Brief Communication / Comunicación Breve

A preliminary study of insect fauna present in a cow carcass found in northeastern Brazil

Un estudio preliminar sobre la fauna de insectos presente en un cadáver de vaca encontrado en el noreste de Brasil

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Abstract. Animal carcasses in natural environments are a food source for various organisms, such as fungi, bacteria, and insects. Insects also utilize this ephemeral resource for oviposition and development of their offspring, with the immatures being responsible for the greatest consumption of the resource. Thus, the aim of this study was to characterize the fauna of necrophagous insects associated with a cow carcass *Bos taurus* Linnaeus found in the municipality of São Raimundo das Mangabeiras, Maranhão, Brazil. Insects were sampled using forceps and entomological nets for eight days, with the immatures being taken to the Laboratory of Entomology and Vectors (LEV) and subsequently reared at room temperature. A total of 1,149 specimens were sampled, comprising 141 adults and 1,017 immatures. Among the adults, the species *Chrysomya albiceps* (Widemann), *Necrobia rufipes* (Fabricius), and *Dermestes maculatus* (De Geer) were prominent. Regarding immatures, all insects that emerged were of the species *C. albiceps*. Additionally, we report the first record of *Chrysomya putoria* (Widemann), *Cochliomyia hominivorax* (Coquerel), and *Hemertia illucens* (Linnaeus) visiting bovine carcasses in the Neotropical region. The results reveal a late colonization of *C. albiceps* in advanced stages, confirming the importance of research with carcasses in the context of forensic entomology.

Key words: Beetles; Calliphoridae; decomposition; necrophagous insects.

Resumen. Las carcasas animales en entornos naturales son una fuente de alimento para diversos organismos, como hongos, bacterias e insectos. Los insectos también utilizan este recurso efímero para la oviposición y el desarrollo de sus crías, siendo los inmaduros responsables del mayor consumo del recurso. Por lo tanto, el objetivo fue conocer la fauna de insectos necrófagos asociados a una carcasa de vaca *Bos taurus* Linnaeus encontrada en el municipio de São Raimundo das Mangabeiras, Maranhão, Brasil. Los insectos se muestrearon con la ayuda de pinzas y redes entomológicas durante ocho días, y los inmaduros se llevaron al Laboratorio de Entomología y Vectores (LEV) y posteriormente se criaron a temperatura ambiente. Se obtuvo un total de 1.149 especímenes, siendo 141 adultos y 1.017 inmaduros. Entre los adultos, destacaron las especies *Chrysomya albiceps* (Widemann), *Necrobia*

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rufipes (Fabricius) y *Dermestes maculatus* (De Geer). En cuanto a los inmaduros, todos los insectos que emergieron fueron de la especie *C. albiceps*. Además, se reporta el primer registro de *Chrysomya putoria* (Widemann), *Cochliomyia hominivorax* (Coquerel) y *Hemertia illucens* (Linnaeus) visitando una carcasa bovina en la región neotropical. Nuestros resultados revelan una colonización tardía de *C. albiceps* en etapas avanzadas, confirmando la importancia de la investigación con carcasas en el contexto de la entomología forense.

Palabras clave: Calliphoridae; descomposición; escarabajos; insectos necrófagos.

When animals die, the process of decomposition begins instantly, releasing a plethora of odorous cues that attract a diversity of insects (Vass *et al*. 2008). This occurs because the body of a dead animal functions as a temporary nutrient island for microorganisms, vertebrates, and invertebrates (Barton *et al*. 2013). Among the invertebrates, the primary visitors are necrophagous or sarcosaprophagous insects (Diptera and Coleoptera) that feed on the tissue or exudates of the carcass (Catts and Goff 1992). However, other groups may be significant in the taphonomic context, as they can cause post-mortem injuries, such as termites, ants, and wasps (Huchet *et al*. 2011; Barbosa *et al*. 2015; Ramón and Donoso 2015).

