

Ecology

Diversity of flesh flies (Diptera: Sarcophagidae) in an Atlantic forest fragment in Rio das Ostras, RJ, Southeastern Brazil

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FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa no Estado do Rio de Janeiro; CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) Abstract. Sarcophagidae is a family of Diptera with medical-veterinary importance, which have species with sarcosaprophagous, necrophagous or causing-myiasis larvae, among others. The Brazilian state of Rio de Janeiro has 92 municipalities, but only 26 have sarcophagid records and the municipality of Rio das Ostras is one of the least registered, with only nine species recorded. This works aimed to increase the knowledge of flesh fly diversity in a conservation unit of this municipality, the "Área de Relevante Interesse Ecológico de Itapebussus". The study was conducted in restinga and semideciduous forest. We used modified Van Someren-Rydon traps baited with decayed fish, and the samples were taken in dry and rainy seasons. A total of 446 male sarcophagines of 18 species of the genera Oxysarcodexia, Peckia, Ravinia, Sarcofahrtiopsis, and Titanogrypa were collected. We found 13 new records for the sampled municipality. The forest presented higher richness than the restinga and the most abundant species was Oxysarcodexia amorosa (Schiner). Diversity and equitability showed similar values in the two areas (H'f=2.193, H'r=2.027, J'f=0.7908 and J'r=0.7682) and the cluster analysis suggest high similarity. PERMANOVA did not present significant results for any source of variation. The presence of synanthropic and asynanthropic species in the fragment demonstrates that it is already a somewhat human-impacted environment. Marked increase in the number of sarcophagid records obtained in this small sample in the studied municipality (from nine to 22 species) shows the importance of taxonomic surveys in poorly studied areas.

Keywords: Biodiversity; Inventory; Neotropical; Lowland Forest; Restinga.

arcophagidae, a family of Calyptratae Diptera, include dull gray muscoid dipterans with three longitudinal black stripes on mesonotum, abdomen checkered, a row of setae in the meron, and undeveloped subscutellum (SHEWELL 1987). There are more than 3,000 described species and they are distributed in all biogeographic areas (PAPE 1996; PAPE et al. 2011). Sarcophaginae, the most diverse of the three subfamilies, shows also a high habit diversity, mainly saprophagy (PAPE 1996). Sarcossaprophagous sarcophagids play a fundamental role in nutrient cycling in ecosystems and the decomposition of carcasses (Byrd & CASTNER 2001). Thus, they are important to forensic entomology and have been the subject of many works of applied science (CARVALHO & LINHARES 2001; OLIVEIRA-COSTA et al. 2001; BARROS et al. 2008; PUJOL-LUZ et al. 2008; ROSA et al. 2009; ALVES et al. 2014; DIAS et al. 2015). Moreover, some species are synanthropic, i.e., they can survive in the human-impacted environments (LINHARES 1981; YEPES-GAURISAS et al. 2013) and can be used as potential bioindicators (BARBOSA et al. 2017; Sousa et al. 2014).

Rio das Ostras is a municipality in the region of "Baixadas Litorâneas", state of Rio de Janeiro, Southeastern Brazil, a coastal region known for intense tourist activities that have generated high environmental degradation (RIBEIRO 2003; MASCARENHAS 2004). The municipality is located in the Atlantic rainforest biome and has original vegetation cover of lowland and submontane forest (MAYERHOFER & TOLEDO 2004). Atlantic rainforest is a worldwide hotspot and actually has only 7% of its original coverage, with high fragmentation (MYERS *et al.* 2000; TABARELLI *et al.* 2005). Rio das Ostras has two federal

and three municipal Conservation Units (UCs), including the municipal Area of Relevant Ecological Interest of Itapebussus (MAYERHOFER & TOLEDO 2004).

The state of Rio de Janeiro has 92 municipalities, but only 26 have records on sarcophagid diversity, with the Region of "Baixadas Litorâneas" one of the least registered. Only nine species of five genera have scattered records from the municipality of Rio das Ostras (MELLO-PATIU *et al.* 2009) and none of these are recorded from Conservation Units. Accordingly, data about insects from the Region of "Baixadas Litorâneas" in the state of Rio de Janeiro, including muscoid Diptera, are quite incipient and we aimed to contribute with the knowledge of Sarcophagidae biodiversity in the Brazilian Atlantic Forest for fomenting its preservation.

MATERIAL AND METHODS

The experiment was performed in Fazenda Itapebussus, municipality of Rio das Ostras, Rio de Janeiro state, Brazil, part of the municipal "Área de Relevante Interesse Ecológico de Itapebussus" (Area of Relevant Ecological Interest of Itapebussus), which will be abbreviated from now on as ARIE Itapebussus. The farm had fragments of restinga vegetation and semideciduous forest, besides pasture lands and wetlands (MAYERHOFER & TOLEDO 2004). The annual rainfall and daily temperature average are 1,150 mm and 23.5°C, respectively (MAYERHOFER & TOLEDO 2004).

