

Sarcosaprophagous dipterans associated with a *Leopardus tigrinus* (Schreber) carcass: First record of *Peckia* (*Peckia*) *pexata* (Wulp, 1895) colonizing a wild animal carcass

R.C. Leite ^{ab,*}, T.M. Barbosa ^c, W.L. Silva ^{ad}, T.F. Soares ^a

^a Laboratório de Entomologia e Vetores (LEV), Instituto Federal do Maranhão, CEP 65840-000, São Raimundo das Mangabeiras-Maranhão, Brasil

^b Departamento de Ciências Biológicas, Universidade Estadual do Maranhão, CEP 65620-000, Coelho Neto-Maranhão, Brasil

^c Secretária de Educação e Esportes, GRE Agreste Centro Norte, EREM Severino Cordeiro de Arruda, 55790-000, Taquaritinga do Norte -Pernambuco, Brasil

^d Secretaria Municipal de Educação, Ciência, Tecnologia, Educação e Inovação (SEMECTI), CEP 65895-000, Loreto - Maranhão, Brasil

*Endereço de e-mail para correspondência: rafaelbiocosta@gmail.com. Tel.: +55-99-984594007.

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Resumo

O Brasil possui muitos casos de animais silvestres atropelados. Esses animais, após o atropelamento podem ser utilizados como recurso alimentar por diversos animais, inclusive moscas sarcosaprófagas das famílias Calliphoridae e Sarcophagidae. Todavia, a decomposição desses animais é rápida e exigem que animais carniceiros a localizem e a utilizem de forma eficiente, sendo as moscas os primeiros visitantes. Este estudo objetivou descrever a entomofauna de dípteros sarcosaprófagos associada a uma carcaça de *Leopardus tigrinus* (Schreber) encontrada em uma rodovia do estado do Maranhão, Brasil. Quatro espécies de Calliphoridae foram registradas visitando a carcaça, *Chrysomya albiceps* (Wiedemann, 1819) (n= 20), *Chrysomya putoria* (Wiedemann, 1830) (n= 1), *Cochliomyia macellaria* (Fabricius, 1775) (n= 2) e *Cochliomyia hominivorax* (Coquerel, 1858) (n= 1). Todavia, apenas duas larvas completaram o ciclo biológico na carcaça, identificadas como pertencentes a espécie *Peckia* (*Peckia*) *pexata* (Wulp, 1895). Além disso, este foi o primeiro registro de *P. (P.) pexata* colonizando uma carcaça de animal silvestre, reforçando a importância da família Sarcophagidae como grupo colonizador de carcaças silvestres.

Palavras-Chave: Calliphoridae; Sarcophagidae; fauna silvestre.

Abstract

Brazil has a high incidence of wildlife roadkill. These carcasses can serve as a food resource for various animals, including sarcosaprophagous flies of the families Calliphoridae and Sarcophagidae. However, the decomposition of these animal remains is rapid, requiring scavengers to locate and utilize them efficiently, with flies being the first visitors. The objective of this work was to report the fauna of sarcosaprophagous dipterans associated with the carcass of *Leopardus tigrinus* (Schreber) found on a highway in the state of Maranhão, Brazil. Four species of Calliphoridae were recorded visiting the carcass, *Chrysomya albiceps* (Wiedemann, 1819) (n= 20), *Chrysomya putoria* (Wiedemann, 1830) (n= 1), *Cochliomyia macellaria* (Fabricius, 1775) (n= 2), and *Cochliomyia hominivorax* (Coquerel, 1858) (n= 1). However, only two larvae completed the cycle, belonging to the species *Peckia* (*Peckia*) *pexata* Wulp (Sarcophagidae). Furthermore, this is the first record of *P. (P.) pexata* colonizing a carcass of a wild animal. This study confirms that carcasses are a temporary resource for dipterans, besides reinforcing the importance of the family Sarcophagidae as a colonizing group of wild carcasses.

Keywords: Calliphoridae; Sarcophagidae; wildlife fauna.

1. INTRODUCTION

Highways are widespread throughout the Brazilian territory and their implementation results in several negative impacts on wildlife [1, 2]. Among the harmful

effects of roads, we can highlight habitat loss, fauna isolation, and high rates of roadkill [2, 3, 4].

