

## Development of flesh flies (Diptera: Sarcophagidae) of medico-legal importance on pig carcass in the Cerrado of Brazil

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### Resumo

Com o intuito de contribuir com dados sobre moscas (Diptera) para auxiliar em processos forenses na área médico-legal, o presente estudo investigou quais são as espécies de moscas da carne (Diptera: Sarcophagidae) que se desenvolvem em carcaças de suínos e em quais estágios de decomposição elas se dispersam no Cerrado na região Nordeste do Brasil. As larvas e pupas foram coletadas diariamente por meio de bandejas com serragem que foram encaixadas debaixo de gaiolas contendo carcaça suína. O material coletado foi criado em laboratório em condições naturais de temperatura e umidade relativa do ar para a emergência dos adultos. Os espécimes adultos machos foram posteriormente identificados. Os experimentos foram realizados nos meses de julho e agosto de 2010, na estação seca. Foram obtidos 140 espécimes adultos, pertencentes a cinco espécies. *Peckia (Squamatodes) trivittata* (Curran, 1927) (n = 109) e *Peckia (Squamatodes) ingens* (Walker, 1849) (n = 28) foram as espécies mais abundantes e se dispersaram das carcaças a partir dos estágios de putrefação escura (ou estágio coliquativo) e fermentação butírica. *Helicobia neuzalmeidae* Silva, Brasil, De-Souza & Carvalho-Filho, 2022, e *Oxysarcodexia timida* (Aldrich, 1916) deixaram as carcaças no estágio de putrefação escura. *Peckia (Euboettcheria) collusor* (Curran & Walley, 1934) foi obtida apenas do estágio seco. *Oxysarcodexia timida* é pela primeira vez registrada se desenvolvendo em carcaça de suíno. Os imaturos de *P. (S.) trivittata* e *P. (S.) ingens* podem auxiliar na estimativa do tempo de morte de cadáveres encontrados no Cerrado, na região Nordeste do Brasil.

Palavras-Chave: Entomologia Forense; Larvas de Moscas; Sarcophaginae.

### Abstract

With the aim of contributing data on flies (Diptera) to assist in forensic processes in the medical-legal area, this study investigated species of flesh flies (Diptera: Sarcophagidae) that develop in pig carcass and at what stages of decomposition they disperse from the carcass in the Cerrado of the Northeast region of Brazil. Larvae and pupae were collected daily using sawdust trays placed beneath cages containing pig carcass. The material collected was allowed to complete development to adults in the laboratory under natural conditions of temperature and relative humidity. Adult male specimens were subsequently identified. The experiments were conducted in July and August 2010, in the dry season. A total of 140 adult specimens belonging to five species were obtained. The most abundant species were *Peckia (Squamatodes) trivittata* (Curran, 1927) (n = 109) and *Peckia (Squamatodes) ingens* (Walker, 1849) (n = 28), that dispersed from the carcasses during the dark putrefaction (or coliquative stage) and butyric fermentation stages. *Helicobia neuzalmeidae* Silva, Brasil, De-Souza & Carvalho-Filho, 2022, and *Oxysarcodexia timida* (Aldrich, 1916) left carcasses at the dark putrefaction stage as well. *Peckia (Euboettcheria) collusor* (Curran & Walley, 1934) was obtained during the dry stage only. For the first time, *Oxysarcodexia timida* was observed developing in a pig carcass. The immatures of *P. (S.) trivittata* and *P. (S.) ingens* can aid in determining the time of death of corpses discovered in Brazil's Northeastern Cerrado.

Keywords: Forensic Entomology; Fly Larvae; Sarcophaginae.

## 1. INTRODUCTION

Flesh flies, as most flies in the Sarcophagidae family (Diptera) known for their scavenging habits, are very significant in forensic studies because their species are found colonizing the carcasses and cadavers [1-3]. Adult specimens of several sarcophagid species are pioneers in locating ephemeral resources and carry out a succession process during the stages of decomposition, as evidenced by preliminary studies on carcasses used as models for decomposing human bodies [3-7]. Females, particularly gravid pioneers, drawn to the odors of gases emanating from carcasses and corpses, use these resources for larviposition and, as a result, as a developmental site for their larvae [8]. Sarcophagid larvae feed on decomposing organic matter to complete their development, passing through first, second, and third instar stages [9,10]. After storing food reserves, the larvae in the last instar leave the carcasses and corpses to look for a place to pupate [9,10].

