Genetic Identification of Necrophagous Insect Species (*Diptera*) of Forensic Importance Sampled from Swine Carcasses in Mato Grosso, Midwestern Brazil

Elisangela Santana de Oliveira Dantas[3,4], Diniz Pereira Leite Junior[1,4*], Junio Damasceno de Souza[5], Reginaldo Rossi do Carmo[2], Flavia Galindo Silvestre Silva[2] and Patricia Pasquali Parisi Maltempi[3]

[1]UFMT – Federal University of Mato Grosso - Av. Fernando Correa da Costa, Brazil

[2]POLITEC – Official and Technical Expertise Identification, Government of Mato Grosso - Av. Gonçalo Antunes de Barros, Carumbe, Brazil

[3]UNESP – State University of Sao Paulo, Institute of Biosciences, Rio Claro Campus, Technical Section Post Graduate, Brazil

[4]UNIVAG – University Center of Varzea Grande, Av. Dom Orlando Chaves, Brazil

[5]UniBH - Centro Universitario de Belo Horizonte, PUC Minas, Rua Diamantina, Brazil

*Corresponding author: Junior DPL, Federal University of Mato Grosso, Faculty of Medicine, Research Laboratory, Av. Fernando Correa da Costa, 2367, Boa Esperanca – Cuiaba, MT 78060-900, Brazil, Tel: +55(65)3615-8809; E-mail: djbiologico@gmail.com

Abstract

Forensic entomology is the science that uses insects found on bodies to set the Post Mortem Interval (PMI) among other investigations. The present work used DNA barcode based on the use of a standardized fragment of Cytochrome C Oxidase subunit I (COI). We undertook analysis of the taxa associated with carcasses, representing 15 dipterans species. Carcasses of pigs during the decomposition process were used, using a cutoff value of 3% for intra-and interspecific differentiation proves efficient for the dipteran of forensic interest. Species identification is more accurate in well studied and reviewed groups. It was observed that specific taxonomic groups were well separated. In the larger group a molecular study, including other mitochondrial genes and nuclear genes is necessary. The greater differentiation in *Lucilia eximia* probably requires review studies of the group.

Keywords: Forensic science; Forensic entomology; Barcode; DNA; *Diptera*; Mato grosso

Introduction

Necrophagous insects are ubiquitous insects occurring in anthropogenic ecosystems. Among them we can highlight the flies which have great medical importance due to their participation in carrion decomposition, facultative parasitism of vertebrate tissues and mechanical transmission of various pathogenic microorganisms [1-3]. In recent decades, there has although there are insect knowledge of the use records for forensic application, historically, the early works were influenced by studies by Jean Pierre Megnin in the second half of the nineteenth century [4]. Currently several publications on the subject has been discussed by several authors [5-8]. But in the center west of Brazil, the state of Mato Grosso; this issue is rarely addressed. This research is the initial registration of this activity.

After a lengthy hiatus of over 50 years new studies were produced propelled by the rise of new research groups in forensic entomology since 1991 [9]. Currently in Brazil, more than 20 scientists and dozens of forensic experts with field training are carrying out research projects related to forensic entomology spread along all Brazilian states [10]. Ironically, Brazilian cities suffering from the highest rates of violence have been neglected in field inventories? Of forensically important species [11,12]. Partly due to convoluted mechanisms of Brazilian legal system and lack of investment in intelligence procedures for the police modus operandi [10].

A variety of necrophagous insect species occurs on or around cadavers and carcasses depending on their preference for a given stage of decomposition [13]. Systematized and pioneer studies of cadaver entomofauna were published in Mégnin's book [14]; the author listed the chronological sequence of body decomposition and insect colonization. The colonization sequence was described as eight waves of invasion whose we were able to recognize the orders: *Diptera*, *Coleoptera*, *Lepidoptera* and *Acarina.*

According to Oliveira-Costa [15], the entomological succession follows an initially predictable pattern since every phase of decomposition processing the stratum is colonized by specific groups which in turn, attract different stages making it possible to associate specific species to specific phases of the decomposition process.