This intimate relationship of insects with carcasses and cadavers is the subject of forensic entomology, which primarily utilizes species development data to determine the post-mortem interval (PMI) in cases of violent deaths (Amendt *et al*. 2007). Although it is a burgeoning research field in northeast Brazil (Oliveira and Vasconcelos 2010; Alves *et al*. 2014a; Meira *et al*. 2020; Silva *et al*. 2023a), much of the data on the diversity of necrophagous or sarcosaprophagous fauna originates from studies with bait (Barbosa *et al*. 2017; Oliveira *et al*. 2016; Medeiros *et al*. 2023).

Therefore, studies involving carcass decomposition (Carvalho and Linhares 2001; Moretti *et al*. 2008; Vasconcelos *et al*. 2013) provide valuable information for the application of forensic entomology in Brazil, which is often neglected in some states, including Maranhão, where studies on the diversity of necrophagous flies are just over a decade old (Sousa *et al*. 2015, 2016; Leite *et al*. 2023), with the use of pig carcasses being the most recent (Silva *et al*. 2023a, b).

Studies with carcasses are important in the context of entomological succession, as they allow us to understand the diversity of visitors in different stages of decomposition. According to Cruz and Vasconcelos (2006), visitation begins with dipterans in the early stages, and as time progresses, other groups (*e.g*., beetles) appear. According to Vasconcelos *et al.* (2013), flies from different families are specialists in finding carcasses in the first hour's post-mortem, and therefore, they form the basis for calculating the PMI in many cases (Thyssen *et al*. 2018; Meira *et al*. 2020).

Thus, the present study aims to list the species of visitors and colonizers found in a bovine carcass (*Bos taurus* Linnaeus, 1758) victim of a roadkill on BR-230, located in Southern Maranhão, in order to contribute to forensic entomology in the state and consequently in Brazil.

The present study was conducted at the Laboratory of Entomology and Vectors (LEV) of the Federal Institute of Maranhão - São Raimundo das Mangabeiras Campus, located in Southern Maranhão, Brazil. Adult and immature insects were collected from a carcass of Nelore breed cow (*Bos taurus* Linnaeus, 1758), which was a victim of a roadkill beside BR 230 - "07°02'21.1"S and 045°31'22.1"W" (Fig. 1). The cow was found dead on September 5, 2023, and exhibited an advanced stage of decomposition based on Bornemissza's classification (1957).