According to Campos and Silva [4], wildlife roadkill is commonplace in the southern region of Brazil, where over 260 animals belonging to 53 taxa were recorded as

roadkill victims. Among these animals, mammals were the most common victims, followed by birds, reptiles, and amphibians [4].

A similar pattern was observed for highways in the semiarid northeast region of Brazil, where about 250 vertebrates fell victim to vehicle accidents, with amphibians, reptiles, and mammals being the most common groups [2].

In this context, carcass of dead animals can represent an ephemeral resource for scavenging animals, including necrophagous.

Flies from the Calliphoridae and Sarcophagidae families have been recorded colonizing carcasses of vertebrates killed by roadkill [5, 6] and carcasses of wild mammals and reptiles [7, 8]. Furthermore, they are attracted to a carcass within hours of death [9], these insects can be useful to estimate the time of death and the approximate time of the roadkill [5].

Species of the genus *Peckia* have been recorded on a wide variety of resources such as animal baits (sardine and chicken), rat and pig carcasses, and human cadavers [10,11]. However, bionomic and ecological information for the species *Peckia (Peckia) pexata* (Wulp, 1895) is still scarce, although it is known that the species is associated with the decomposition of sardine, pig, and rat carcasses [10,12] and exhibits a long-life cycle, particularly its intrapuparial development [13].

Although knowledge of the abundance and richness of sarcosaprophagous Diptera associated with carcasses of wild animals is still scarce, especially when compared to ephemeral baits [14] and animal models [15], it is a fact that these insects can provide important data on roadkill and death of wildlife victims of vehicle accidents on Brazilian roads. Additionally, this study can strengthen areas that are underexplored in Brazilian Forensic Science, such as Legal Veterinary Medicine [16].

Within the context of Legal Veterinary Medicine, insects that colonize wildlife carcasses can aid to determine the cause, time and circumstances of an animal's death, helping to solve crimes against wildlife [17].

This case report aimed to describe the species of sarcosaprophagous flies associated with a Northern Tiger Cat carcass, *Leopardus tigrinus* (Schreber), victim of roadkill.

2. MATERIALS E METHODS

This study was authorized by license number (SISBIO) 90841-1. The carcass of *L. tigrinus* (Figures 1A and 1B) was found on August 23, 2022, on the margins of highway MA-374 (Coordinates: 7°00'26"S; 45°11'04"W, Figure 1C), in the municipality of Loreto, Maranhão state, northeast region of Brazil.

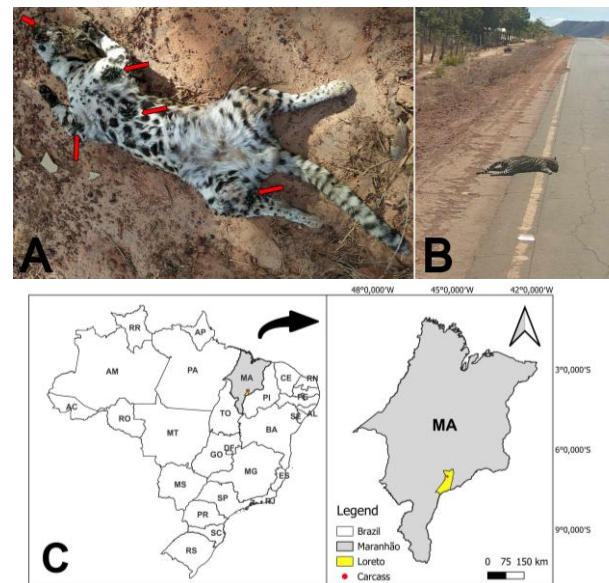


Figure 1. Northern Tiger Cat, *Leopardus tigrinus* (Schreber), victim of roadkill. A) Red arrows showing blowflies visiting the carcass. B) Moment it was found on the road. Source: Authors, 2022. C). Location where the *L. tigrinus* carcass was found.

After the discovery, the carcass was photographed, and entomological samples (larvae and adults) present on the body of the animal were collected. Adult samples were collected using entomological nets and dipteran larvae were collected using tweezers. The immatures samples were placed in plastic containers containing a little soil from the location and transported to the Entomology and Vectors Laboratory (LEV) of the Federal Institute of Maranhão, located in the municipality of São Raimundo das Mangabeiras, while the adults were killed in ethyl acetate and placed in containers containing 70% ethanol.

