) and *D. buzzatii* Pavan and da Cunha (1947) in flooded areas and the prevalence of *D. immigrans* Sturtevant (1921), *D. ornatifrons* Duda (1927) and *D. mediopunctata* Dobzhansky and Pavan (1943) in nonflooded areas explained more than 77% of the difference. In fact, *D. ornatifrons* (indval = 1.00, $p = 0.001$, freq = 7), *D. mediopunctata* (indval = 0.71, $p = 0.008$, freq = 5) and *D. onca* Dobzhansky and Pavan (1943) (indval = 0.63, $p = 0.045$, freq = 8) presented significant indval estimates for nonflooded woods.

Discussion

This study provides the first attempt to address the dissimilarities in Drosophilidae species composition between nonflooded and flooded wooded areas, and despite shortages related to the temporal (2010–2011 and 2015–2016, respectively), seasonal (most collections in spring × winter, respectively) and spatial distribution of the sampling points (interior × coast, respectively), it suggested the existence of significant differences between both assemblages. In fact, different composition patterns between flooded and nonflooded areas were supported by Multivariate analyses of NMS and ANOSIM.

At a first sight, this difference seems to be related to the lower diversity values presented by swamps. In this sense, general richness values were higher for nonflooded areas, and multiplicative partitioning of species diversity consistently presented these as detainers of higher alpha, beta and gamma diversities, at least when Shannon's entropy is employed. Nevertheless, beta diversity patterns between flooded and nonflooded areas approached when only richness is considered, suggesting the difference between environments is mainly of quantitative source, which is also supported by general dominance comparisons. This result is possibly related to the low plasticity presented by several Drosophilidae species against environmental variations such as humidity, temperature, precipitation, and other environmental factors, as suggested by Tidon (2006) and Poppe et al. (2013) and even demonstrated by Kellermann et al. (2012a,b). In this sense, as flooded areas generally present a more open environment, they possibly impose a higher variation in climatic conditions, added to a lower protection against intense precipitation and wind speed, constituting a more hostile environment for entry and, mainly, for the persistence of different Drosophilidae species. Moreover, swamps are much more homogeneous in regard to resource availability, presenting lower abundance and richness of plant species (Scarano, 2006), which could restrict attractiveness for several frugivorous drosophilid species, as those sampled here. After all, the more plants, the more potential niches to be colonized (Cuevas-Reyes et al., 2004).

In this sense, the collection point with the higher Euclidean distance to the others (flooded and nonflooded) in the NMS scatterplot was just TA (average Euclidian distance of 0.48 to flooded and nonflooded areas), which encompasses a large area of protected swamps (Gomes et al., 1987). So, in agreement with the hypothesis depicted above, the higher homogeneity in vegetation and the climatic variability presented by larger swamp areas also presented a negative impact on frugivorous species diversity. So, this area was possibly "out of reach" for several Drosophilidae species, to the exception of the exotic species, acknowledged as more plastic in a variety of traits (Davidson et al., 2011). This result contrasts with the general higher diversity found for several more perturbed areas, as the LV protection area, and highlights the interference of different processes in wetlands and mangroves, which were suggested to act as sink habitats for drosophilids populations (Schmitz et al., 2010).

Table 4
Group-wise hierarchical diversity estimates multiplicatively partitioned into alpha (within group), beta (among groups) and gamma (total) components according to Shannon's entropy measures. Uncertainty estimates were approximated using 999 bootstrap replicates, whose 0.95 quantiles are presented at the bottom of each table. L2 patterns, for points 1–8 and 9–15 refer to flooded areas and nonflooded areas, respectively, whereas L3 patterns were encountered for the total set of sampling points.

