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Diptera survey in human corpses in the north of the state of Santa Catarina, Brazil

Levantamento de dípteros em cadáveres humanos na região norte do estado de Santa Catarina, Brasil

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ABSTRACT
Aiming to verify the species associated with the decomposition process carried out by necrophagous insects in human bodies, 11 species of dipterans were collected in 10 distinct cadavers from April 2014 to March 2016, resulting in individuals of the families Calliphoridae (*Calliphora lopesi* (Mello, 1962), *Chrysomya megacephala* (Fabricius, 1794), *Chrysomya albiceps* (Wiedemann, 1819), *Hemilucilia segmentaria* (Fabricius, 1805), *Hemilucilia semidiaphana* (Rondani, 1850), *Lucilia cuprina* (Wiedemann, 1830) and *Lucilia eximia* (Wiedemann, 1819)), Sarcophagidae (*Peckia (Euboettcheria*) *australis* (Fabricius, 1805) and *Peckia (Sarcodexia) lambens* (Wiedemann, 1830)), Muscidae (Muscidae sp.) and Stratiomyidae (*Hermetia illucens* (L. 1758)). Regarding the seasonality, dipterans were found in corpses in the four seasons, with distinct richness in each one. Dipterans were observed in corpses in all phases of decomposition (coloration, gaseous, colliquative and remains), the greater richness being verified in the gaseous phase. The data demonstrate differences in ecological succession, evidencing specialization of the insects found in relation to seasonality and the decomposition phase.
Keywords: decomposition phases; forensic entomology; necrophagous insects.

RESUMO
Visando verificar o processo de decomposição realizado por insetos necrófagos em corpos humanos, foram coletadas 11 espécies de dípteros em dez cadáveres distintos, no período de abril de 2014 a março de 2016, tendo sido obtidos indivíduos das famílias Calliphoridae (*Calliphora lopesi* (Mello, 1962), *Chrysomya megacephala* (Fabricius, 1794), *hrysomya albiceps* (Wiedemann, 1819), *Hemilucilia segmentaria* (Fabricius, 1805), *Hemilucilia semidiaphana* (Rondani, 1850), *Lucilia cuprina* (Wiedemann, 1830) e *Lucilia eximia* (Wiedemann, 1819)), Sarcophagidae (*Peckia* (Euboettcheria) *australis* (Fabricius, 1805) e *Peckia (Sarcodexia) lambens* (Wiedemann, 1830)), Muscidae (Muscidae sp.) e Stratiomyidae (*Hermetia illucens* (L. 1758)). Com relação à sazonalidade, foram encontrados dípteros em cadáveres nas quatro estações, com riquezas distintas em cada uma. Observaram-se dípteros em cadáveres em todas as fases de decomposição (coloração, gasosa, coliquativa e restos), sendo obtida maior riqueza na fase gasosa. Os dados demonstram diferenças na sucessão ecológica, evidenciando especialização dos insetos encontrados com relação à sazonalidade e à fase de decomposição.
Palavras-chave: entomologia forense; fases de decomposição; insetos necrófagos.

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INTRODUCTION

Forensic Entomology (FE) is responsible for analyzing insects and other arthropods that colonize decaying carcasses as they are important biological indicators for estimating the time of death, also known as the *Postmortem* Interval (PMI) (CATTS & GOFF 1992), and its consequences since knowing the entomological fauna of the place of study and the pace by which it changes in a predictable sequence, during the process of decomposition (MARQUES, 2008), make it possible, through the analysis of the insects, to diagnose the cause of death or to measure the PMI.

The use of insects in forensic practices has been frequent (OLIVEIRA-COSTA & MELLO-PATIU, 2004; PUJOL-LUZ *et al.*, 2008) and the information obtained on the biological characteristics of the species may be the basis for analysis.