In the laboratory, the larvae were reared in same 500 mL plastic container covered with voile fabric, containing a smaller container with raw ground beef and sawdust for pupation [18], under ambient conditions similar to the collection site (average temperature = $27 \pm 1^\circ\text{C}$ and relative humidity = $50\% \pm 5\%$).

All material was subsequently identified with the aid of a stereomicroscope and taxonomic keys [19, 20, 21]. Climatological data were collected from the nearest weather station, located in the municipality of Balsas, Maranhão, Brazil.

Throughout the study, daily data on the development of immature stages collected from the carcass until emergence were recorded. These data served as a preliminary basis for estimating the minimum post-mortem interval (PMI), following the study conducted by Ramos-Pastrana et al. [13] and personal biological data (unpublished data).

3. RESULTS AND DISCUSSION

At the time of carcass encounter, 24 adult specimens of 4 species belonging to the family Calliphoridae were collected: *Chrysomya albiceps* (Wiedemann, 1819) (n = 20), *C. putoria* (Wiedemann, 1830) (n = 1), *Cochliomyia macellaria* (Fabricius, 1775) (n = 2), and *C. hominivorax* (Coquerel, 1858) (n = 1) (Table 1).

Regarding the immature specimens (LII stage) of the eight collected from the natural orifices of the carcass, only two flies emerged. The species was identified as *Peckia* (*Peckia*) *pexata* (Figure 2).

All species recorded in this study are common in studies with baits or animal carcasses [10]. Furthermore, the numerical prevalence of Calliphoridae specimens is

also a common pattern in forensic entomology studies, including cases involving human corpses [18,22,23].

Table 1. Species of sarcosaprophagous flies (Calliphoridae and Sarcophagidae) recorded on the carcass of *Leopardus tigrinus* (Schreber) victim of a roadkill in the municipality of Loreto, Maranhão, Brazil.

Family	Species	Abundance	%	Stage collected
Calliphoridae	<i>Chrysomya albiceps</i>	20	76,9	Adult
	<i>Chrysomya putoria</i>	1	3,8	Adult
	<i>Cochliomyia macellaria</i>	2	7,7	Adult
	<i>Cochliomyia hominivorax</i>	1	3,8	Adult
Sarcophagidae	<i>Peckia</i> (<i>Peckia</i>) <i>pexata</i>	2	7,7	Larvae LII

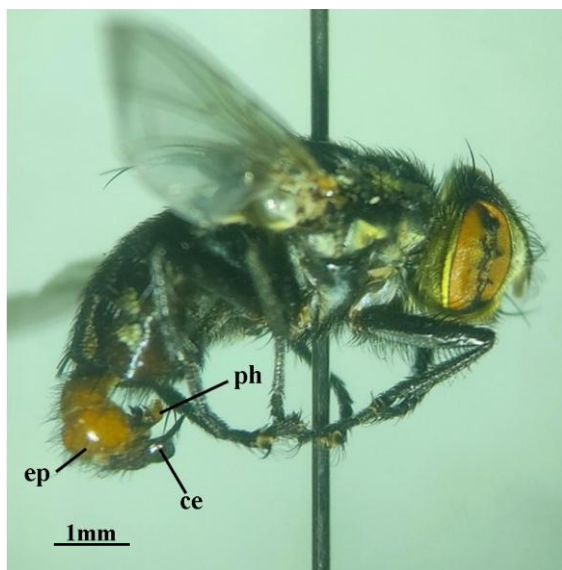


Figure 2. Adult of the species *Peckia* (*Peckia*) *pexata* (Diptera: Sarcophagidae) in lateral view, with emphasis on the phallic characters. Legend: ep = epandrium, ce = cerci, ph = phallus. Source: Authors, 2022.

The chronological analysis demonstrated that the immatures collected on August 23, 2022, emerged on September 5, 2022 (Figure 3), completing their biological cycle in 336 hours (14 days) after collection. The Accumulated Degree Day (ADD) method described by Oliveira-Costa [24] was not used because detailed bionomic studies for *Peckia* (*Peckia*) *pexata* Wulp were not found.