Insects can be useful for crime investigation because they provide an estimation of the time elapsed after death known as Post Mortem Interval (PMI). PMI is based on the time which an insect takes to develop from an egg to the stage in which it is found on the body. After identification of the cadaveric fauna several clues about the crime can be inferred by linking these bugs or fragments found on victim such as where the crime took place and the suspects of the crime [16-18]. Due to their keen sense of smell dipterans rapidly locate dead bodies by their scent then colonizes this resourceful habitat as a reproduction site for oviposition also for nutritional source [16,19,20].

There are studies conducted in other countries that combine a large database on the succession pattern of insects on bodies. However, due to differences in climate conditions along with huge territorial extension, these data cannot be safely used in our forensic examinations [21].

Therefore, the present study has the objective of describing *Diptera* entomological species succession in different carcasse decomposition phases on climate conditions of Cuiaba/MT. We used DNA barcode to

assemble an easy access database to be utilized by forensic experts in criminal investigations.

Materials and Methods

Sample collection

The study was conducted in an area inside POLITEC. The public institution responsible for official forensic examinations in the state of Mato Grosso, Brazil (GPS: L 06-01-88-4/N 82-77-22-8). As substrate, we used swine carcasses *Sus scrofa* (*Suidae*) inside a metallic cage (60 × 90 × 45 cm high) until their complete decomposition.

Experimental models of carcasses used in the research were large white domestic pigs with a body weight of about 15-20 kg. Carcasses of pigs were acquired through traders and merchants who slaughtered pigs in farms near the city of Cuiaba. The animals were slaughtered at 06:00 am and brought to the worksite two hours late where the decomposition process and consequently the collection of specimens *Diptera* later occur. The requirements on ethics permits were not required at the time of the experiment and the ethical committees were not available for any evaluations accordingly; as the case of a legal and common practice in the local trade fairs in the state of Mato Grosso.

The climate is characteristic of the semiarid region, with a mean annual rainfall of 1469.4 mm and average annual temperature of 25°C to 40°C. Despite this inequality, the region is well supplied with rain and seasonality is typically tropical, with maximal temperatures in summer and minimal in winter.

Four experiments were conducted over the seasons. One experiment for each season of the year between January 2013 and December 2013. The region has a hot sub-humid tropical climate with the summer high temperature and the winter minimum temperature around 17°C. The winters are excessively dry because rainfall is very rare. Spring and fall are unstable. The study area are surrounded by open arboreal vegetation.

The phases of decomposition were observed until complete skeletonization. Where decomposition phases being defined as fresh, bloat, active decay, advanced decay and dry remains phase. The carcasses were monitored every day at 08:00 a.m. and 10:00 a.m. until complete decomposition of the individual. For entomofauna collection, larvaes was collected with a tablespoon every three days in three different regions of the animal choosing only well-developed bigger larvaes.

For identification part of collected specimens was transported alive to the forensic entomological lab at POLITEC where larvaes completed their life cycles necessary for identification through morphology. A second part of larvaes were fed for three days in plastic containers containing sawdust. After that they were washed in water and stored in 96% ethanol in a -20°C freezer. This material was stored and then used in following molecular processes.

DNA extraction and molecular analysis

The DNA was extracted only from the immature stages. The extractions were carried out from a whole individual, as follows: For the Mix we used 60 μl of EDTA (C=0.5M; pH 8.0) and 250 μl of Nucleus Lysis Solution for each sample. The Mix was chilled on ice becoming cloudy and gelatinous. We distributed the Mix in big eppendorf tubes and macerated the individual larvae. We added 10μl of Proteinase K (20 mg/ml) to the solution and agitated it. The tubes