Taxonomic studies of dipterans are still incipient in FE. Currently, the identification of most of the immature is carried out making the rearing of the specimens in laboratory until the emergence of adults. However, in criminal cases, fast determination of PMI is crucial to the investigation and there is often no time to rear the larvae until the adult stage, the specimens die eventually before identification at a specific level occurs, leading to loss of important criminal evidence (LITJENS *et al.*, 2001; SCHROEDER *et al.*, 2003).

The dipterans are the most interesting insects in FE, as they present a wide diversity of species that follow each other in the carcass (KEH, 1985) and this is due mainly to the scope of this group in tropical regions and its great attractiveness for decomposing organic matter (BARBOSA *et al.*, 2006).

The faunistic survey of necrophagous insects that is proposed here complements the data already obtained in the region of Joinville with an animal model, in 2013, that was the first study of FE carried out in Santa Catarina State (SC), Brazil. In this study, a survey of scavenging insects was carried out in a submontane rain forest, using an animal model (pig – *Sus scrofa* Linnaeus, 1758) (GAEDKE & MOUGA, 2013).

This work aimed to know the dipteran fauna associated with cadavers and verify the list of species sampled with the stage of decomposition and to assemble an entomological collection of specimens collected directly from humans in an advanced state of decomposition. Such a study constitutes an essential step in obtaining reliable data for future comparisons, thus facilitating the elucidation of cases that require FE as a tool.

MATERIAL AND METHODS

The work was carried out from April 2014 to March 2016. Insects were collected from 10 human cadavers found in an advanced stage of decomposition, from expert analysis carried out by Instituto de Pericias (IGP) of Joinville, in an area of approximately 2,919,911 square kilometers. Six bodies were found in the city of Joinville, state of Santa Catarina, Brazil (data are presented in chronological order of appearance): body 01 on 16 April 2014 (26°12'18.9"S 48°47'18.7"W), body 03 on 18 May 2014 (26°16'14.0"S 48°49'04.5"W), body 04 on 23 June 2014 (26°21'25.4"S 48°49'56.2"W), body 05 on 19 October 2015 (26°19'04.3"S 48°53'30.9"W), body 06 on 23 November 2015 (26°18'39.4"S 48°50'16.8"W), body 08 on 05 December 2015 (26°12'04.7"S 48°47'13.1"W). In the city of São Francisco do Sul, SC, Brazil, there were found three bodies: body 07 on 4 January 2016 (26°14'28.7"S 48°35'46.0"W), body 09 on 8 February 2016 (26°09'31.6"S 48°37'53.8"W) and body 10 on 26 March 2016 (26°12'37.9"S 48°32'16.9"W). In the city of Itapoa, SC, Brazil only one corpse was recovered: body 02 on 10 May 2014 (25°58'09.0"S 48°38'18.6"W) (figure 1).

