

Muscidae (Diptera) of medico-legal importance associated with ephemeral organic substrates in seasonally dry tropical forests

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Abstract. Muscidae (Diptera) comprises one of the most important taxa in medical, veterinary and forensic entomology, especially due to their association with decomposing carcasses and cadavers. Yet, knowledge on their distribution and behaviour is still incipient in several biomes, which is the case of dry tropical forests. This study aimed to evaluate the attractiveness of different organic substrates to species of Muscidae in areas of seasonally dry forests (Caatinga) in Northern Brazil. Sampling was carried out in five Caatinga areas between 2015 and 2017, using suspended traps baited with bovine spleen, fish or human faeces. When all samplings were combined, 3,176 adults of nine species were collected. The assemblages of muscids had higher richness and abundances on bovine spleen, which attracted 66% of all specimens, when compared to the other substrates. *Musca domestica* was the most abundant species, and it was dominant in assemblages associated with spleen and fish. Assemblages attracted to spleen and fish had higher similarity, differing from those associated with faeces. Differences in food attractiveness are presented for species of medical interest as pathogen vectors (e.g., *Synthesiomyia nudiseta* (Wulp, 1883)) and of forensic relevance as colonizers of human corpses (e.g., *Ophyra aenescens* (Wiedemann, 1830)).

Key-Words. *Atherigona orientalis*; Caatinga; Carrion; Forensic entomology; *Synthesiomyia nudiseta*.

INTRODUCTION

Muscidae (Diptera) comprises one of the most important taxa in medical, veterinary and forensic entomology (Skidmore, 1985; Grzywacz *et al.*, 2017). Yet, knowledge on their distribution and behaviour is still incipient in several biomes, which is the case of dry tropical forests (DTF), a complex set of environments that cover over one million km² in Africa, Americas, Asia and Oceania (Miles *et al.*, 2006). In broad terms, DTF's are characterized by limited and irregular rainfall, high temperature throughout the year, low air and soil humidity and a set of specializations of local fauna and flora to adapt to xeric conditions (Bullock *et al.*, 1995). Sudden shifts in resource availability in dry forests, regulated mostly by pulses in water availability, act as a selective force that favours insects that can promptly locate and colonize patchy substrates, such as carrion.

Dry tropical forests are among the most threatened environments in the world due to climate change, deforestation, fire and conversion to agriculture, particularly in Brazil (Miles *et al.*, 2006; Santos *et al.*, 2011). The Brazilian seasonally dry tropical forest (SDTF), known as *Caatinga*, covers ca. 800,000 km² and it is exposed to severe droughts that can last decades. It is the least studied biome in the Neotropics (Santos *et al.*, 2011), which encourages research on insect diversity and

behaviour in order to understand the dynamics of assemblages in patchy, ephemeral resources.

Besides their ecological role in nutrient cycling, Muscidae species can act as vectors of helminths, bacteria and viruses (e.g., *Synthesiomyia nudiseta* (Wulp, 1883)), cause myiasis (e.g., *Musca domestica* L., 1758) and feed on livestock (e.g., *Stomoxys calcitrans* Linnaeus, 1758) (Skidmore, 1985). Additionally, several species, such as *Ophyra aenescens* (Wiedemann, 1830), colonize human cadavers and have increasing forensic relevance in criminal investigations (Grzywacz *et al.*, 2017). For instance, the presence of puparia and adult fragments of *Ophyra capensis* (Wiedemann, 1818) in the oesophagus of a mummy from the early XIX century helped to reconstitute the *post-mortem* conditions (Couri *et al.*, 2009).

In this study, we performed a short-term survey of Muscidae species associated with decomposing substrates in fragments of SDTF. Specifically, we aimed to compare the attractiveness of different baits to muscids, with emphasis on species of medico-legal importance.

MATERIALS AND METHODS

Field experiments were performed in five fragments of SDTF in the states of Paraíba and Pernambuco, Northeastern Brazil, separated from

one another by at least 100 km, in the municipalities of Afogados da Ingazeira (lat 07°45'03"S, long 37°38'20"W), Buíque (lat 08°29'56"S, long 37°22'14"W), Betânia (lat 08°16'29"S, long 38°02'03"W), Petrolina (lat 09°23'39"S, long 40°30'35"W) and Boqueirão (lat 08°25'35"S, long 36°08'06"W) (Fig. 1). To maximize collection, sampling was performed always in the rainy season in all fragments, and each area was sampled twice. During the three-year study, insect collection was performed in 2015 and 2016 in Buíque, Betânia and Petrolina, while the SDTF fragments in Afogados da Ingazeira and Boqueirão were sampled in 2016 and 2017. Despite intrinsic variations, vegetation in all fragments consists mostly of shrub native species, with predominance of Cactaceae, and sparsely distributed trees such as the native *Ziziphus joazeiro* (Rhamnaceae), *Spondias tuberosa* (Anacardiaceae) and the introduced *Prosopis juliflora* (Leguminosae). Vertebrate fauna comprises mostly small to medium-sized species of mammals, reptiles and birds, whose population can diminish considerably in the dry periods.

