

# Diversity of Diptera species associated with pig carcasses in a Brazilian city exposed to high rates of homicide

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**Abstract.** Cities in northeastern Brazil experience extreme rates of unsolved homicides, a situation that stimulates innovative procedures in the police work, such as forensic entomology. We surveyed necrophagous insects associated with carrion in a city exposed to high rates of homicides in Northeastern Brazil. The experiments were carried out in a rainforest fragment located in Recife, State of Pernambuco. Two pig carcasses were used as models, one in the dry and the other in the rainy season. The collection of adults was performed daily until the complete skeletonization of the carcasses. At least 32 Diptera species from the families Calliphoridae, Muscidae, Sarcophagidae, Fanniidae, Phoridae, Anthomyiidae, Piophilidae, and Stratiomyidae were registered, some of which have been previously documented on cadavers. A high richness of Diptera species was registered in all stages of decomposition. A strong overlap in the occurrence of most species was observed, which invalidates a defined entomological succession on the carcasses. Two species stood out in terms of abundance: *Ophyra chalcogaster* (Muscidae) and *Chrysomya albiceps* (Calliphoridae). The ubiquity of *Hemilucilia semidiaphana* (Calliphoridae) seems to confirm its preference for forest fragments exposed to low anthropogenic action. Our data contribute to expand the knowledge on the geographical distribution of forensically relevant species in the region and confirm the rapid dissemination of invasive *Chrysomya* species in forested areas.

**Key-Words.** Calliphoridae; *Chrysomya*; Forensic entomology; Muscidae, *Ophyra*.

## INTRODUCTION

Few areas in the world are as appropriate to develop forensic entomology as Northeastern Brazil. Depending on the criminality indices considered, 12 out of the 50 most violent big cities in the world (in countries not experiencing war) are located in that region (Mexico, 2017). Nevertheless, forensic entomology's local development is hampered by the scarcity of research on the diversity and life cycle of necrophagous species. So far, 14 insect species from five Diptera families (Calliphoridae, Sarcophagidae, Fanniidae, Muscidae, and Phoridae) have been recorded from human cadavers in the region (Andrade *et al.*, 2005; Oliveira & Vasconcelos, 2010; Vasconcelos *et al.*, 2014, 2019), a figure that is overly underestimated.

Species of Diptera from at least 11 families are known to colonize human corpses at different decomposition stages (Byrd & Castner, 2010). Some of them have been demonstrated to locate carrion a few minutes after the death (Vasconcelos *et al.*, 2013). Bionomical data on blow fly species (Calliphoridae) have been used to determine the minimum post-mortem interval (minPMI) in several countries (Arnaldos *et al.*, 2001; Kosmann *et al.*, 2011), and it is expected that a growing body of quantitative data will help to expand this utility in South America.

Since the geographical region where a corpse is found affects not only the structure of the necrophagous community but also the temporal pattern of colonization, entomological evidence obtained in one area cannot always be extrapolated

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to estimate the minPMI in a different region (Tomberlin *et al.*, 2012). Checklists of necrophagous species in distinct environments can be useful to determine if there was *post-mortem* transfer of the corpse or, under a conservationist approach, to assess the potential of some species as biological indicators of anthropogenic action (Cabrini *et al.*, 2013; Carmo & Vasconcelos, 2016, Barbosa *et al.*, 2017). Variations in the structure and composition of insect assemblages throughout the year also help in associating the likelihood of cadaver colonization by insects to a particular season, as evidenced in Argentina (Horenstein *et al.*, 2012), Brazil (Carvalho & Linhares, 2001), and Japan (Tachibana & Numata, 2006).

We investigated the diversity of forensically relevant Diptera species in a peri-urban fragment of rainforest located in Recife, capital of the State of Pernambuco, which has a shocking homicide rate of 33/100,000 inhabitants (Waiselfisz, 2016). Specifically, we aimed to: (a) survey necrophagous species of Diptera associated with carrion using pig carcasses as models; (b) relate the occurrence of species to the stage of carcass decomposition; (c) detect the occurrence of invasive species in the environment; and (d) identify species with potential use for forensic entomology in the region.

