



# Case report of a preserved male corpse: estimation of post-mortem interval based on four Dipteran species of four different families

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**Abstract.** Case reports are extremely valuable in forensic entomology and very rare in Brazil. In this report we describe a case of multiple colonization of a preserved male cadaver found indoors in Santa Catarina State, southern Brazil, by four dipterans species of four different families: *Fannia canicularis* (Linnaeus, 1761) (Diptera: Fannidae), *Microcerella halli* (Engel, 1931) (Diptera: Sarcophagidae), *Muscina stabulans* (Fallén, 1817) (Diptera: Muscidae) and *Sarconesia chlorogaster* (Wiedemann, 1830) (Diptera: Calliphoridae). The development time data of the species were used to estimate the minimum postmortem interval (PMI). Considering the methodology applied in this study and the values calculated for the species development, it was possible to estimate a minimum PMI of 24 days. Besides the diversity of dipteran species colonizing a single human body in an indoor environment, this case report reinforces the importance of these species as forensically indicator to estimate the time of death.

Keywords: Calliphoridae; Fanniidae; Forensic entomology; Muscidae; Sarcophagidae.

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Evidence produced from necrophagous insects can objectively demonstrate several circumstances in death investigations, mainly to determine the minimum postmortem interval (minPMI) (Byrd & Sutton 2020; Dias Filho *et al.* 2022). Dipterans usually are the first necrophagous insects to detect a dead body through the process of ecological succession and some species present preference for certain types of environment, seasonality and specific decomposition stages (Linhares 1981a, 1981b; Monteiro-Filho & Penereiro 1987; Souza & Linhares 1997; Carvalho *et al.* 2000; Campobasso *et al.* 2001; Mello *et al.* 2007).

Forensic entomology has been developing in Brazil in recent years (Pujol-Luz *et al.* 2008a; Oliveira-Costa 2013; Vairo & Moura 2021). Brazil is a country of continental dimensions with great entomological diversity, and the knowledge on cadaveric entomofauna of different regions is essential for forensic entomology applications (Oliveira-Costa 2011, 2013; Vairo & Moura 2021). However, forensic entomology case reports are still very rare in many regions of the country (*e.g.*, Pujol-Luz *et al.* 2006, 2008a, 2008b; Vasconcelos *et al.* 2013, 2017).

Specifically, the southern region of Brazil (subtropical climate) has few published studies on forensic entomology surveys and case reports (Moura et al. 1997, 2005; Vianna et al. 2004; Souza et al. 2008; Soligo & Panigalli 2013; Gaedke & Mouga 2017; Corrêa et al. 2019; Madeira-Ott et al. 2022). In municipality of Xanxerê, Santa Catarina State, Soligo & Panigalli (2013) collected 3,226 insect specimens, including several species of flies classified as forensically indicator, such as Chrysomya albiceps (Wiedemann, 1819), Chrysomya megacephala (Fabricius, 1794), Hemilucilia segmentaria (Fabricius, 1805), Lucillia eximia (Wiedemann, 1819) (Diptera: Calliphoridae); Musca domestica Linnaeus, 1758, Muscina stabulans (Fallén, 1817), Anthomyia aenescens Wiedemann, 1830 and Stomoxys calcitrans (Linnaeus, 1758) (Diptera: Muscidae). In a singular study carried out in Joinville region, northern of Santa Catarina State, Gaedke & Mouga (2017) observed dipterans in all decomposition stages of 10 human corpses from April 2014 to March 2016, collecting 11 species of four families: seven species of Calliphoridae (Calliphora lopesi Mello, 1962, C. albiceps, C. megacephala, H. segmentaria, Hemilucilia semidiaphana (Rondani, 1850), Lucillia cuprina (Wiedemann, 1830) and L. eximia); two species of Sarcophagidae [Peckia (Euboettcheria) australis (Townsend, 1927) and Peckia (Sarcodexia) lambens (Wiedemann, 1830)], one Muscidae (Muscidae sp.) and one Stratiomyidae [Hermetia illucens (Linnaeus, 1758)].