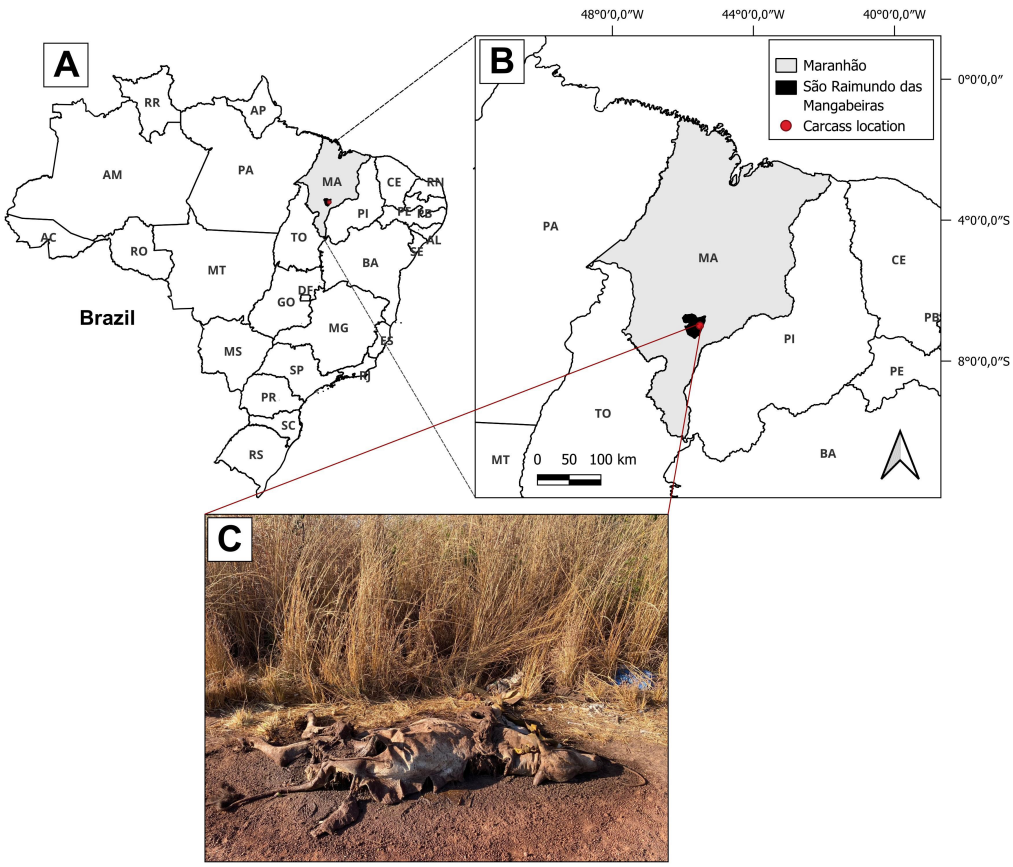

Figure 1. Location of the study area where the cow carcass (*Bos taurus* Linnaeus) was found in the Brazilian Cerrado. A= Brazil and the state of Maranhão, B= São Raimundo das Mangabeiras and C= carcass of cow *Bos taurus*. Source: IBGE (2023) (Modified by Leite (2024)). / **Figura 1.** Ubicación del área de estudio donde se encontró la carcasa de vaca (*Bos taurus* Linnaeus) en el Cerrado brasileño. A= Brasil y el estado de Maranhão, B= São Raimundo das Mangabeiras y C= carcasa de vaca *Bos taurus*. Fuente: IBGE (2023) (Modificado por Leite (2024)).

The sampling of insects was carried out through daily manual collections using entomological forceps and entomological nets during the morning shift. The carcass was examined from 09/05/23 to 09/12/2023, totaling eight days of collections. The collected adult insects were euthanized and stored in 250 ml plastic containers containing 70% alcohol as a preservative.

The immatures were taken alive to the Laboratory of Entomology and Vectors, where they were placed in plastic containers (5 liters) containing feeding substrate (bovine meat) and covered with voile fabric. They were monitored daily until the final development of the biological cycle, with the emergence of the adult. Subsequently, the emerged adults were euthanized with ethyl acetate and stored in Falcon tubes (15 and 50 ml) containing 70% alcohol.

All material was sorted and identified to the lowest possible taxonomic level. Dichotomous keys and taxonomic revisions of specific groups were used for identification (Snyder 1924; Constantino 1995; Háva 2004; Marchi and Melo 2006; Carvalho and Mello-Patiu 2008; Almeida and Mise 2009; Grella *et al*. 2015; Rafael *et al*. 2024). A digital thermo-hygrometer was used for the collection of abiotic factors (temperature and humidity).

It is worth noting that the collections carried out were supported by authorization from SISBIO (Biodiversity Authorization and Information System) under Ordinance No. 748/2022, with the number 90841-1, which allows the collection and transportation of biological samples of animals for scientific purposes.

To characterize the sampled fauna, we calculated the frequency of occurrence (FO) and dominance (D), following the formulas used by Oliveira and Vasconcelos (2010). The frequency of occurrence (FO) of each species was calculated as: FO = [number of samples containing species 'X' / total number of samples] x 100. Species were classified as: very frequent when FO > 50%; frequent for 25% < FO < 50%; and infrequent if FO < 25%. Dominance was calculated as: D = [abundance of species i / total abundance of specimens] x 100. When D > 5% = dominant species; if 2.5% < D < 5%, accessory species; and when D < 2.5%, occasional species.