were left in a water bath at 65°C for 2 hours. It was optional to add 1.5 μl of RNAse to the solution, mixing the sample by inverting the tube 2-5 times. We incubated the samples at 37°C for 30 minutes. After, we let the samples cool to room temperature for 5 minutes. 100μl of Protein Precipitation Solution was added (Promega) and vortexed for 20 seconds. The samples were chilled on ice for 5 minutes. We centrifuged the samples for 10 minutes at 13,000-16,000×g. The protein will form a white pellet. We transferred the DNA containing supernatant to a large eppendorf already containing 300 μl of isopropanol (room temperature). We mixed the solution inverting the tubes and centrifuged it for 2 minutes at 13,000-16,000×g. DNA will form a white pellet. We carefully removed the supernatant. We added 300 μl of 70% Ethanol and carefully inverted the tube several times to wash the DNA. We centrifuged the sample again for 2 minutes at 13,000-16,000×g, carefully removing the supernatant after that. We left the samples in a water bath (40°C) for 30 minutes in order for the alcohol to evaporate. We added 60-100 μl of autoclaved Milli-Q H2O. The samples were, then, incubated overnight at 4°C (or room temperature). We briefly and carefully vortexed the samples and then store them in a freezer.

DNA from whole specimens stored in 96% ethanol was extracted. The samples were macerated in lysis solution containing SDS/Proteinase K (480 μl of 50 mM Tris, 50 mM EDTA, pH 8.0; 20 μl of 20 mg/mL proteinase K) and incubated for 3 hours in a water bath at 55°C. Afterwards, we added 500 μl of EZDNA KIT (Biosystems®) followed by a new incubation at room temperature for 12 hours. The samples were centrifuged and the supernatant was washed 3 times in 95-100% ethanol for DNA precipitation. After the alcohol evaporated, DNA was resuspended in 70 μl of TE (10 mM Tris, 50 mM EDTA, ultrapure H2O), quantified in a spectrophotometer and stored in a freezer.

Amplification

The mitochondrial DNA region analyzed was the final portion of the cytochrome c oxidase subunit I (COI), corresponding to the Barcode DNA region [22]. This segment was amplified through the initiators set LCO1490-L (18 μl) and HCO2198-L (18 μl) [23]. Amplification was performed using: 40 ng of DNA, 25 mM of dNTP, 20 mM of each initiator, 2.5 U of Taq and 3-4 mM of MgCl2 (Biosystems). PCR temperature cycles consisted in an initial denaturation step at 94°C for two minutes, followed by 30 denaturation cycles at 94°C for 30 seconds, annealing at 46C for 30 seconds and elongation at 72°C for 2 minutes. The last cycle was followed by a 5 minute incubation at 72°C for a final elongation [23].

Sequencing

Amplicons were visualized in a 1.5% agarose gel electrophoresis, stained with ethidium bromide. Then, purified according to protocol based on PolyEthylene Glycol (PEG) and stored in a freezer. Sequencing reaction was carried out with 40 ng of DNA; 1.6 μM of the same PCR initiator and specific sequencing kit.

Amplification was conducted in a thermocycler in which cycles consisted of initial denaturation at 96°C for 1 minute, 35 denaturation cycles at 96°C for 10 seconds, annealing at 50C for 5 seconds and elongation at 60°C for 4 minutes [23]. Sequencing was done at Politec-MT forensic laboratory.

Data entry in the database

Once we obtained the genetic sequence of the questioned sample, analysis and editing are necessary for submitting the sequence to the database for similarity search. Gross data from the sequencer are called base calling.

Allignment

Consensus sequences were aligned in Clustal W software [8] using default parameters then manually checking and editing them. Blast's major utility in this study was to compare between nucleotides sequences provided by the user (query) with other sequences inside the database. Sequence comparison was analyzed through pair by pair alignment where every nucleotide pair receives a score determined by a score matrix in which values are empirically determined. When there is no gap in a sequence it is represented in a graph by a continuous diagonal line. Otherwise that line would be interrupted one or more times [24].

Result

Of the 220 individuals initially selected only 50% had their DNA amplified by the COI region. This because their supporting amounts, result in a 50% success rate amplification. Because of the low value of the core samples at the time of recognition of the species; generating ambiguous samples. The ones that were successfully amplified 13 species could be identified through 613 bp sequences.

