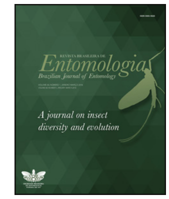
Revista Brasileira de Entomologia 62 (2018) 292–303

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution
www.rbentomologia.com

SOCIEDADE BRASILEIRA DE ENTOMOLOGIA FUNDADA EM 1937



Biology, Ecology and Diversity

Houseflies speaking for the conservation of natural areas: a broad sampling of Muscidae (Diptera) on coastal plains of the Pampa biome, Southern Brazil

Ândrio Zafalon-Silva[a,*], Frederico Dutra Kirst[b], Rodrigo Ferreira Krüger[c]

[a] *Universidade Federal do Paraná, Departamento de Zoologia, Curitiba, PR, Brazil*
[b] *Universidade Federal de Minas Gerais, Departamento de Zoologia, Minas Gerais, MG, Brazil*
[c] *Universidade Federal de Pelotas, Departamento de Microbiologia e Parasitologia, Pelotas, RS, Brazil*

ARTICLE INFO

Article history:
Received 9 February 2018
Accepted 10 September 2018
Available online 5 October 2018
Associate Editor: Gustavo Graciolli

Keywords:
Atlantic forest
Diptera
Ecology
Guilds
Grasslands
Muscidae

ABSTRACT

The Brazilian Coastal Plain of the Pampa Biome (CPPB), has suffered fragmentation caused by resource extraction and cattle raising. In turn, conservation proposals are needed to prevent the anthropisation of Pampa natural areas. The first step towards conservation proposals by using insects is fauna inventories, providing data support for legislators. Thus, we undertook a regional and broad-scale sampling survey to investigate the diversity of Muscidae flies in protected and non-protected areas of CPPB. In addition, we carried out an ecological guild diversity analysis as a metric approach of bioindication. The Muscidae sampling resulted in 6314 specimens, 98 species taxa in 31 genera. Based on diversity estimators, our sampling represents 70–86% of all muscids of CPPB. The highest diversity occurs in Pelotas streams (non-protected) and Taim Ecological Station (a huge protected area). Despite the fact these areas are more diversified and present more predatory muscid species than others, invasive species associated with livestock were observed at a higher level, providing evidence of the impact of livestock proximity to protected areas. Based on biological characters of Muscidae species and ecological guild analysis, we were able to identify: (i) high diversity of carnivorous species associated with forested and more preserved areas and (ii) a high level of a few saprophagous species as indicator of anthropisation process. In general, our results represent a significant step towards understanding Muscidae in Southern Brazil, and we demonstrate how the population ecology of muscid flies supports data to conservation proposals.

Introduction

The conversion of tropical forests, natural fields, and grasslands into agricultural and urban areas is the primary driver of the biodiversity crisis currently observed (Foley et al., 2005). Approximately 43% of the terrestrial world surface has been disturbed, and the original vegetation has been converted into anthropogenic new habitats (Barnosky et al., 2012). Tropical countries, such as Brazil, follow the same pattern showing an increasing trend as agricultural frontiers are still expanding (Ferreira et al., 2012). Extinction rates in this century are estimated to be more than double in comparison to previous ones (Pereira et al., 2010).

In the state of Rio Grande do Sul (RS), the Pampa biome is subjected to high anthropic pressure (Pillar et al., 2009), and knowledge on biodiversity is particularly neglected (Overbeck et al., 2007). Among areas of biological importance, we can highlight the Coastal Plain of the Pampa Biome (CPPB) which has a great diversity of habitats (wetlands, floodplains, riparian forests, and sands) (Overbeck et al., 2007).

Fragmentation process in the CPPB mainly occurs via the replacement of native grasslands with agriculture and exotic pastures and the destruction of wetlands by drainage or barring. Additionally, frequent fires, mischaracterisation of habitat by livestock overgrazing and trampling, hunting, and biological invasions are also significant (Bencke, 2009; Fontana et al., 2003; Machado et al., 2008; Mikich and Bérnils, 2004). In recent years, habitat loss has intensified (Bencke, 2009; Crawshaw et al., 2007), influencing some groups of birds and mammals (Bilenca et al., 2012; Filloy and Bellocq, 2006; Gonzalez and Merino, 2008; Krapovickas and Di Giacomo, 1998). However, knowledge about the impact on insect communities in Pampa biome is incipient, despite the efforts to survey the diversity of Lepidoptera (Paz et al., 2013), Coleoptera (Da Silva et al., 2013), Formicidae (Hymenoptera) (Rosado et al., 2012, 2013), and Diptera. Among studied flies

* Corresponding author.
E-mail: andrio.zafalon@gmail.com (Â. Zafalon-Silva).

https://doi.org/10.1016/j.rbe.2018.09.002

diversity we can list the following families: Drosophilidae (Hochmüller et al., 2010; Poppe et al., 2012, 2013) Muscidae (Krüger et al., 2010), Sciomyzidae (Kirst et al., 2015), Syrphidae (Kirst et al., 2017) and Tabanidae (Krüger and Krolow, 2015).

The Pampa biome is a highly significant area for biodiversity conservation (MMA, 2004), but among the regions of CPPB, data on insect biodiversity are scarce, with the exception of the municipality of Pelotas and Capão do Leão, for Muscidae, Calliphoridae, and Tabanidae flies (Azevedo and Krüger, 2013; Krüger and Krolow, 2015; Krüger et al., 2010). Studies on the species richness of Sciomyzidae (Kirst et al., 2015) and the diversity of Syrphidae (Kirst et al., 2017) were the first to report data on insect fauna in most protected areas in the CPPB.

The first step towards insect conservation proposals is to increase knowledge on fauna, mainly by species inventories. Thus, this study involved a regional and broad sampling survey to determine the diversity of Muscidae on CPPB (including the transition zone to the Atlantic forest in the north of RS). Furthermore, this study aimed to establish measures of diversity (Magurran, 2004) to evaluate the success of sampling in protected and non-protected areas. A limitation of these estimates is the high dependence on sampling effort, mainly in the measures using abundance and richness (Bunge and Fitzpatrick, 1993). Therefore, for conservation proposals, determination of species richness is as essential as the number of species to be discovered (Santos et al., 2010). In this regard, nonparametric estimators of diversity were used to extrapolate species richness in the studied habitats (Gotelli and Colwell, 2001; Willie et al., 2012).

The importance of Muscidae to the CPPP habitats is that this family presents an evolutionary history in the Pampa biome, its Southern forests were an area of high diversification of this family (Löwenberg-Neto and Carvalho, 2009), and today along with the Atlantic forest, it has many areas of endemism (Carvalho et al., 2003; Nihei and Carvalho, 2005). Furthermore, the Muscidae show excellent taxa properties, which make them suitable for diversity studies in this biome. They present broad occupancy, high species richness (Carvalho et al., 2005) and wide habitat range (Ferrar, 1987; Skidmore, 1985). Due to these reasons, one can consider muscids flies assemblages comprising many species with fundamentals of bioindicator taxa. The expression 'bioindicator' is an aggregate term referring to all sources of biotic and abiotic reactions to ecological changes. Instead of merely working as gauges of natural change, taxa are utilised to show the impacts of surrounding natural changes, or environmental changes (Parmar et al., 2016). In this sense, the following may be expected from Muscidae: the presence of synanthropic species as indicator of disturbance in native forested areas (e.g. *Musca domestica* Linnaeus, *Hydrotaea* Robineau-Desvoidy species, *Stomoxys calcitrans* (Linnaeus), among others) (Greenberg, 1971; Krüger et al., 2010; Skidmore, 1985; Zafalon-Silva et al., 2014). On the other hand, the presence of species that do not tolerate habitat anthropisation could be an indicator of forested "healthy" environments (e.g. *Cyrtoneurina* Giglio-Tos, *Limnophora* Robineau-Desvoidy, *Neomuscina* Townsend, *Neodexiopsis* Malloch, among others) (Krüger et al., 2010; Skidmore, 1985; Werner and Pont, 2006).

We can argue towards the ecological guilds as a bioindicator approach in Muscidae as the efficiently discrete delimitation of trophic levels are mainly based on data of larval morphology (Skidmore, 1985), and by the discussion gathered in Rodríguez-Fernández et al. (2006) and Krüger et al. (2010). This survey aims at complementing the guild descriptions by analyzing the number of species per guild in each region.

It also aims at answering the following questions: (i) which is the diversity of Muscidae assemblages in protected and non-protected areas of CPPB? (ii) Is it possible to use ecological guilds of Muscidae as a tool to check environmental aspects?

Material and methods

Study sites

The forested areas surveyed included the Grasslands of the Pampa biome, and forests in the northeastern Coastal Plain of the Pampa Biome (CPPB) in the transition zone to the Atlantic forest of the Rio Grande do Sul state (RS), Brazil. The sampling areas in the CPPB (Table 1 and Fig. 1) were chosen according to the high priority indicated for the conservation of invertebrates (MMA, 2000). The Ministry of Environment (MMA) indicates areas around the coastal lagoons of the Patos, Mirim–Mangueira complex, and the lagoons of North Coast of RS (MMA, 2000).

Fig. 1 and Table 1 show the study sites. Region 1 is made up of areas around the Pelotas stream (points 1–3), Corrientes stream (points 4 and 5), and Turuçu stream (points 6 and 7). Region 2 contains the Lami Biological Reserve (points 8 and 9), Camaquã river (point 10), and the private natural heritage reserve (RPPN) Barba Negra (points 11–14). Region 3 contains the Taim Ecological Station (points 15–21), which is a significant area of national protection. The Itapuã State Park (points 22–24), Tupancy Natural Municipality Park (point 25), José Lutzenberger State Park (known as Guardhouse Park, point 26), and Itapeva State Park (points 27 and 28) make up Region 4 on the northern coast of CPPB. Region 5 is the Lagoa do Peixe National Park (points 29–35).

The characterizations of the sampled areas and their protection status by law are summarised in Table 1. For a complete characterisation of all areas, including conservation unities, the reader is referred to Waechter (1985, 2002), IBGE (1986, 2004), Mello-Filho et al. (1992), SEMA (1996, 2013), Meira and Porto (1998), ICMBIO (1999), Burger (2000), Leite (2002), Celulose Riograndense (2012), Venzke (2012), and Witt (2013).

Sampling design

The Muscidae flies were collected in five regions of the CPPB using 140 Malaise traps, model Townes (1972) with a modified collection container (Duarte et al., 2010). Seven areas were sampled in each region (Table 1 and Fig. 1) and four traps were installed in each area. The traps remained in the field for eight days. On the eighth day, collector containers carrying material preserved in 70% alcohol were sent to triage and pinning in the laboratory. The periods of collection campaigns were the following: (i) region 1 between October and November of 2011; (ii) region 2 in November of 2011; (iii) region 3 in December of 2011; (iv) region 4 in January of 2012; (v) region 5 in February of 2012.