A total of 1,149 specimens were collected from the carcass, comprising 141 adults and 1,017 immatures. Among the adults, Coleoptera was the most abundant order with 122 individuals collected, followed by Diptera (14), Hymenoptera (2), and Blattodea (1) (Tab. 1). Regarding the number of recorded species, Diptera was the richest order with four species from the family Calliphoridae (*Chrysomya albiceps* (Widemann, 1919), *Chrysomya putoria* (Widemann, 1818), *Cochiomyia macellaria* (Fabricius, 1775), and *Cochliomyia hominivorax* (Coquerel, 1858) and one species from Stratiomyidae (*Hermetia illucens* Linnaeus, 1758). Additionally, two species of Coleoptera were recorded, *Necrobia rufipes* (Fabricius, 1781) (Cleridae) and *Dermestes maculatus* (De Geer, 1774) (Dermestidae), along with one unidentified specimen. There was also a record of *Trigona spinipes* (Fabricius, 1793) (Apidae) and *Syntermes molestus* (Burmeister, 1839) (Termitidae) and one unidentified Apidae.

Table 1. Absolute and relative abundance, frequency of occurrence and dominance of adult insects collected from cow carcass *Bos taurus* Linnaeus, 1758 in the state of Maranhão 2023. / **Tabla 1.** Abundancia absoluta y relativa, frecuencia de ocurrencia y dominancia de insectos adultos recolectados de la carcasa de vaca *Bos taurus* Linnaeus, 1758 en el estado de Maranhão 2023.

Order	Family	Species	A	%	FO	Dominance
Diptera	Calliphoridae	*Chrysomya albiceps* (Widemann)	9	6.38	VF	D
		Chrysomya putoria (Widemann)	2	1.42	LF	O
		Cochliomyia hominivorax (Coquerel)	1	0.71	LF	O
		Cochliomyia macellaria (Fabricius)	1	0.71	LF	O
	Stratiomyidae	*Hermetia illucens* (Linnaeus)	1	0.71	LF	O
Hymenoptera	Apidae	*Trigona spinipes* (Fabricius)	3	2.13	LF	O
		sp. 1	1	0.71	LF	O
Coleoptera	Cleridae	*Necrobia rufipes* (Fabricius)	35	24.82	VF	D
	Dermestidae	*Dermestes maculatus* (De Geer)	86	60.99	VF	D
	undetermined	sp. 1	1	0.71	LF	O
Blattodea	Termitidae	*Syntermes molestus* (Burmeister)	1	0.71	LF	O
		Total	141	100		

Legend: A= Abundance, FO= Frequency of occurrence, D= Dominant, O= Occasional, VF= Very Frequent and LF= Less common. / Leyenda: A= Abundancia, FO= Frecuencia de ocurrencia, D= Dominante, O= Ocasional, VF= Muy frecuente y LF= Menos común.

On the other hand, among the immatures, all emerged adults belonged to the species *C. albiceps*. Among the adults, the species *C. albiceps*, *N. rufipes*, and *D. maculatus* were considered very frequent and dominant (Tab. 1). Meanwhile, the species *C. putoria* was classified as infrequent and occasional, along with the other collected species (Tab. 1). Regarding the immatures, collections were only made during the first five days, with the first two days concentrating more than 80% of the immatures collected (Fig. 2).

This study also reports for the first time in the literature the occurrence of the species *C. putoria*, *C. hominivorax*, and *H. illucens*, visiting bovine carcasses in the Neotropical region. Thus, we expand the food guild of these species, although frequency of occurrence analyses classified the taxa as infrequent and occasional (Tab. 1).