Thus, knowing the time spent by each species in every stage of larval development, the time required for each species to disperse from the resource to pupate, and the time elapsed for the emergence of adult individuals is important data for forensic entomology and has been used to infer cadaver post-mortem interval, that is, the time elapsed from death until the cadaver is found [1,3,11-14]. In Brazil, sarcophagid species information is still scarce, and only a small number of studies have been conducted in the laboratory and/or field using small to medium-sized animal models, such as rats and pigs [2,11,15-19]. Some of the species that have been found colonizing these carcasses (e.g. *Peckia (Pattonella) intermutans* (Walker, 1861), *Peckia (Peckia) chrysostoma* (Wiedemann, 1930), *Peckia (Sarcodexia) lambens* (Wiedemann, 1930), *Peckia (Squamatodes) ingens* (Walker, 1849), *Peckia (Squamatodes) trivittata* (Curran, 1927), *Oxysarcodexia modesta* Lopes, 1946, and *Sarcophaga (Liopygia) ruficornis* (Fabricius, 1794) (Diptera: Sarcophagidae), have also been observed colonizing human cadavers in the country [14,20-23]. This highlights the importance of sarcophagid species in forensic entomology and emphasizes the need for further research on immatures in the country's various biomes and regions in order to create a database that can be used by the medical-legal community [3].

In this context, the Cerrado biome stands out as the second largest in Brazil, occupying approximately 24% of the territory, primarily in the central part and extending to a portion of the Northeast Region [24]. Furthermore, it is the only biome that borders four of the other four biomes (Amazon Forest, Atlantic Forest, Caatinga, and Pantanal) [24]. When compared to other biomes, the diversity of some taxonomic groups in the Cerrado is relatively low, nevertheless it contains many endemic species [19,25-29].

The number of Sarcophagidae species recorded for the Cerrado, on the other hand, is high [4,7,19,28-36], as several species of the group prefer open and sunny places [32,35,37]; however, this fauna remains poorly studied in the Northeast Region.

Given the importance and need for research on flies that develop in carcasses and cadavers which can aid forensic processes, the study sought to determine for the first time in Brazil's Northeastern Cerrado which flesh flies species develop in pig carcasses and learn from what stages of decomposition carcasses the larvae disperse.

## 2. MATERIAL AND METHODS

The field experiments were carried out in a Cerrado area in the municipality of Caxias, state of Maranhão, Northeastern Brazil, in the Inhamum Municipal Environmental Protection Area (*Área de Proteção Ambiental Municipal do Inhamum – APA do Inhamum*; coordinates 4°53'54.8" S, 43°26'32.9" W), which has 3,564 ha [25], in July and August of 2010. The study was authorized by the Municipal Department for the Environment and Preservation of Natural Resources of Caxias, MA (*Secretaria Municipal de Meio Ambiente e Preservação dos Recursos Naturais de Caxias, MA – SEMUMA*). The collection period was marked by 1.7 mm of total rainfall [38], an average temperature of 27.5°C, and an average relative humidity of 71% (data measured with a digital thermo-hygrometer at the experiment site).

Three pigs (*Sus scrofa* Linnaeus, 1958) were used in total, and they were slaughtered at the experimental sites by shooting a firearm in the front of the head, reducing suffering and pain (according to the recommendations of the Ethics Committee on Animal Use – CEUA). Following the classification and characterization of Bornemissza (1957) [39], the carcasses were observed for 10 days for the recognition and dating of their decomposition stages (initial, putrefaction, dark putrefaction (or coliquative stage), butyric fermentation, and dry). The carcasses were placed in cages approximately 500 meters apart in areas with tree shade. The pupating larvae were collected using an aluminum tray in the shape of a sliding drawer (1.10 m x 85 cm x 5 cm) containing sawdust, which was fitted to the bottom of each cage containing a 12 kg pigs' carcass (Figure 1). The collection was authorized through the License (No. 12417) issued by the Biodiversity Authorization and Information System (*Sistema de Autorização e Informação em Biodiversidade – SISBIO*).