Samples were obtained from two areas: semideciduous forest and restinga forest. The semideciduous forest was present

on slopes and hilltops, occupying most of the Itapebussus ARIE, while the restinga forest was in the whole post-beach areas (MAYERHOFER & TOLEDO 2004). The first experimental area, named "forest", was a fragment of lowland forest with a closed canopy, near some wetlands (Figure 1) and the second, named "restinga", was located near the beach, with the predominance of herbaceous and shrubby vegetations and intense light incidence (Figure 1). These areas are 761m away from the houses and pastures, 565m apart and spatially separated by a lagoon.



Figure 1. Studied area in forest and restinga of the Area of Relevant Ecological Interest of Itapebussus, municipality of Rio das Ostras, state of Rio de Janeiro, Brazil (images modified from Google Earth Pro; A, B, C, D = georeferenced location of traps).

Specimens were collected in modified Van Someren-Rydon traps, suspended approximately 1.20 m from the ground, baited with decayed fish, previously kept at room temperature for 24 h. They were exposed in the field for 48 hours and the specimens were collected every 24 hours and were installed about 50-100 m apart from each other, depending on the type of terrain and vegetation. In each area, two traps were installed: forest 1 [S 22°28.878' W41°52.951'], forest 2 [S22°29.129' W41°53.161'], restinga 1 [S22°29.076' W41°53.218'] and restinga 2 [S22°29.093' W41°53.197'] (Figure 1, points A, B, C, and D, respectively). Two expeditions were conducted, one in the dry season (July 2016) and the other in the rainy season (January 2017), totalizing eight samples.

Trapped adult flesh flies were collected, pinned, and males had their terminalia exposed to allow identification. All male specimens were identified up to the species level, by using an identification key (CARVALHO & MELLO-PATIU 2008) and by comparison with material from the "Coleção Entomológica do Museu Nacional/UFRJ" (MNRJ). Species identified were deposited at the same collection (MNRJ), but, unfortunately, all specimens were lost in the fire at the "Museu Nacional" in September 2018. Weather data from the nearest station were obtained through the INMET portal (Station A608, Macaé, RI), available on https://www.gov.br/agricultura/pt-br/assuntos/ inmet. Diversity and equitability were calculated using Shannon-Wiener (H') and Pielou (J') index, respectively. Statistical analyses were performed using the Primer software. PERMANOVA was performed to test if the types of environment (forest or restinga) or period (dry or rainy season) influence sarcophagids abundance and richness. To investigate the similarity between the species composition, a non-metric multidimensional scaling (nMDS) based on a Bray-Curtis matrix and cluster analysis was carried out. The efficiency of the sampling in the studied area was verified using species accumulation curves derived from a secondorder nonparametric Jackknife procedure. Analyzes were performed using a significance level of 5%.

RESULTS

In total, 446 male specimens were collected, representing 5 genera, and 18 species, all belonging to the subfamily Sarcophaginae (Table 1). *Oxysarcodexia* and *Peckia* were the most representative genera, with 8 and 7 species, respectively. Other genera were represented by a single species, with less than 5% of total abundance. Five species were the most abundant, representing 82.73% of trapped flesh flies: *Oxysarcodexia amorosa* (Schiner), *Oxysarcodexia intona* (Curran & Walley), *Oxysarcodexia thornax* (Walker), *Oxysarcodexia timida* (Aldrich), and *Peckia* (*Peckia*) chrysostoma (Wiedemann).

The sampled areas were similar in richness but differed in species composition and abundance. The forest presented 218 specimens and 16 species. *Oxysarcodexia xanthosoma* (Aldrich), *Peckia (Squamatodes) ingens* (Walker), *Peckia (Euboettcheria) anguilla* (Curran & Waley), *Sarcofahrtiopsis cuneata* (Townsend), and *Titanogrypa (Cucullomyia) larvicida* (Lopes) were restricted to the forest. The most abundant species in this area was *O. amorosa*, followed by *O. thornax*. We collected 228 specimens representing 14 species in restinga, with two exclusive species: *Oxysarcodexia avuncula* (Lopes) and *Oxysarcodexia fringidea* (Curran & Walley). The most abundant species in this area was *O. thornax*, followed by *P. chrysostoma*.

The individuals were more abundant in July (n = 279, 62.6% of total), the season that presented the lowest temperature value (21.7 °C) and higher relative humidity (84%). The January expedition, with higher temperatures (27.5 °C), showed a high rate of bait decay by the largest number of deposited larvae, which may have caused lower adults' attractiveness to traps.