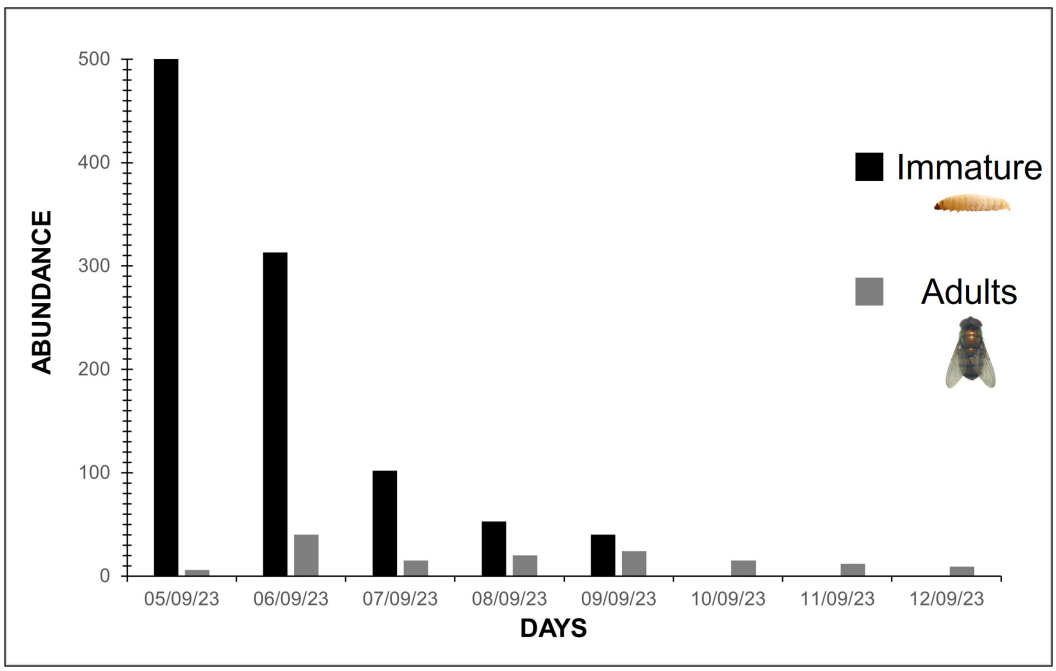

Figure 2. Abundance of immatures and adults collected from cow carcass *Bos taurus* during the 5th to 12th of September 2023. / **Figura 2.** Abundancia de inmaduros y adultos recolectados de la carcasa de vaca *Bos taurus* durante el 5 al 12 de septiembre 2023.

It is also worth noting that throughout the collections, the abiotic conditions were extreme and typical of the Northeast region, with an average temperature of 37.1 °C and relative humidity around 27%, which resulted in the loss of attractiveness of the resource and desiccation, producing a mummified appearance of the carcass.

In recent years, the number of studies involving animal carcasses has been increasing in northeast Brazil (Alves *et al*. 2014a; Vasconcelos *et al*. 2016; Cruz *et al*. 2021; Silva *et al*. 2023a), contributing to the construction of a database on the diversity of necrophagous and sarcosaprophagous species. However, most of the data have focused on dipterans, with few studies inventorying other groups, such as beetles (Mayer and Vasconcelos 2013; Santos and Alves 2016). In this scenario, our data corroborate the diversity pattern commonly found in studies with carcasses and cadavers, with the occurrence of groups such as Diptera, Coleoptera, and Hymenoptera (Cruz and Vasconcelos 2006; Meira *et al*. 2020).

Furthermore, we present the first record of the species *C. putoria*, *C. hominivorax*, and *H. illucens* visiting bovine carcasses in the Neotropical region. Our assertion was based on the

most current compilation of Diptera species with forensic importance for the Neotropical region (Alves *et al*. 2014b), which lists these species associated with decomposition and their preference for living tissues, as the species is frequently associated with cases of animal and human myiasis (Costa-Junior *et al*. 2019).