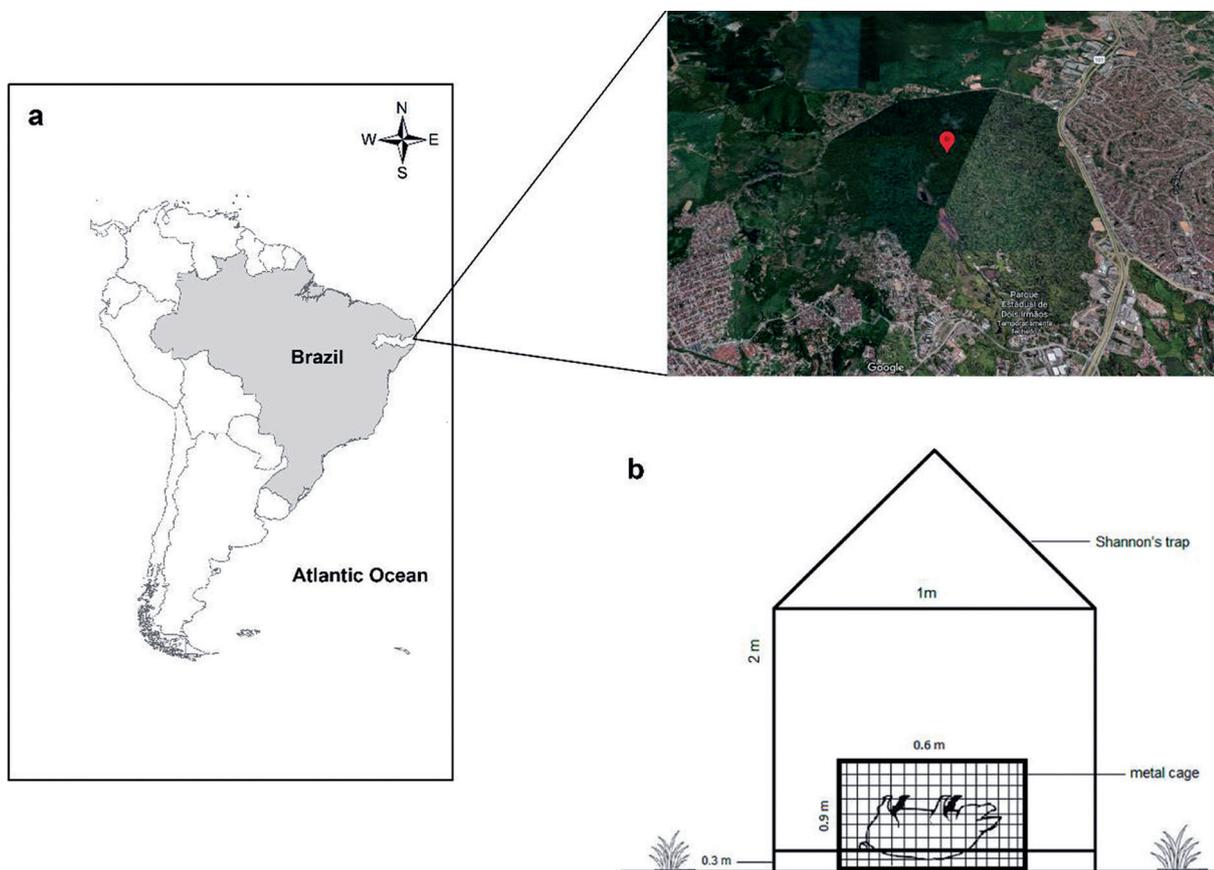
## MATERIAL AND METHODS

The field study took place in Dois Irmãos State Park, a 388-ha preserved rainforest fragment located in

Recife (08°07'30"S, 34°52'30"W, altitude 40 m) (Fig. 1A). The local climate is hot and humid, with mean rainfall *ca.* 2,500 mm/year, the average annual temperature *ca.* 25.6°C, and two well defined seasons: dry (October-February) and rainy (March-September). Vegetation is classified as dense ombrophyllous forest, with sparse clearings (Machado *et al.*, 1998). The area was chosen due to its proximity to urban settings (*ca.* 2 km) and because it is similar to common repositories used for clandestine dumping of cadavers resulting from homicides.

