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Insect Fauna Associated with Exposed Pig Carcasses in Southern Brazil

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Abstract. The knowledge of insect patterns visiting decomposing carcasses and the analysis of parameters related to their presence help determine the *post-mortem* interval (PMI). This information depends on regional studies because the diversity of insects and the environmental conditions interfere in this process. The aim of the study was to analyze the insect fauna that colonizes carcasses of pigs exposed in different stages of decomposition in the study area. The experiment was conducted in southern Brazil using three domestic pigs (*Sus scrofa* Linnaeus) that were killed on site. Adult insects associated with carcasses were sampled daily using an insect net, manual collection and pitfall traps. Statistical tests were performed to evaluate the diversity of insects. During the process of decomposition (14 days), Scarabaeidae (25%) and Calliphoridae (23%) species were the most abundant of all of the samples. The diversity of insects is distinguishable when all parameters are analyzed. The Black Putrefaction (IV) stage exhibited the highest diversity according to the applied methods. The succession pattern was established from the species dominance index: Fresh stage - *Lucilia eximia* Wiedemann (Diptera, Calliphoridae); Chromatic and Bloat - *Chrysomya albiceps* (Wiedemann) (Diptera, Calliphoridae); Black Putrefaction and Skeletonization - Aphodiinae sp. 1 (Coleoptera, Scarabaeidae).

Keywords: Colonization; forensic entomology; postmortem interval; scavenger insects; *Sus scrofa*.

Entomofauna Associada a Carcaças de Suínos Expostas no Sul do Brasil

Resumo. O conhecimento dos padrões de insetos que visitam carcaças em decomposição e a análise dos parâmetros relacionados a sua presença auxilia na determinação do intervalo *post-mortem* (IPM). Estas informações dependem de estudos regionais, pois a diversidade de insetos e as condições ambientais interferem neste contexto. O objetivo do estudo foi analisar a entomofauna que coloniza carcaças expostas em diferentes estágios de decomposição na área de estudo. O experimento foi conduzido no sul do Brasil utilizando três porcos domésticos (*Sus scrofa* Linnaeus), abatidos no local. Insetos adultos associados às carcaças foram amostrados diariamente com rede entomológica, coleta manual e armadilhas *pitfall*. Foram realizados testes estatísticos para avaliar a diversidade de insetos. Durante o processo de decomposição (14 dias), espécimes de Scarabaeidae (25%) e Calliphoridae (23%) foram os mais abundantes, considerando-se a totalidade das amostras. A diversidade de insetos é distinguível quando são analisados todos os parâmetros. A fase de Putrefação negra (IV) exibiu a maior diversidade de acordo com os métodos aplicados. O padrão de sucessão foi estabelecido a partir do índice de dominância das espécies: fase Fresca - *Lucilia eximia* Wiedemann (Diptera, Calliphoridae), fase Cromática e de Inchamento – *Chrysomya albiceps* (Wiedemann) (Diptera, Calliphoridae), e fase de Putrefação Negra e de Esqueletização – Aphodiinae sp. 1 (Coleoptera, Scarabaeidae).

Palavras-chave: Colonização; entomologia forense; intervalo *post-mortem*; insetos necrófagos; *Sus scrofa*.

The use of insects in criminal investigations relies on the fact that they are present at all stages of decomposition and in a predictable sequence (Payne 1965; Dillon 1997). In addition, certain insect species are characteristic of certain geographic regions (Payne 1965; Anderson & VanLaerhoven 1996). Many species participate in the insect colonization of the carcass, triggering a process of succession; if the succession pattern is constant and know, it can be highly informative (Mise *et al.* 2007). Thus, the study of insects associated with dead bodies may assist in solving crimes and their surrounding circumstances (Smith 1986; Catts & Haskel 1990; Anderson & Hobischak 2004).

