



Direct Injury, Myiasis, Forensics

Ecological succession of blow flies (Diptera: Calliphoridae) in the decomposition of pig carcasses in Cerrado areas of Brazil

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Subject Editor: Jason Byrd

Received on 27 March 2023; revised on 21 June 2023; accepted on 14 September 2023

This research aims to describe the process of ecological succession by associating the decomposition stages of pig carcasses with flies from the Calliphoridae family (Diptera). For this, 6 pig carcasses were exposed in Maranhão's Cerrado, utilizing metal cages with sawdust trays to catch immature specimens and “suspended traps” to capture adults. Adults of the *Hemilucilia benoisti* Séguéy, and *Hemilucilia townsendi* Shannon species were only associated with the swelling stage. *Chrysomya megacephala* (Fabricius) was associated with 2 stages (black putrefaction and fermentation). The species *Chloroprocta idioidea* (Robineau-Desvoidy), *Chrysomya albiceps* (Wiedemann), *Cochliomyia macellaria* (Fabricius), and *Lucilia eximia* (Wiedemann) were associated with 4 of the 5 stages of decomposition, the latter being the only 1 associated with the initial stage. The larvae abandoned the carcasses to pupate from the second stage of decomposition, with *L. eximia* being the only 1 leaving the carcasses in the swelling stage, and *C. albiceps* the only 1 associated with both the fermentation and dry stages. Our findings indicate that calliphorid species can help forensic investigators estimate the post-mortem interval of cadavers in situations similar to those detailed in this study. Since there was a link between adult and immature species and certain stages, they can be used as indicators in future forensic investigations.

Key words: Brazilian savanna, forensic entomology, Oestroidea, post-mortem interval estimation

Introduction

While being a continuous process, the decomposition of a carcass or corpse can be separated into periods or phases (stages), each with its own characteristics, attracting different scavenger species, and with durations that vary according to the climatic and faunal conditions of the region (Goff 1993, Amendt et al. 2010). Diptera are the most common and abundant insects on carcasses, making them crucial in forensic investigations (Souza and Linhares 1997, Moretti et al. 2008, Rosa et al. 2011, Scaglia 2014, Faria et al. 2018, Ramos-Pastrana et al. 2018, Meira et al. 2020, Vairo and Moura 2021, Silva et al. 2023). Calliphoridae, or blow flies, is one of the most studied Diptera families under the forensic aspect, because they are pioneers and abundant in carcasses in the decomposition stage, which are

used as a food source, copulation site, and/or substrate for the development of their immature stages (Wolff et al. 2001, Amendt et al. 2010, Oliveira-Costa 2011, Silva et al. 2014, Ramos-Pastrana et al. 2018, Meira et al. 2020, Cruz et al. 2021).

Even though Calliphoridae species can be found at all stages of carcass decomposition, they are often associated with specific stages (Byrd and Castner 2001, Pérez et al. 2005, Ramos-Pastrana et al. 2018, Meira et al. 2020, Cruz et al. 2021). Therefore, identifying the occurrence of each species by stage is an essential source of information in calculating the post-mortem interval (PMI) of human corpses, mainly when they are in an advanced condition of decomposition and traditional approaches are ineffective (Catts and Haskell 1991, Byrd and Castner 2001, Oliveira-Costa 2011, Meira et al. 2020).

In Brazil, information on the post-embryonic development time of calliphorid species have already been used to estimate the PMI in the Southeast (Oliveira-Costa and Mello-Patiu 2004, Scaglia 2014), North (Pujol-Luz et al. 2006, Souza et al. 2014), Central-West (Kosmann et al. 2011, Scaglia 2014) and in the Northeast regions (Oliveira-Costa 2013, Vasconcelos et al. 2014, Meira et al. 2020). On the other hand, due to the scarcity of studies, the method of ecological succession of insect species has not yet been used to estimate the PMI in the country.