As a model, we used pigs (*Sus scrofa*, *ca.* 15 kg), which were killed *in loco* with a gunshot to the occipital region, a procedure authorized by the Research Ethics Committee of the Federal University of Pernambuco (protocol No. 23076.030693/2009-23). After the death, the carcass was immediately placed in a metal cage (0.9 m × 0.6 m × 0.5 m) to prevent disturbance by large scavengers (Fig. 1B). Around the cage, a Shannon's inverted trap (Fig. 1B), made up of a metal frame (2 m high × 1 m long × 1 m wide) covered with a fine white mesh fabric, was placed to trap insects that visited the carcass. A 30 cm gap between the bottom of the net and the soil was left, through which insects could access the carcass.

To maximize the collection of insects from different species and respecting the legal and ethical restraints imposed by local committee rules, two pigs were sacrificed; one in the dry season (January) and the other in the rainy season (July). Sampling took place at every 24 h until the 11<sup>th</sup> day, and at every 48 h after that until complete skeletonization of the carcass. The decomposition



**Figure 1.** (A) Location of sample sites in the Dois Irmãos State Park in Recife and (B) structure used in the attraction and collection of insects.

stages were classified according to Rodriguez & Bass (1983). Flies trapped in the mesh structure were collected by sweeping an entomological net (20 cm diameter) for 20 minutes on each sampling day, always between 12:00h and 14:00h. Insects were killed using ethyl acetate and identified using taxonomical keys (Mc Alpine *et al.*, 1981; Carvalho *et al.*, 2002; Carvalho & Mello-Patiu, 2008; Disney & Aguiar, 2008; Rochefort *et al.*, 2015). All specimens were deposited in the Entomological Collection at the Federal University of Pernambuco, Brazil (Curator: L. Iannuzzi). Data on the overall stage of decomposition, presence of insects, and abiotic factors – temperature, relative humidity, weather conditions, and rainfall – were collected *in loco* every day sampled.

The composition and the structure of the assemblages were examined under the following variables: richness, abundance, diversity, dominance, and evenness, all of which were refined per season and stage of decomposition. A Chi-square test was used to analyze the difference in the richness between seasons and stages. We estimated Shannon-Weiner's diversity ( $H'$ ), Pielou's evenness ( $J$ ), and Simpson's dominance ( $D$ ) for each stage of decomposition in both seasons. We built a ranking of species' dominance in each season using a logarithmic transformation of the abundance of all species. The assemblage of dipterans was analyzed in terms of structure (abundance) and composition (presence/absence) using ordination analysis constructed through the Bray Curtis matrix. To evaluate the similarity among the decomposition stages, a grouping analysis was performed to visualize graphical differences. All ecological and statistical analyses were carried out using Primer® 6.0, BioEstat® 5.3, and SigmaPlot with a significance level of 5%.

## RESULTS

Throughout the experiment, mean diurnal temperature varied from 29.6°C (min = 28.1°C, max = 30.9°C) in the dry to 25.4°C (min = 23.5°C, max = 26.9°C) in the rainy season. Overall weather conditions were sunny in the dry season, with only two cloudy days during the entire decomposition period. In the rainy season, on the other hand, sunny days were sporadic; rainy and/or cloudy days corresponded to 25 of the 30 days of the experiment. Relative humidity varied from 61% to 73% (mean = 66.7%) in the dry and from 71% to 93% (mean = 81.2%) in the rainy season. Accumulated rainfall during the experiment was 25 mm in the dry and 230 mm in the rainy season.