The decomposition of a corpse is characterized by a continuous process that starts at the time of death and ends when the carcass is reduced to a skeleton. Although continuous, the process is divided into stages to facilitate research studies (Goff 1993; Bornemissza 1956). Each stage of decomposition provides a favorable microenvironment for certain insect species, causing certain groups to colonize the carcass; this establishes a predictable chronological sequence (Payne 1965; Bornemissza 1956; Vasconcelos & Araujo 2012).

The recognition of entomological species that colonize carcasses and their development at different temperatures provide an estimated time of death (Smith 1986; Greenberg 1991). Catts & Haskell (1990) emphasized the influence of several factors that should be considered when estimating the *post-mortem* interval; these factors may change the arrival and colonization of insects. The weather, particularly the temperature and humidity, exerts great influence on the decomposition and insect succession pattern (Shean *et al.* 1993; Archer 2004). Besides, scavenger insects have different synanthropy indices, determined by their abundance in a particular ecological area; this classification system was established by Gregor & Polvony (1958). For example, representatives of Calliphoridae, in general, are typical of urban environments (Ferreira 1978), which have a higher incidence of homicide (Anderson 2011).

Studies on the succession of insects colonizing decaying carcasses have been conducted in tropical and subtropical areas (Martinez *et al.* 2007), and show that the composition and diversity of species varies in relation to geographic region and abiotic factors (Goff 1993).

In the last two decades, the homicide rate in Brazil grew by 41%, with an average of 27.1 deaths per 100,000 inhabitants

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(IBGE 2013). Many cases remain unsolved due to difficulties in the criminal investigation procedures. Complementing the advances in biological research, tools that utilize ecological and entomological data are being routinely implemented by forensic scientists in Brazil (Vasconcelos & Araujo 2012).

Considering the need for regional information to support criminal expertise, as many factors influence the process of animal tissue decomposition, we examined the successional pattern of adult insects associated with carcasses at various stages of decomposition in southern Brazil.

MATERIAL AND METHODS

The study investigating the fauna that colonize carcasses of domestic pigs was conducted in December 2011 in Viamão, the metropolitan area of Porto Alegre in Rio Grande do Sul, southern Brazil. This location has an altitude of 52 meters and coordinates of 30° 02'10, 47" S, 51° 01'19, 05" O. Carcasses of domestic pigs (*Sus scrofa* Linnaeus) were placed in pasture area at the Station Research and Production Fine Waters of the State Foundation for Agricultural Research, which belongs to the Secretariat of Agriculture, Livestock and Agribusiness of the State of Rio Grande do Sul; the area had a size of 150 ha. The study encompasses the ecoclimatic region of the Central Depression (Maluf & Caiaffo 2001), where the climate is classified as Cfa type according to Koppen (subtropical, humid climate with hot summers). The average maximum temperature is 28ºC, and the average minimum is 20ºC in the summer (Kuinchtner & Buriol 2001). The average annual rainfall is 1,322 mm, and the rainfall is well distributed throughout the year (Moreno 1961). The soil is classified as typic Paleudalf soil (Embrapa 1999).

The characteristic vegetation in the Experimental Station includes fields with native *Ficus organencis* (Micah) Mic, and vegetation in recovery are marked by grasses and bromeliads *Eryngium horridum* Malme. In the subtropical forest region, *Syagrus romanzoffiana* (Cham.) Glassman is abundant, and *Erythryna cristagalli* L can be found in the wetland regions. The land in the Station is used for agricultural and livestock activities and its surrounding areas are dominated by urban centers.

We used three domestic pigs (*S. scrofa*) for our animal model. All of the pigs were male and weighed approximately 16 kg. The age and color of all three pigs were similar. They were slaughtered with a .38 caliber firearm using a shot to the occipital region, and they died immediately. Anesthesia and sedatives were not used because these drugs influence the rate of carcass decomposition (Introna *et al.* 2001) and the development of insect colonies on the substrate, changing the estimated *post-mortem* interval (PMI) (Goff & Lord 1994).