Forensic entomology studies have revealed differences in the composition and/or succession of calliphorid in the decomposition of pig corpses between areas and even within the same region, demonstrating the importance of conducting studies in different environments (Souza and Linhares 1997, Carvalho et al. 2004, Souza 2009, Barbosa et al. 2010, Biavati et al. 2010, Ururahy-Rodrigues et al. 2013, Faria et al. 2018). However, few studies have used this approach in the Cerrado biome, which is limited to the Southeast and Central-West (Marchiori et al. 2000, Ribeiro 2003, Rosa et al. 2009 2011, Biavati et al. 2010, Faria et al. 2018). In this sense, this study aims to describe the pattern of ecological succession and evaluate the relationship of adults visiting and larvae that leave to pupate of blow flies along the decomposition stages of pig carcasses in Cerrado areas, Northeastern Brazil.

Materials and Methods

The research was conducted in Cerrado areas of the Inhamum Municipal Environmental Protection Area (Área de Proteção Ambiental Municipal do Inhamum—*Inhamum APA*; 04°53'45"S

43°26'21"W), in the municipality of Caxias, state of Maranhão, Brazil (Fig. 1). The experiments were carried out during July and August of 2010 (dry season) and March and April of 2011 (rainy season). Six pigs (*Sus scrofa* Linnaeus) with a biomass of 12 kg were used to assess the pattern of ecological succession of blow flies in the carcass. Each pig was put down at the experimental site with a shotgun blast to the front of the head. The stages of decomposition of pig carcasses were identified using Bornemissza's (1957) categorization, which separates it into 5 stages: initial, swelling (or putrefaction), black putrefaction, butyric fermentation, and dry. The initial stage of carcass decomposition begins with death and ends with the onset of swelling. In the beginning of the swelling stage, an accumulation of gases occurs within the carcasses, caused by bacterial activity. As a result, the pig legs move apart, giving the carcass a distended appearance resembling that of a "boxer." In the subsequent black putrefaction stage, the carcass skin disintegrates due to gas leakage and the feeding activity of larvae. Furthermore, exposed areas become blackened, accompanied by a pronounced putrid odor. During the butyric fermentation phase, the carcass dries out as a result of the fermentation process. In the subsequent dry phase, or skeletonization, only the skin, leather, and bones of the carcasses remain. The investigation of the chronological sequence of decomposition stages was conducted 3 times per day: in the early morning (between 7 and 8 AM), around midday (11:30–12:30 PM), and in the late afternoon (17–18 PM).

The pig carcasses were placed in metal cages (110 × 85 × 85 cm; with a 3 × 3 cm mesh screen) with a removable tray (110 × 85 × 5 cm) underneath filled with sawdust that functioned as a pupariation area for the immatures, which left the carcasses. A "suspended trap"