Regarding environmental conditions, the pattern of low humidity and high temperatures may have influenced the rapid desiccation of the carcass, reducing its attractiveness, especially considering that during the study, an atypical phenomenon called El Niño was occurring worldwide (Andrade *et al*. 2023). The rapid loss of biomass from the carcass in arid environments was also observed by Santos and Alves (2016) when inventorying the beetle fauna associated with bovine carcasses in northeast Brazil. Aballay *et al*. (2012), in an arid environment in Argentina, also observed rapid decomposition of pig carcasses due to low humidity, which facilitated tissue desiccation, making them unsuitable for consumption by fly larvae.

These facts may justify the numerical overlap of beetle specimens, as the carcass was in an advanced stage of decomposition, which is the preferred phase for the recorded species, *N. rufipes* and *D. maculatus* (Mise *et al*. 2007). These species also stood out in another study with bovine carcasses in the semi-arid region of Brazil (Santos and Alves 2016). However, these authors did not observe adult and immature dipterans, which differs from our results.

The observations regarding dipterans in this study are intriguing, especially due to the dominance of *C. albiceps* immatures on the carcass, a species more commonly found in the early stages of cadaveric decomposition (Aballay *et al*. 2012). The presence of over 1,000 larvae also diverges from the study by Santos and Alves (2016), which did not report the presence of fly larvae in the face of the rapid decay of carcass biomass. We believe that the high number of *C. albiceps* larvae could be justified by the predatory behavior of the species (Barbosa *et al*. 2021), or it may reflect a second generation, as the carcass was in an advanced stage of decomposition. The former point may also explain the absence of other common dipteran groups in carcass studies (*e.g*., Muscidae and Sarcophagidae) (Silva *et al*. 2023a, b).

Another noteworthy point is the occurrence of Hymenoptera and Isoptera specimens, which according to the literature, can cause post-mortem injuries and induce errors in real cases (Huchet *et al*. 2011; Barbosa *et al*. 2015). However, further studies are still necessary to understand the true forensic potential of these groups.

The results of this study reinforce the importance of research involving carcasses to gain a clearer understanding of the effects of environmental conditions on the decay of animal organic matter, even in specific cases such as the present study. Additionally, the findings reveal an atypical colonization pattern with a pool of *C. albiceps* immatures in an advanced stage of decomposition, which may result from a second generation. These atypical patterns are important in the forensic scenario and need to be reported to alert forensic experts and entomologists to potential cases.

We also highlight the occurrence of Hymenoptera and Isoptera species that deserve attention, and future research may help understand their forensic potential in the context of forensic taphonomy. The high abundance of *N. rufipes* and *D. maculatus* also reinforces the importance of studies involving carcasses, as these species occur in the most advanced stages of decomposition (Mise *et al*. 2007). Finally, the Calliphoridae species recorded here are common in carcasses and cadavers, which reinforces the forensic and necrophagic potential of the taxon.

This research confirms that the order Coleoptera is the predominant group visiting animal carcasses in advanced stages of decay, with the species *D. maculatus* and *N. rufipes* considered very frequent and dominant. We demonstrated a late colonization of the carcass by the species *C. albiceps*, which may indicate colonization by a possible second generation on the carcass, or it may reflect its predatory habit towards other immature forms.

We also reported the occasional occurrence of Hymenoptera and Isoptera species on the carcass. These insects can potentially cause injuries to the carcass, leading to errors in the calculation of the PMI. However, we did not observe any injuries due to the advanced state of decomposition, and further studies are needed to achieve a better understanding of this dynamic, especially regarding this specific fauna, which is often overlooked in studies.

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Author Contributions

RCL: Identification of specimens, preparation of maps, writing, editing and review of the final manuscript. **TMB:** Writing, drafting, editing and reviewing the final manuscript. **SSS**: Identification of specimens and review of the final manuscript. **GSC:** Identification of specimens and review of the final manuscript. **TFS:** Identification of specimens, writing, editing and review of the final manuscript.

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