This systematic approach provides the initial infrastructure for the next generation of biodiversity assessment and environmental monitoring adults of necrophagous blowflies. It can lead to more effective understanding, appreciation, and management of these complex ecosystems. A geographical distribution of the species is shown in Table 1.

Super-family /Family	Subfamily	Tribe	Genus	Species	Geographical distribution
Muscoide Muscidae	Muscinae	Muscini	*Musca*	House fly *M. domestica* Linnaeus, 1758 [29]	Cosmopolitan
	Azeliinae	Azeliini	*Ophyra*	Dung fly *O. albuquerquei* (Loper, 1985)	Brazil, Argentina
	Azellinae	Azeliini		*O. aenescens* (Wiedemann, 1830)	USA, Nicaragua, Bahamas, Bermuda, Greater and Lesser Antilles, French Polinesia, Hawaii, Neotropical Region
Oestroidea Calliphoridae	Toxotarsinae	Sarconesiini	*Sarconesia*	Blow fly *S. chlorogaster* (Wiedemann, 1831)	Peru, Brazil, Paraguay, Uruguay, Argentina, Chile, Juan Fernandez Islands and Easter Islands
	Chrysomyinae	Chrysomyini	*Chrysomya*	*C. albiceps* (Wiedemann, 1819)	Africa, Mediterraneam, North American and South American and Brazil
				C. megacephala (Fabricius, 1794)	Africa, Australasia, Palearctic and Oriental regions until Southern Africa and Afrotropical islands
				C. putoria (Wiedemann, 1818)	Argentina, Bolivia, Brazil, Colombia, Panama, Paraguay, Peru
	Calliphorinae	Luciliini	*Lucilia = Phaenicia**	Green-bottle fly *L. sericata* (Meigen, 1826)	Southern Canada, Argentina, Bermuda, Brazil, Chile, Colombia, Peru, Venezuela
				L. cluvia (Walker, 1849)	Southern USA, Cuba, Puerto Rico, Bahamas, Argentina, Colombia, Guatemala, Honduaras, Martinique, Mexico, Nicaragua
				L. porphyrina (Walker, 1856)	Sri-Lanka, China, India, Malasya, Nepal and Philipines

				L. cuprina (Wiedemann, 1830)	Tropical Regions, southern USA, Argentina, Brazil, Colombia, Cuba, Haiti, Jamaica, peru, Uruguay, Venezuela
				L. eximia (Wiedeman, 1819)	Southern USA, Mexico, Peru, Venezuela, Brazil, Argentina, Bahamas, Nicaragua, Chile, Colombia
				L. mexicana (Macquart, 1843)	Southern USA, Mexico, Guatemala, southern Brazil
				L coeruleiviridis (Macquart, 1855)	U.S.A., Maryland, Baltimore, Neotropical: Cuba, Guatemala
Oestroidea Sarcophagidae	Sarcophaginae	Sarcophagiini	Peckia (*Pattonella*)	Flesh fly *P. intermutans* (Walker, 1861)	concentrated in regions of tropical and warm temperate climate and in Neotropical region
This information and classification is modeled on the basis of previous reports by Kosmann et al. [51]; ITIS Report [50]*; Whithworth [25] and Rognes [52], respectively.					

Table 1: Necrophage insects collected in swine (*Sus scrofa L.*) carcasses in Cuiabá/MT region.