On January, 2022, the Forensic Entomology Division was formally established at Scientific Police of Santa Catarina. However, forensic entomology is still incipient in Santa Catarina State and more dipteran fauna knowledge are necessary for database construction and practical applications.

In this singular case of multiple colonization, we estimate minPMI based on entomological evidence collected from a preserved male corpse found indoors in northern of Santa Catarina State.

## MATERIAL AND METHODS

**Case Report.** On September 09, 2022, a preserved old-male corpse, presenting orange skin, was found in an urban area in São Bento do Sul city, northern region of Santa Catarina State (-26.248472, -49.3861). There was no information about when the victim was last seen alive. This study was approved by Santa Catarina Scientific Police ethical committee (process PCI n° 4492/2024).

The body was found indoors lying down on his own bed, wearing sweater and pants, and covered by fungus in face, hands and feet. No *antemortem* wounds were observed in the cadaver and there were no signs of bloodstains or struggle at the death scene. The body was identified by necropapilloscopic examination. No samples were taken for toxicological analysis due to putrefaction conditions. *Causa mortis* was considered undetermined.

**Entomological evidence.** The concentration of maggot masses was observed inside the head during autopsy. There were no larvae and/or pupae at the death scene. No coleopterans specimens were observed at the corpse. Insect evidence (larvae of Diptera) was collected by forensic experts from Santa Catarina Scientific Police using sterilized tweezers and sent to the Forensic Entomology Division, located at Joinville city, northern region of the State.

In order to estimate the age of each larva collected on the cadaver and to identify the colonizing species, all sampled larvae were separated by morphological types and reared in plastic recipients containing raw ground beef at  $25 \text{ °C} \pm 1^{\circ} \text{ C}$ ,  $70 \pm 10\%$  RH and 12 h of photofase. Pupae were transferred to plastic recipients containing vermiculite and maintained in a dark room. After emergence, the flies were identified according to specific taxonomic keys and consulting specialists (Carvalho & Ribeiro 2000; Carvalho *et al.* 2005; Carvalho & Mello-Patiu 2008; Wendt & Carvalho 2009; Grzywacz *et al.* 2015).

**Environmental data and minimum PMI estimation.** Mean daily temperature, rainfall and relative humidity values of the months prior to the corpse's discovery were obtained from meteorological station near to the death scene. Based on species' life cycle and data obtained, the minPMI was estimated using the accumulated degree-day (ADD) method (Oliveira-Costa 2011):

# $ADD = D x (T_m - T_b)$ , where:

D = duration in days;  $T_m$  = mean temperature (°C);  $T_b$  = basal temperature (°C).

Thus, the minimum time (in days) required for each species to reach adulthood was based on the mean temperature values obtained closest to the values obtained in this study and ADD based on previous studies (Raffi *et al.* 2011; Nassu *et al.* 2014; Lecheta *et al.* 2015; Grzywacz 2019).

#### RESULTS

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All insect samples collected were third instar larvae (L3). Four species of four different families were identified: *Fannia canicularis* (Linnaeus, 1761) (Diptera: Fanniidae), *Microcerella halli* (Engel, 1931) (Diptera: Sarcophagidae), *Muscina stabulans* (Fallén, 1817) (Diptera: Muscidae) and *Sarconesia chlorogaster* (Wiedemann, 1830) (Diptera: Calliphoridae).

Meantemperature data (°C), rainfall (mm) and relative humidity values (%) are presented in Table 1 and entomological data obtained for each species to estimate minPMI are presented in Table 2.

Adults emergence of *F. canicularis* occurred 09 days of postcollection. According to Grzywacz (2019), *F. canicularis* took an average of 36 days to complete its biological cycle (egg to adult) at 18° C. ADD value was 288, obtaining a minPMI estimation of 24 days. *S. chlorogaster* took an average of 590.51  $\pm$  11.29 hours (~24.60 days) to complete its biological cycle at 20 °C (Lecheta *et al.* 2015). So, ADD value obtained was 246, obtaining a minPMI estimation of 22 days.