Treatment of the animals followed the recommendations set forth by the National Council for Control of Animal Experimentation (CONCEA) and legislation (Arouca Law No. 11.794, of 08/10/2008), in addition to Resolution 714 of the Federal Council of Veterinary Medicine. The Ethics Committee for Animal Use (CEUA) at the Pontifical Catholic University of Rio Grande do Sul approved the experiment (registration 152 dated of 28/11/2011).

Immediately after death, the carcasses were placed in right lateral decubitus in metal mesh boxes (1.5 cm²) with dimensions of 100 cm x 70 cm x 60 cm. Mesh boxes were used to repel vertebrate carnivores and to allow insect fauna to access the carcasses. These boxes were placed in an area with herbaceous vegetation, approximately 1 m from the edge of a remnant native forest. The boxes were spaced out, such that 10 m separated them, and all boxes were placed under similar exposure conditions. Six *pitfall* traps (plastic cups 300 mL) were placed around each box to collect insects (Figure 1). These traps contained water and a drop of detergent to break the surface tension of the water (Kearns & Inouye 1993), and the contents were replaced daily after collection. These traps were placed 10 cm from the box and were equidistant from each other.

The experiment was conducted between December 7-20, 2011, and the several activities were performed daily: a) observation of carcasses and characterization of decomposition stages from putrefaction phenomena (Gomes 1997), b) record of carcasses and insect fauna present using a FujiFilm Finepix S4500 camera and c) collection of insects. Three sampling methods were used, and the cages were removed to allow sampling. Hand nets were placed on each carcass for 10 minutes, and used in order to

Figure 1. Picture of the 1.5 cm² wire mesh box and the arrangement of the pitfall traps (*) around the box.

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capture adult insects that were in flight near the carcasses. The collected insects were transferred to killing chambers containing ethyl acetate. For manual collection, carcasses were inspected for 10 minutes, and adult insects present on and around the carcasses were collected using tweezers. Finally, insects in the *pitfall* traps were harvested. Samples were collected at the same time (12:00 to 13:00 h) each day and by the same operator, minimizing variations in sampling. Insects collected manually and from the *pitfall* traps were preserved in 70% alcohol. The insects were incorporated into the Insect Collection of the Museum of Science and Technology (MCT), Pontifical Catholic University of Rio Grande do Sul. The specimens were identified using taxonomic keys, the reference collection of insects, and when necessary, with the help of experts.

Daily records for the experimental period (07/12/2011 to 20/12/2011), including temperature, relative humidity and rainfall, were obtained from the 8th District of Meteorology of Porto Alegre / National Institute of Meteorology (INMET 2012).

Given the proximity of the metal cages, we applied the Kruskal-Wallis test using Palaeontological Statistics (PAST), version 2.17b (Hammer *et al.* 2001), to compare the diversity of insects in the three pig carcasses during the decomposition process to determine whether the data from the treatments could be pooled.

The diversity of the insect fauna colonizing the decomposing pig carcasses during the experiment was evaluated using the Margalef, Shannon, Simpson and Pielou Equitability diversity index. All diversity index calculations were performed from the number of records for each family of insects and for each decomposition stage of the carcasses; these indices were calculated using PAST software, version 2.17b (Hammer *et al.* 2001).

The constancy term represents the proportion of days in which the insect species in question was sampled and the total number of days sampled was based on the study by Silveira Neto *et al.* (1976). Also based on the Silveira Neto *et al.* study (1976), the following categories were used: constant = >50%, accessory = >25-50% and accidental = <25%. The dominant species was estimated according to the parameters established by Friebe (1983); these parameters are: eudominant > 10% dominant > 5-10%, subdominant > 2-5%, recessive > 1-2% and rare <1%. To express the abundance of various sampled insect species, the following calculation was used: D% = (i / t) x 100, where *i* is the total number of individual species, and t is the total number of collected individuals.

A Pearson correlation for parametric data was performed using the Statistical Package for the Social Sciences (SPSS 2006). This analysis utilized the raw insect abundance data for each collection day and daily average temperature and relative humidity.