The flies were identified with specific keys of Neotropical Muscidae (Carvalho and Couri, 2002; Costacurta and Carvalho, 2005; Costacurta et al., 2005; Couri and Carvalho, 2002; Marques and Couri, 2007; Nihei, 2005; Nihei and Carvalho, 2007; Pereira-Colavite and Carvalho, 2012; Schüehli and Carvalho, 2005), and from the original descriptions of some species. Identification of many specimens was confirmed by comparing with material from the *Coleção de Entomologia Padre Jesus Santiago Moure, Universidade Federal do Paraná* (DZUP).

The vouchers are deposited at DZUP and *Coleção de Diptera do Laboratório de Ecologia de Parasitos e Vetores*, *Universidade Federal de Pelotas* (LEPAV).

Data analysis

All analyses were performed using the statistical program R (R Development Core Team, 2015) and with the statistical packages *vegan* and *iNEXT* (Hsieh et al., 2016; Oksanen et al., 2015). The species with specimens which were much damaged to identify

Table 1
Description of the 35 points sampled in five regions of the Coastal Plain on Pampa Biome (CPPB). Locality: sampled areas (four Malaise trap per area) inside the regions; LP: law-protected area; coordinates: central coordinate of the sampled area; site description: a general description of the locality, including it phytophysiognomy and geology; climate: average temperature and relative humidity during the sampling period. APL, Pelotas stream; ACS, Corrientes stream; ATU, Turuçu stream; LAMI, Lami Biological Reserve; RCMQ, Camaquã river; RPPN, private natural heritage reserve Barba Negra farm; TAIM, Taim Ecological Station; ITPA, Itapuã State Park; PMTY, Tupancy Natural Municipality Park; PEJL, José Lutzenberger State Park; PEVA, Itapeva State Park; LPXE, Lagoa do Peixe National Park; points 1–7, region 1 (Oct–Nov, 2011); points 8–14, region 2 (Nov, 2011); points 15–21: region 3 (Dec, 2011); points 22–28, region 4 (Jan, 2012); points 29–35: region 5 (Feb, 2012).

Point	Locality	LP	Latitude and longitude		Regional description
1	APL	No	−31.72175	−52.25422	Atlantic forest remains with pioneer vegetation and fluvial influence. The general classification is a semideciduous seasonal forest. Sandbank, riparian, and gallery forests predominate. Abundant marshes and river streams from Patos Lagoon system.
2	APL	No	−31.67118	−52.21764	
3	APL	No	−31.62781	−52.35285	
4	ACS	No	−31.55675	−52.14568	
5	ACS	No	−31.56435	−52.13951	
6	ATU	No	−31.43295	−52.12173	
7	ATU	No	−31.49678	−52.00834	
8	LAMI	Yes	−30.23630	−51.09601	Riparian forests with shrubby marshes, herbaceous marshes, sandbanks, and wet fields.
9	LAMI	Yes	−30.25632	−51.10126	
10	RCMQ	No	−31.12159	−51.79237	Transition border between the Atlantic forest and Pampa biomes. Abundant riparian forests. The general classification is a semideciduous seasonal forest.
11	RPPN	Yes	−30.43135	−51.23704	Farming forests covered by abundant non-indigenous *Eucalyptus* species. Heavy groves of native species covering dunes, internal beaches, and all borders of RPPN.
12	RPPN	Yes	−30.41323	−51.21834	
13	RPPN	Yes	−30.42993	−51.14240	
14	RPPN	Yes	−30.39651	−51.12725	
15	TAIM	Yes	−32.55568	−52.50060	Several internal lagoons and forested marshes. The predominant phytophysiognomy is a coastal field with savanna-like grasses. The landscape is composed of a mosaic of lagoons, marshes, sandbank shrubberies, dunes, and riparian forests. Farming and cattle raising in the surrounding park area.
16	TAIM	Yes	−32.53865	−52.53732	
17	TAIM	Yes	−32.56059	−52.50975	
18	TAIM	Yes	−32.53347	−52.52538	
19	TAIM	Yes	−32.53845	−52.53713	
20	TAIM	Yes	−32.59684	−52.56837	
21	TAIM	Yes	−32.63511	−52.48655	
22	ITPA	Yes	−30.38372	−51.02095	Several wooded foothills with herbaceous vegetation and shrubby fields. The phytophysiognomy varies from shrubby/wooded fields to dense forests. The mosaic landscape is composed of internal lagoon beaches, riparian forests, and mobile dunes.
23	ITPA	Yes	−30.38291	−51.03008	
24	ITPA	Yes	−30.36819	−51.02620	
25	PMTY	Yes	−29.48945	−49.84442	Psammophile forested area, low-height wood profile. Highest trees are about 10-m tall. General physiognomy is characterised by a small riparian forest, three internal lagoons, one dune of 9 m height, and urban areas surrounding the park.
26	PEJL	Yes	−29.35508	−49.73529	Dense ombrophilous forest influenced by sea proximity. Paleodunes fixed by dense forest cover. Mobile dunes are covered only by herbaceous and shrub vegetation. Several marshes with swamp-like vegetation.
27	PEVA	Yes	−29.37978	−49.76215	
28	PEVA	Yes	−29.37819	−49.75909	
29	LPXE	Yes	−31.05366	−50.80873	High seasonal variation of salinity in soil, causing seasonal patterns in phytophysiognomy. Lagoon complex of developed riparian forests, savanna-like fields, and xerophytic shrubberies on fixed paleodunes. Farming and cattle raising in the surrounding park area.
30	LPXE	Yes	−31.05613	−50.81078	
31	LPXE	Yes	−31.06581	−50.81776	
32	LPXE	?	−31.21367	−50.96057	
33	LPXE	?	−31.21715	−50.96621	
34	LPXE	?	−31.21911	−50.97516	
35	LPXE	?	−31.43412	−51.17502	

or to recognise as morphospecies (e.g. *Coenosia* sp., *Polietina* sp., *Pseudoptilolepis* sp.) were removed from data analyses.

Dissimilarity in the southern assemblages of flies

The dissimilarity of fly communities between regions (1–5) and between Southern Brazil muscid assemblages (Costacurta et al., 2003; Krüger et al., 2010; Rodríguez-Fernández et al., 2006; and the present study) were subjected to multivariate analysis with agglomerative hierarchical classification by unweighted pair-group method using arithmetic averages (UPGMA), with complete linkage (Borcard et al., 2011; Sneath and Sokal, 1973).

The UPGMA clustering method was selected by the best Pearson's correlations index (Table 2) for verification between regions (1–5). The index was obtained comparing the cophenetic matrices of various hierarchical clustering methods. The abundance was standardised by Hellinger's transformation, and dissimilarities between pairs of samples were calculated using the Bray–Curtis dissimilarity coefficient (Borcard et al., 2011). UPGMA clustering of Muscidae assemblages from studies in Southern Brazil was performed by Jaccard index of dissimilarity using the binary matrices, as not at all the analysed studies contained data on abundance. This cluster was generated by comparing only species with specific names with morphospecies which are not considered in all studies.

Table 2
Results of the cophenetic matrices test. The choice of cluster method was based on the highest score in the Pearson's correlations index. UPGMA, unweighted pair-group method using arithmetic averages; UPGMC, unweighted pair-group method using centroids; WARD, Ward's minimum variance clustering; WPGMA, weighted pair-group method using arithmetic averages; WPGMC, weighted pair-group method using centroids; PCI, Pearson's correlation index.

Cluster method	PCI
UPGMA	0.8846567
WPGMA	0.8842323
WARD	0.8827867
WPGMC	0.7437148
UPGMC	0.6925564

Sampling effort

Sampling sufficiency was verified using a species accumulation curve (SAC), which was built using the *vegan* package, with

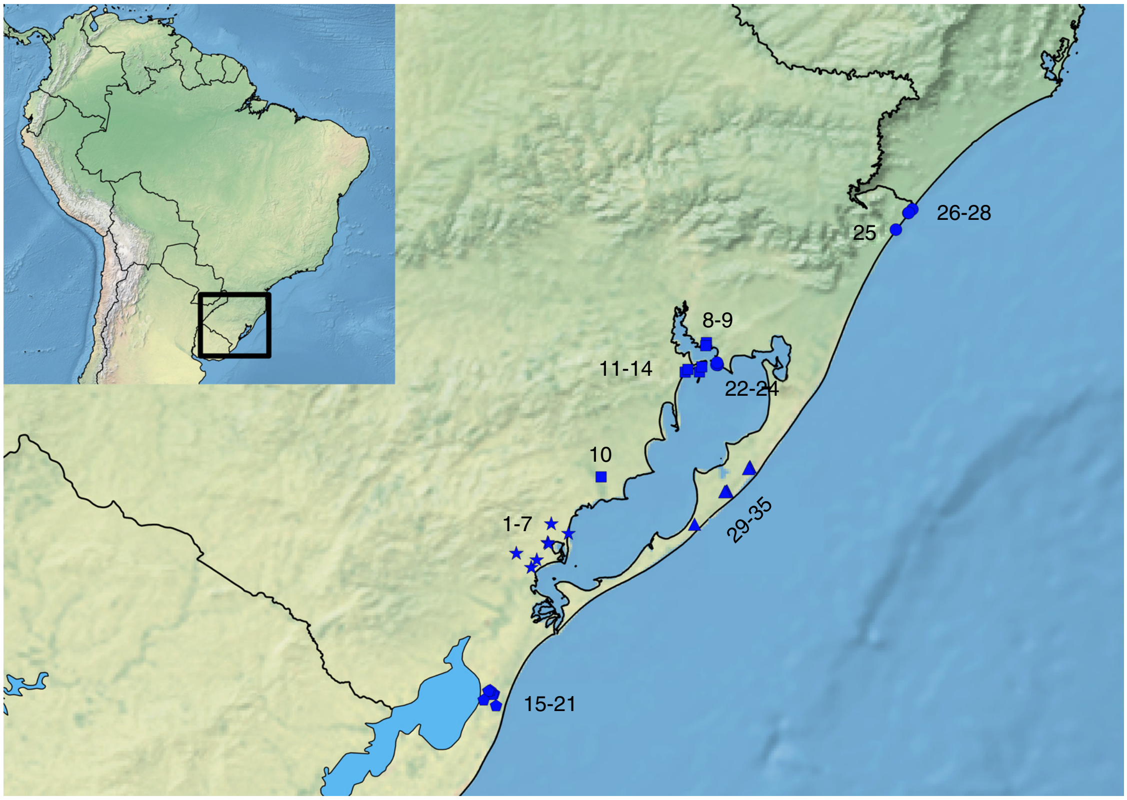


Fig. 1. Partial map of South America, with details of the Coastal Plain of Pampa Biome, Rio Grande do Sul (Brazil). The marked points refer to all 35 collection areas (groups of four traps). Descriptions of each area are provided in Table 1. Stars: region 1; squares: region 2; pentagons: region 3; circles: region 4; triangle: region 5.

the *specaccum* function. This function produces the SAC or the number of species at a given number of sampled points (Malaise trap). Within the *specaccum* function, the "exact" method was used, which obtained the expected (mean) species richness. The "exact" method finds the expected SAC using the method independently developed by Ugland et al. (2003), Colwell et al. (2004), and Kindt et al. (2006).