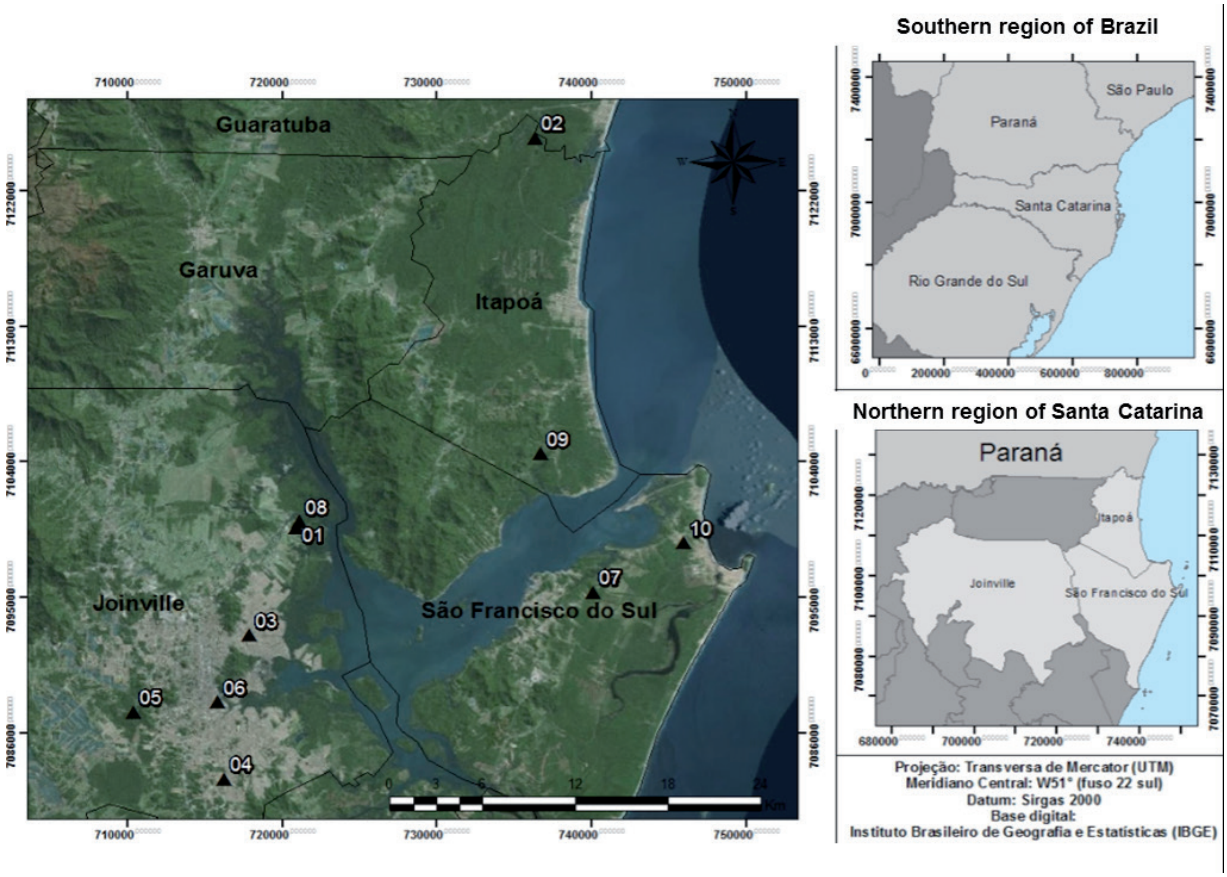

Figure 1 – Map with location of the corpses found in the cities of Itapoa, Joinville and São Francisco do Sul, Santa Catarina, Brazil from April 2014 to March 2016. Legend: 1-10) sampled points.

After finding the corpse, procedures according to Oliveira-Costa (2011) were performed, a photographic survey of the area was carried out to characterize the environment (which is described in the Results and Discussion) and the decay stage in which the body was found was recorded. After the initial survey of the scene, specimens of adult dipterans were collected, as described by Catts & Haskell (1990), using entomological nets for sampling. After the sampling of adult insects, immatures and pupae found on the body and in its surroundings, were collected and stored in transport pots as described by Oliveira-Costa (2011).

After finishing the sampling at the place where the body was found, the body was again analyzed in the Legal Medical Institute (MLI) of Joinville, where the remaining immature insects, not seen in the field, that were in orifices and between the victim's clothing, were recovered. The collected adult specimens were sacrificed in the laboratory, using the dry way with gauze and ethyl acetate (ALMEIDA *et al.*, 1998), then mounted on entomological pins and identified with specific taxonomic keys (CARVALHO & RIBEIRO, 2000; OLIVEIRA-COSTA, 2011; VAIRO, 2011) and the help of specialists of UFSC (Federal University of Santa Catarina). In relation to the immature ones, about 50% of these were placed in breeding pots adapted from Oliveira-Costa (2013) (which included a bigger pot with sawdust for pupation and a smaller one inside the first with rotten meat as a food substrate, which were kept in a stove at 25°C, without humidity or photoperiod control until the emergence of the adults). The remaining sampled immature were stored in 70% alcohol solution and, afterwards, the characteristics of their posterior spiracle were analyzed to define the larval instar. The insects were identified with the help of experts and compared with the entomological collection of Gaedke & Mouga (2013). Afterwards, the insects were placed in entomological boxes and conserved in the entomological collection of the Zoology Laboratory of Univille. Information obtained was inserted in a database available at the Univille (University of the Region of Joinville).