To assess whether the sampling was adequate, we used a curve rarefaction (Krebs 1999), comparing the insect species richness with the number of insects sampled. Indices were calculated using Sobs, Chao 1 and 2, Jacknife first and second order and Bootstrap corrections. The richness estimators and rarefaction curves were obtained using the PRIMER statistical program, version 6.0 (Clarke & Gorley 2006). A significance level of 5% was used for all statistical analyses.

For classification of decomposition stages used in this study, please refer to the provision by Gomes (1997) for Neotropical countries.

RESULTS

Decomposition process of carcasses and environmental conditions. The decomposition of the carcasses lasted fourteen days, and five stages of decomposition were observed: Fresh (I), Chromatic (II), Bloat (III), Black Putrefaction (IV) and Skeletonization (V).

The duration of each decomposition stage was determined according to the physical changes observed in the exposed pig carcasses (Table 1).

The process of decay curve showed an asymmetric distribution in the number of insects associated with the carcasses; the peak was observed at day 5, coinciding with the Bloated stage (Figure 2). During this period, the total precipitation was 31.7 mm, characteristic of a dry and hot summer. The average daily temperature (24.2°C ± 1.59°C) and relative humidity (67.1% ± 6.49%) were not correlated with the number of sampled insects (r = 0.221, p = 0.448 and r = -0.345, p = 0.227 for temperature and humidity, respectively) during the studied carcass decomposition period.

Table 1. Stages of decomposition and physical characteristics observed in pig carcasses exposed in Viamão, Brazil.

Stages of decomposition	Physical characteristics	Days *post-mortem*
Fresh (I)	Fresh appearance, no odor	0
Chromatic (II)	Emergence of abdominal green stains and the beginning of gas accumulation	1
Bloat (III)	Swelling of the abdomen and the appearance of Circulation Posthumous Brouardel	3
Black Putrefaction (IV)	Complete deflation and intense carcass odor	6
Skeletonization (V)	Decreased odor; carcass consists primarily of bones, skin and hair.	11 to 13

Faunal composition and ecological succession. The Kruskal-Wallis test indicated that there were no significant differences (p> 0.05) among the three *S. scrofa* carcasses used in the study with respect to the diversity of insects at each decomposition stage. Due to this finding, the data were grouped to determine the pattern insect fauna succession for the three carcasses.

The sampling next to the pig carcasses produced 569 adult insects belonging to 29 families, 55 genera and 68 morphospecies. Representatives of Scarabaeidae (25%), Calliphoridae (23%), Muscidae (14.2%), Formicidae (9.6%) and Sarcophagidae (8%) were the most abundant (Table 2). The presence of insects next to the carcasses was observed from one hour after death until the end of the decomposition process.

Among the species collected, the Eudominants (>10% prevalence) were *Chrysomya albiceps* (Wiedemann), *Ophyra aenescens* (Wiedemann) and Aphodiinae sp. 1. The only dominant species (> 5% prevalence) observed was Sarcophagidae sp. 1. There were six subdominant (> 2% prevalence species collected: *Musca domestica* Linnaeus; Piophilidae sp. 1; *Euspilotus nigrita* Blanchard; *Camponotus rufipes* Fabricius; *Iridomyrmex* sp. 1; and Rhopalidae sp. 1. All of the other species were classified as rare or recessive, and eight morphospecies were classified as constant, present in more than 50% of sampling days: *C. albiceps*, *O. aenescens*, *Ophyra albuquerquei* Lopes, Sarcophagidae sp. 1, *E. nigrita*, Aphodiinae sp. 1, Scarabaeinae sp. 1 e *Iridomyrmex* sp. 1 (Table 2).

The Black Putrefaction stage (IV) had the greatest diversity of insect species, with the presence of 24 insect families and 271 individual insects. The Fresh stage (I) was the least rich, with only 4 families and 13 individuals collected (Table 2).