To estimate species richness (S), which is specific for each treatment, Rarefaction, and Chao2 were used (Chao, 1987; Chao et al., 2009; Chiu et al., 2014; Colwell and Coddington, 1994; Gotelli and Colwell, 2001). These are non-parametric methods, used to estimate S based on the incidence of species (presence/absence). The individual-based rarefaction computes the expected number of species in a subsample of "*n*" individuals drawn at random from a single representative sample from an assemblage. The values of Rarefactions and Chao2 were obtained using the function "*Poolaccum*," which estimates the extrapolated richness index, as well as the number of species for the random ordination of sampling units.

Ecological guilds in Muscidae

Before defining the ecological guilds, it is essential to determine how muscid flies explore environmental resources. The most notable study on Muscidae biology was Skidmore (1985), in which the author indicated larval habit mainly by the morphology of the cephalo-pharyngeal skeleton and the anal spiracles. Based on the anatomical features of larvae, we can suggest three definitions for the biology of Muscidae. The first one is the saprophagous larvae, characterised by well-developed suction mechanisms, lacking oral accessory sclerites, and large anal spiracles. The second one is the saprophagous larvae with predatory facultative behaviour in the third instar or parasitic behaviour, as in some genera (e.g. *Charadrella* Wulp, *Philornis* Meinert), characterised by well-developed oral hooks, the presence of oral accessory sclerites, the presence of a suction mechanism, and large anal spiracles. The third is the obligate carnivorous larvae, characterised by well-developed and well-sclerotised oral hooks and accessory sclerites, the absence of suction mechanisms, and small anal spiracles. For additional details of the species, morphology, and substrates used by immatures see Skidmore (1985) and Ferrar (1987).

The habits of adults could be identified by the hematophagous with labium modified for penetration and suction. The second type is lickers in saprophagous species, with labella that are not reduced and with a more considerable lateral expansion membranous. The last one is the predators, with reduced labella, developed prestomal teeth, and sclerotised prementum to penetrate the exoskeleton of their prey (Coelho, 1997; McAlpine, 1981).

Therefore, we defined an ecological guild as a group of species which exploit the same class of resources in a similar manner (Root, 1967). In Muscidae, we recognise four ecological guilds or functional groups (*sensu* Blondel, 2003) combining trophic roles of larvae and adult stages (Krüger et al., 2010). The species of CPPB are classified according to the following: saprophagous larvae and saprophagic/hematophagous adults species (SS); facultative predators/parasitic larvae and saprophagous adults species (SPS); predatory larvae and saprophagous adults species (OS): predatory larvae and adults species (PP).

Region-guild diversity proportionality

We tested the influence of region and guild of Muscidae on the proportional richness and proportional abundance using ANOVA and *F* test, considering $P < 0.05$ as significant.

Table 3
List of Muscidae (Insecta, Diptera) species collected in five regions of the Coastal Plain of Pampa Biome (CPPB), Rio Grande do Sul, Brazil. Sampled CPPB areas include R1: region 1, where: APL: Pelotas stream; ACS: Corrientes stream; ATU: Turuçu stream. R2: region 2, where: LAMI: Lami Biological Reserve; RCMQ: Camaquã River; RPPN: private natural heritage reserve Barba Negra farm. R3: region 3, where: TAIM: Ecological Station of Taim. R4: region 4, where: ITPA: Itapuã State Park; PMTY: Tupancy Natural Municipality Park; PEJL: José Lutzenberger State Park; PEVA: Itapeva State Park. R5: region 5, where LPXE: Lagoa do Peixe National Park.

Taxa	R1			R2			R3	R4				R5	(∑)
	APL	ACS	ATU	RPPN	RCMQ	LAMI	TAIM	ITPA	PEVA	PMTY	PEJL	LPXE	
Biopyrellia bipuncta (Wiedemann, 1830)	0	0	0	0	4	0	13	8	0	0	0	3	28
Bithoracochaeta annulata Stein, 1911	0	0	0	0	0	1	0	0	0	0	0	33	34
Bithoracochaeta calopus Bigot, 1885	120	44	98	7	43	6	160	0	1	0	0	26	505
Bithoracochaeta flavicoxa[a] Malloch, 1934	1	12	2	0	10	1	4	1	0	3	0	0	34
Bithoracochaeta plumata Albuquerque, 1955	0	0	0	0	0	0	0	1	0	0	0	0	1
Bithoracochaeta sp. 2	0	0	0	0	0	0	0	1	0	0	0	6	7
Coenosia sp.	1	0	0	0	0	0	0	1	0	0	0	0	2
Coenosia sp. 1	1	0	0	0	0	0	1	0	0	0	0	0	2
Coenosiini Genus A	3	0	0	0	0	0	2	0	0	0	0	0	5
Coenosiini Genus B	0	0	0	0	0	0	1	0	0	0	0	0	1
Cyrtoneurina costalis (Walker, 1853)	5	3	0	0	10	0	2	2	0	0	0	1	23
Cyrtoneuropsis brunnea (Hough, 1900)	1	0	0	0	2	1	1	0	0	0	0	1	6
Cyrtoneuropsis maculipennis[a] (Macquart, 1843)	10	4	4	11	0	10	0	0	0	1	11	0	51
Cyrtoneuropsis pararescita (Couri, 1995)	0	0	0	0	0	0	1	0	0	0	0	9	10
Cyrtoneuropsis incognita[a] (Snyder, 1954)	1	0	0	2	0	17	0	18	4	0	0	0	42
Cyrtoneuropsis sp. 2	0	0	0	2	0	6	0	0	0	0	0	0	8
Dolichophaonia cacheuta[a] (Snyder, 1957)	0	0	0	0	0	1	0	0	0	0	0	0	1
Dolichophaonia plaumanni (Carvalho, 1983)	0	0	0	0	0	0	0	0	0	0	0	1	1
Dolichophaonia trigona (Shannon and Del Ponte, 1926)	0	5	1	0	0	0	1	1	0	0	0	0	8
Dolichophaonia elongata[a] (Albuquerque, 1958)	0	0	0	0	0	0	1	0	0	0	0	1	2
Graphomya auriceps Malloch, 1934	2	0	1	0	0	0	21	0	0	0	0	0	24
Gymnodia delecta (Wulp, 1896)	0	0	0	0	0	0	15	0	0	0	0	0	15
Gymnodia quadristigma (Thomson, 1869)	0	0	0	0	0	0	9	1	0	0	0	0	10
Gymnodia sp.	0	0	0	0	0	0	1	0	0	0	0	0	1
Haematobia irritans (Linnaeus, 1758)	0	0	0	0	0	0	20	0	0	0	0	11	31
Helina sp. 1	1	5	2	0	0	0	2	0	0	0	0	0	10
Helina sp. 2	26	4	3	0	2	0	6	0	0	0	0	0	41
Helina sp. 3	8	0	1	0	0	1	4	1	0	1	0	0	16
Helina sp. 4	0	6	1	0	0	0	4	0	0	0	0	0	11
Helina sp. 6	2	5	1	5	0	4	1	5	0	0	0	24	47
Limnophora aurifacies Stein, 1911	14	7	7	3	5	15	19	0	0	0	0	0	70
Limnophora sp. 1	4	0	0	0	1	1	12	0	0	0	0	1	19
Limnophora sp. 2	13	2	2	0	1	0	50	1	0	0	0	0	69
Limnophora sp. 3	3	0	0	0	0	0	7	0	0	0	0	0	10
Limnophora sp. 4	99	11	8	6	5	5	155	2	0	1	4	2	298
Limnophora sp. 5	59	36	22	8	9	3	216	1	1	0	1	0	356
Limnophora sp. 6	0	1	0	0	0	0	2	0	0	0	0	0	3
Lispe serotina Wulp, 1896	1	0	0	0	0	2	0	0	0	0	0	0	3
Micropotamia minuscula (Albuquerque, 1955)	7	8	0	2	0	0	16	0	0	0	1	0	34
Morellia humeralis (Stein, 1918)	0	0	0	0	0	0	2	17	0	0	0	0	19
Morellia paulistensis Pamplona and Mendes, 1995	0	0	0	8	58	5	40	6	1	0	0	1	119
Morellia sp. 1	0	2	1	0	8	0	3	0	0	0	0	0	14
Morellia sp. 2	1	1	0	1	2	0	1	0	0	0	0	0	6
Musca domestica Linnaeus, 1758	0	0	0	0	0	0	0	0	0	1	0	0	1
Mydaea plaumanni Snyder, 1941	29	20	5	2	3	0	1	2	0	0	0	0	62
Myospila obscura (Shannon and Del Ponte, 1926)	1	7	0	1	6	0	13	5	1	0	2	0	33
Myospila sp. 1	4	1	0	0	0	0	4	0	0	1	1	1	12
Neodexiopsis erecta Costacurta, Couri and Carvalho, 2005	0	0	0	0	0	0	0	0	0	1	0	0	1
Neodexiopsis rustica Albuquerque, 1956	2	20	0	1	0	0	1	0	0	0	0	1	25
Neodexiopsis sp.	0	0	0	0	0	0	0	1	0	0	0	0	1
Neodexiopsis sp. 1	3	2	0	0	0	0	75	0	0	0	0	0	80
Neodexiopsis sp. 2	0	1	0	0	2	0	2	0	0	0	0	0	5
Neodexiopsis sp. 3	1	0	4	0	0	1	108	0	0	0	1	0	115
Neodexiopsis sp. 4	98	29	79	0	1	1	101	0	0	0	0	0	309
Neodexiopsis sp. 5	2	13	3	5	0	0	18	0	1	0	0	0	42
Neodexiopsis sp. 6	0	0	0	0	0	0	1	0	0	0	0	0	1
Neodexiopsis sp. 7	28	14	5	6	9	3	7	5	0	0	1	0	78
Neodexiopsis sp. 8	0	0	0	0	0	0	2	1	0	0	0	0	3
Neodexiopsis sp. 9	154	88	86	4	31	8	259	3	5	0	2	4	644
Neodexiopsis sp. 10	0	0	0	0	0	3	1	0	0	0	0	0	4
Neodexiopsis sp. 11	12	5	1	0	3	2	0	0	0	0	0	4	27
Neodexiopsis sp. 12 aff *vulgaris*[a] Couri and Albuquerque, 1979	0	0	0	1	0	0	0	0	0	0	0	0	1
Neodexiopsis sp. 13	0	0	1	0	0	0	0	0	0	0	0	0	1
Neomuscina aff *douradensis*[a] Lopes and Khouri, 1996	1	0	0	0	0	0	0	0	0	0	0	0	1
Neomuscina aff. *Goianensis*[a] Lopes and Khouri, 1995	0	0	0	0	1	0	0	0	0	0	0	0	1
Neomuscina inflexa (Stein, 1918)	0	0	0	0	0	0	0	0	0	0	0	1	1
Neomuscina neosimilis Snyder, 1949	0	0	0	0	0	0	0	1	0	0	3	0	4
Neomuscina pictipennis pictipennis (Bigot, 1878)	1	0	0	0	2	0	0	2	1	0	4	0	10
Neomuscina sanespra Snyder, 1949	0	0	0	0	1	0	0	0	0	0	0	0	1