Decomposition of both carcasses occurred fast, and only four phases of decomposition were discernible: fresh, bloated, decay, and dry (Tables 1 and 2). The fresh stage lasted 24 hours in the dry season and 72 hours in the rainy season; the bloated stage and the decay stage lasted 48 hours each in both experiments. After 25 days (dry season) and 30 days (rainy), there were not soft tissues left in the carcass, and adult dipterans were virtually absent.

When the numbers of individuals from the two experiments were added, a total of 10,410 insects be-

longing to 19 families were registered. Of these, 10,116 belonged to eight families whose species exhibit an association with decomposing carcasses: Calliphoridae, Sarcophagidae, Muscidae, Fanniidae, Piophilidae, Mesembrinellidae, Anthomyiidae, Phoridae, and Stratiomyidae (Tables 1 and 2). Species from the families Neriidae, Tabanidae, Asilidae, Milichiidae, Drosophilidae, Micropezidae, Dixidae, and Ropalomeridae were also collected in small amounts in both seasons. Because of their limited abundance (individuals from these eight families summed up 3.7% of total) and the debatable nature of their necrophagy, they were not considered for this study.

The families with the highest number of species were Calliphoridae (7 species) and Muscidae (5) (Tables 1 and 2). Fanniidae and Piophilidae were represented by four and three species, respectively. Due to the absolute prevalence (> 90%) of females in the samples and difficulties in identifying, specimens from Sarcophagidae were treated as a single taxon. Whenever possible, sarcophagids were identified to a specific level, and at least 10 species were registered: *Blaesoxipha (Gigantotheca) plinthopyga* Wiedemann, 1830; *Oxysarcodexia modesta* Lopes, 1946; *Oxysarcodexia fluminensis* Lopes, 1946; *Oxysarcodexia riograndensis* Lopes, 1946; *Oxysarcodexia intona* Curran & Walley, 1934; *Oxysarcodexia avuncula* Lopes, 1933; *Oxysarcodexia* sp.; *Peckia (Squamatoses) ingens* Walker, 1849; *Oxyvinia excisa* Lopes, 1950 and *Tricharaea (Sarcophagula) sp.*

Two families stood out in the experiment performed during the dry season, Calliphoridae and Sarcophagidae, as their combined abundances represented over 80% of all specimens. Muscidae (61.2% of all adults) and Calliphoridae (22.8%) were the most abundant families in the rainy season. Two species had the highest abundances: *Ophyra chalcogaster* (Wiedemann, 1824) (Muscidae), particularly in the rainy season (59.5% of all adults), and *Chrysomya albiceps* (Wiedemann, 1819) (Calliphoridae) in the dry season (38.4%). Codominance occurred between two invasive blow fly species, *C. albiceps* and *Chrysomya megacephala* (Fabricius, 1794) in the dry season, while *O. chalcogaster* (Muscidae) and *Hemilucilia semidiaphana* (Rondani, 1850) were codominant in the rainy season (Fig. 2).

High diversity of necrophagous species of Diptera was associated with the carcasses throughout the entire decomposition (Tables 1 and 2). The number of carrion-associated species did not seem to differ markedly throughout the decomposition ( $\chi^2 = 1.156$ ; d.f. = 3;  $P > 0.05$ ). For example, when data from the two carcasses were combined, 21 species were reported at the fresh stage; 20 at the bloated stage, 15 at the decay stage, and 20 at the dry stage.