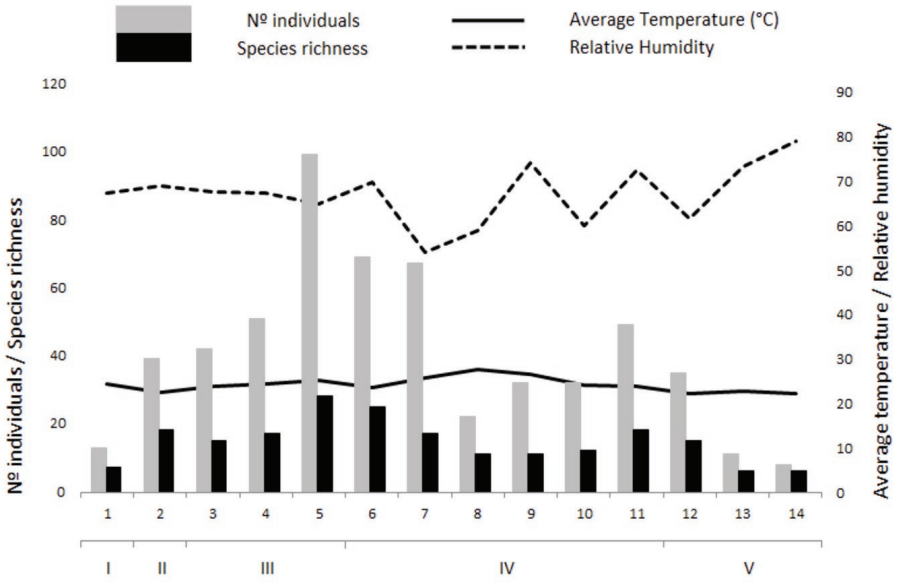

Figure 2. The number of individuals and insect species richness observed in pig carcasses during the 5 stages of decomposition (I – Fresh, II - Chromatic, III – Bloat, IV - Black Putrefaction and V - Skeletonization) relative to the daily average temperature and relative humidity values during the study period (07 to 20 December 2011 in Viamão, Brazil).

Table 2. Insects collected from pig carcasses exposed in Viamão, Brazil. The number of individuals (NI) were recorded for each carcass decomposition stage (SD: I, Fresh; II, Chromatic; III, Bloat; IV, Black Putrefaction; and V, Skeletonization). The frequency (F), Dominance (D), Constance (C) and status (A, accessory; Ac, accidental; C, constant; ED, eudominant; SD, subdominant; D, dominant; Rc, recessive; and R, rare) are also provided.

Taxon	NI	SD	F (%)	D	C
DIPTERA					
CALLIPHORIDAE			23.02		
Calliphora vicina Robineau-Desvoidy	1	I	0.18	R	Ac
Chrysomya albiceps (Wiedemann)	109	I, II, III, IV, V	19.16	ED	C
Chrysomya megacephala (Fabricius)	1	IV	0.18	R	Ac
Chrysomya putoria (Wiedemann)	3	III	0.53	R	Ac
Cochliomyia macellaria (Fabricius)	6	I, II, III	1.05	Rc	A
Hemilucilia semidiaphana (Rondani)	3	I, III, IV	0.53	R	Ac
Lucilia eximia (Wiedemann)	8	I, III, IV	1.41	Rc	Ac
FANNIDAE			1.93		
Fannia sp. 1	9	III, IV	1.58	Rc	Ac
Fannia pusio (Wiedemann)	1	IV	0.18	R	Ac
Fannia trimaculata (Stein)	1	IV	0.18	R	Ac
LAUXAMIIDAE			0.18		
Lauxamiidae sp. 1	1	IV	0.18	R	Ac
MUSCIDAE			14.24		
Biopyrellia bipuncta (Wiedemann)	2	III, IV	0.35	R	Ac
Musca domestica Linnaeus	12	II, III, IV	2.11	SD	A
Ophyra aenescens (Wiedemann)	57	II, III, IV, V	10.02	ED	C
Ophyra albuquerquei Lopes	9	II, III, IV	1.58	Rc	C
Ophyra solitaria Albuquerque	1	II	0.18	R	Ac
PIOPHILIDAE			3.16		
Piophilidae sp. 1	13	III, IV	2.28	SD	A
Piophilidae sp. 2	4	III, IV	0.70	R	Ac
Piophilidae sp. 3	1	III	0.18	R	Ac
SARCOPHAGIDAE			8.08		
Blaesoxipha denieri (Blanchard)	3	IV	0.53	R	Ac
Blaesoxipha lanei (Lopes)	1	V	0.18	R	Ac
Microcerella halli (Engel)	2	IV, V	0.35	R	Ac

to be continued...