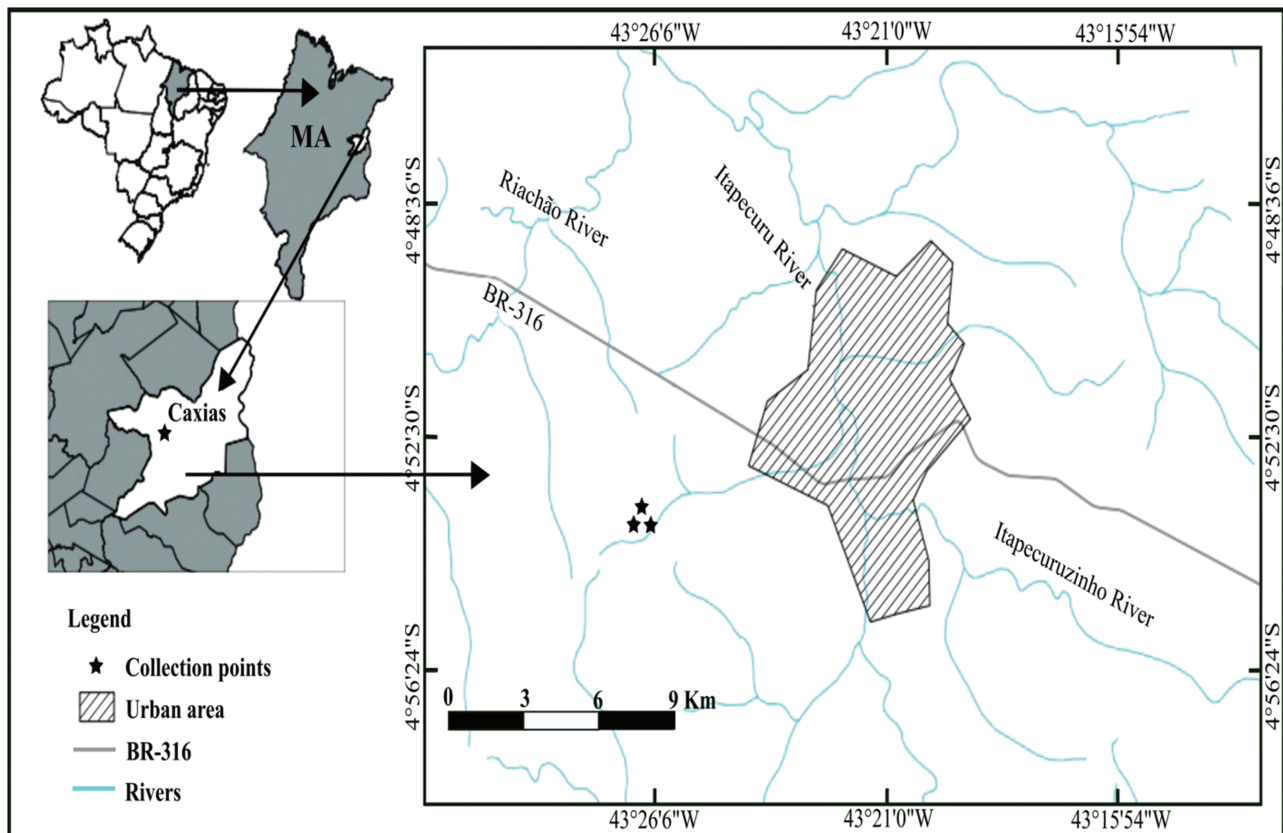


Fig. 1. Collection sites of blow flies species in pig carcasses in Cerrado areas in the Área de Proteção Ambiental Municipal do Inhamum, in the municipality of Caxias, Maranhão, Brazil. Source: IBGE (2010, modified by P. R. C. Santos 2021).

(Rafael and Gorayeb 1982) was set 30 cm from the ground over each cage, devoid of the lower septum, pyramidal in shape, and with a square base (1.60 m high by 1.50 m side edge), with a collecting flask on its top for the retention of adult insects. Adults and immature blow flies were collected at 24-hour intervals between 07:00 and 10:00. The immatures captured in the trays were placed in five-liter plastic bottles sealed with netting and stored at room temperature in the laboratory to obtain adults.

The blow flies species were identified using taxonomic keys from Dear (1985), Mello (2003), De Carvalho and Mello-Patiu (2008), Amat (2009), and Whitworth (2010). In some cases, the genitalia were taken from the abdomen and bleached in cold 70% KOH

for 24 h before being rinsed in water and alcohol for further study under a microscope. The specimens were deposited in the Maranhão Zoological Collection (Coleção Zoológica do Maranhão – CZMA), on the Caxias Campus, of the State University of Maranhão (Universidade Estadual do Maranhão – UEMA), in Caxias (MA), and in the Entomological Collection of the Emilio Goeldi Paraense Museum (Coleção Entomológica do Museu Paraense Emílio Goeldi—MPEG), in Belém (PA).