Table 3 (*Continued*)

Taxa	R1			R2			R3	R4				R5	(∑)
	APL	ACS	ATU	RPPN	RCMQ	LAMI	TAIM	ITPA	PEVA	PMTY	PEJL	LPXE	
Neomuscina zosteris (Shannon and Del Ponte, 1926)	33	89	4	0	4	0	9	1	0	0	0	4	144
Neomuscina sp. 1	0	0	0	1	0	0	0	0	0	0	0	0	1
Neomuscina sp. 2	2	1	0	1	0	0	0	0	0	0	0	0	4
Neomuscina sp. 3	1	0	0	1	1	0	0	0	0	0	0	0	3
Neomuscina sp. 4	1	1	0	0	0	0	0	0	0	0	0	0	2
Neomuscina sp. 5	0	0	0	0	0	0	0	1	1	0	1	0	3
Neurotrixa felsina (Walker, 1849)	3	0	0	0	1	0	21	0	0	0	0	0	25
Neurotrixa marinonii[a] Costacurta and Carvalho, 2005	1	9	2	0	0	0	5	2	0	0	0	4	23
Neurotrixa sulina Costacurta and Carvalho, 2005	1	3	0	1	0	0	8	1	0	0	0	0	14
Phaonia advena Snyder, 1957	0	0	0	4	0	0	0	3	2	1	0	0	10
Phaonia annulata (Albuquerque, 1957)	9	21	0	0	0	0	0	4	0	0	1	0	35
Phaonia bigoti[a] (Albuquerque, 1957)	0	0	0	0	0	0	0	1	0	0	0	0	1
Phaonia grajauensis (Albuquerque, 1957)	6	13	1	10	2	7	1	10	2	0	5	0	57
Phaonia nigriventris (Albuquerque, 1954)	18	18	0	8	3	1	160	20	1	0	2	69	300
Phaonia praesuturalis (Stein, 1904)	0	0	0	0	2	0	0	9	0	0	0	0	11
Phaonia similata (Albuquerque, 1957)	1	0	0	8	4	3	1	3	2	0	3	1	26
Phaonia trispila (Bigot, 1885)	7	20	18	2	50	0	64	0	0	0	0	7	168
Phaonia sp. 1	8	10	1	2	5	5	24	5	11	2	10	9	92
Phaonia sp. 2	0	4	1	1	0	3	316	0	0	0	0	13	338
Phaonia sp. 3	22	0	6	1	9	2	0	0	0	0	0	0	40
Phaonia sp. 4	0	0	0	0	0	1	0	0	0	0	0	0	1
Philornis seguyi[a] Garcia, 1952	1	0	0	0	0	0	0	0	0	0	0	0	1
Philornis sp. 2	0	0	0	0	1	0	0	0	0	0	0	0	1
Plumispina sp. 1	0	0	0	0	0	0	1	0	0	0	0	0	1
Polietina orbitalis (Stein, 1904)	21	16	5	3	9	0	16	4	2	0	3	17	96
Pseudoptilolepis nudapleura[a] Snyder, 1949	0	0	0	5	0	0	0	0	0	0	0	0	5
Pseudoptilolepis sp.	2	0	1	0	0	0	0	0	0	0	0	0	3
Sarcopromusca pruna (Shannon and Del Ponte, 1926)	5	2	3	0	43	1	903	0	0	0	0	18	975
Stomopogon argentina (Snyder, 1957)	10	30	3	0	1	1	398	0	0	0	0	0	443
Stomoxys calcitrans (Linnaeus, 1758)	0	0	0	0	0	0	2	0	0	0	0	3	5
Trichomorellia seguyi[a] (Pamplona, 1983)	0	0	0	0	1	0	22	0	0	0	0	0	23
(∑)	871	593	383	123	355	121	3337	151	36	12	56	276	6314

[a] New record for RS state.

Results

Muscidae of the Coastal Plain of Pampa Biome

We collected 6314 specimens, 98 species in 31 genera of Muscidae on the CPPB (Table 3). The most abundant species were *Sarcopromusca pruna* (Shannon and Del Ponte) ($n = 934$), *Neodexiopsis* sp. 9 ($n = 641$), *Bithoracochaeta calopus* (Bigot) ($n = 503$), *Stomopogon argentina* (Snyder) ($n = 388$), *Limnophora* sp. 5 (356), *Phaonia* sp. 2 ($n = 338$), *Neodexiopsis* sp. 4 ($n = 309$), *Phaonia nigriventris* (Albuquerque) ($n = 300$), *Limnophora* sp. 4 ($n = 298$).

Among the 98 identified species of Muscidae, 41 did not fit descriptions of valid species, indicating that there may be many new species in the studied regions. Other data show that 34 taxa were unique to at least one location (Table 3). The geographical distribution of 13 species of Muscidae was expanded, and they were registered for the first time in the state of Rio Grande do Sul, based on the distributional catalogue of South American muscids (Löwenberg-Neto and Carvalho, 2013).

Regarding the number of species as a function of the samples (areas) in CPPB, the SAC exhibited an upward trend (Fig. 2), although the Chao2 diversity estimator report was collected between 70% and 86% Muscidae of CPPB (Table 4).

The diversity of muscid flies through CPPB regions and dissimilarity with Southern Brazil fauna

The species richness was the highest in regions 1 and 3, where there was a greater abundance of flies. When rarefaction by region was applied, computing the expected number of species in a subsample of n individuals ($n = 255$, corresponding to region 4), regions 2 and 4 had the highest estimated richness (Table 4). Rarefaction by area was computed to a subsample of $n = 8$ individuals, and the highest rarefied richness was observed in areas of regions 2, 4, and 1 (Table 4).

The most abundant species were found to vary in each region. In region 1, the most abundant species were *Neodexiopsis* sp. 9, *Neodexiopsis* sp. 4, and *B. calopus*, which are small predators in larvae and adult stages, and their combined abundance represented 38% of the collected specimens from this region. Singletons and doubletons accounted for 31.9% of the total species gathered in this region. In region 2, the most abundant species (28.9%) were *Morellia paulistensis* Pamplona and Mendes, *Phaonia trispila* (Bigot), and *B. calopus*. In this region, 44.4% of the species were singletons and doubletons. More than 52% of the total abundance of species in the CPPB was in region 3 (Taim Ecological Station). In this region, 46.8% of the specimens were *S. pruna*, *S. argentina*, and *Phaonia* sp. 2. We collected 26 species (36.62%) of singleton and doubletons in this region. In region 4, *Phaonia* sp. 1, *Phaonia nigriventris* (Albuquerque), and *Cyrtoneuropsis incognita* Malloch were the most abundant species (28.6%). Region 4 contained 48.9% of singleton and doubleton species. In region 5 (Lagoa do Peixe National Park), the most abundant species, corresponding to 55.5%, were *P. nigriventris*, *B. calopus*, *B. annulata* Stein and *Helina* sp. 6. Singleton and doubleton species accounted for 40% of the species richness (Table 3).

Differences in species composition between regions are shown by the cluster analysis (Fig. 3). The first cluster group is composed by regions 1, 2, and 3. The assemblage dissimilarity between groups from regions 1 and 2 was more than 40%. The difference between the cluster formed by regions 1 and 2 and formed

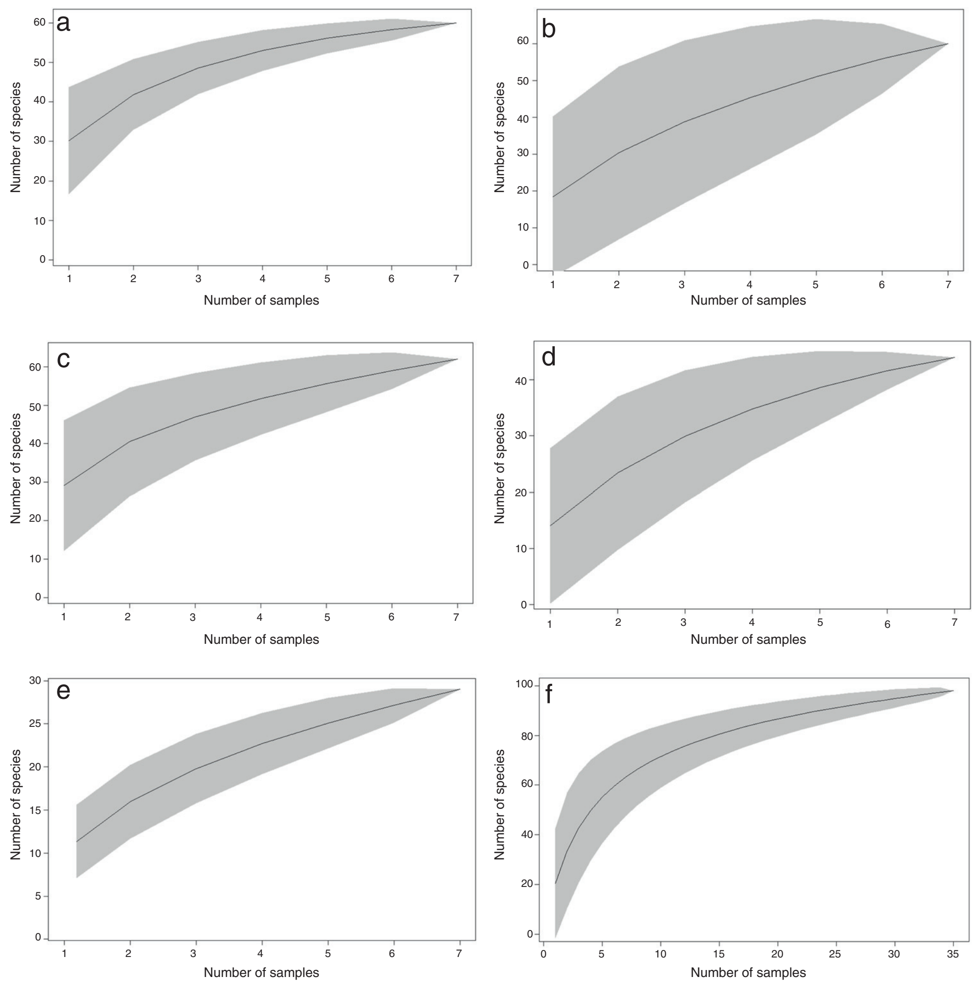


Fig. 2. Sample coverage curves based on the number of individuals of Muscidae per region in the Coastal Plain (CPPB) of Rio Grande do Sul, Brazil. The grey shaded area represents the confidence intervals. (a) region 1; (b) region 2; (c) region 3; (d) region 4; (e) region 5; (f) CPPB total.