Species		1	2	3	4	5	6	7	8	9	10	11	12	13	
		0.01	0.009	0.009	0.009	0.01	0.011	0.009	0.009	0.008	0.014	0.014	0.013	0.012	
L. cluvia	0.074		0.007	0.011	0.011	0.008	0.009	0.012	0.011	0.011	0.014	0.015	0.014	0.013	
L.coeruleiviridis	0.056	0.03		0.009	0.009	0.007	0.008	0.011	0.009	0.009	0.013	0.013	0.013	0.013	
L. cuprina	0.05	0.08	0.062		0.001	0.01	0.011	0.01	0.003	0.008	0.015	0.013	0.012	0.011	
L.cuprinahawaii	0.05	0.08	0.061	0.001		0.01	0.011	0.01	0.003	0.008	0.015	0.013	0.012	0.011	
L. eximia	0.084	0.06	0.048	0.082	0.081		0.005	0.012	0.01	0.01	0.014	0.013	0.013	0.012	
L. mexicana	0.074	0.05	0.044	0.073	0.072	0.032		0.012	0.011	0.011	0.015	0.014	0.014	0.013	Standard deviation
L. porphyrina	0.055	0.09	0.079	0.071	0.071	0.1	0.096		0.011	0.01	0.015	0.015	0.014	0.014	
L. sericata	0.049	0.08	0.058	0.007	0.006	0.082	0.072	0.074		0.008	0.015	0.013	0.012	0.011	
L. thatuna	0.044	0.08	0.054	0.038	0.038	0.077	0.07	0.069	0.039		0.013	0.013	0.013	0.011	
M.domestia	0.105	0.12	0.103	0.123	0.123	0.135	0.136	0.13	0.12	0.107		0.013	0.014	0.013	
O.aenescens	0.113	0.13	0.109	0.106	0.107	0.133	0.127	0.128	0.103	0.117	0.103		0.01	0.014	
O.albuquerquei	0.107	0.13	0.103	0.093	0.095	0.13	0.121	0.124	0.09	0.098	0.124	0.063		0.012	
S.chlorogaster	0.086	0.11	0.097	0.081	0.081	0.116	0.109	0.116	0.077	0.073	0.106	0.123	0.101		
Genetic difference															

Table 2: Genetic distance of the analyzed species.

The present study is the first to report the analysis of the obtained results made it possible to verify the succession pattern of these species in the decomposition process of pigs (*Sus scrofa*) (Table 2).

Members of the family *Calliphoridae* were the most commonly isolated. This genus includes numerous *saprophagous* species formerly *Phaenicia* [25]. A common visitor to carrion, feces and garbage plays an important role in forensics, medical science and veterinary medicine.

In the fresh phase, *Calliphoridae* was dominant, especially *Lucilia eximia* (Wiedemann, 1819) followed by *Chrysomya megacephala* (Fabricius, 1794) and *Musca domestica*. In the bloat phase, we observed *L. eximia*, *C. albiceps* (Wiedemann, 1819), *C. putoria* (Wiedemann, 1818), *Peckia* (*Pattonella*) *intermutans* (Walker, 1861), *Ophyra aenescens* (Wiedemann, 1830) and *O. albuquerquei* (Lopes, 1985). During active decay phase we observed immatures of: *C. megacephala*, *C. albiceps*, *C. putoria* (Wiedemann, 1818), *P.*

intermutans, *M. domestica*, *L. cuprina* (Wiedemann, 1830), *L. sericata* (Meigen, 1826), *L. cluvia* (Walker, 1849), *L.coeruleiviridis* (Macquart, 1855), *L. mexicana* (Macquart, 1843) and *L. porphyrina* (Walker, 1856). In advanced decay phase, it was possible to observe C. albiceps, *C. megacephala*, *Sarconesia chlorogaster* (Wiedemann, 1830) and *M. domestica*. During the dry remains phase, we could observe *M. domestica*, *C. megacephala* and *C. albiceps*. Other insects from other orders, like: *Coleoptera*, *Hemiptera* and *Dermaptera* were predominant during this period.

With respect to the succession patterns observed in the different seasons of the year we could evaluate that they are extremely similar varying only with regard to the time duration of the fresh and dry remains phases. During the rainy season (November to March) complete decomposition occurred in twelve days. On the other hand, during dry season (April to September) this process was accelerated with total decomposition reaching an average of nine days.