The experiments were conducted in the APA of Inhamum with permission from the Municipal Secretary of Environment and Preservation of Natural Resources of Caxias, MA (Secretaria Municipal de Meio Ambiente e Preservação dos Recursos Naturais

Table 1. Composition, abundance, and ecological succession of collected adults and created immatures (which emerge to pupate) of blow flies species in different stages of decomposition of pig carcasses in the respective seasons in Cerrado areas in the Área de Proteção Ambiental Municipal do Inhamum, Caxias, MA, Brazil

Species	Decomposition stages	Season						Total A + Im
		Dry		Rainy		Total		
		A	Im	A	Im	A	Im	
<i>Cochliomyia macellaria</i> (Fabricius)	I	0	0	2	0	2	0	2
	II	46	0	488	47	534	47	581
	III	6,664	60	2,588	23	9,252	83	9,335
	IV	2,346	1,351	858	3	3,204	1,354	4,558
	V	163	0	92	1	255	1	256
<i>Chrysomya albiceps</i> (Wiedemann)	I	0	0	0	0	0	0	0
	II	5	0	211	104	216	104	320
	III	1,457	735	1,741	2,019	3,198	2,754	5,952
	IV	605	1,347	1,073	6,014	1,678	7,361	9,039
	V	77	9,068	120	5,191	197	14,259	14,456
<i>Chrysomya megacephala</i> (Fabricius)	I	0	0	0	0	0	0	0
	II	0	0	4	0	4	0	4
	III	4	0	16	0	20	0	20
	IV	7	2	23	0	30	2	32
	V	3	0	1	0	4	0	4
<i>Chloroprocta idioidea</i> (Robineau-Desvoidy)	I	2	0	22	0	24	0	24
	II	55	0	569	0	624	0	624
	III	402	0	2,567	2	2,969	2	2,971
	IV	263	0	1,121	0	1,384	0	1,384
	V	36	0	338	0	374	0	374
<i>Lucilia eximia</i> (Wiedemann)	I	6	0	38	0	44	0	44
	II	27	0	327	38	354	38	392
	III	89	11	351	106	440	117	557
	IV	31	0	137	2	168	2	170
	V	3	0	26	0	29	0	29
<i>Hemilucilia benoisti</i> Séguy	I	0	0	0	0	0	0	0
	II	0	0	16	0	16	0	16
	III	1	0	4	0	5	0	5
	IV	0	0	6	0	6	0	6
	V	0	0	0	0	0	0	0
<i>Hemilucilia segmentaria</i> (Fabricius)	I	0	0	0	0	0	0	0
	II	0	0	2	0	2	0	2
	III	1	0	3	0	4	0	4
	IV	0	0	5	0	5	0	5
	V	1	0	0	0	1	0	1
<i>Hemilucilia townsendi</i> Shannon*	I	0	0	0	0	0	0	0
	II	0	0	29	0	29	0	29
	III	1	0	17	0	18	0	18
	IV	0	0	12	0	12	0	12
	V	0	0	3	0	3	0	3
Total		12,295	12,574	12,810	13,550	25,105	26,124	51,229

A = adults, Im = created immature, I = initial, II = swelling, III = black putrefaction, IV = butyric fermentation, V = dry. New record for Brasil (*).

de Caxias, MA—SEMUMA). Blow flies were collected without suffering or pain from experiments with dead pig carcasses, in compliance with the general rules of the Commission for Ethics in Animal Use (Comissão de Ética no Uso de Animais—CEUA). Moreover, they were collected and transferred with the permission of the Biodiversity Authorization and Information System (Sistema de Autorização e Informação em Biodiversidade—SISBIO; License No. 12417).

Statistical Analysis

Each day of collection in each pig carcass was considered as a sampling unit. The Indicator Value Method (IndVal) index was used to assess the association of calliphorid species, visiting adults and immatures that left the carcasses to pupate, with the stages of decomposition of pig carcasses (Dufrene and Legendre 1997, De Cáceres and Legendre 2009, De Cáceres et al. 2010). This indicator combines a species' relative abundance with its relative frequency of occurrence at different stages. A perfectly associated species must appear exclusively in samples within a particular stage (specificity) and in all samples from that stage (reliability) (Dufrene and Legendre 1997). Monte Carlo tests with 10,000 randomizations were used to confirm the significance of the indicator value. Values were considered significant when $P \leq 0.05$. The species association was only investigated for populations bigger than 20 individuals. High associations had an indicator value of 70% or higher, while moderate associations had an indicator value of 50% to 70%.