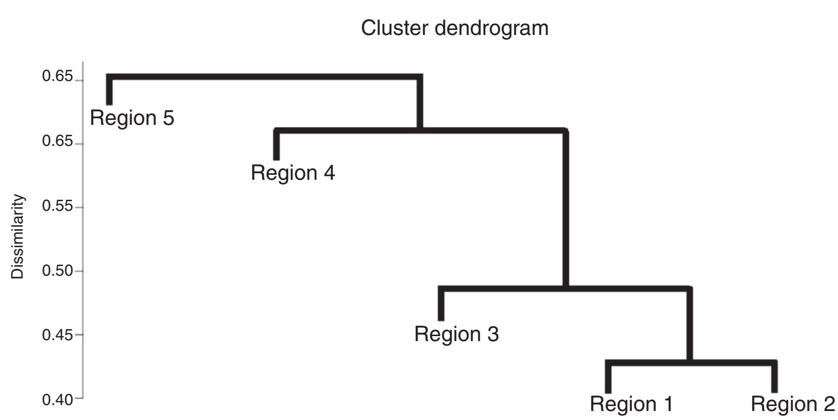


Fig. 3. Compositional differences between Muscidae (Insecta, Diptera) assemblages of all five regions of the Coastal Plain of Pampa Biome (Rio Grande do Sul, Brazil) shown by the unweighted pair-group method using arithmetic averages (UPGMA) cluster analysis.

by region 3 was also low, at about 45%. More significant differences between assemblage clusters were observed between group 1 (regions 1–3) and regions 4 and 5, with more than 60% dissimilarity. Among the species of muscids in Southern Brazil, including those in this study, there was high dissimilarity between clusters with a trend to increase/decrease based on geographic distance (Fig. 4).

Diversity pattern by trophic guilds of Muscidae

The proportion of species and abundance by guild was not consistent throughout the CPPB; this difference was influenced by collection region (Tables 5 and 6). In the first three regions, the highest species richness was found in a guild of PP settings followed by OS settings; in the former, both larvae and adults are predators,

Table 4
Richness estimators by location and region of collection. Non identified specimens were pruned from data matrices before analysis. R1: region 1, where: APL: Pelotas stream; ACS: Corrientes stream; ATU: Turuçu stream. R2: region 2, where: LAMI: Lami Biological Reserve; RCMQ: Camaquã river; RPPN: private natural heritage reserve Barba Negra farm. R3: region 3, where: TAIM: Taim Ecological Station. R4: region 4, where: ITPA: Itapuã State Park; PMTY: Tupancy Municipality Natural Park; PEJL; José Lutzenberger State Park; PEVA: Itapeva State Park. R5: region 5, where LPXE: Lagoa do Peixe National Park. CPPB, Coastal Plains on Pampa Biome; Traps, number of traps per location; S, species richness; Abund, abundance; Chao2, Chao2 non-parametric diversity estimator; Srar, rarefaction; SE, standard error. In the rarefaction data by location, the standard number of individuals was 12 (PMTY), and by region the standard number of individuals was 254 (R4).

	Traps	S	Abund	Chao2 ± SE	Srar ± SE
R1					
APL	12	55	870	81.96 ± 17.6	8.08 ± 1.36
ACS	8	42	592	46.49 ± 4.79	8.80 ± 1.34
ATU	8	34	383	51.95 ± 14.35	6.22 ± 1.35
Total R1	28	62	1845	104.23 ± 38.5	38.40 ± 2.65
R2					
LAMI	8	31	121	54.8 ± 20.03	9.07 ± 1.27
RCMQ	4	39	355	46.12 ± 5.88	8.04 ± 1.36
RPPN	16	32	123	40.26 ± 6.85	9.62 ± 1.17
Total R2	28	60	599	72.78 ± 8.36	46.15 ± 2.60
R3					
TAIM	28	62	3333	74.5 ± 8.46	30.11 ± 2.59
R4					
ITPA	12	37	150	72.88 ± 25.7	9.01 ± 1.30
PMTY	4	9	12	31.45 ± 28.54	9.00
PEVA	8	15	36	22.77 ± 7.29	7.62 ± 1.26
PEJL	4	18	56	26 ± 8.13	8.08 ± 1.26
Total R4	28	46	254	86.34 ± 28.44	46
R5					
LPXE	28	29	276	78.82 ± 59.37	28.19 ± 0.84
CPPB total	140	98	6307	181.65 ± 58.58	–

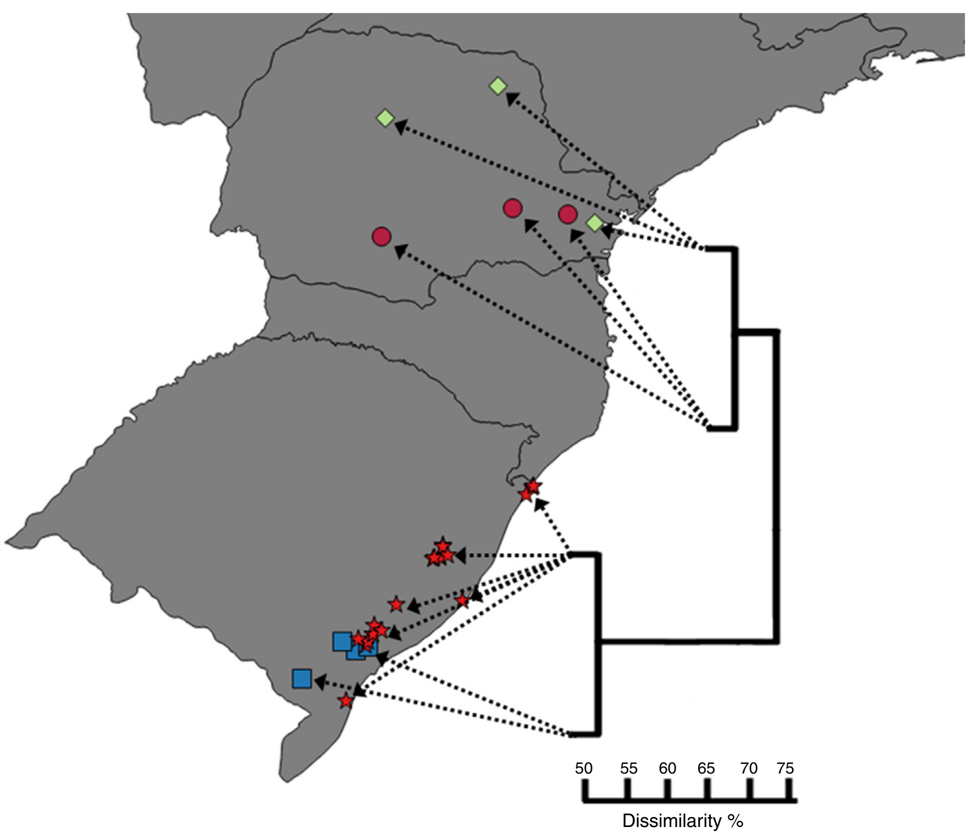


Fig. 4. The UPGMA cluster between Muscidae (Insecta, Diptera) assemblages of Southern Brazil. The dissimilarity analysis was performed using the Jaccard index with binary matrices. In Paraná state: green diamonds, Rodríguez-Fernández et al. (2006); pink circles, Costacurta et al. (2003). In the Rio Grande do Sul state: red stars, current study; blue squares, Krüger et al. (2010).

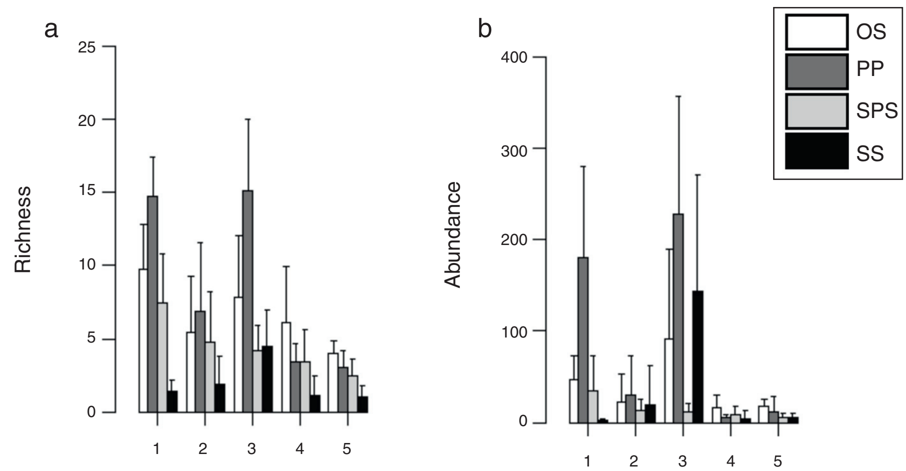


Fig. 5. Graphical representation of proportional richness (a) and abundance (b) by the guild in the five regions of Coastal Plain of Pampa Biome (Rio Grande do Sul, Brazil). SS, saprophagous larvae and saprophagic/hematophagous adults; SPS, facultative predators/parasitic larvae and saprophagous adults; OS, predatory larvae and saprophagous adults; PP, predatory larvae and adults.

Table 5
Analysis of variance (ANOVA) and *F* test, testing the influence of region and ecological guilds of Muscidae on the proportional richness. Df, degrees of freedom; Sum Sq, sum of squares; Mean Sq, mean of squares.