The contents of each tray were collected only once every morning from 7:30 to 10 a.m., to reduce the loss of larvae dispersing after the temporary removal of the tray, from the dark putrefaction stage (72h) until the second day of the carcasses' dry stage (240h). Because the first

two days are insufficient for the larvae to reach the third instar of development and feed enough to leave the carcasses to pupate, collections began at the stage of dark putrefaction. The contents of the trays were transferred to 5 L plastic pots with the upper opening sealed with fine mesh fabric and elastic alloy so that the third-instar larvae could pupate and reach adulthood in the laboratory at room temperature and relative humidity and protected from direct sunlight (Figure 2). Furthermore, this procedure protects the larvae from predation by ants (Hymenoptera: Formicidae) and beetles (Coleoptera) as well as parasitoidism by wasps (Hymenoptera).



**Figure 1.** Aluminum tray containing sawdust fitted to the bottom of a cage containing a pig carcass to collect larvae and pupae of sarcophagids.



**Figure 2.** Plastic pots utilized in the rearing of sarcophagids immatures collected in pig carcass.

The time required for the adults to emerge in the laboratory was 16 days longer than that needed for the immature collection activities on the carcasses in the field, that is, until the beginning of August 2010. The newly emerged adults were collected daily between 7:00 and 8:00 p.m. and then killed in vials containing ethyl

acetate. They were kept in 92.8% alcohol in 60 mL jars or on pins in boxes inside entomological drawers. 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*) and the Entomological Collection of the Emílio Goeldi Paraense Museum (*Coleção Entomológica do Museu Paraense Emílio Goeldi – MPEG*) in Belém, PA, both in Brazil. Because the identification is based on structures of the male genitalia, only male specimens were identified to a specific level. To identify the species, the genitalia were exposed with entomological tweezers, submerged in alcohol inside a petri dish, and analyzed with a stereomicroscope, with the works of Carvalho & Mello-Patiu [40], Pape & Dahlem [41], Buenaventura & Pape [42], and Souza *et al.* [43] as references. Due to a lack of keys and description works that could provide the necessary support for species identification, we did not identify and count the female specimens.

### 3. RESULTS

A total of 140 adult male Sarcophagidae from three genera and five species were identified and studied. The most species-rich genus was *Peckia* Robineau-Desvoidy 1830: *Peckia (Euboettcheria) collusor* (Curran & Walley, 1934), *P. (S.) ingens* and *P. (S.) trivittata*. The other two genera, *Helicobia* Coquillett, 1895, and *Oxysarcodexia* Townsend, 1917, were represented by only one species: *Helicobia neuzalmeidae* Silva, Brasil, De-Souza & Carvalho-Filho, 2022, and *Oxysarcodexia timida* (Aldrich, 1916), respectively. *Oxysarcodexia timida* was found to develop in a pig carcass for the first time. *Peckia (S.) trivittata* and *P. (S.) ingens* were the most common, accounting for 78% and 20% of the total sample, respectively. While the other three species (*H. neuzalmeidae*, *O. timida* and *P. (E.) collusor*) accounted for only 2% of all individuals collected (Table 1).

The immatures of *P. (S.) trivittata* and *P. (S.) ingens* were obtained after an average of 10 days of carcass decomposition, leaving to pupate from about 120 hours of decomposition, that is, from the dark putrefaction stage (which lasted from the third to the fifth day) until the eighth day, during the fermentation stage (which took place from the sixth to the eighth day). On the fourth day of decomposition, in the dark putrefaction stage, *H. neuzalmeidae* and *O. timida* emerged to pupate. *Peckia (E.) collusor* was only discovered on the tenth day, when the carcass was already dry (Table 1).

**Tabela 1.** Species of Sarcophagidae and abundance of male specimens obtained as larvae and pupae from pig carcass at different stages of decomposition in the Cerrado in the Área de Proteção Ambiental Municipal do Inhamum, Caxias, MA, Brazil.

SPECIES	DECOMPOSITION STAGES					TOTAL
	I	II	III	IV	V	
<i>Helicobia neuzalmeidae</i> Silva, Brasil, De-Souza & Carvalho-Filho, 2022	-	-	1	0	0	1
<i>Oxysarcodexia timida</i> (Aldrich, 1916) *	-	-	1	0	0	1
<i>Peckia (Euboettcheria) collusor</i> (Curran & Walley, 1934)	-	-	0	0	1	1
<i>Peckia (Squamotodes) ingens</i> (Walker, 1849)	-	-	21	7	0	28
<i>Peckia (Squamotodes) trivittata</i> (Curran, 1927)	-	-	82	27	0	109
<b>TOTAL</b>	-	-	<b>105</b>	<b>34</b>	<b>1</b>	<b>140</b>

Note: Carcass decomposition stages: I = initial (1st day), II = putrefaction (2nd day), III = dark putrefaction (3rd-5th day); IV = fermentation (6th-8th day); V = dry (9th-10th day). No collection was carried out (-). First record of development in pig carcass (\*).