Adult flies were collected using traps described by Oliveira *et al.* (2016), a low-cost device that relies on olfactory cues and positive phototropism. The traps were suspended 1.0 m above the soil and contained decomposing matter as bait (150 g, after 48 h exposure to 25°C). Three types of baits were used: bovine spleen, fish (sardine) and human faeces. Sixteen traps of each treatment separated from one another by 25 m were exposed in the field for 48 h in each environment, after which all traps were taken to the laboratory and adults were identified (Carvalho *et al.*, 2002).

The assemblage of Muscidae associated with each substrate was characterized by: richness, relative frequency, Shannon-Wiener's diversity index, Simpson's dominance and Pielou's equity index, while abundance was compared using a Chi-square test (minimum of 100 adults/treatment). To test for similarities in the assemblages on each bait, we built a similarity matrix, through the index of Bray Curtis, after data transformation $\log(x + 1)$. The statistical packages Primer 5.0 and Biostat 5.0 were used, with 5% significance level.

RESULTS

When all samplings were combined, 3,176 adults of nine species were collected (Table 1). Overall, the most abundant species were *M. domestica*, *Atherigona orientalis* Schiner, 1868 and *S. nudiseta*, corresponding to 64.0%, 18.3% and 12.6% of all specimens, respectively. Insect abundance differed between treatments ($\chi^2 = 52.39$; d.f. = 2; $P < 0.01$), as spleen was associated with the highest number of flies (66.0% of total). Higher abundance on spleen was observed for *M. domestica* and *A. orientalis*, while faeces were the most attractive substrate to *S. nudiseta* (Table 1). The number of female adults was significantly higher than their male counterparts for all species in all substrates ($P < 0.0001$) (Fig. 2).

Diversity indices ranged from 0.86 (spleen) to 1.16 (fish) and dominance varied from 0.39 (fish) to 0.50 (spleen), which is explained by the numerical dominance (73.2%) of *M. domestica* on spleen. Equity indices varied from 0.41 (spleen) to 0.68 (fish), showing higher unifor-