	Df	Sum Sq	Mean Sq	*F* value	*P*
Guild	3	1.51893	0.50631	61.9787	<0.001
Region	4	0.00010	0.00002	0.0030	1.000
Guild:region	12	0.51359	0.04280	5.2392	<0.001
Residuals	120	0.98029	0.00817	–	–

Table 6
Analysis of variance (ANOVA) and *F* test, testing the influence of region and ecological guilds of Muscidae on the proportional abundance. Df, degrees of freedom; Sum Sq: sum of squares; Mean Sq: mean of squares.

	Df	Sum Sq	Mean Sq	*F* value	Pr(>*F*)
Guild	3	1.8376	0.61254	21.9057	<0.001
Region	4	0.0048	0.00119	0.0426	0.997
Guild:region	12	2.0160	0.16800	6.0079	<0.001
Residuals	120	3.3555	0.02796	–	–

and in the latter, larvae are predators and adults are saprophagous (Fig. 5A). In regions 4 and 5, this relationship was reversed, with a predominance of OS settings species followed by PP guild (Fig. 5A). In all regions, guild species with saprophagous habits in the larval and adult stages is present in the lowest proportion of the total fauna, except in region 3 (Taim), which exhibited increased richness compared with the SPS guild (Fig. 5A). The same pattern was observed for the relative abundance within each guild by region. Contrary to the relationship between the proportion of species per guild and region, the abundance of the SS guild greatly contributes to the fauna in regions 3 and 5 (Fig. 5B).

Discussion

The Muscidae in Southern Brazil, fauna similarity, and possible areas of endemism

In Southern Brazil, major taxonomic surveys of Muscidae were carried out, and a trend of greater or lesser similarity between the areas of collections was observed with increasing or decreasing distance. There was greater similarity between the data collected in CPPB and those inventoried by Krüger et al. (2010) in the Rio Grande do Sul over a 1-year collection period. We share 36 species without the morphospecies, and there was a tendency of similarity among the most abundant species in the two studies. Surveys conducted in the Paraná state (Costacurta et al., 2003; Rodríguez-Fernández et al., 2006), which is about 1000 km from the study site in the CPPB, found that the proportion of species was lower, with 17 and 23 species, and there is a high dissimilarity with CPPB (Fig. 4). Some studies (Löwenberg-Neto and Carvalho, 2009; Nihei and Carvalho, 2005) have indicated areas of endemism and diversification for Muscidae in the Paranaense Forest (Paraná state) and the Pampa. Therefore, our data indicate that the CPPB regions may represent new areas of endemism, since there is: (i) significant Muscidae richness; (ii) new records of occurrence; (iii) high rates of particular species per region; (iv) high dissimilarities with the Paraná Forest communities.

Sampling effort in the areas and conservation units in the CPPB

Differences between the samplings by Costacurta et al. (2003), Rodríguez-Fernández et al. (2006), and Krüger et al. (2010) and those in the present study include the exposure time of Malaise traps versus the number of traps installed by area. The studies by Costacurta et al. (2003), Rodríguez-Fernández et al. (2006), and Krüger et al. (2010) addressed the sampling of Muscidae assemblies on a temporal scale at the expense of a spatial scale. In contrast, we sought to directly sample on a spatial scale over time, with many replicates (traps) and a short period of exposure.

While representing a considerable sampling effort (140 Malaise traps in 35 areas), our species accumulation curve possesses no asymptote. We can argue that (i) the restricted use of Malaise traps is not problematic for the sampling of Muscidae diversity, as once compared with traps containing decomposing bait (Carvalho et al., 1984), the Malaise trap has higher efficiency in the capture of Diptera richness (Brown, 2005; Costacurta et al., 2003; Duarte et al., 2010; Krüger et al., 2010; Rodríguez-Fernández et al., 2006). Species collected with baits are often, though not always, gathered in Malaise traps; (ii) the ascending pattern of the SAC shows that more focus should be placed on traps in the large fragments than in the small fragments. It is possible that the richness has been underestimated in larger areas as the collection effort was the same in large and small areas. Therefore, small areas showed more richness in the CPPB (e.g., Pelotas and Corrientes streams, Camaquã river, Taim ecological Station, Itapuã state park). (iii) In regions 4 and 5, an increase in the number of traps per se cannot reverse the upward trend of the SAC. In such areas, it is necessary to move the collection period before January (the beginning of summer in the Southern Hemisphere) due to climatic conditions of high temperatures and low relative humidity. These conditions have probably prevented the movement of adult specimens, or else caused their death, preventing them from being collected by Malaise traps. Despite this fact, analysis with Chao2 revealed that 75% of Muscidae species were collected in some regions (Table 4), such as the areas around the Pelotas, Corrientes, and Turuçu streams. There was more richness in region 1 than at the Taim Ecological Station (Tables 3 and 4). The areas in region 1 are not protected and suffer high exploitation due to extractive practice and agricultural production practices in their surroundings.

In general, singletons and doubletons species represent 29.7% of the richness, and their proportion ranges from 31.9 to 48.9% of the species collected in different regions, which is a typical pattern for insect communities in tropical forests (Erwin, 1998; Basset and Kitching, 1991; Novotný, 1993).

Muscidae ecological guilds as indicators of priority areas for conservation

Ecological/trophic guilds of Muscidae can be used to assess environmental health as they respond to specific features. For example, one of the factors closely linked to the conservation of natural areas is the abundance of predatory species of *Neodexiopsis* and *Limnophora*. The species of these genera occur in areas where there is high availability of freshwater sources for the development of larvae, and a high abundance of prey for the larvae and adults of these species (Werner and Pont, 2006). The preys recorded in the literature include chironomids, simulids, and other small flies that do not tolerate disturbed areas (Werner and Pont, 2006). The PP guild, which includes the predators, was dominant in most areas, especially in regions 1–3 (Fig. 4). In these regions, there are many marshes, small rivers, streams, and other flooded areas, which are the surrounding physiognomy of the Patos lagoon and Mirim–Mangueira lagoon, and priority areas for conservation according to MMA (2000). Therefore, the high richness and abundance proportional to the PP guild indicate potential areas for permanent protection, such as the Pelotas streams (Pelotas, Corrientes, Turuçu) and the Camaquã river.

Other guilds may be indications of a disturbance in natural areas, specifically the SS guild, containing larvae and saprophagous adults. The species in the SS guild developed preferentially in wild and domestic animal faeces; therefore, this would be expected a priori for high diversity and abundance in Taim Ecological Station, which is a refuge for a wide variety of birds, reptiles, and mammals. Proportionality analysis showed that the SS guild contains a higher abundance in region 3. This result is not inconsistent with expectations, as the dominant species in this guild is *Sarcopromusca pruna*. The larvae of this species are associated with animal faeces (Pedroso-De-Paiva, 1996), while adults feed on wounds induced by bloodsucking species such as horseflies, horn flies (*Haematobia irritans*), and stable flies (*Stomoxys calcitrans*). These species are commonly associated with and recognised as vectors of many pathogens and eggs of parasites (Azevedo et al., 2007). The biology of the horn fly should be noted, whereby the adult constantly remains on the host to feed, and females move away from the host only for oviposition on newly deposited faeces, as they are dependent on high humidity in dung pats (McLintock and Depner, 1954). This peculiar behaviour should limit their capture in Malaise traps since *H. irritans* do not often fly, confirming the traffic of cattle within fragments of Taim. The presence of species of the SS guild, *S. pruna*, *H. irritans*, and *S. calcitrans*, along with the occurrence of *Gymnodia* species (OS guild), which also occurs in the stools of production ungulates, are an indication of disturbance.

The OS guild containing predatory larvae and saprophagous adults generally contains generalist predatory larvae in their microhabitats (*Phaonia*, *Helina*, *Graphomya*), and often the faeces of herbivorous vertebrates (*Gymnodia*) (Skidmore, 1985). The presence of species of this genus indicates a disturbance in the natural environment due to the association with cattle dung, unlike indigenous species of *Phaonia*, *Helina*, and *Graphomya*, where the larvae are predators in humus and mosses. The high proportion of the OS guild in Lagoa do Peixe (region 5), without the occurrence of *Gymnodia* species, is indicative of areas of diverse resources, consistent low anthropised area.

Closing remarks

Our results show in general that some habitats of CPPB are suffering from invasions by non-indigenous species closely associated with livestock. The invasions present a danger to the indigenous species which occupy the same space as these invaders, such as herbivorous faeces. This topic raises discussion about the implementation of buffer zones and monitoring the anthropic activities surrounding protected areas (Allan et al., 2017; Weisse and Naughton-Treves, 2016).

Conversely, the non-protected areas in Pelotas streams and Camaquã River need more legal attention by legislators to become protected areas due to the high diversity of flies and high proportionality of guilds of predators (indicators for healthy environments). Despite our sampling efforts in CPPB, the results reveal the need for additional surveys in these regions, as many more species remain to be discovered. The CPPB may be considered a hotspot for the conservation of Muscidae species, in turn, new biogeographical approaches are needed to identify new areas of endemism in the Pampa biome.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

All authors have contributed substantially to the conception, design, analysis, and/or interpretation of the data in this manuscript and will take public responsibility for the content. AZS was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and by the National Council of Technological and Scientific Development (CNPq) (process no. 161907/2015-6). We also thank CNPq for financing the project "Efeito da redução da área na diversidade de Muscidae (Insecta: Diptera) da planície costeira do Rio Grande do Sul" (process no. 473949/2010-5). We thank Juliano Lessa Pinto Duarte, PhD, for assistance in the genus sorting of material; Claudio José Barros de Carvalho, PhD (Universidade Federal do Paraná) for providing help with identification. We also thank the administrators of protected areas where collecting was conducted and to "Sistema de Autorização e Informação em Biodiversidade" (SISBIO) for providing the collection license (process no. 29229). RFK is supported by CNPq (process no. 308908/2016-3).

References

Allan, J.R., Venter, O., Maxwell, S., Bertzky, B., Jones, K., Shi, Y., Watson, J.E.M., 2017. Recent increases in human pressure and forest loss threaten many Natural World Heritage Sites. Biol. Conserv. 206, 47–55.

Azevedo, R.R., Krüger, R.F., 2013. The influence of temperature and humidity on abundance and richness of Calliphoridae (Diptera). Iheringia. Ser. Zool. 103, 145–152.

Azevedo, R.R., Duarte, J.L.P., Ribeiro, P.B., Krüger, R.F., 2007. Occurrence of *Sarcopromusca pruna* (Diptera) in Southern Brazil as a vector of *Dermatobia hominis* (Diptera) eggs. Arq. Bras. Med. Vet. Zootec. 59, 1348–1350.