Adults of *P. (S.) ingens* collected as immatures in the dark putrefaction stage emerged in the laboratory after 17-18 days ( $\pm 2$  days), while those collected in the fermentation stage emerged after 15 days ( $\pm 2$  days). Adults of *P. (S.) trivittata* collected as immature in the dark putrefaction stage emerged after 18-19 days ( $\pm 3$  days), while those collected in the fermentation stage emerged after 16 days ( $\pm 3$  days). *Peckia (E.) collusor*, *O. timida*, and *H. neuzalmeidae* adults emerged in 13, 7, and 8 days, respectively.

#### 4. DISCUSSION

The five flesh flies species found colonizing pig carcass in the Cerrado of Northeastern Brazil (*P. (E.) collusor*, *P. (S.) ingens*, *O. timida*, *P. (S.) trivittata* and *H. neuzalmeidae*) are only found in the Neotropical Region [30,32,42,44-46]. Except for *O. timida*, which does not occur in cold, humid subtropical climates such as the Pampa (*Campo Sulino*) of Southern Brazil, Argentina, Paraguay, and Uruguay, *P. (E.) collusor*, *P. (S.) ingens*, and *O. timida* have a wide spatial distribution across several biomes and countries from Central America to southern South America [30,32,42,44-46]. *Peckia (S.) trivittata* is only found in Brazil, and only in regions and biomes with hot climates, such as the Cerrado, Cacaís forests, and Amazon and Atlantic forests [30,32,42,44-46]. As a result, *P. (S.) trivittata* is also not registered in the country's southern region [48]. So far, *H. neuzalmeidae* has only been found in the Cerrado of northeastern Brazil (state of Maranhão). This species was recently described based on specimens discovered during the current study's field research [19].

Even though the species *P. (E.) collusor*, *P. (S.) ingens*, *P. (S.) trivittata*, and *O. timida* have been identified as being of forensic importance for the Neotropical region, and more specifically for South America [5,30,40], little is known about the natural history of these species in the context of rearing in pig carcass and bodies human. The majority of the

information on these species comes from field studies that collected and determined the seasonal preferences and/or succession patterns of adult specimens that visited pig carcass during the stages of decomposition where they were abundant and, in some cases, pioneers [4,7,48-50].

Other forensic studies carried out in the Brazil, like this one, obtained a few species and male adult specimens from larvae and pupae of flesh flies that colonize carcass, such as pigs, showing differences in species composition [2,7,17,47]. In a study conducted with four pig carcass in areas of Cerrado *stricto sensu* and *campo sujo* vegetation during the dry season in the Southeast region of the country (in the state of Minas Gerais), Rosa et al. (2009) [2] obtained only 43 adults of three species of the same genus: *P. (P.) intermutans*, *P. (S.) lambens*, and *P. (S.) trivittata*. Paseto et al. [7] obtained 163 male specimens of three species using four pig carcass in pasture and Cerrado forest areas during the dry season in the same state and region where Rosa et al. [2] conducted their studies: *Oxysarcodexia avuncula* (Lopes, 1933), *Oxysarcodexia diana* (Lopes, 1933), and *P. (P.) intermutans*.

Salviano et al. [51] confirmed that the larval stages of *P. (S.) trivittata* last approximately four days at a controlled temperature of 27°C. Additionally, Ferraz [11] discovered that the larval stages of *P. (S.) ingens* last approximately three days, under controlled temperature of 27°C. This information on larval development times for these two species suggests that gravid females of *P. (S.) trivittata* and *P. (S.) ingens* visited and larviposited on the carcass on the second and third days, respectively, during the putrefaction stage and the dark putrefaction stage. Obtaining larvae leaving to pupate from the fifth to the eighth day of carcass decomposition, that is, over four days, demonstrates that individuals from different larval instars of each of these two species overlapped. Thus, the use of immatures of *P. (S.) trivittata* and *P. (S.) ingens* to help estimate the dating of cadaver post-mortem intervals should be done, while always taking care to collect and use as a reference the older individuals, which often have

already abandoned their bodies to pupate, representing the first individuals that were larviposited, as they will provide more accurate and reliable estimates from their developmental times [3,5].