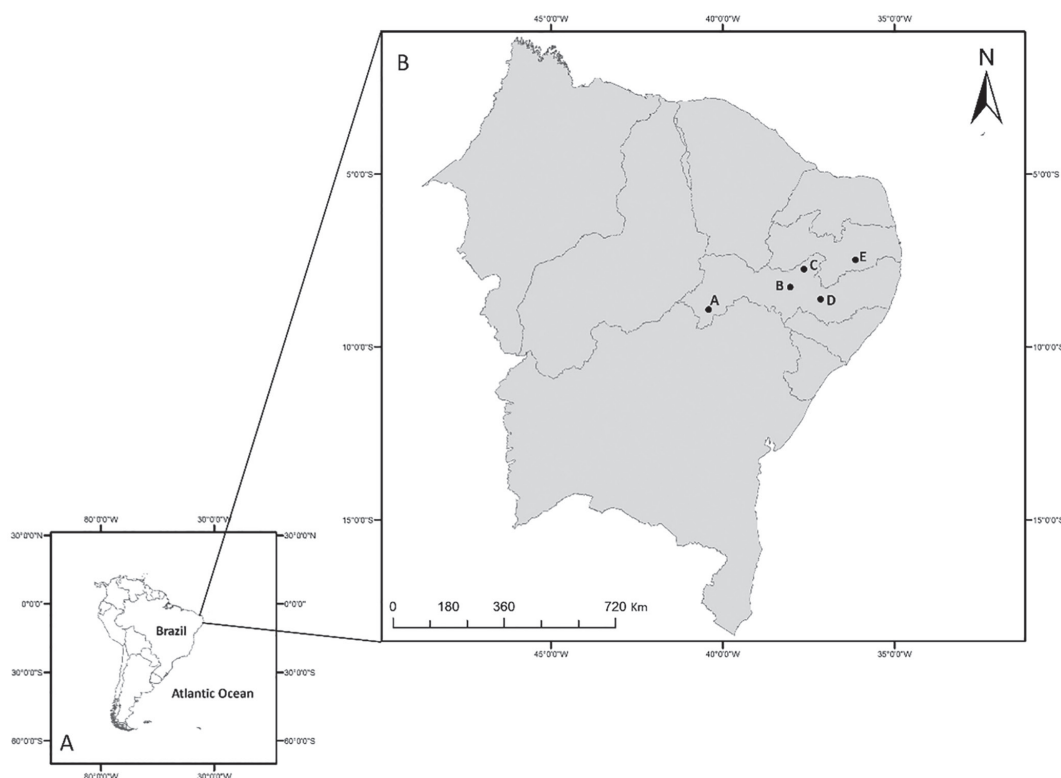


Figure 1. Location of sample sites along the seasonally dry tropical forest (*Caatinga*) in Northeastern Brazil. (A) Petrolina; (B) Betânia; (C) Afogados da Ingazeira; (D) Buíque; (E) Boqueirão.

Table 1. Abundance and relative frequency (%) of Muscidae species registered on three types of bait in fragments of seasonally dry forests in Brazil.

Species	Spleen		Fish		Faeces		Total
	N	%	N	%	N	%	
<i>Atherigona orientalis</i>	350	16.57	155	20.58	76	24.44	581
<i>Atherigona</i> sp.	33	1.56	81	10.76	1	0.32	115
<i>Gymnodia debilis</i>	4	0.19	—	—	2	0.64	6
<i>Graphomya maculata</i>	6	0.28	—	—	—	—	6
<i>Graphomya</i> sp.	—	—	—	—	2	0.64	2
<i>Musca domestica</i>	1,546	73.20	436	57.90	50	16.08	2,032
<i>Ophyra aenescens</i>	24	1.14	7	0.93	—	—	31
<i>Ophyra chalcogaster</i>	1	0.05	2	0.27	—	—	3
<i>Synthesiomyia nudiseta</i>	148	7.01	72	9.56	180	57.88	400
Total	2,112	100	753	100	311	100	3,176

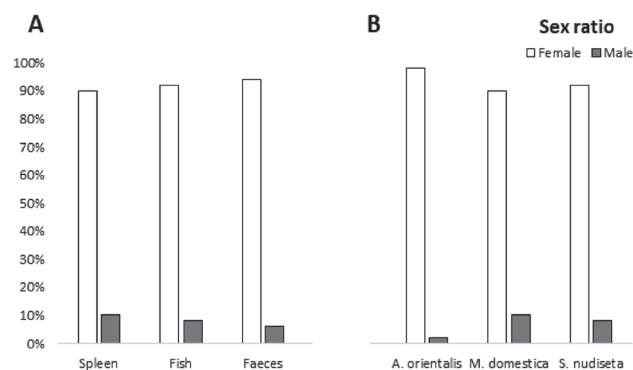
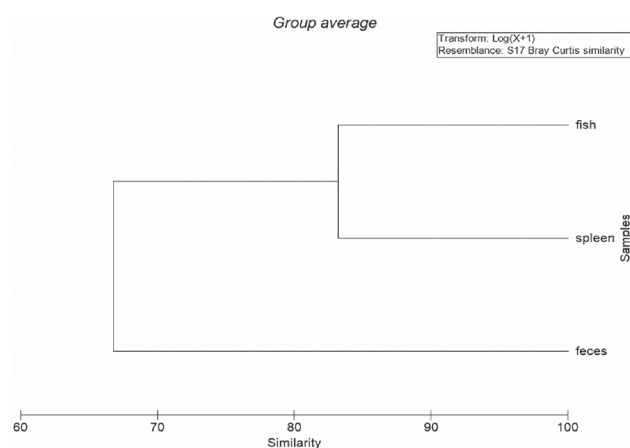
mity in the relative frequency of the species on the latter. Cluster analysis evidenced high similarity between muscid assemblages on spleen and fish (Fig. 3).

DISCUSSION

The number of species registered in this study did not differ much from other studies performed in rainforest fragments, urban areas and agroecosystems in South America (Vasconcelos & Araujo, 2012) and was similar to that recorded in longer studies using large vertebrate carcasses as baits in fragments of seasonally dry tropical forests (Vasconcelos *et al.*, 2016). This suggests that small baits can be surrogates for short-term field inventories of diversity of copronecrophagous Muscidae, without the ethical, logistical and economical restraints imposed by the use of animal carcasses.