Thirteen species were not previously recorded in Rio das Ostras and 12 were not known from the region of "Baixadas Litorâneas" (Table 1). Although new records of 13 species (Table 1) for the municipality correspond to approximately 72% of the sampled species (18 species), the species accumulation curve was still rising at the end of the study and the estimators calculated that there is a difference of at least four species not collected (Figure 2B). The number of species represents 72.58% of expected richness according to the Jackknife estimator (Table 2).

Equitability (J'f = 0.7952; J'r = 0.7395) and the diversity index (H'f = 2.773; H'r = 2.155) showed that richness and abundance were similar in both areas. No source of variation (phytophysiognomy, period and phytophysiognomy x period) exhibited significant results for the samples (p >0.05). In addition, the phytophysiognomy presented p = 0.058 (Table 3) and nMDS analysis with abundance data not presented a clear grouping between areas (Figure 2A).

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Table 1. Absolute (N) and relative abundance (RF) of Sarcophagidae species collected from the Area of Relevant Ecological Interest of Itapebussus, municipality of Rio das Ostras, state of Rio de Janeiro, Brazil, in July 2016 and January 2017 (only males are included). Species in bold are new records for the municipality.

Emocion	Forest		Restinga		Total	
species	Ν	RF (%)	N	RF (%)	N	RF (%)
Oxysarcodexia amorosa (Schiner)	43	9.64%	9	2.02%	52	11.66%
Oxysarcodexia avuncula (Lopes)	0	0.00%	1	0.22%	1	0.22%
Oxysarcodexia fringidea (Curran & Walley)		0.00%	1	0.22%	1	0.22%
Oxysarcodexia intona (Curran & Walley)	20	4.48%	32	7.17%	52	11.66%
Oxysarcodexia morretesi Tibana & Mello	2	0.45%	15	3.36%	17	3.81%
<i>Oxysarcodexia thornax</i> (Walker)	39	8.74%	61	13.68%	100	22.42%
Oxysarcodexia timida (Aldrich)	39	8.74%	39	8.74%	78	17.49%
Oxysarcodexia xanthosoma (Aldrich)	2	0.45%	0	0.00%	2	0.45%
Peckia (Peckia) chrysostoma (Wiedemann)	35	7.85%	52	11.66%	87	19.51%
Peckia (Sarcodexia) lambens (Wiedemann)	9	2.02%	2	0.45%	11	2.47%
Peckia (Squamatodes) ingens (Walker)	2	0.45%	0	0.00%	2	0.45%
Peckia (Euboettcheria) anguilla (Curran & Walley)	3	0.67%	0	0.00%	3	0.67%
Peckia (Euboettcheria) collusor (Curran & Walley)	6	1.35%	1	0.22%	7	1.57%
Peckia (Pattonella) intermutans (Walker)	4	0.90%	1	0.22%	5	1.12%
Peckia (Peckia) pexata (Wulp)	4	0.90%	6	1.35%	10	2.24%
Ravinia belforti (Prado & Fonseca)	4	0.90%	8	1.79%	12	2.69%
Sarcofahrtiopsis cuneata (Townsend)	5	1.12%	0	0.00%	5	1.12%
Tytanogrypa (Cucullomyia) larvicida (Lopes)	1	0.22%	0	0.00%	1	0.22%
TOTAL	218	48.88%	228	51.12%	446	100%



Moreover, the cluster analysis revealed that point Restinga 1 is most similar to both forest points rather than point Restinga 2 (Figure 2C), differing from the expected, which would be a higher similarity between the subsamples from each area, if the phytophysiognomy was the source of variation.

Table 2. Abundance (N) and richness (S) observed and estimated byJackknife 2.

Area	N	s	Jackknife 2 estimator	Proportion to estimator
Forest	218	16	18.4	86.96%
Restinga	228	13	19.2	67.71%
Total	446	18	24.8	72.58%

Table 3. PERMANOVA results based on Bray-curtis similarity matrix. DF = degree of fredoom

Source of variation	DF	Pseudo-F	Р
Phytophysiognomy	1	2.6623	0.058
Period	1	2.2439	0.086
Phytophysiognomy x Period	1	1.2483	0.337
Residue	4	-	-
Total	7	-	-

Figure 2. A= Species accumulation curves for the collected Sarcophagidae from the Area of Relevant Ecological Interest of Itapebussus, municipality of Rio das Ostras, state of Rio de Janeiro, Brazil, with expected number of species by Jackknife 2 estimator and collected number of species. B = Representation of non-metric multidimensional scaling (nMDS) of the relationships between the abundance in each sample area. Resemblance: S17 Bray Curtis similarity. C = Similarity analysis (Bray Cluster's dendrogram) of the diversity of Sarcophagidae from each sample area. Source: the authors.