Table 1. Mean	temperature	(°C),	rainfall	(mm)	and	relative
humidity values	(%).					

Day	Mean Temperature (°C)	Rainfall (mm)	Relative Humidity (%)
09/09/2022	21.2	1.4	77.2
09/08/2022	17.3	0.2	87
09/07/2022	14	20.8	98.1
09/06/2022	12.4	20.4	98.8
09/05/2022	10.7	0.6	92.8
09/04/2022	8.9	0	85.8
09/03/2022	12.2	19.2	98.4
09/02/2022	14.2	3.2	93.5
09/01/2022	13	0	83.2
08/31/2022	11.4	0	87.4
08/30/2022	9.1	0	92.5
08/29/2022	9.4	1.6	85.2
08/28/2022	14.7	2.2	88.6
08/27/2022	17.8	0	78.1
08/26/2022	16.4	0	85.9
08/25/2022	15.7	0	87.2
08/24/2022	13.8	0	87.9
08/23/2022	12.9	0	90.2
08/22/2022	12.4	0	92.5
08/21/2022	10	0	94.1
08/20/2022	7.1	0	87.2
08/19/2022	6.1	0	70.9
08/18/2022	14.2	36.2	96.2
08/17/2022	15.5	1.6	95.9
08/16/2022	15.4	8.8	90.3
08/15/2022	18.2	0	77.9

According to Raffi *et al.* (2011), *M. stabulans* took an average of 20 days to complete its biological cycle at 20 °C (ADD of 200), obtaining a minPMI estimation of 15 days. Lastly, *M. halli* reached pupae stage two days of post-collection. According

Table 2. Data on species of flies analyzed in this case report.

Species	Family	Adult emergence (days)	T°C	ADD	Minimum PMI (days)	Study
Fannia canicularis	Fanniidae	09	18	288	24	Grzywacz (2019)
Microcerella halli	Sarcophagidae	021	15	71.65	11	Nassu <i>et al</i> . (2014)
Muscina stabulans	Muscidae	09	20	200	15	Raffi <i>et al</i> . (2011)
Sarconesia chlorogaster	Calliphoridae	11	20	246	22	Lecheta <i>et al</i> . (2015)

<sup>1</sup>Pupation. No record of adult emergence.

to Nassu *et al.* (2014), ADD calculated was 71.65 at 15 °C, obtaining a minPMI estimation of 11 days.

# DISCUSSION

Determining the time of death could be essential in criminal investigations and entomological evidence for PMI estimation requires rigorous information collection of death conditions (Vasconcelos *et al.* 2013; Byrd & Sutton 2020). In our record, entomological data were essential to estimate minimum PMI of a preserved body. However, PMI estimation may be underestimated since corpses located indoors can be colonized later and by a more limited number of species (Reibe & Madea 2010).

So far, the main dipteran families collected in Santa Catarina were Calliphoridae, Muscidae and Sarcophagidae, contributing with important information on the patterns of corpses colonization (Soligo & Panigalli 2013; Gaedke & Mouga 2017). In this particular case report, we estimate minPMI based on four dipteran species (*F. canicularis, M. halli, M. stabulans* and *S. chlorogaster*) of four different families (Calliphoridae, Fanniidae, Muscidae and Sarcophagidae), in a case of multiple colonization of a preserved male cadaver found indoors in an urban area at northern of Santa Catarina State.

Considering dipterans survey in southern Brazil, Corrêa *et al.* (2019) also found *S. chlorogaster* and *M. halli* colonizing human remains in Paraná State. The species *S. chlorogaster* and *M. halli* that we found in the reported case are often collected from corpses in closed environments (Nassu *et al.* 2014; Vairo *et al.* 2015, 2017). Both Neotropical species occurred associated with cold climate regions (Nassu *et al.* 2014; Vairo *et al.* 2015).