Results

The carcasses decomposed in an average of 10 days, with no notable difference between the dry and rainy seasons. The initial, swelling, black putrefaction, butiric fermentation, and dry stages took an average of 1, 2, 2, 3, and 2 days to decompose, respectively. A total of 51,229 blow flies specimens were collected on the carcasses, 25,105

as adults and 26,124 as immatures, being represented by 5 genera and 8 species (Table 1).

Among the collected adults, the species *Cochliomyia macellaria* (Fabricius), *Chloroprocta idioidea* (Robineau-Desvoidy), and *Lucilia eximia* (Wiedemann) were present in all 5 stages. The other species occurred in 4 stages, except for *Hemilucilia benoisti* Séguy, which was also not present in the dry stage (Table 1).

The IndVal approach detected an association of the species *H. benoisti* and *Hemilucilia townsendi* Shannon only to the swelling stage. The species *H. townsendi* (Figs. 2 and 3) was recorded in Brazil for the first time. *Chrysomya megacephala* (Fabricius) was only associated with the stages of black putrefaction and butiric fermentation. *Lucilia eximia* was associated with the initial stages, swelling, black putrefaction, and butiric fermentation. *Lucilia eximia* was the only species associated with the initial stage and the only 1 unrelated to the dry stage. The species *C. macellaria*, *C. idioidea*, and *Chrysomya albiceps* (Wiedemann) were associated with the last 4 stages: swelling, black putrefaction, butiric fermentation, and drying (Table 2).

Regarding the immature species, *C. idioidea* and *C. megacephala* were obtained when they left the carcasses to pupate during the black putrefaction and butiric fermentation stages, respectively. However, only 2 specimens of each of these 2 species were obtained. Specimens of *C. albiceps*, *C. macellaria*, and *L. eximia* did not leave the carcasses to pupate in the initial stage. In addition to this stage, only the species *L. eximia* was not obtained in the dry stage. Species of the genus *Hemilucilia* were the only ones that did not develop in pig carcasses (Table 1).

Only 2 species, *L. eximia* and *C. albiceps*, were shown to have a significant association with the stages of decomposition of pig carcasses during larval stage, when they left it to pupate, using the IndVal index. Only swelling and black putrefaction were related with *L. eximia*. *Chrysomya albiceps* was associated with black putrefaction, butiric fermentation, and dry stages (Table 3).