Barnosky, A.D., Hadly, E.A., Bascompte, J., Berlow, E.L., Brown, J.H., Fortelius, M., Getz, W.M., Harte, J., Hastings, A., Marquet, P.A., Martinez, N.D., Mooers, A., Roopnarine, P., Vermeij, G., Williams, J.W., Gillepsie, R., Kitzes, J., Marshall, C., Matzke, N., Mindell, D.P., Revilla, E., Smith, A.B., 2012. Approaching a state shift in Earth's biosphere. Nature 486, 52–58.

Basset, Y., Kitching, R.L., 1991. Species number, species abundance and body length of arboreal arthropods associated with an Australian rainforest tree. Ecol. Entomol. 16, 391–402.

Bencke, G.A., 2009. Diversidade e conservação da fauna dos Campos do Sul do Brasil. In: Pillar, V.P.P., Müller, S.C., Castilhos, Z.M.S., Jacques, A.V.A. (Eds.), Campos Sulinos: Conservação e Uso Sustentável da Biodiversidade. Ministério do Meio Ambiente, Brasília, pp. 101–121.

Bilenca, D., Codesido, M., González-Fischer, C., Carusi, L.P., Zufiaurre, E., Abba, A., 2012. Impactos de la transformación agropecuaria sobre la biodiversidad en la provincia de Buenos Aires. Rev. Mus. Argent. Cienc. Nat. 14, 189–198.

Blondel, J., 2003. Guild or functional groups: does it matter? Oikos 100, 223–231.

Borcard, D., Gillet, F., Legendre, P., 2011. Numerical Ecology with R. New York, Springer-Verlag, http://dx.doi.org/10.1007/978-1-4419-7976-6.

Brown, B.V., 2005. Malaise trap catches and the crisis in neotropical dipterology. Am. Entomol. 513, 180–183.

Bunge, J., Fitzpatrick, M., 1993. Estimating the number of species: a review. J. Am. Stat. Assoc. 88, 364–373.

Burger, M.I., 2000. Situação e ações prioritárias para a conservação de banhados e áreas úmidas da zona costeira. ANP, Brasília, Available at: https://www.brasil-rounds.gov.br/round7/arquivos_r7/PERFURACAO_R7/refere/Banhados.pdf (accessed 14.02.17).

Carvalho, C.J.B., Couri, M.S., 2002. Part I. Basal groups. In: Carvalho, C.J.B. (Ed.), Muscidae (Diptera) of Neotropical Region: Taxonomy. Editora Universidade Federal do Paraná, Curitiba, pp. 17–132.

Carvalho, C.J.B., Almeida, J.R., Jesus, C.B., 1984. Dípteros sinantrópicos de Curitiba e arredores (Paraná, Brasil). I. Muscidae. Rev. Bras. Entomol. 28, 551–560.

Carvalho, C.J.B., Bortolanza, M., Silva, M.C.C., Soares, E.D.G., 2003. Distributional patterns of the Neotropical Muscidae (Diptera). In: Morrone, J.J., Bousquets, J.L. (Eds.), Una perspectiva Latinoamericana de la Biogeografía. , 1ed. Las Prensas de Ciencia, Facultad de Ciencias Universidad Autónoma de México, México, pp. 263–274.

Carvalho, C.J.B., Couri, M.S., Pont, A.C., Pamplona, D., Lopes, S.M., 2005. A catalogue of the Muscidae (Diptera) of the Neotropical Region. Zootaxa 860, 1–282.

Celulose Riograndense, 2012. Plano de Manejo da RPPN Barba Negra, Available at: http://www.celuloseriograndense.com.br/responsabilidade/ (accessed 26.06.15).

Chao, A., 1987. Estimating the population size for capture–recapture data with unequal catchability. Biometrics 43, 783–791.

Chao, A., Colwell, R.K., Lin, C.W., Gotelli, N.J., 2009. Sufficient sampling for asymptotic minimum species richness estimators. Ecology 90, 1125–1133.

Chiu, C.H., Wang, Y.T., Walther, B.A., Chao, A., 2014. An improved nonparametric lower bound of species richness via a modified good-turing frequency formula. Biometrics 70, 671–682.

Coelho, S.M.P., 1997. Aspectos morfológicos da Probóscide de Muscoidea (Diptera, Muscomorpha). Rev. Bras. Zool. 14, 245–253.

Colwell, R.K., Coddington, J.A., 1994. Estimating terrestrial biodiversity through extrapolation. Philos. Trans. R. Soc. London Ser. B 345, 101–118.

Colwell, R.K., Mao, C.X., Chang, J., 2004. Interpolating, extrapolating, and comparing incidence-based species accumulation curves. Ecology 85, 2717–2727.

Costacurta, N.C., Carvalho, C.J.B., 2005. Taxonomy of *Neurotrixa* Shannon e Del Ponte (Diptera: Muscidae) with description of new species from southern Brazil. Neotrop. Entomol. 34, 927–932.

Costacurta, N.C., Couri, M.S., Carvalho, C.J.B., 2005. Descriptions of new species with a key to identification of the genus *Neodexiopsis* Malloch (Diptera, Muscidae) in Brazil. Rev. Bras. Entomol. 49, 353–366.

Costacurta, N.C., Marinoni, R.C., Carvalho, C.J.B., 2003. Fauna de Muscidae (Diptera) em três localidades do Estado do Paraná, Brasil, capturada por armadilha Malaise. Rev. Bras. Entomol. 47, 389–397.

Couri, M.S., Carvalho, C.J.B., 2002. Part II. Apical groups. In: Carvalho, C.J.B. (Ed.), Muscidae (Diptera) of Neotropical Region: Taxonomy. Editora Universidade Federal do Paraná, Curitiba, pp. 133–286.

Crawshaw, D., Dall'agnol, M., Cordeiro, J.L.P., Hasenack, A., 2007. Caracterizacão dos campos sul-rio-grandenses: uma perspectiva da ecologia da paisagem. Bol. Gaúcho Geogr. 33, 233–252.

Da Silva, P.G., Vaz-De-Mello, F.Z., Di Mare, R.A., 2013. Diversity and seasonality of Scarabaeinae (Coleoptera: Scarabaeidae) in forest fragments in Santa Maria, Rio Grande do Sul, Brazil. An. Acad. Bras. Cienc. 85, 679–697.

Duarte, J.L.P., Krüger, R.F., Carvalho, C.J.B., Ribeiro, P.B., 2010. Evidence of the influence of Malaise trap age on its efficiency in the collection of Muscidae (Insecta, Diptera). Int. J. Top. Insect. Sci. 30, 115–118.

Erwin, T.L., 1998. The tropical forest canopy. The heart of biotic diversity. In: Wilson, E.O. (Ed.), Biodiversity. National Academy Press, Washington, pp. 109–127.

Ferrar, P., 1987. A Guide to the Breeding Habits and Immature Stages of Diptera Cyclorrhapha. Scandinavian Science Press, Copenhagen.

Ferreira, J., Pardini, R., Metzger, J.P., Fonseca, C.R., Pompeu, O.S., Sparovek, G., Louzada, J., 2012. Towards environmentally sustainable agriculture in Brazil: challenges and opportunities for applied ecological research. J. Appl. Ecol. 49, 535–541.

Filloy, J., Bellocq, M.I., 2006. Spatial variations in the abundance of *Sporophiia* seedeaters in the southern Neotropics: contrasting the effects of agricultural development and geographical position. Biodivers. Conserv. 15, 3329–3340.

Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N., Snyder, P.K., 2005. Global consequences of land use. Science 309, 570–574.

Fontana, C.S., Bencke, G.A., Reis, R.E., 2003. Livro vermelho da fauna ameaçada de extinção no Rio Grande do Sul. EDIPUCRS, Porto Alegre.

Gonzalez, S., Merino, M.L., 2008. Ozotoceros bezoarticus. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2, Available at: http://www.iucnredlist.org (accessed 18.03.14).

Gotelli, N.J., Colwell, R.K., 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecol. Lett. 4, 379–391.

Greenberg, B., 1971. Flies and Disease. Ecology, Classification and Biotic Associations. Princeton University Press, New Jersey.

Hochmüller, C.J., Lopes-Da-Sila, M., Valente, V.L.S., Schmitz, H.J., 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.

Hsieh, T.C., Ma, K.H., Chao, A., 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). Methods Ecol. Evol. 7, 1451–1456.

IBGE – Instituto Brasileiro de Geografia e Estatística, 1986. Vegetação, Uso Potencial da Terra. In: IBGE, Levantamento de Recursos Naturais, Rio de Janeiro, pp. 1–796.

IBGE – Instituto Brasileiro de Geografia e Estatística, 2004. Mapa de vegetação do Brasil, Brasília, Available at: https://ww2.ibge.gov.br/home/presidencia/noticias/21052004biomas.shtm (accessed 30.01.18).

ICMBIO – Instituto Chico Mendes de Conservação da Biodiversidade, 1999. Plano de Manejo do Parque Nacional da Lagoa do Peixe, Available at: http://www.icmbio.gov.br/portal/images/stories/imgs-unidades-coservacao/parna_lagoa-do-peixe.pdf (accessed 02.08.16).

Kindt, R., Van-Damme, P., Simons, A.J., 2006. Tree diversity in western Kenya: using profiles to characterize richness and evenness. Biodivers. Conserv. 15, 1253–1270.

Kirst, F.D., Marinoni, L., Krüger, R.F., 2015. New distribution records for Sciomyzidae species (Insecta, Diptera) from Rio Grande do Sul, Brazil. Check List 11, 1–5.

Kirst, F.D., Marinoni, L., Krüger, R.F., 2017. What does the Southern Brazilian Coastal Plain tell about its diversity? Syrphidae (Diptera) as a model. Bull. Entomol. Res. 107, 645–657.

Krapovickas, S., Di Giacomo, A.S., 1998. Conservation of pampas and campos grasslands in Argentina. Parks 8, 47–53, Available at: https://www.iucn.org/sites/dev/files/import/downloads/parks_oct98_1.pdf (accessed 30.01.18).

Krüger, R.F., Krolow, T.K., 2015. Seasonal patterns of horse fly richness and abundance in the Pampa biome of southern Brazil. J. Vector Ecol. 40, 364–372.

Krüger, R.F., Carvalho, C.J.B., Ribeiro, P.B., 2010. Assembly rules in muscid fly assemblages in the grasslands biome of Southern Brazil. Neotrop. Entomol. 39, 345–353.

Leite, P.F., 2002. Contribuição ao conhecimento fitoecológico do sul do Brasil. Ciência and Ambiente 24, 52–73.