RESULTS AND DISCUSSION

During the study period 10 cadavers were obtained from different locations. It was possible to characterize three different environments with four bodies (03, 04, 06 and 10) located in closed areas and with protection against climatic interferences such as sun and rain. The others, found in external environments, were in dense rain forest, which we divided into two areas. The first, a shrub area, had a predominance of shrubs and some trees, allowing a humid and shaded environment for the corpse and, in this environment, specimens of two different bodies (01 and 07) were captured. The other environment was characterized as herbaceous, being of open areas with presence of herbaceous plants which allowed a greater exposure to the sun, where four bodies (02, 05, 08 and 09) were found.

RICHNESS OF DIPTERANS

Data are summarized in table 1. During the studied period, dipterans of four families were obtained: Calliphoridae with seven species: *Calliphora lopesi* (Mello, 1962), *Chrysomya albiceps* (Wiedemann, 1819), *Chrysomya megacephala* (Fabricius, 1794), *Hemilucilia segmentaria* (Fabricius, 1805), *Hemilucilia semidiaphana* (Rondani, 1850*), Lucilia cuprina* (Wiedemann, 1830) and *Lucilia eximia* (Wiedemann, 1819); Muscidae with one species (Muscidae sp.); Sarcophagidae with two species (*Peckia (Euboettcheria) australis* (Fabricius, 1805) and *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830) and Stratiomyidae with one species (*Hermetia illucens* (L. 1758)).

The richness found corresponds to that cited by Gennard (2012), which describes Calliphoridae, Sarcophagidae, Muscidae, Fanniidae and Stratiomyidae among the most representative families in FE. Among the genera considered to be of major importance, Oliveira-Costa (2011) points out *Chrysomya, Hemilucilia, Lucilia* and *Cochliomyia.* =*Chrysomya* is considered an exotic *taxon*, which was introduced in the Brazil in the mid – 1970s (GUIMARÃES *et al.*, 1978).

Table 1 – List of families and species sampled during the phases of decomposition (COL – coloration, GAS – gaseous, COLLI – colliquative, REM – remains), environment where the corpse was found (H – herb, A – shrub, R – residence), season of the year (Win – winter, Aut – autumn, Spri – spring, Sum – summer), development phase of immatures (O – egg, 1st Instar, 2nd Instar, 3rd Instar, P – pupae, PP – pre-pupae,) and number of corpses (n).

Family/Species	COL	GAS	COLLI	REM	Environment	Season	Stage
Calliphoridae							
Hemilucilia segmentaria	–	X	X	–	H, A, R	Sumr/Aut	2, 3, P, PP
Hemilucilia semidiaphana	–	X	–	–	R	Aut	2 and 3
Calliphora lopesi	–	X	–	–	R	Aut	2 ad 3
Lucilia. cuprina	X	-	–	–	H	Spr	3º
Lucilia. eximia	–	X	–	–	R	Sumr/Aut	A e 3
Chrysomya megacephala	–	X	–	–	R	Spr	3
Chrysomya albiceps	–	X	–	–	A, R	Sumr/Aut	2, 3, P
Sarcophagidae							
Peckia (*Euboettcheria*) *australis*	–	-	X	–	A, H	Autt	A and P
Peckia (Sarcodexia) lambens	–	-	X	–	A, H	Aut	A and 3
Muscidae							
Muscidae sp.	–	X	–	–	R	Win	O and 1
Stratiomyidae							
Hermetia illucens	–	–	X	X	H	Sum	A and 3

In relation to species richness (figure 2), the first body had one species (*P. australis*), the second two (*P. australis* and *H. segmentaria*), the third two (*H. semidiaphana* and *C. lopesi*), the fourth one (Muscidae sp), the sixth one (*C. megacephala*), the seventh four (*C. albiceps, H. segmentaria, L. eximia* and *P. lambens*), the eighth one (*H. illucens*), the ninth three (*H. segmentaria, P. lambens* and *H. illucens*) and the tenth three (*C. albicepis, H. segmentaria* and *L. eximia*). *H. segmentaria* was the most frequent species in the cadavers, being found in four of them. These data differ from the surveys conducted by Cerigatto (2009) in Bauru / SP and Oliveira & Vasconcelos (2010) in Pernambuco, which performed fauna surveys at the Medical Legal Institute of their respective regions.