There was no difference in the richness of species between the stages of decomposition in the dry stage ( $\chi^2 = 2.342$ ; d.f. = 3;  $P = 0.504$ ), but the fresh and the dry stages harbored a significantly higher number of species in the rainy season ( $\chi^2 = 11.043$ ; d.f. = 3;  $P < 0.05$ ). Overall abundance also varied between the stages in both dry ( $\chi^2 = 1,582$ ; d.f. = 3;  $P < 0.001$ ) and rainy seasons ( $\chi^2 = 5,480$ ; d.f. = 3;  $P < 0.001$ ). The highest relative fre-

**Table 1.** Diversity and abundance of Diptera associated with a pig carcass in the dry season in a forest fragment in Pernambuco State, Brazil.

Family	Species	Fresh	Bloated	Decay	Dry	Total
Calliphoridae	<i>Chrysomya megacephala</i> (Fabricius, 1794)	81	268	12	23	384
	<i>Chrysomya albiceps</i> (Wiedemann, 1819)	415	1045	46	160	1,666
	<i>Chrysomya putoria</i> (Wiedemann, 1818)	10	16	—	9	35
	<i>Hemilucilia semidiaphana</i> (Rondani, 1850)	23	13	9	44	89
	<i>Hemilucilia segmentaria</i> (Fabricius, 1805)	14	4	4	30	52
	<i>Cochliomyia macellaria</i> (Fabricius, 1775)	—	2	—	1	3
	<i>Lucilia eximia</i> (Wiedemann, 1819)	1	—	—	—	1
Mesembrinellidae	<i>Mesembrinella bellardiana</i> Aldrich, 1922	6	4	9	25	44
Muscidae	<i>Parapyrellia maculipennis</i> (Macquart, 1846)	11	40	17	13	81
	<i>Morelia humeralis</i> (Stein, 1918)	6	52	18	14	90
	<i>Musca domestica</i> Linnaeus, 1758	1	2	—	1	4
	<i>Biopyrellia pipuncta</i> (Wiedemann, 1830)	—	3	5	1	9
	<i>Ophyra chalcogaster</i> (Wiedemann, 1824)	6	29	11	19	65
Fanniidae	<i>Fannia obscurinervis</i> (Stein, 1900)	1	—	—	—	1
	<i>Fannia</i> sp.1	—	—	1	—	1
Piophilidae	<i>Dasyphlebomyia stylata</i> Becker, 1914	—	2	—	3	5
	<i>Piophila bipunctata</i> (Fallen, 1810)	—	1	—	1	2
	<i>Piophila casei</i> (Linnaeus, 1758)	—	3	7	16	26
Phoridae	<i>Megaselia scalaris</i> (Loew, 1866)	—	7	5	10	22
	Phoridae spp.	—	1	—	5	6
Stratiomyidae	<i>Hermetia illucens</i> (Linnaeus, 1758)	—	1	3	23	27
Sarcophagidae	Unidentified species	192	734	605	213	1,744
<b>Total</b>		<b>767</b>	<b>2,227</b>	<b>752</b>	<b>611</b>	<b>4,357</b>

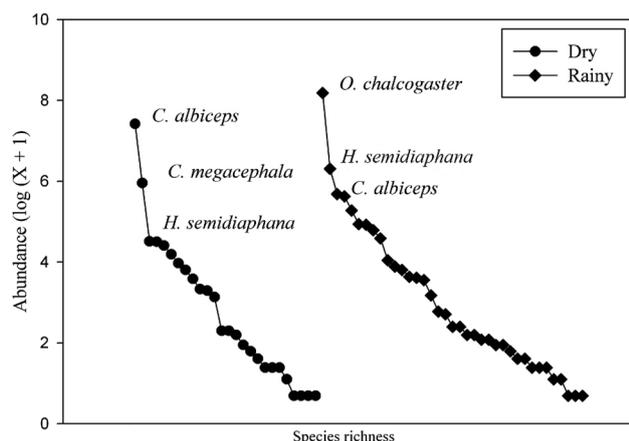
**Table 2.** Diversity and abundance of Diptera associated with a pig carcass in the rainy season in a forest fragment in the State of Pernambuco, Brazil.