L3 patterns: points 1–15					
	Alpha	Beta	Gamma	Turnover	Homogeneity
Points 1–15	3.50	1.13	3.95	0.13	0.77
SE	1.29	0.13	1.83	0.13	0.17
q0.025	2.23	1.01	2.28	0.01	0.28
q0.975	7.11	1.56	9.18	0.56	0.99
L2 patterns: points 1–8 (flooded area)					
	Alpha	Beta	Gamma	Turnover	Homogeneity
Points 1–8	2.17	1.52	3.28	0.07	0.61
SE	0.40	0.14	0.79	0.02	0.08
q0.025	1.59	1.13	1.85	0.02	0.55
q0.975	3.13	1.64	4.84	0.09	0.87
L2 patterns: points 9–15 (nonflooded area)					
	Alpha	Beta	Gamma	Turnover	Homogeneity
Points 9–15	3.98	2.74	10.90	0.29	0.26
SE	0.76	0.32	1.87	0.05	0.08
q0.025	2.71	1.59	5.14	0.10	0.25
q0.975	5.52	2.83	12.37	0.30	0.57

Table 5
Summary of the results provided by SIMPER analysis, listing the seven species that are primarily responsible for the differences between flooded and nonflooded areas in decreasing order of contribution.

Taxon	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance in flooded areas	Mean abundance in nonflooded areas
D. simulans	44.460	52.580	52.58	468.0	249.0
D. immigrans	5.102	6.033	58.61	4.2	33.1
Z. indianus	4.450	5.262	63.87	54.3	1.3
D. ornatifrons	4.325	5.114	68.99	0.0	11.3
D. buzzatii	3.786	4.477	73.46	24.6	0.7
D. mediopunctata	3.739	4.421	77.88	0.0	10.0
D. paraguayensis	3.426	4.051	81.93	0.9	13.6

Drosophila simulans was by far the most abundant species in both habitats, representing 72% of the Drosophilidae species sampled in this study, which is in agreement with the high abundance presented by this species in different environments, from mangroves (Schmitz et al., 2010), to forest (Hochmüller et al., 2010; Poppe et al., 2012) and urban habitats (Gottschalk et al., 2007; Hochmüller et al., 2010). This species is generally associated with disturbed environments, together with *Z. indianus* (Ferreira and Tidon, 2005). Nevertheless, in agreement with the scenario depicted above, both exotic species were more abundant in the swamps, and this quantitative difference explained almost 58% of the variation between both kinds of environments. Although abundant in flooded areas, *D. simulans* abundance values varied among sampling sites, with both extremes detected in swamps: the lowest abundance was observed in TA (only six individuals), and the highest in LV (1814 individuals). This is possibly related to differences in vegetation cover (TA is more open and unprotected in relation to LV), suggesting that open areas represent a challenge even for this cosmopolitan generalist species.

Among the other eight species that explain most of the differences between flooded and nonflooded environments, *D. buzzatii* and *D. mercatorum* Patterson and Wheeler (1942) also presented higher abundance values in swamps, which may be explained by the distribution of flooded points in coastal areas, where the cactophylic resources exploited by these species may be especially profitable (Franco et al., 2006). Moreover, *D. mercatorum* is generally associated with open vegetation or anthropic actions (Ferreira and Tidon, 2005; Gottschalk et al., 2007). In contrast, *D. onca*, *D. immigrans* Sturtevant (1921) and *D. polymorpha* Dobzhansky and Pavan (1943) presented larger abundance and fidelity values to nonflooded areas, whereas *D. ornatifrons* Duda (1927) and *D. mediopunctata* Dobzhansky and Pavan (1943) were exclusive and presented significant indicator values for such environments. Among these, *D. polymorpha* was previously suggested as tolerant in regard to urbanization levels (Gottschalk et al., 2007), which might also be the case for other traits. Conversely, *D. mediopunctata* seems to be more restricted, having been collected only in nonflooded environments, especially in the points sampled during autumn and winter, confirming the patterns found by several authors in other environments (Franck and Valente, 1985; De Toni et al., 2007; Cavasini et al., 2014). The other species exclusive to nonflooded woods was *D. ornatifrons*, which is frequently reported in forest remnants with low anthropogenic action, mainly in the Atlantic Forest (Gottschalk et al., 2007; Cavasini et al., 2014).

So, our results suggest the presence of compositional differences between nonflooded and flooded woods, with lower

diversity being reported for the latter, mainly in regard to abundance and dominance differences. So, several native species were registered in swamps only as occasional samplings, in contrast to some exotic species that are more plastic and were quite abundant in such areas. Since the diversity gradient detected in flooded areas was not directly associated to the conservation status of each sampling point, our results also question the use of Drosophilidae species as bioindicators of environmental disturbance and anthropic influence in such open and homogeneous habitats.

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.2017.11.005.

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