Löwenberg-Neto, P., Carvalho, C.J.B., 2009. Areas of endemism and spatial diversification of the Muscidae (Insecta: Diptera) in the Andean and Neotropical regions. J. Biogeogr. 36, 1750–1759.

Löwenberg-Neto, P., Carvalho, C.J.B., 2013. Muscidae (Insecta: Diptera) of Latin America and the Caribbean: geographic distribution and check-list by country. Zootaxa 3650, 1–147.

Machado, A.B.M., Drummond, G.M., Paglia, A.P., 2008. Livro Vermelho da Fauna Brasileira Ameaçada de Extinção. Biodiversidade 19.

Magurran, A.E., 2004. Measuring Biological Diversity. Oxford Blackwell Science, Oxford.

Marques, B., Couri, M.S., 2007. Taxonomia e morfologia de espécies neotropicais de *Graphomya* Robineau-Desvoidy (Diptera, Muscidae). Rev. Bras. Entomol. 51, 436–444.

McAlpine, J.F., 1981. Morphology and terminology: adults. In: McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R., Wood, D.M. (Eds.), Manual of Neartic Diptera. Ottawa, pp. 9–63.

McLintock, J., Depner, K.R., 1954. A review of the life-history and habits of the horn fly, *Siphona irritans* (L.) (Diptera: Muscidae). Can. Entomol. 86, 20–33.

Meira, J.R., Porto, M.L., 1998. Reserva Biológica do Lami: a vida na beira do lago. In: Menegat, R. (Ed.), Atlas Ambiental de Porto Alegre. Editora UFRGS, Porto Alegre, pp. 89–92.

Mello-Filho, L.E., Somner, G.V., Peixoto, A.L., 1992. Centuria Plantarum Brasiliensium Exstintionis Minitata. Sociedade Botânica do Brasil/Ibama, Brasília.

Mikich, S.B., Bérnils, R.S., 2004. Livro Vermelho da Fauna Ameaçada no Estado do Paraná, Available at: http://www.meioambiente.pr.gov.br/arquivos/File/cobf/livro_fauna_extincao.pdf (accessed 14.02.17).

MMA – Ministério do Meio Ambiente, 2000. Avaliação e ações prioritárias para a conservação da biodiversidade da Mata Atlântica e Campos Sulinos, Available at: http://www.mma.gov.br/estruturas/sbf_chm_rbbio/_arquivos/Sumario%20Mata%20Atlantica.pdf (accessed 17.02.17).

MMA – Ministério do Meio Ambiente, 2004. Federal Decree n° 5.092, May 21, 2004 , Available at: http://www2.camara.leg.br/legin/fed/decret/2004/decreto-5092-21-maio-2004-532394-publicacaooriginal-14437-pe.html (accessed 14.02.17).

Nihei, S.S., 2005. A review of the Neotropical genus Sarcopromusca Townsend (Diptera: Muscidae) with a key to species and a redescription of *S. sarcophagina* (Wulp). Zootaxa 1004, 51–64.

Nihei, S.S., Carvalho, C.J.B., 2005. Distributional patterns of the Neotropical fly genus *Polietina* Schnabl and Dziedzicki (Diptera, Muscidae): a phylogeny-supported analysis using panbiogeographic tools. Pap. Avulsos Zool. 45, 313–326.
Nihei, S.S., Carvalho, C.J.B., 2007. Systematics and biogeography of *Polietina* Schnabl and Dziedzicki (Diptera, Muscidae): Neotropical area relationships and Amazonia as a composite area. Syst. Entomol. 32, 477–501.
Novotný, V., 1993. Spatial and temporal components of species diversity in Auchenorrhyncha (Insecta: Hemiptera) communities of Indochinese montane rain forest. J. Trop. Ecol. 9, 93–100.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Wagner, H., 2015. vegan: Community Ecology Package. R Package Version 2.3-0, Available at: https://github.com/vegandevs/vegan (accessed 07.08.18).
Overbeck, G.E., Muller, S.C., Fidelis, A., Pfadenhauer, J., Pillar, V.D., Blanco, C.C., Boldrini, I.I., Both, R., Forneck, E.D., 2007. Brazil's neglected biome: the South Brazilian Campos. Plant Ecol. Evol. Syst. 9, 101–116.
Parmar, T.K., Rawtani, D., Agrawal, Y.K., 2016. Bioindicators: the natural indicator of environmental pollution. Front Life Sci. 9, 110–118.
Paz, A.L.G., Romanowski, H.P., de Morais, A.B.B., 2013. Distribution of *Satyrini* (Lepidoptera, Nymphalidae) in Rio Grande do Sul State, southern Brazil. Ecol. Res. 28, 417–426.
Pedroso-De-Paiva, D., 1996. Aspectos da biologia de adultos de *Sarcopromusca pruna* (Shannon and Del Ponte, 1926) (Diptera: Muscidae). Rev. Bras. Biol. 56, 183–190.
Pereira, H.M., Leadley, P.W., Proença, V., Alkemade, R., Scharlemann, J.P.W., Fernandez-Manjarrés, J.F., Araújo, M.B., Balvanera, P., Biggs, R., Cheung, W.W.L., Cini, L., Cooper, H.D., Gilman, E.L., Guénette, S., Hurtt, G.C., Huntington, H.P., Mace, G.M., Oberdorff, T., Revenga, C., Rodrigues, P., Scholes, R.J., Sumaila, U.R., Walpole, M., 2010. Scenarios for Global Biodiversity in the 21st Century Science. Science 330, 1496–1501.
Pereira-Colavite, A., Carvalho, C.J.B., 2012. Taxonomy of Neomuscina Townsend (Diptera, Muscidae) from Brazil. Zootaxa 3504, 1–55.
Pillar, V.P.P., Müller, S.C., Castilhos, Z.M.S., Jacques, A.V.A., 2009. Campos Sulinos: Conservação e Uso Sustentável da Biodiversidade. Ministério do Meio Ambiente, Brasília.
Poppe, J.L., Valente, V.L.S., Schmitz, H.J., 2012. Structure of Drosophilidae assemblage (Insecta, Diptera) in Pampa Biome (São Luiz Gonzaga, RS). Pap. Avulsos Zool. 52, 185–195.
Poppe, J.L., Valente, V.L.S., Schmitz, H.J., 2013. Population dynamics of drosophilids in the pampa biome in response to temperature. Neotrop. Entomol. 42, 269–277.
R Development Core Team, 2015. R: A Language and Environment for Statistical Computing, Avaiable at: http://www.R-project.org (accessed 26.11.13).
Rodríguez-Fernández, J.I., Carvalho, C.J.B., Moura, M.O., 2006. Estrutura de assembléias de Muscidae (Diptera) no Paraná: uma análise por modelos nulos. Rev. Bras. Entomol. 50, 93–100.
Root, R., 1967. The niche exploration pattern of the blue-gray gnatcatcher. Ecol. Monogr. 37, 317–350.
Rosado, J.L.O., Gonçalves, M.G., Dröse, W., Silva, E.J.E., Krüger, R.F., Feitosa, R.M., Loeck, A.E., 2012. Epigeic ants (Hymenoptera: Formicidae) in vineyards and grassland areas in the Campanha region, state of Rio Grande do Sul, Brazil. Check List 8, 1184–1189.
Rosado, J.L.O., Gonçalves, M.G., Dröse, W., Silva, E.J.E., Krüger, R.F., Feitosa, R.M., Loeck, A.E., 2013. Effect of climatic variables and vine crops on the epigeic ant fauna (Hymenoptera: Formicidae) in the Campanha region, state of Rio Grande do Sul, Brazil. J. Insect Conserv. 17, 1113–1123.
Santos, A.M.C., Whittaker, R.J., Triantis, K.A., Jones, O.R., Borges, P.A.V., Quicke, D.L.J., Hortal, J., 2010. Are species–area relationships from entire archipelagos congruent with those of their constituent islands? Global Ecol. Biogeogr. 19, 527–540.
Schüehli, G.S., Carvalho, C.J.B., 2005. Revision and cladistics of the Neotropical genus *Pseudoptilolepis* Snyder (Diptera, Muscidae). Rev. Bras. Entomol. 22, 23–34.
SEMA – Secretaria Estadual do Meio Ambiente, 1996. Plano de Manejo do Parque Estadual Itapuã, Available at: http://www.sema.rs.gov.br/upload/arquivos/201610/15171153-plano-manejo-peitapua.pdf (accessed 14.02.17).
SEMA – Secretaria Estadual do Meio Ambiente, 2013. Parques Estaduais, Available at: http://www.sema.rs.gov.br/conteudo.asp?cod_menu=174 (accessed 02.08.15).
Skidmore, P., 1985. The Biology of Muscidae of the World. Junk Publishers, Dordrecht.
Sneath, P.H., Sokal, R.R., 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification. W.H. Freeman and Company, San Francisco.
Townes, H.A., 1972. A light-weight Malaise trap. Entomol. News 83, 239–247.
Ugland, K.I., Gray, J.S., Ellingsen, K.E., 2003. The species accumulation curve and estimation of species richness. J. Anim. Ecol. 72, 888–897.
Venzke, T.S., 2012. Florística de comunidades arbóreas no Município de Pelotas, Rio Grande do Sul. Rodriguésia 63, 571–578.
Waechter, J.L., 1985. Aspectos ecológicos da vegetação de Restinga no Rio Grande do Sul, Brasil. Comun. Mus. Cienc. Tecnol. Pucrs. Ser. Bot. 33, 49–68.
Waechter, J.L., 2002. Padrões geográficos na flora atual do Rio Grande do Sul. Ciência and Ambiente 24, 93–108.
Weisse, M.J., Naughton-Treves, L.C., 2016. Conservation beyond park boundaries: the impact of buffer zones on deforestation and mining concessions in the Peruvian Amazon. Environ. Manage. 58, 297–311.
Werner, D., Pont, A.C., 2006. The feeding and reproductive behaviour of the Limnophorinii (Diptera: Muscidae). In: Proceedings of the International Symposium on Simuliidae. Supplement. Stud. Dipterol. 14, pp. 79–114.
Willie, J., Petre, C.A., Tagg, N., Lens, L., 2012. Evaluation of species richness estimators based on quantitative performance measures and sensitivity to patchiness and sample grain size. Acta Oecol. 45, 31–41.
Witt, P.B.R., 2013. Fauna e Flora da Reserva Biológica Lami José Lützenberger. Secretaria Municipal do Meio Ambiente, Porto Alegre.
Zafalon-Silva, Â., Hoffmeister, C.H., Anjos, V.A., Ribeiro, P.B., Krüger, R.F., 2014. Necrophagous Diptera associated with wild animal carcasses in southern Brazil. Rev. Bras. Entomol. 58, 337–342.