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Table 2. continued...

Taxon	NI	SD	F (%)	D	C
Oxysarcodesia thornax (Walker)	6	III, IV	1.05	Rc	Ac
Oxysarcodesia avuncula (Lopes)	1	V	0.18	R	Ac
Ravinia belforti (Prado & Fonseca)	4	III, IV, V	0.70	R	A
Sarcophagidae sp. 1	29	II, III, IV, V	5.10	D	C
SIRPHIDAE			0.18		
Toxomerus sp. 1	1	V	0.18	R	Ac
STRATIOMYIDAE			0.53		
Hermetia illucens (Linnaeus)	3	III, IV, V	0.53	R	Ac
TABANIDAE			0.53		
Tabanus sp. 1	3	I, IV, V	0.53	R	Ac
COLEOPTERA					
CARABIDAE			0.18		
Cicindelinae sp. 1	1	IV	0.18	R	Ac
CLERIDAE			0.53		
Necrobia ruficollis (Fabricius)	1	V	0.18	R	Ac
Necrobia rufipes (De Geer)	2	IV	0.35	R	Ac
CURCULIONIDAE			0.18		
Naupactus auricinctus (Boheman)	1	V	0.18	R	Ac
DERMESTIDAE			0.70		
Dermestes maculatus (De Geer)	4	IV, V	0.70	R	Ac
ELATERIDAE			0.53		
Conoderus abbreviatus (Germar)	4	II, III, IV	0.70	R	Ac
Elateridae sp. 1	3	II, III	0.53	R	Ac
HISTERIDAE			2.99		
Euspilotus nigrita (Blanchard)	17	III, IV	2.99	SD	C
HYDROPHILIDAE			0.18		
Hydrophilidae sp. 1	1	IV	0.18	R	Ac
SCARABAEIDAE			25.13		
Aphodiinae sp. 1	126	II, III, IV, V	22.14	ED	C
Canthon sp. 1	1	III	0.18	R	Ac
Canthon mutabilis (Lucas)	1	IV	0.18	R	Ac
Eurysternus parallelus (Castelnau)	1	II	0.18	R	Ac
Onthophagus sp. 1	1	IV	0.18	R	Ac
Scarabaeinae sp. 1	7	III, IV, V	1.23	Rc	C
Uroxys sp. 1	2	III	0.35	R	Ac
SCIRTIDAE			0.18		
Scirtidae sp. 1	1	IV	0.18	R	Ac
SILPHIDAE			1.76		
Oxelytrum discicolle (Brullé)	10	III, IV	1.76	Rc	A
STAPHYLINIDAE			0.18		
Aleocharinae sp. 1	1	IV	0.18	R	Ac
HYMENOPTERA					
APIDAE			0.70		
Scaptotrigona bipunctata (Lepeletier)	4	III, IV	0.70	R	Ac
FORMICIDAE			9.67		
Acromyrmex sp. 1	4	II, IV, V	0.70	R	A
Atta sp. 1	2	II, IV, V	0.35	R	Ac
Camponotus sp. 1	8	III, IV	1.41	Rc	A
FORMICIDAE (Continued)					
Camponotus rufipes Fabricius	14	II, III, IV	2.46	SD	A
Ectatomma sp. 1	3	II, III	0.53	R	Ac
Formicidae sp. 1	8	III, IV, V	1.41	Rc	Ac

to be continued...

Table 2. continued...