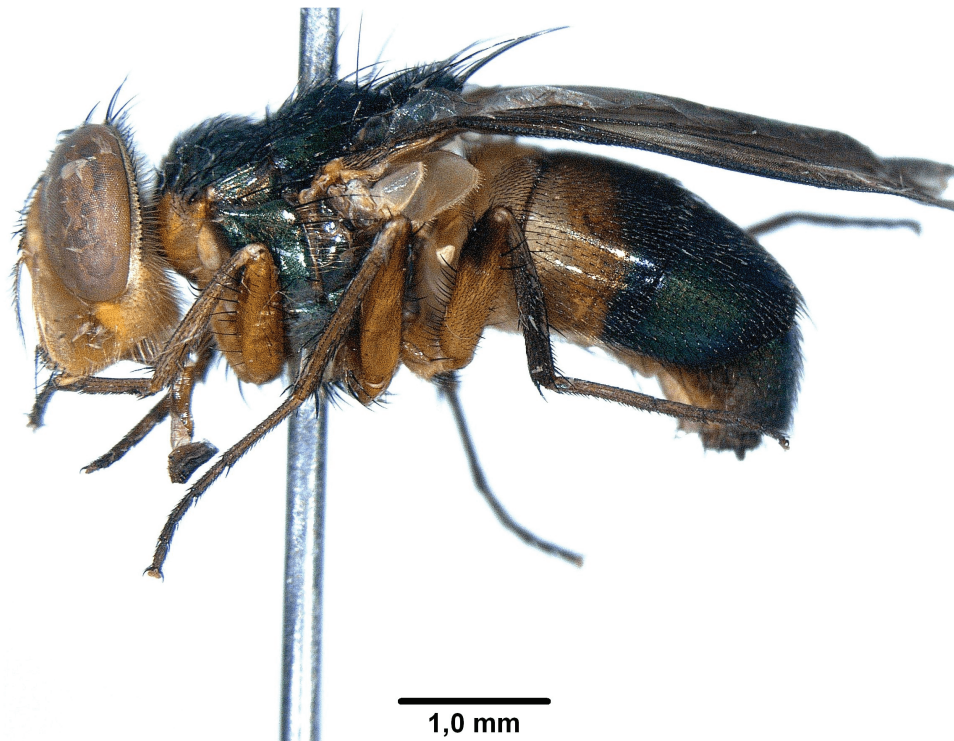


Fig. 2. *Hemilucilia townsendi* Shannon, 1926, left lateral view.



Fig. 3. *Hemilucilia townsendi* Shannon, 1926, dorsal view.

Table 2. Individual Indication Value (*IndVal*) of adults collected from Calliphoridae species during the decomposition stages of pig carcasses in Cerrado areas of the Área de Proteção Ambiental Municipal do Inhamum, Caxias, MA, Brazil

Species	Association	<i>IndVal</i>	<i>P</i>
<i>H. townsendi</i>	Swelling	0.655	0.033
<i>H. benoisti</i>	Swelling	0.522	0.025
<i>C. megacephala</i>	Black putrefaction + fermentation	0.694	0.016
<i>L. eximia</i>	Initial + swelling + black putrefaction + fermentation	0.955	0.003
<i>C. idioidea</i>	Swelling + black putrefaction + fermentation + dry	0.995	0.001
<i>C. albiceps</i>	Swelling + black putrefaction + fermentation + dry	0.981	0.001
<i>C. macellaria</i>	Swelling + black putrefaction + fermentation + dry	0.981	0.001

Time lapse in days partitioned according to the decomposition stage of pig carcasses: initial = 1st day; swelling = 2nd–3rd days; black putrefaction = 4–5th days; butyric fermentation = 6–8th days; dry = 9–10th days.

Discussion

Adults Calliphoridae specimens visit carcasses at all stages of decomposition, while some are associated with only 1, 2, or all 4 stages. *Lucilia eximia* is associated with the first stage, emphasizing its

pioneering position among insects that colonize carcasses (Carvalho et al. 2000, Carvalho and Linhares 2001, Barbosa et al. 2010, Ururahy-Rodrigues et al. 2013). Furthermore, no association of this species with the last dry stage was found in our investigation,

Table 3. Individual Indication Value (*IndVal*) of immature Calliphoridae collected during the decomposition stages of pig carcasses in Cerrado areas of the Área de Proteção Ambiental Municipal do Inhamum, Caxias, MA, Brazil

Species	Association	<i>IndVal</i>	<i>P</i>
<i>L. eximia</i>	Swelling + black putrefaction	0.779	0.001
<i>C. macellaria</i>	Swelling + black putrefaction + fermentation	0.718	0.240
<i>C. albiceps</i>	Black putrefaction + fermentation + dry	0.958	0.001

Time lapse in days partitioned according to the decomposition stage of pig carcasses: initial = 1st day; swelling = 2nd–3rd days; black putrefaction = 4–5th days; butyric fermentation = 6–8th days; dry = 9–10th days.

making its absence in this stage significant evidence for establishing the elapsed time, post-mortem interval, or even the end of the butyric fermentation stage. Similar results were also found by Ribeiro (2003), bolstering the robustness and effectiveness of the pattern presented in the study.