The consumption of several types of decomposing substrates by Muscidae reported in the literature (Savage, 2002) is corroborated in this study, especially for *M. domestica*, *A. orientalis* and *S. nudiseta*, which are widely distributed and show adaptations to the harsh condition of the *Caatinga* (Carvalho, 2002). The high similarity of muscid assemblages associated with fish and spleen have been reported to occur in other environments such as rainforest and agroecosystems (Vasconcelos *et al.*, 2015). Volatile organic compounds (VOC's) released by decomposing fish are thought to attract a high diversity of necrophagous dipterans of Muscidae and other families, including also Calliphoridae and Sarcophagidae (Carmo *et al.*, 2017). Faeces, on the other hand, are nutritionally poorer than carcasses and tend to be used as food substrate for the development of fewer species (D'Almeida & Almeida, 1998). According to Blackith & Blackith (1993), flies visit faecal matter to oviposit, to gain water from fresh dung in dry climates, and to gain nourishment from the dissolved or finely particulate substances. This is critical in the *Caatinga*, where resources are short-lived and erratic. Considering the scarcity of large native mammals, goats raised in small-scale livestock tend to provide the main input of faecal matter.

The low attractiveness of faeces to *M. domestica* reported here diverges from previous records that suggest

**Figure 2.** Sex ratio (female/male) of muscids collected in SDTF's fragments in Brazil, according to the type of bait (A) and species (B).**Figure 3.** Cluster analysis for the similarity between muscid assemblages, according to the type of bait.

strong colonization of faeces by this species (Greenberg, 1971). Although *S. nudiseta* have been frequently reported on fish carcasses (D'Almeida & Almeida, 1998), this study reinforces the use of faecal matter as an alternative and/or complementary substrate. The overall low abundance of *Ophyra aenescens* and *Ophyra chalcogaster* (Wiedemann 1824) is probably due to their preference for large carcasses (Barbosa *et al.*, 2009). *A. orientalis* was strongly attracted to animal baits in this study but it has also been shown to feed on decaying plant material (D'Almeida & Almeida, 1998). Interestingly, the species is reported in association with carcasses in urban and forested areas (Barbosa *et al.*, 2009; Vasconcelos *et al.*, 2016).

Coprophagy is a common feeding habit of *Gymnodia* species (Uribe-M. *et al.*, 2010) and it is confirmed for *Gymnodia debilis* (Williston 1896) in this study. The low abundance of *G. debilis* in the five dry forest fragments in Brazil may reflect a stronger association of the species with urban/modified environment. The low abundance of *Graphomya maculata* (Scopoli, 1763) may be due to the fact that its larvae often prey upon other dipteran larvae and adults are mostly flower feeders (Skidmore, 1985; Marques & Couri, 2007). The numerical dominance of females found in the *Caatinga* has been a common pattern in surveys based on baits and carrion performed elsewhere (Mulieri *et al.*, 2015; Carmo *et al.*, 2017) and reflects the selection of the substrates for the development of the immature stages. Furthermore, the capture of few

male specimens compromises the register of a few species of Muscidae whose identification depends on the examination of the male genitalia.

Data presented here contribute to expand the knowledge on the biogeography of Muscidae species, especially given the shortage of information on DTF's in the Americas and other continents. The unfavourable conditions of DTF's (e.g., severe drought) act as selective forces for the development of physiological traits to cope with water-stressed environments and ephemeral resources. In the case of coprophagous species, the scarcity of large vertebrates in the *Caatinga* further stimulates fierce competition for patchy resources.

The non-discriminatory feeding behaviour of *S. nudisetata*, *M. domestica* and *A. orientalis* and their synanthropy highlights their medical importance due to their role as mechanical vectors of pathogens and as causal agents of myiasis (Greenberg, 1971; Skidmore, 1985; Uribe-M. *et al.*, 2010). Brazilian DTF's suffer a radical transformation caused by urbanization and intensive agriculture so that field surveys are crucial for the monitoring and control of species of medical and veterinary importance. Unsanitary conditions observed in many breeding systems are likely to maximize the potential of muscid in causing myiasis on goats in *Caatinga* fragments (Barbosa & Vasconcelos, 2015).

Finally, according to Grzywacz *et al.* (2017), Muscidae have received relatively little attention in forensic field studies although they can colonize bodies in urban and forested areas, under sunny and shade conditions, in exposed and concealed situations. Recently, *O. aenescens* was found on the soil below a hanged cadaver in Northeastern Brazil (Vasconcelos *et al.*, 2017), which suggests that the actual role of Muscidae in forensic entomology is far from being properly appraised. In this scenario, databases on species-habitat association may help in the evaluation of the potential of species as indicators of site of death.

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