*Peckia (S.) trivittata* and *P. (S.) ingens* were found colonizing human corpses for the first time in Northeastern Brazil. Ramos et al. [23] discovered *P. (S.) trivittata* colonizing a charred corpse, that found inside a metal deposit in a *restinga* vegetation area in the Brazilian state of Bahia. Guimaraes et al. [14] revealed *P. (S.) ingens* colonizing a corpse found inside a house in Paraiba, Brazil, in the Atlantic Forest biome. This information emphasizes the importance of these two species in forensic processes throughout the country, where they have a wide spatial distribution.

Only one adult individual of *O. timida* and *P. (E.) collusor* were collected, both as immature in the dark and dry putrefaction stages, respectively. Adult specimens of *P. (E.) collusor* have been collected in traps baited with rotting animal (vertebrate and invertebrates) and vegetal (fruits) material matter decay [16,46,47,52,53]. The larvae of this species have been reared from dead insects and vertebrates [46], including pig carcasses, as reported by Faria et al. [17], based on few specimens (n = 4) obtained from the dark putrefaction stage in a Cerrado area in the state of Minas Gerais, in southeastern Brazil. However, according to D'Almeida [53], this species prefers to larviposit on human feces.

Carmo et al. [18] obtained the only record of *O. timida* larval development in carcass so far, when they collected an individual larva that developed to the adult stage in a rat carcass in an Atlantic Forest fragment in the state of Pernambuco, Northeastern Brazil. As a result, we present for the first time a record of *O. timida* developing in pig carcass, which is the most commonly used model in forensic studies due to anatomical and physiological similarities with humans [1,5,54]. The fact that we found an immature of *O. timida* pupating on the fourth day of carcass decomposition, in the dark putrefaction stage, suggests that the gravid female progenitor of this specimen was one of the first species to find the carcass. However, because only one adult specimen was obtained, we believe additional research is needed to confirm the potential use of immature specimens of this species in estimating the minimum postmortem interval of cadavers.

Most species and individuals leave the dark putrefaction stage to pupate (*P. (S.) ingens*, *P. (S.) trivittata*, *O. timida* e *H. neuzalmeidae*; 75% of the total sample), and continue, but in a smaller number of species and individuals (*P. (S.) ingens* e *P. (S.) trivittata*; 24% of the total sample), on the days corresponding to the fermentation stage. This which can be a strategy to avoid competition [10,55]. It could also be an escape behavior to avoid predation, as demonstrated by larvae of

*Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae) [10,55], as many *C. albiceps* larvae were observed emerging to pupate from the dark putrefaction stage to the dry stage in the same carcass where this study was conducted. Denno & Cothran [8] also demonstrated that the presence and quantity of blow flies (Diptera: Calliphoridae) influence flesh flies behavior and abundance by artificially reducing the number of these flies, resulting in an increase in the number of flesh flies individuals.

Other insects such as ants (Hymenoptera: Formicidae) [56,57], personal observation of the first author], wasps (Hymenoptera: Vespidae) and beetles (Coleoptera: Histeridae, Silphidae) may have contributed to the reduced number of species and specimens of sarcophagids colonizing the carcasses, as well as parasitoidism by wasps (Hymenoptera: Braconidae, Chalcidae, Pteromalidae) [1,3,10,58]. Furthermore, the heat produced by the larvae agglomeration in the carcasses may have resulted in the death of specimens [1]. Because the two families were not split into separate pots, there was most likely predation by *C. albiceps* larvae in the rearing pots used to obtain adults [59].

## 5. CONCLUSION

The information presented by this study on the five species of sarcophagids (*P. (S.) ingens*, *P. (S.) trivittata*, *O. timida*, *P. (E.) collusor* and *H. neuzalmeidae*) that were raised in carcass of pigs, such as knowledge about the moment in which they leave the carcass to pupate (mainly the stages of dark putrefaction and fermentation in relation to the species *P. (S.) ingens* and *P. (S.) trivittata*) in the Cerrado in the Northeastern Brazil, are very important entomology tools for the forensic area. Information on *P. (S.) trivittata* and *P. (S.) ingens* may be considered in some forensic cases to help estimate cadaver postmortem interval, observing the appropriate proportions and environmental conditions equivalent to those of the present study.

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