The association of adult *C. megacephala* individuals with only 2 stages of decomposition, and *H. benoisti* and *H. townsendi* individuals with only 1 stage can aid in estimating the post-mortem interval for the region. Adults of *H. townsendi* and *H. benoisti* can be used to estimate the swelling stage, whereas *C. megacephala* can be used to identify the stages of black putrefaction and butyric fermentation.

Adults of *C. megacephala* had already been recorded in the black putrefaction and butyric fermentation stages in Southeast Brazil (Campinas (SP)) by Carvalho et al. (2004), and in the swelling and black putrefaction stages in Central-West region by Biavati et al. (2010). This variation in the occurrence patterns of this species along the stages of decomposition highlights the importance of conducting decomposition research in different parts of Brazil. Because changes in environmental conditions are intimately tied to the particularities of each region, identifying and understanding these variations is critical in order to increase the use of Calliphoridae species in forensic entomology.

Hemilucilia townsendi is only found in South America, with records for Peru (James 1970, Dear 1985) and Colombia (Amat 2009); however, it had not yet been recorded for Brazil. Thus, this is the first time *H. townsendi* and *H. benoisti* have been recorded visiting decomposing pig carcasses in Brazil.

Chloroprocta idioidea, *C. macellaria*, and *C. albiceps* were not found in the initial stage visiting pig carcasses in the Southeast (Carvalho et al. 2004, Barbosa et al. 2010) and Central-West (Biavati et al. 2010) regions of Brazil. Yet, Carvalho and Linhares (2001) recorded *C. albiceps* in the early stages visiting pig carcasses in the Campinas (state of São Paulo) region and considered it a pioneer species because it was one of the first to colonize the carcasses.

As expected, no immatures were detected leaving pig carcasses in the initial stages. Most species left carcasses to pupate from the swelling stage on. Only *L. eximia* was associated, leaving the carcasses, to swelling and black putrefaction, demonstrating and confirming its evidencing attitude and quick immature development. In general, this species was associated with the less advanced stages of decomposition, whereas *C. albiceps* was the only 1 associated with the more advanced stages of butyric fermentation and dry. As a result of competition and predation by larvae of this exotic species of *Chrysomya* (Andrade et al. 2002, Rosa et al. 2006), *L. eximia* was predominantly reared in stages previous to those used by *C. albiceps* larvae.

Although we collected 101 adult specimens of *Hemilucilia* visiting the carcasses, we did not find any immature specimens of this genus feeding on them. Immature stages of *Hemilucilia segmentaria* have previously been observed to develop from human cadavers in Cerrado areas in Southeast Brazil, which were also used to estimate the post-mortem interval (Kosmann et al. 2011). This suggests that *Hemilucilia* larvae likely migrated away from the trays to pupate at a distance from the carcasses in our study (Rosa et al. 2011). Another possibility is that *Hemilucilia* immatures may have fallen prey to other insects such as Coleoptera (Silphidae, Histeridae) and Hymenoptera (Formicidae, Vespidae), or become parasitized by wasps belonging to Hymenoptera (Chalcidae, Pteromalidae, Braconidae) (Catts and Goff 1992, Marchiori et al. 2005, Oliveira-Costa 2011, Andrade-Silva et al. 2016, Sales et al. 2016). Additionally, the immatures might have been attacked by larvae of the exotic species *C. albiceps* in the rearing pots, as previously documented with *C. macellaria* (Faria et al. 1999, Sebastião and Castro 2019). Furthermore, the immatures could have been killed inside the carcasses due to the heat generated by the crowding and friction among the larvae (Catts and Goff 1992).