Taxon	NI	SD	F (%)	D	C
Formicinae sp. 1	1	III	0.18	R	Ac
Iridomyrmex sp. 1	13	III, IV, V	2.28	SD	C
Odontomachus sp. 1	1	V	0.18	R	Ac
Ponerinae sp. 1	1	III	0.18	R	Ac
VESPIDAE			0.18		
Polybia sericea (Olivier)	1	IV	0.18	R	Ac
HEMIPTERA					
LYGAEIDAE			0.18		
Lygaeidae sp. 1	1	IV	0.18	R	Ac
RHOPALIDAE			2.99		
Rhopalidae sp. 1	17	I, II, III, IV, V	2.99	SD	Ac
ORTHOPTERA					
ACRIDIDAE			0.53		
Acrididae sp. 1	1	III	0.18	R	Ac
Dichroplus sp. 1	1	III	0.18	R	Ac
Ronderosia sp. 1	1	IV	0.18	R	Ac
GRYLLIDAE			0.18		
Gryllidae sp. 1	1	II	0.18	R	Ac
ODONATA					
LIBELLULIDAE			1.05		
Libellulidae sp. 1	6	I, II, III, IV, V	1.05	Rc	A
LEPIDOPTERA					
NYMPHALIDAE			0.18		
Diaethria clymena meridionalis (H. Bates)	1	III	0.18	R	Ac

The diversity of insects found in each carcass decomposition stage is distinguishable when all parameters are analyzed. The Black Putrefaction (IV) stage exhibited the greatest number of individuals and species, as well as the highest diversity according to the methods of Simpson (0.860), Shannon (2.779) and Margalef (8.211). In contrast, the lowest levels of diversity were observed in the Fresh stage (Margalef value = 2.339). The Fresh stage also had the highest Pielou's Equitability index (0.838) due to reduced species diversity and high dominance (0.462). The Skeletonization (V) stage was characterized by a decrease in species abundance and species richness. This final stage was also defined by decreased diversity indices (Margalef = 5.014 and Shannon = 2.380), which resulted in an increased Pielou's Equitability index (0.782). These data suggest that the first and last decomposition stages are the most similar with respect to distribution diversity (Table 3).

The Berger-Parker dominance analysis was able to identify the dominance of particular morphospecies during each stage of carcass decomposition. The following key species were identified: *Lucilia eximia* Wiedemann – Fresh stage, *C. albiceps* - Chromatic and Bloat stages and Aphodiinae sp. 1 - Black Putrefaction and Skeletonization stages.

The projection of insect species richness in decomposing carcasses using multiple richness estimators (Sobs, Chao 1 and 2, Jackknife first and second order, and Bootstrap) does not reach an asymptote, according to rarefaction curve, suggesting that the number of samples was insufficient to observe rare species (Figure 3).

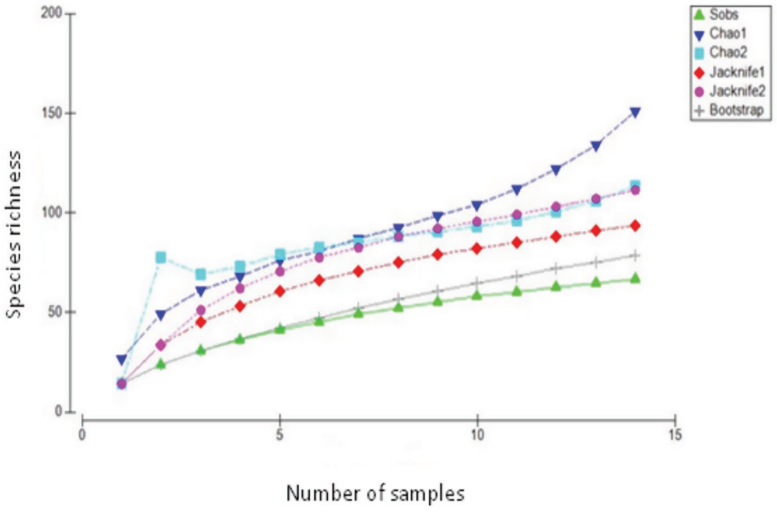

Figure 3. Estimates of insect species richness associated with pig carcasses in Viamão, Brazil.