This work apparently presented lower richness in relation to the work done before in the region, with the use of an animal model (GAEDKE & MOUGA, 2013) although this last study had been done on different substrates and with different decomposition times and these variables, especially the length of exposition, interfere in the species richness.

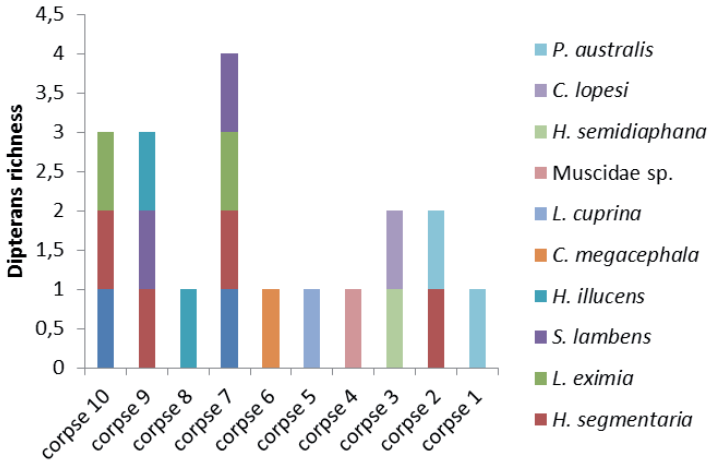

Figure 2 – Richness of dipterans and corpses found.

PHASES OF DECOMPOSITION

In relation to the decomposition phase, four phases of decomposition were considered: coloration (chromatic), that is the first one to appear and is distinguished by the appearance of spots, especially in the abdominal and inguinal regions; gaseous (swelling), when the presence of gases originating from the bacterial action provokes stuffing and characteristic odors; colliquative (deterioration) when the soft parts are reduced to a liquefied state causing anatomical deformity; and remains (skeletonization), the final phase when the appearance of the bone parts occurs (BANDARRA & SEQUEIRA, 1999).

Of the 10 corpses found (figure 3), one was in the coloration phase where a specimen of *L. cuprina* (Calliphoridae) was found; six were in the gaseous phase when nine species were collected, six of which were from Calliphoridae (*C. albiceps*, *C. lopesi*, *C. megacephala*, *H. segmentaria*, *H. semidiaphana*, *L eximia*), one of Muscidae (Muscidae sp.), one of Stratiomyidae (*H. illucens*) and one from Sarcophagidae (*P.* (*S.*) *lambens*); in the colliquative phase there were two bodies, each one with one species, namely *H. segmentaria* (Calliphoridae) and *P.* (*E.*) *australis* (Sarcophagidae); in the remains phase, there was only one body which presented only the species *H. illucens* (Stratiomyidae).

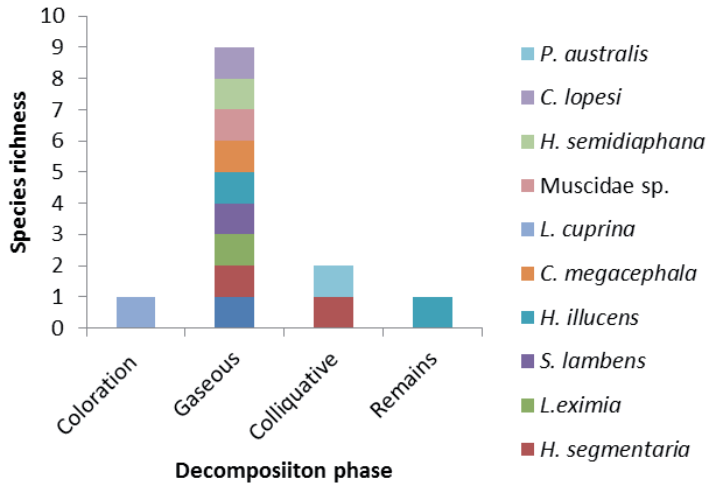

Figure 3 – Species richness and decomposition phases.