Family	Species	Fresh	Bloated	Decay	Dry	Total
Calliphoridae	<i>Chrysomya megacephala</i>	95	89	2	9	195
	<i>Chrysomya albiceps</i>	164	101	7	20	292
	<i>Chrysomya putoria</i>	8	4	—	3	15
	<i>Hemilucilia semidiaphana</i>	293	145	36	74	548
Mesembrinellidae	<i>Hemilucilia segmentaria</i>	23	15	7	3	48
	<i>Cochliomyia macellaria</i>	—	—	—	1	1
	<i>Lucilia eximia</i>	—	—	—	1	1
	<i>Mesembrinella bellardiana</i>	54	146	9	68	277
Muscidae	<i>Parapyrellia maculipennis</i>	4	12	20	23	59
	<i>Morelia humeralis</i>	2	4	8	31	45
	<i>Musca domestica</i>	1	—	—	1	2
	<i>Ophyra chalcogaster</i>	540	2,977	30	43	3,590
Anthomyiidae	<i>Craspedochoeta punctipennis</i>	8	—	—	—	8
	<i>Hylemyiodes plurinervis</i>	2	—	—	—	2
	<i>Hylemyiodes aureficies</i>	1	—	—	—	1
Fanniidae	<i>Fannia obscurinervis</i>	4	—	—	—	4
	<i>Fannia</i> sp.1	6	—	—	—	6
	<i>Fannia</i> sp.2	6	1	—	—	7
	<i>Fannia</i> sp.3	6	—	—	—	6
Piophilidae	<i>Dasyphlebomyia stylata</i>	23	9	3	2	37
	<i>Piophila bipunctata</i>	7	2	1	—	10
	<i>Piophila casei</i>	41	55	2	21	119
Phoridae	<i>Megaselia scalaris</i>	55	63	15	16	149
	Phoridae spp.	20	8	—	6	34
Stratiomyidae	<i>Hermetia illucens</i>	1	3	3	16	23
Sarcophagidae	Unidentified species	21	93	74	92	280
<b>Total</b>		<b>1,385</b>	<b>3,727</b>	<b>217</b>	<b>430</b>	<b>5,759</b>

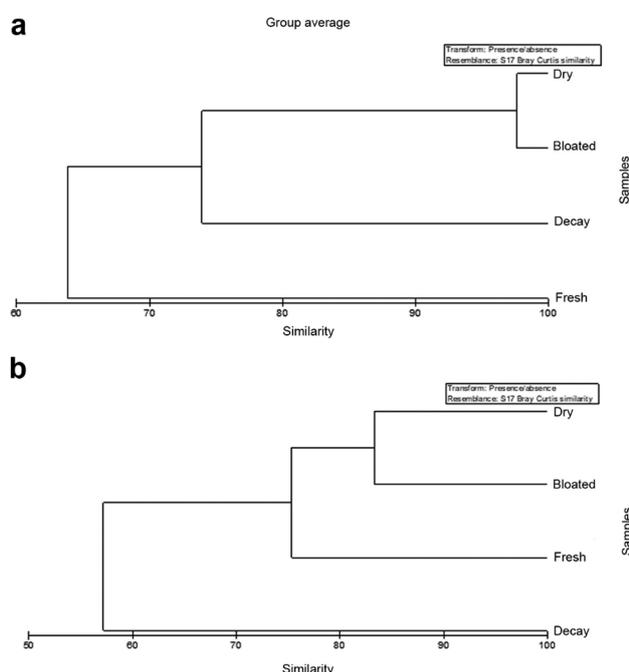
quency of necrophagous insects occurred at the bloated stage (51% of all specimens in the dry and 64% in the rainy season).