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DISCUSSION

MELLO-PATIU et al. (2009) identified the North and Northeast of Rio de Janeiro as priority areas for carrying out inventories of flesh flies since most municipalities in both regions have little or no collection records. The Rio das Ostras municipality had nine previously recorded species of Sarcophagidae (MELLO-PATIU et al. 2009). Five of them, also sampled in the present study, O. amorosa, O. thornax, O. timida, P. chrysostoma, and Ravinia belforti (Prado & Fonseca) had already been collected in the metropolitan region of Rio de Janeiro (LEANDRO & D'ALMEIDA 2005), indicating that they can be also common in the urban area. The other four species with previous records to Rio das Ostras municipality, Oxysarcodexia modesta Lopes, Oxysarcodexia terminalis (Wiedemann), Oxyvinia excisa (Lopes), and Tricharaea (Sarcophagula) occidua (Fabricius) were not collected in this study. The locality shown on the label of these specimens was only "Rio das Ostras" with no more details.

The region of "Baixadas Litorâneas" gathers 10 municipalities, but only four had flesh fly records, totaling 12 known species. In the present study, 12 new records were obtained for this region, increasing this knowledge by 100%. In the municipality of Rio das Ostras, the knowledge of Sarcophagidae fauna increased from nine to 22 species, 13 new records obtained only in the present study.

Taxonomic works are fundamental to the knowledge about true flies' fauna in several ecosystems whose biodiversity has never been investigated. Data on true flies' fauna in restingas are still scarce, even in the state of Rio de Janeiro, where many works were carried out in this environment, but few of them involved inventories, especially entomological ones. Particularly on Sarcophagidae, is evident the absence of knowledge on species that inhabit restingas and the closest works involving flesh flies' inventories were carried out in beach environments in Northeastern Brazil, but not in restinga vegetation (BARBOSA *et al.* 2017).

Oxysarcodexia amorosa, O. thornax, O. timida, and *P. chrysostoma* have the potential for forensic entomology since they are recurrent in works using decaying carcasses in the state of Rio de Janeiro (OLIVEIRA-COSTA *et al.* 2001; BARBOSA *et al.* 2009). Two of these species, *P. chrysostoma* and *O. thornax,* were also reported in human cadavers (OLIVEIRA-COSTA *et al.* 2001).

Oxysarcodexia thornax is widely distributed in the state of Rio de Janeiro (MELLO-PATIU et al. 2009). It is commonly found in South America, including in regions with temperature and humidity considerably distinct (MULIERI et al. 2011; SOUSA et al. 2014). D'Almeida (1984) classified O. thornax as a positive synanthropic because of the preference for inhabited areas. In other regions, it was considered independent in preference (LINHARES 1981). Peckia intermutans (Walker) and Peckia collusor (Curran & Walley) are first recorded in the Northern and Northeastern Rio de Janeiro state. Both species are potential bioindicators of environmental quality, as they were classified as asynanthropic species (Linhares 1981; D'Almeida 1984; Yepes-GAURISAS et al. 2013). Furthermore, P. intermutans was also found on a sandy beach with low degree of anthropization in the state of Pernambuco, Northeaster Brazil (BARBOSA et al. 2017).

Although both sampled areas are somewhat humanimpacted, our results tend to indicate a higher degradation on restinga, since *O. thornax* (positive synanthropic) showed a higher abundance in this area, while *P. intermutans* and *P. collusor* (asynanthropics) had a higher abundance in the forest. This is consistent with observations made during sampling expeditions: in restinga areas bathers have unrestricted access to the beach by trails, while access to the forest is in privately owned, with controlled access. Restinga remains found in the state of Rio de Janeiro are mostly small and highly degenerate areas (Rocha *et al.* 2007). The small size and the high isolation rate of these fragments threaten their long-term survival, highlight the urgency to a better knowledge of these habitats and the need to invest in their conservation (TABARELLI *et al.* 2005; ROCHA *et al.* 2007).

The results herein obtained demonstrated that the presence of sarcophagid species considered as urban and synanthropic in Rio de Janeiro in the Atlantic Forest of Itapebussus ARIE emphasize that, despite being in a conservation unit, the fragments are somehow impacted. However, it is also possible to observe the presence of species related to a low rate of anthropization, showing that the Conservation Unit has been playing an essential role in the preservation of microenvironments in the municipality of Rio das Ostras. This confirms the need for more scientific investigation in this area, especially under conservation aspect, to understand the fauna diversity in this ARIE and assist in the formulation of public conservation policies.

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