The values found with respect to the decomposition phases point to a more concentrated configuration of dipterans in the gaseous phase, especially Calliphoridae. Moura *et al.* (1997), who carried out a study in Curitiba using *Sus scrofa* Linnaeus 1758, also found a greater abundance of this family in the gaseous phase. According to Mello-Patiu & Santos (2001), the blowflies (Calliphoridae) present as the group of more interest for the FE and are eventually used for calculations of PMI.

In relation to the specificity of the dipterans for the colonized phase (figure 4), the only species recovered in two distinct phases were *H. segmentaria* and *H. illucens*. Oliveira-Costa (2013) emphasize, in an experiment conducted in the municipality of Rio de Janeiro with the use of an animal model, the presence of *H. segmentaria* in all phases of decomposition, with a higher incidence in the gaseous and colliquative phases.

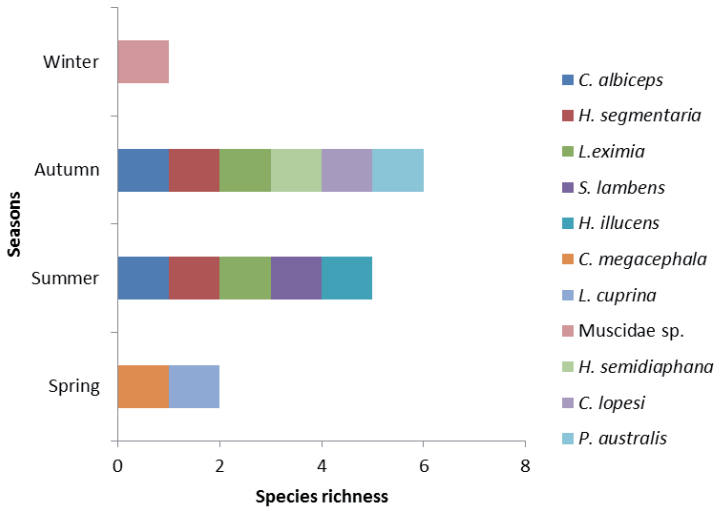

Figure 4 – Species richness and seasonality.

In relation to families, Sarcophagidae seems to have a higher preference for more advanced stages of decomposition (CARVALHO & LINHARES, 2001; GENNARD, 2012), data also observed for *P. australis* and *P. lambens* species, found only in the colliquative phase. With regard to the Muscidae family, specimens were found only in the gaseous phase, data similar to those obtained by Moura *et al.* (1997) with the use of *Rattus norvegicus* carcasses in a forest area in Curitiba. Stratiomyidae family showed the lowest richness as only *H. illucens* was collected in the final stage of decomposition. Carvalho *et al.* (2000), in Campinas / SP, Brazil, found adults and larvae of this species in human cadavers and in animal models, always in later stages of decomposition and in wild environments. Ferrari *et al.* (2009) described the presence of this species in buried *Rattus norvegicus* carcasses and its attractiveness by steroid hormones, in a study carried out in the city of Ribeirao Preto / SP, Brazil. In the present study, the corpses where the specimens were found were in open environments, with herbaceous and shrub vegetation, one of them being buried.

SEASONALITY

Oliveira-Costa (2013) emphasize, in an experiment conducted in the municipality of Rio de Janeiro with the use of an animal model, the appearance of *H. segmentaria* in all seasons of the year. In the present work, this species was captured in two seasons, autumn and summer. According to Souza and Linhares (1997), this species occurs exclusively to perform oviposition.