This analysis allowed us to infer that the roadkill may have occurred at least 36 hours prior. This hypothesis was

based on previous laboratory data, which showed that *P.* (*P.*) *pexata* species completes the larva-to-adult cycle in approximately 16 days at a temperature of $27 \pm 1^\circ\text{C}$, humidity of 60% and fed with ground beef (unpublished data), corroborated by Ramos-Pastrana et al. [13] studying the intrapuparial development of this species, these authors observed adult emergence after 15.5 days at a temperature of 24°C .

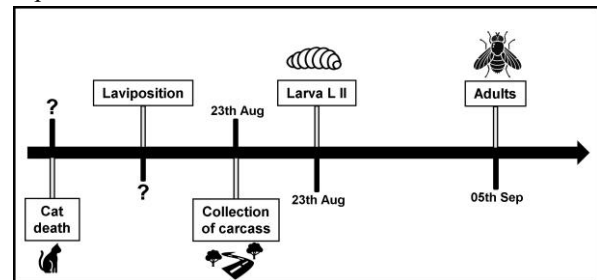


Figure 3. Timeline of northern Tiger Cat, *Leopardus tigrinus* (Schreber) carcass colonization by *Peckia* (*Peckia*) *pexata*.

Although the carcass used in this study resulting from animal-vehicle collisions, it allows us to foster important data to solve crimes against wildlife. According to Baptista et al. [25], a high rate of crimes against wildlife remains unsolved due to the lack or inadequate identification of crime evidence, with Forensic Entomology being a tool that can change this scenario.

In this context, Botteon et al. [16], for example, using specimens of *Co. hominivorax* demonstrated that negligence on the part of a domestic dog's guardian contributed to the animal's death. The use of this species was also important due to its ability to cause obligatory

myiasis, with the entomological analysis being crucial to understand that the animal had a previous lesion before death. The screwworm fly was also recorded in this study, however, only one adult visited the carcass and there was no collection of immatures colonizing the carcass.

The use of Calliphoridae species was also fundamental to estimate PMI of rhinos illegally hunted in the Republic of South Africa [26]. Based on these studies, we can state that the species recorded here can be key pieces in determining the post-mortem interval in future cases of crimes against wildlife [26]. The successful use of entomological data in the Republic of South Africa to estimate the time of death of wild animals and build a case against poachers [26] highlights the importance of studies on wild animals, even if these are case-specific studies involving victims of vehicle collisions.

The most important finding of this study was the first record of *Peckia* (*Peckia*) *pexata* colonizing a wild carcass. Since Alves et al. [10] recently complicating the resources colonized by this species lists only pig carcasses and rats. *Peckia* (*P.*) *pexata* is a species commonly associated with animal baits and carcasses in studies conducted in several kinds of environments in Brazil [11,23, 27]. In addition to visiting baits and carcasses, this species has been recorded colonizing rat carcasses in Atlantic Forest environments as an experimental model and not in wild carcasses [28].

Interestingly, no Calliphoridae larvae were collected in this case, a group of high forensic importance and considered primary colonizers. The absence of Calliphoridae larvae may be associated with the fact that adults are oviparous and require some time after feeding to oviposit [29], unlike Sarcophagidae species that deposit first instar larvae on food substrate [30, 31]. The ability to lay larvae may be an advantage in the colonization process of roadkill animals or simply Sarcophagidae species arrived before Calliphoridae species, following an entomological succession pattern similar to that recorded by Vairo et al. [32].

We also highlight that, although calliphorids are the most common colonizers in studies with animal carcasses [33], sarcophagids appear to excel in colonizing wild carcasses [7, 8]. This may be associated with their ability to rapidly colonize the resource due to being larviparous, especially considering that wildlife carcasses are exposed to large scavengers in nature. However, further studies are necessary to corroborate these hypotheses.

This study reinforces the importance of monitoring wildlife roadkill on highways, especially to understand more about diversity of sarcosaprophagous flies present on these animal carcasses, and reinforces the forensic potential of *P. (P.) pexata* to estimate the PMI.

4. CONCLUSION

This study reported the occurrence of the flies *C. albiceps*, *C. putoria*, *Co. macellaria*, and *Co. hominivorax* visiting a *L. tigrinus* carcass victim of a roadkill.

Additionally, this case reported for the first time the species *P. (P.) pexata* colonizing *L. tigrinus* carcasses on Brazilian highways. These findings indicate the potential forensic utility of *Peckia* (*P.*) *pexata*.

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