With sequence alignment obtained from COI we obtained a 613 bp sequence matrix. from which genetic distance analysis was carried out using the Tamura-Nei method [26]. Purine and pyrimidine frequency composition analysis for COI segments is summed up in Table 3.

SPECIES	T	C	A	G	Total
L. caeser	37.7	16	30.2	16.6	613
L. coeruleiviridis	37.4	17	29.2	16.5	613
L. cuprina	37.5	16	30.5	16.5	613
L. cuprina hawaii	37.5	16	30.3	16.5	613
L. eximia	37.2	17	28.7	17.1	613
L. mexicana	36.1	18	29.2	16.7	612
L. porphyrina	35.6	18	30.3	16.5	613
L. sericata	37.8	15	30.5	16.3	613
L. thatuna	37.8	15	30.3	16.5	613
M. domestica	38.2	15	29.5	17	613
O. aenescens	36.1	18	29.2	16.5	613
O. albuquerquei	38.7	15	30.3	16.2	613
S. chlorogaster	39.3	14	29.7	16.6	613
Average	38	16	30	16.5	613

Table 3: Purine and pyrimidine frequencies in COI segments.

Discussion

In study area the average annual rainfall is 1.300 mm to 1.450 mm, with maximum intensity in January, February and March. The mean maximum temperature reaches 25°C to 35°C. But the absolute maximum can reach 40C in the warmer months; on rainy days. These meteorological data confirm that the climate of Cuiaba is Cwa. according to the climatic classification of Köppen-Geiger created in 1928 [27]. A Cwa climate presents subtropical characteristics, with a dry winter and a rainy summer.

The Order *Diptera* is divided into two suborders: *Nematocera* which includes mosquitoes along other flies with long antennae and *Brachycera* covering the robust and compact flies with short antennas. Among the *Brachycera*, scavenger species of the families *Calliphoridae*, *Sarcophagidae*, *Muscidae*, *Fanniidae* and *Stratiomyidae* are among the representatives of most forensic relevance [7,28,29].

DNA barcoding has become a fairly useable method of choice for rapid species identification in the last decade. Due to the existence of a taxonomic crisis (relative to taxonomic identification of immature specimens that can result in wrong identifications) of the bar code to identify eukaryotic DNA, based on use of a standardized cytochrome fragment (COI); this method has been the subject of much debate [30-33]. The DNA barcoding; It is still the subject of numerous criticisms of its applicability. As opposed to the use of morphology and identification of species and other taxa. Regarding this regard. the identification of forensic interest dipteran. in this work was to record the relevant samples *Calliphoridae* and *Muscidae*, becoming 13 identified species.

Among these proposals, DNA barcoding has been particularly successful in the identification and delineation of new species of various groups and has received greater acceptance because it is simple and affordable [34-38]. Species identification of the adults of necrophagous blowflies is usually not difficult. and numerous keys are currently available for potential users [25,39-42]. However, some characters must nonetheless be treated with caution.

The approach for intra and interspecific differentiation using a 3% cutoff value has shown to be valid for forensically important *diptera*: *Ophyra albuquerquei* and *O. aenescens* as well as *Sarconesia chlorogaster*, *Lucilia thatuna* (Shannon, 1926*)*, *L. cluvia*, *L. porphyrina* and *L. Caesar* (Linnaeus, 1758) [29] were separated from the rest. Gender differences were higher than 3% in all cases. But in some cases, mitochondrial DNA sequence monophilia analyzed here was not proved. It was not possible to separate *L. cuprina* and *L. sericata* that behaved as if they belonged to the same species, as well as *L. coeruleiviridis* and *L. mexicana* that were grouped with different haplotypes comparing to *L. eximia*.