With regard to the Muscidae family, Moura *et al.* (1997) indicate that their presence is related to lower temperatures in seasons such as autumn and winter. In the present work, specimens were collected only in winter.

Due to the fact that there are distinct distributions of corpses among the seasons, the abundance data may not correspond to the seasons, but to the availability of corpses.

In relation to seasonality, autumn presented the greatest richness with six species, followed by summer with five, spring with two and winter with one. This pattern of succession can be explained by the number of bodies found in each season: in autumn, four bodies; in summer, three; in spring, two and in winter, one. Thus, the richness found seasonally was related to the pattern of abundance of cadavers recovered in each season.

DEVELOPMENTAL STAGES

In relation to the immatures, all phases (egg, larvae of 1st, 2nd and 3rd instars, pre-pupae and pupae) were considered and collected. They are represented in figure 5 (except the egg phase).

Relating the instars with the decomposition phases, it was observed that the coloration phase showed only immatures *of L. cuprina* in the 3rd larval instar. This larval stage in a coloration phase of decomposition can be explained by the fact that the studied corpse was in a partially flooded and charred area, which caused a delay in relation to the advancement of the cadaveric decomposition. According to Gennard (2012), *L. cuprina* performs oviposition already in the first 24 hours after death.

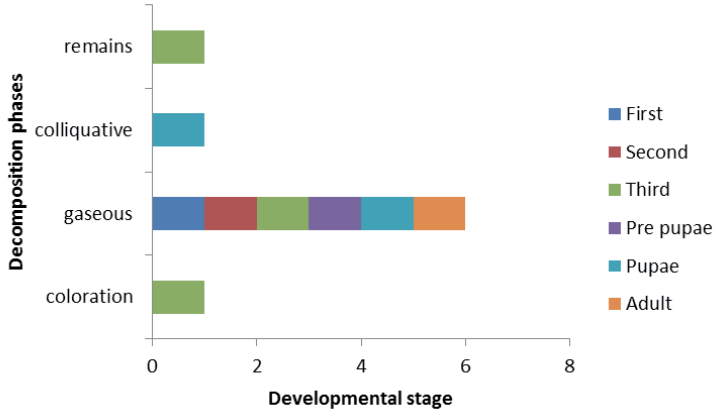

Figure 5 – Developmental stage and decomposition phases.

The gaseous phase presented a greater richness, with a total of seven species and individuals were found in all stages of development, with predominance of Calliphoridae, from which larvae of the 2nd and 3rd instar, pre-pupae and pupae were obtained. Stages of 1st instar or egg were not found in the gaseous phase. This can be due to the fact that the gaseous phase begins approximately 3 to 4 days after death and Calliphoridae females tend to carry out the ovoposition at the beginning of the decomposition, besides the fact they have a short life cycle (VASCONCELOS *et al.*, 2013). Differently, the Muscidae species found was in the egg stage and the 1st instar. This late colonization can be explained as the cadaver in question was recovered inside a residence, which a delayed colonization (GOFF *et al.*, 1991). According to Pohjoismaki *et al.* (2010), there may be a delay of three to four days for the colonization of muscoid dipterans in confined corpses. Moura *et al.* (1997) state that another factor which can interfere is the low temperature, because the encounter of the body occurred in winter, which possibly slowed down the decomposition.

In relation to the colliquative phase, an immature of Calliphoridae (*H. segmentaria*) was found in the pupae stage and two of Sarcophagidae (*P.* (*E.*) *australis* 3rd.instar and *P.* (*S.*) *lambens* pupae). Both colonized corpses were found in a shrub and herbaceous outdoor environment. Adult specimens were also found in the corpse, suggesting that these species are indicative for the estimation of the postmortem interval (PMI).