The highest values of diversity and evenness were registered in the dry stage for both rainy ( $H' = 2.657$ ;  $J' = 0.815$ ) and dry seasons ( $H' = 2.244$ ;  $J' = 0.842$ ), although

these indices varied little across the stages (Table 3). The similarity of the assemblages across decomposition stages was as follows: in the dry season, two major groups were formed: one related to the fresh stage and the other comprising bloated, decay, and dry, with practically 100% of the species co-occurring in the bloated and dry



**Figure 2.** Dominance ranking for dipterans species according to the season (Dry or Rainy).



**Figure 3.** Similarity analysis (Clusters dendrogram) of the diversity of necrophagous Diptera species between decomposition stages: (A) in the season dry and (B) season rainy.

stages (Fig. 3A). In the rainy season, assemblages in the bloated and dry stages were also highly similar, but a separate group formed by insects in the fresh, bloated, and dry stages (Fig. 3B).

### DISCUSSION

Abiotic conditions, that is, high temperature and humidity (and intense rainfall in the rainy season), resulted in fast decomposition of the carcasses, a pattern that was found in other studies performed in the Neotropical region (Carvalho & Linhares, 2001; Souza & Linhares, 1997). However, the extremely short duration of the decay phase – two days in both carcasses – was more drastic than what was observed in studies performed in other rainforest fragments in South America (Moura et al., 2005; Barbosa et al., 2010). This phenomenon is associ-

**Table 3.** Ecological indices of assemblages of necrophagous Diptera according to the decomposition stage; S = richness of species, N = abundance, J' = equity, H' = Shannon's diversity; D = Simpson's dominance.

Season/Stage	S	N	J'	H'	D
<b>Dry</b>					
Fresh	12	767	0.404	1.094	0.464
Bloated	17	2,227	0.3522	1.089	0.480
Decay	13	752	0.7369	2.28	0.860
Dry	17	611	0.8421	2.244	0.819
<b>Rainy</b>					
Fresh	22	1,385	0.5877	2.055	0.779
Bloated	15	3,727	0.308	0.9521	0.351
Decay	13	217	0.7496	1.797	0.788
Dry	16	430	0.8155	2.657	0.912

ated with behavioral and physiological traits in necrophagous dipterans that ensure the immediate location and colonization of an ephemeral resource (Hanski, 1987).

The numerical dominance of species of Calliphoridae and Sarcophagidae observed here derives from their intimate association with decomposing carrion as a site for egg and/or larvae laying and the development of immature stages (Carvalho et al., 2012). The dominance of a few blow fly species, such as *C. albiceps*, *C. megacephala*, and *Hemilucilia semidiaphana* Rondani, 1850 is not surprising since these species are frequently associated with carrion to which they are attracted by decomposing tissues and blood (Vasconcelos et al., 2013). Furthermore, *Chrysomya* species may cause deleterious effects on the population of native Calliphoridae, such as *Cochliomyia macellaria* (Fabricius, 1775) (Faria et al., 1999). However, the numerical dominance of the muscid species *O. chalcogaster* had not been described in previous studies in South America. Larvae of *Ophyra* species can occur in extremely diverse habitats, including dung, putrefied meat, and even decomposing vegetal matter, and they are also reported to prey upon larvae of Sarcophagidae, Calliphoridae, and Muscidae species (Skidmore, 1985; Pamplona & Couri, 1989; Krüger et al., 2004).

The high richness and abundance of dipterans reported in all stages of decomposition are typical of *r*-strategist species. Although no difference in richness was observed among the stages, the highest proportion of insects was observed in the bloated stage, which is noticeable considering that this phase only lasted 48 hours, compared to a much longer dry stage duration. Females tend to concentrate their oviposition activity at initial stages due to a preference for soft tissues. In the dry (or remains) stage, there can be a radical reduction in the tissues, muscles, and viscera suitable for larval feeding (Payne, 1965).

The ephemeral nature of the carcass requires an efficient location mechanism by adult females – for instance, *H. semidiaphana* can locate an animal carcass within 30 minutes of the death, a fact that gives them an advantage over other species (Vasconcelos et al., 2013). Contrarily to reports that associate *Hermetia illucens* Linnaeus, 1758 solely to advanced stages of decomposition (Ferrari et al., 2009), the species was registered since the first day

post-death and continued to be collected throughout decomposition.