In another study, Souza *et al.* (2008) reported *S. chlorogaster* and *M. stabulans* in Pelotas region, Rio Grande do Sul State. In this study, the authors collected a total of 5,239 insects, with 1,827 specimens obtained from larvae collected from four rabbit carcasses. Most important and abundant forensic indicators species in this study were: *C. albiceps* and *L. eximia* that occurred in all seasons, *C. megacephala*, *H. semidiaphana*, *H. segmentaria* and *S. chlorogaster* (Calliphoridae); *M. stabulans* and *Synthesiomyia nudiseta* (Wulp, 1883) (Muscidae); *Peckia* (*Pattonella*) *resona* (Lopes, 1935) and *Sarcophaga* (*Lyopygia*) *crassipalpis* Macquart, 1839 (Sarcophagidae); and *Fannia pusio* (Wiedemann, 1830) (Fanniidae). The species *M. halli* and *F. canicularis* were not found in this survey.

Fanniidae species are not commonly found on corpses and not widely reported in cases in southern Brazil (Souza *et al.* 2008). Fanniidae comprises over 285 species belonging to four genera and *Fannia* is the most diverse genus, presenting relevant species in forensic entomology to estimate PMI (Oliveira & Vasconcelos 2010; Carvalho *et al.* 2012; Vasconcelos *et al.* 2013; Vasconcelos *et al.* 2017). In a semiarid area at Mendoza, Argentina, Aballay *et al.* (2012) identified six species of Fanniidae associated to pig carcasses during the winter season: *Euryomma peregrinum* (Meigen, 1826), *Fannia albitarsis* Stein, 1911, *Fannia femoralis* (Stein, 1898), *Fannia fusconotata* (Rondani, 1868), *Fannia heydenii* (Wiedemann, 1830) and *Fannia sanihue* Domínguez & Aballay, 2008, demonstrating the relevance of Fanniidae species during cold weather.

Now considering a case report occurred in Brazil, Vasconcelos *et al.* (2017) reported a death investigation involving an adult male corpse found in incomplete suspension, obtaining specimens of *F. pusio* to estimate minPMI. Similar to our case report, Vasconcelos *et al.* (2013) described a case of six species of Diptera simultaneously colonizing a single male cadaver found indoors in northeast of Brazil: *Chrysomya* 

putoria (Wiedemann, 1818), *C. albiceps, C. megacephala* (Calliphoridae); *Fannia trimaculata* (Stein, 1898) (Fanniidae); *Megaselia scalaris* (Loew, 1866) (Phoridae); and *Peckia (Peckia) chrysostoma* (Wiedemann, 1830) (Sarcophagidae), providing the first record of *F. trimaculata* colonizing a human cadaver. However, reports of *F. canicularis* developing on human cadavers have been scant in literature.

Our case recorded larvae of *F. canicularis* associated on a preserved old-male corpse in southern region of Brazil. The species *F. canicularis* have been recorded and already used as criminal evidence in a case of child neglect in Germany (Benecke & Lessig 2001) and to estimate PMI in death investigation (Hu *et al.* 2020). In a wetland area in Guangdong, China, Hu *et al.* (2020) recorded abundance of *F. canicularis* larvae colonizing an unidentified female corpse found in a soft-shell suitcase. According to these authors, Fanniidae species frequently appear in corpses found in suitcases, preferring a wet and nutrient-rich environment, such as the conditions observed in our case report.

The presence of four dipteran species simultaneously colonizing a single human body highlights the need for more dipteran fauna surveys in the region and more understanding of indoors colonization patterns. Based on entomological evidence, estimation of minPMI varied from 11 days (*M. halli*) until 24 days for *F. canicularis*, dating back to August 16, 2022. In an unusual successional pattern, *F. canicularis* arrived two days earlier than *S. chlorogaster*, which can be explained by the cold and wet environmental conditions previously observed in the period prior to the death. Besides, further studies are necessary to understand the fungus role on the entomological colonization process and the possible influence on the preservation of the body.

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## **AUTHORS CONTRIBUTION**

VWB worked at the death scene. AG identified the species. VWB, AG and VMA analyzed and interpreted the entomological data to estimate minimum PMI. VWB and AG had major contributor in writing the manuscript. All authors read and approved the final manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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