For the remains phase, only larvae of *H. illucens* (Stratiomyidae) were found in 3rd instar larval. This species is of great importance in FE due to the fact that it has a longer life cycle, about 30 days, and its eggs take about 5 to 14 days to hatch, depending on the temperature (OLIVEIRA-COSTA, 2013). In the case of the present study, this species was only found in summer and in the 3rd instar, characterizing a PMI of more than 20 days. Pujol-Luz *et al.* (2008) emphasize its use in cases of deaths exceeding 15 days, when other muscoids have already left the body.

ECOLOGICAL SUCCESSION

Data on ecological succession are presented in figure 6.

It was possible to verify a predominance of species of Calliphoridae family in the initial stages of decomposition (coloration and gaseous). Carvalho *et al*. (2000) report a peak of dipterans in the initial phases of decomposition, even for experiments conducted with pig' carcasses as well as with samples obtained at the MLI of the city of Campinas/SP, Brazil.

With the advancement of the decomposition, it was possible to see a decline in richness with, in the colliquative phase, the predominance of species of Sarcophagidae (two species), with still the presence of *H. segmentaria* in the pupae stage, but without the presence of adults for new colonization. In this phase, an adult of *H. illucens* (Stratiomyidae) was also collected but without the presence of immatures.

In the remains phase, only the species *H. illucens* was seen, characterizing the preference of this species for the later phases (PUJOL-LUZ *et al.*, 2008).

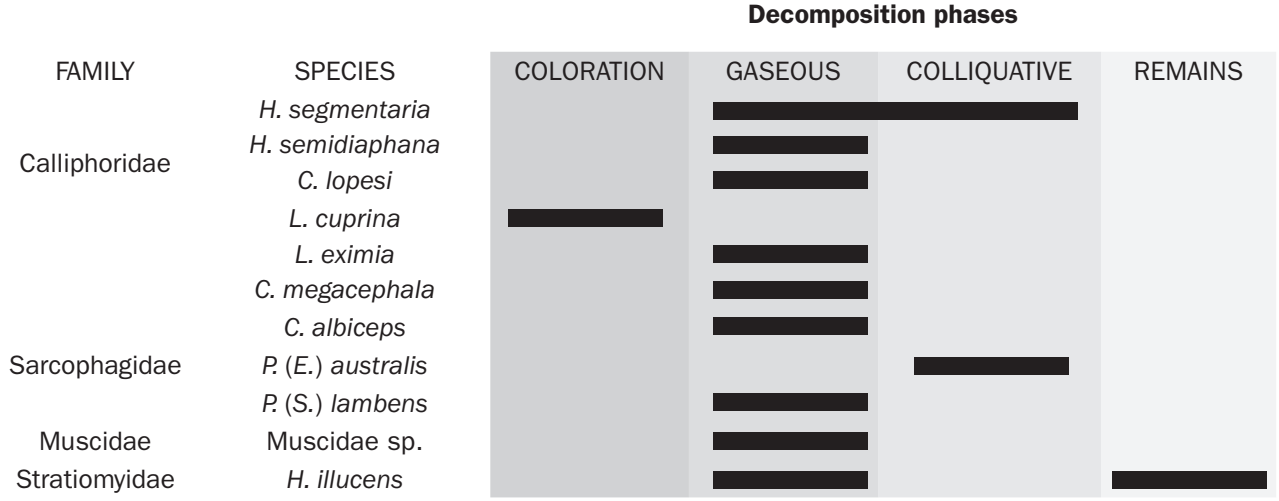

Figure 6 – Ecological succession process and decomposition phases.

CONCLUSION

Data obtained show that, in relation to the decomposition phases, it was possible to verify the difference in the richness found, which exposes the importance of studies about to the ecological succession of these species, this knowledge being indispensable for a better estimative of the PMI. Since the abundance of cadavers varied in each season, data on seasonality are incipient. Regarding the use of the species for the calculation of PMI, the development time of each species was related to the decomposition phase in which it was recovered and data unveiled specificities with respect to the time of decomposition and the taxonomic group. Since this research is pioneer in the use of humans as food substrate for necrophagous dipterans species, further research is needed in the area in order to better understand the physiology and ecological relationship of the arthropods that perform this decomposition.

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