Assemblages of necrophagous insects are modeled by processes such as intra- and interspecific competition, predation, and even cannibalism (Wells & Greenberg, 1994). These interactions can produce a marked dominance of a few species, especially invasive ones (Hanski, 1987; Wells & Greenberg, 1994). According to Schoenly & Reid (1987), the hypothesis of a discrete process would imply a higher similarity in species composition within a given stage of decomposition than between stages. However, this was not observed in this study, given the enormous overlap in the species throughout the decomposition, and this pattern is consistent in both seasons as detected by the Cluster analysis (Figs. 3A, B).

Twelve species reported here have been documented as colonizers of human cadavers: *C. albiceps*, *C. megacephala*, *Chrysomya putoria* Wiedemann, 1819, *C. macellaria*, *H. semidiaphana*, *Hemilucilia segmentaria* Fabricius, 1805, (Calliphoridae), *O. riograndensis* (Sarcophagidae), *Musca domestica* Linnaeus, 1758 and *O. chalcogaster* (Muscidae), *Megaselia scalaris* Loew, 1866 (Phoridae), *Piophilina casei* Linnaeus, 1758 (Piophilidae) and *H. illucens* (Stratiomyidae). This reinforces the importance of forensic relevance of this study since peri-urban fragments of forest are amongst the most frequently used sites for discarding cadavers of victims of homicide.

Species from the genus *Chrysomya*, dominant in this study, are the most frequently used species in the estimation of minimum PMI worldwide (Byrd & Castner, 2010), but information on the presence and age of the native species *H. segmentaria* helped to estimate the PMI in a criminal case in Brazil (Kosmann et al., 2011). In a study performed in Recife, larvae of *H. segmentaria* were recovered from the soil below a cadaver victim of suicide by hanging, which further stimulates investigation on this species' forensic relevance (Vasconcelos et al., 2019).

Although complete identification was not achieved for all Sarcophagidae, the record of at least 10 species reported here reinforces the diversity of the family in Neotropical environments, especially of the genus *Oxysarcodexia* and *Peckia*. The relatively limited forensic application (e.g., PMI) of Muscidae so far may be due to difficulties in rearing and identification, as well as due to their preference for other substrates, such as decomposing plant material. In a recent review, Grzywacz et al. (2017) highlight that although fewer muscids are known to colonize human cadavers than species of Calliphoridae, corpses may be colonized out- or indoors, in sunny or shaded, dry or wet sites, in exposed or concealed situations, and at early and late stages of decomposition. We found five species in association with pig carcasses, and the dominance of *O. chalcogaster* raises questions whether the forensic potential of muscids has been underestimated (Grzywacz et al., 2017).

Field surveys of necrophagous dipterans can also strengthen their potential as biological indicators of environmental conservation or indicators of the site of death. For example, the presence of *Mesembrinella belgardiana* (Aldrich, 1922) and *Hemilucilia* species reflects

an intimate association with forested areas, as they are rare or absent in urban zones (Cabrini et al., 2013; Carmo & Vasconcelos, 2016; Thyssen et al., 2005).

Given the experiments' short duration, our results present a conservative estimate of the actual diversity of necrophagous Diptera in rainforest fragments in Northeastern Brazil. Nevertheless, our data contribute to expand the knowledge on the geographical distribution of forensically relevant species and confirm the rapid dissemination of invasive *Chrysomya* species in forested areas. Further studies should investigate the life cycle of native species and how they respond to environmental differences so that criminal investigators can effectively use entomology databases.

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## AUTHORS' CONTRIBUTIONS

Conceived and designed the project: TMC, SDV. Collected field samples: TMC, SDV. Analyzed the data: TMB, TMC, SDV. Species identification: TMC, PJT. Wrote the paper: TMB, TMC, PJT, SDV. All authors read and approved the final version of the manuscript.

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