Species identification is more accurate in well studied and revised groups. Interspecific differences varied between 13.6% for *L. mexicana* and *M. domestica* and 0.1% for *L. cuprina* and L. *cuprina hawaii*. Regarding intraspecific differences. *L. eximia* has more than 3% differentiation. Despite the COI gene alone is not being enough for a deep phylogenetic study. It gave support for the formation of important clades. We observed that the specific taxonomic groups were generally well separated. In the larger group where we find: *L. eximia*, *L. Mexicana, L. cluvia* and *L. coeruleiviridis* a molecular phylogenetic study including more mitochondrial and nuclear genes might be necessary. Still, L. Mexicana, L. cluvia and *L. coeruleiviridis* are well separated and have a high support index. The high differentiation in *L. eximia* would probably require a group review in future studies.

*Dipter*a constitute along with *Coleoptera* the majority of the necrophage insects representing the largest portion of cadaveric fauna found on carcasses during the decomposition process [43-45] and that was also observed in this study. For this reason, members of *Sarcophagidae*, *Calliphoridae* and *Muscidae* family have been commonly cited in forensic entomology studies [46,47] as observed in this study as well. Adults of the *Calliphoridae* family are the first to colonize the carcasses due to their agility in laying their eggs soon after the death of the animal. In accordance with this study, members of this family have been frequently observed by other authors [45,46,48,49].

The presence of *M. domestica* and *C. megacephala* during the fresh phase (first 18 hours after death) was also reported by Carvalho and researchers [50] that worked with the succession pattern of insects on dead pigs in the state of São Paulo. Chin and collaborators [51] in their studies are found *Calliphoridae* and *Muscidae* visiting carcasses, finding *C. megacephala* as the dominant species. In our study, we found calliphorid fly (*Sarconesia spp., Chrysomya spp.,* and *Lucilia spp.*) and muscid fly (*Ophyra spp.* and *M. domestica*) visiting the floating carcass; with the gender *Lucilia* as the dominant species. The presence of *L. eximia* during this phase corroborates the data obtained by Souza and researchers [48] as well as its presence in all seasons. The results of the fly species found in this study, approach the species identified in the study by Oliveira-Costa et al. [7] in an experiment in the city of Rio de Janeiro, Brazil. More recently, Salem et al. [52] report on their studies in Egypt, the results of *Chrysomyinae* specimens belonging to three forensically important.

The significative presence of members of the *Calliphoridae* family during the bloat phase corroborates the data from Gomes et al. [53] that already proposed their use as an indirect "biological clock" capable of indicating the amount of time elapsed since death to the Discovery of the body. Members of the *Sarcophagidae* family have been observed by Barros et al. [54] during the period of active and advanced decay phases corroborating the data found in the literature. However, different from what Carvalho and Linhares [46] and Barros et al. [54] observed. The abundance of members from *Sarcophagidae* family was small when compared with members of the *Calliphoridae* family. In 2015, Barbosa and colleagues [55] showed the geographical distribution and diversity of the expansion of the flesh flies distributed by the northeast region of Brazil.

In contrast, in northern Brazil amazonense authors [8] studied the composition and temporal distribution one of the must forensic importance insect families. More currently Akbarzadeh and colleagues [56] identified species of *Calliphoridae* of forensic importance. Showing that in recent decades there has been growing interest in research into the biology and ecology of insects with potential forensic applications [13,56]. Therefore, it must be considered that the active decay phase for the decomposition process in Cuiaba region, starts in the third day after death, for the rainy season, and second day after death for the dry season.

The faster decomposition during the dry season when compared to the rainy season has already been reported by Carvalho & Linhares [45] and Oliveira-da-Silva et al. [57-60] that justify it by saying it happens due to the high temperature, as well as the air's relative humidity. However, total decomposition period is different from that observed by the authors mentioned above, who had periods of 24 days for the rainy season and 11 days for the dry season.

In this study, the use of democratic barcode nurtures the easy access of species identification even when dealing with insect fragments. This work is relevant due to taxonomic identification of insects of forensic interest providing additional information needed to further comprehensive study of these scavengers organisms.

Even so when secure opportunities present themselves safe. we encourage future studies and other researchers to continue documenting species of necrophagous dipterous taking place in this part of the world to help facilitate the various applications of these important insects, in view of the species diversity and overlapping habitat of such flies in Mato Grosso state.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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