Hence, we verified that species succession occurs during carcass decomposition, and the pattern of collected visitors adults differs from immature ones, that left to pupate, which is very relevant for investigations in the field of forensic entomology. Intraspecific competition is unlikely to occur between adult and larval blow flies, as they occupy distinct niches. Adults primarily visit carcasses to consume bacteria and other microorganisms, while larvae feed on the decomposing tissues of deceased animals (as observed in the species found in this study) (Oliveira-Costa 2013). For these reasons, the phases (adult and immature) should be treated separately in data analysis. Hence, adults of *L. eximia* can be used to establish the initial stage in estimating the post-mortem interval of corpses with body masses similar to those of the current study in Cerrado areas; except for *C. megacephala*, all species can be used to estimate the swelling stage; all species, except the 2 in the *Hemilucilia* genus, can be used to estimate the stages of black putrefaction and butyric fermentation; and *C. idioidea*, *C. albiceps*, and *C. macellaria* can be used to estimate the dry stage. *Lucilia eximia* immatures leaving the carcasses to pupate can be used to estimate the swelling stage; *L. eximia* and *C. albiceps* leaving the carcasses to pupate can be used to estimate the black putrefaction stage; and *C. albiceps* leaving the carcasses to pupate can be used to estimate the butyric fermentation and dry stages.

Acknowledgments

Thanks to Neuza A. Silva (first author's mother), Eduardo A. Silva (first author's brother), José R. P. Silva Júnior (first author's nephew), Elisvaldo V. Santos, Liliane T. Neves, Alberico A. Santos, Geniana A. Reis, Lazaro C. M. França, Joselma P. Góis, Mariana M. Abreu, Laiana M. Oliveira, Gizélia A. Cunha, Erisleide M. Silva, Aldiléia L. Costa, Luciana S. Santos, Erisvane M. Silva, Irlice G. G. Santos, Cassio L. A. Silva, Rosa C. R. Silva, Deise W. A. Marques, Rafael A. Paula, Deusiano C. Almeida, and Josué J. Sousa, for their contribution to the activities carried out in the field and/or laboratory. We thank Dr. Francisco Limeira-de-Oliveira, for providing the infrastructure of the Laboratory for Invertebrate Studies (Laboratório de Estudos dos Invertebrados—LEI; Campus de Caxias, Universidade Estadual do Maranhão). To the Secretária Municipal de Meio Ambiente e Preservação dos Recursos Naturais de Caxias, MA (SEMUMA), for authorizing this research in the Inhamum APA. The first author would like to thank the Coordination for the Improvement of Higher

Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES) for the 10 monthly research grants for the master's degree. Leandro Juen (process: 304710/2019-9) and Maria C. Esposito (process: 309090/2019-9) thank National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq) for productivity scholarships. We also thank Paulo R. C. dos Santos, for creating the map of the study areas, and Raimundo F. O. Nascimento, for assistance in formatting the figures.

Author Contributions

José Silva (Conceptualization [Lead], Data curation [Lead], Formal analysis [Lead], Funding acquisition [Lead], Investigation [Lead], Methodology [Lead], Project administration [Lead], Resources [Lead], Software [Lead], Supervision [Lead], Validation [Lead], Visualization [Lead], Writing—original draft [Lead], Writing—review & editing [Lead]), Fernando Carvalho-Filho (Conceptualization [Supporting], Data curation [Supporting], Formal analysis [Supporting], Investigation [Supporting], Methodology [Supporting], Project administration [Supporting], Writing—original draft [Supporting], Writing—review & editing [Supporting]), Leandro Juen (Conceptualization [Supporting], Data curation [Supporting], Formal analysis [Lead], Methodology [Supporting], Project administration [Supporting], Writing—original draft [Supporting], Writing—review & editing [Supporting]), and Maria Esposito (Conceptualization [Supporting], Data curation [Lead], Formal analysis [Lead], Funding acquisition [Supporting], Investigation [Supporting], Methodology [Lead], Project administration [Lead], Supervision [Supporting], Visualization [Lead], Writing—original draft [Lead], Writing—review & editing [Lead])

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