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Table 3. Insect diversity, equitability and dominance indices during the pig carcass decomposition stages (I - Fresh stage, II - Chromatic, III - Bloat, IV - Black Putrefaction and V - Skeletonization) over the study period (07 to 20 December 2011 in Viamão, Brazil).

Stages of Decomposition	I	II	III	IV	V
Abundance	13	39	192	271	54
Richness	7	18	37	47	21
Margalef Diversity	2.339	4.640	7.038	8.211	5.014
Shannon	1.631	2.174	2.637	2.779	2.380
Simpson's Diversity	0.734	0.767	0.843	0.860	0.821
Equitability of Pielou	0.838	0.752	0.725	0.722	0.782
Berger-Parker dominance	0.462	0.462	0.359	0.336	0.389

DISCUSSION

In this study, five stages of decomposition were observed in pig carcasses, which supports the studies of Moura *et al.* (1997) and Carvalho *et al.* (2004). The decomposition progressed rapidly in most stages, as is expected during the summer in Neotropical countries (Moura *et al.* 1997; Carvalho & Linhares 2001), and only bones and skin remained by day 14.

The weather conditions during the study were recorded and characteristic of summer in the region. These conditions contributed to the accelerated decomposition process, which is in accordance with the findings of Rodriguez & Bass (1983) and Shean *et al.* (1993). However, according to the Pearson correlation test, the meteorological factors were not associated with the number of individuals collected from the decomposing carcasses. This fact emphasizes that the maximum temperature is more important than the minimum temperature with respect to the rate of carcass decomposition; high relative humidity is also important because it acts directly on the decomposition of the carcasses and promotes the emergence of stage alternative decomposition by butyric fermentation, for example (Moura *et al.* 1997). However, the relationship between abiotic factors and the number of individuals collected is most evident when there is seasonal variation; this finding was proposed by the Carvalho & Linhares (2001) study, which was conducted in São Paulo, southeastern Brazil. The Carvalho & Linhares (2001) study recorded low temperatures during the winter (7.5°C) and found that lower temperatures were related to a low number of individuals; conversely, high temperatures in the summer (29.2°C) were associated with a greater number of insects and more rapid decomposition. Because temperature and relative humidity conditions throughout the study exhibited little variation, it was not possible to demonstrate the influence of these factors on the presence of insects on the carcass or on the decomposition process itself.

The predominance of Scarabaeidae, Calliphoridae, Muscidae and Formicidae families observed during the study confirms the pattern observed by Moura *et al.* (1997), which was a similar study conducted in southern Brazil, but on smaller sized model corpses (rats). Members of these families, mainly Calliphoridae, are considered the main colonizers and consumers of this type of substrate (Campobasso *et al.* 2001). These families encompass the largest number of species due to their frequent use of animal matter, such as decaying site, for their development (Stevens 2003).

The predominance of individuals from the Calliphoridae family during the early stages of decomposition is attributed to the high perception of decay odors at great distances from the carcass (Smith 1986). Martinez *et al.* (2007) conducted a study in Paramo, Colombia, and they noted that the blowfly is observed during stages III and IV but not at the Skeletonization (V) stage. The rapid carcass weight loss is associated with the conversion of the carcass biomass to larval biomass; subsequently, larval insects leave the carcass during pupation. Rodriguez & Bass (1983) indicated that flies from the *Lucilia* genus act as pioneers in the colonization of carcasses in Tennessee. Similarly, in the present study, *L. eximia* was the first species to reach the substrate and could be observed as soon as one hour after death.

The specie *C. albiceps* is originally from Africa and was introduced into Brazil in the 1970s (Guimarães *et al.* 1978). The current distribution of the blowfly encompasses nearly all of the national territory, as it is easily able to adapt and spread (Zumpt 1965; Guimarães *et al.* 1983). The blowfly is also one of the first insects to colonize decomposing bodies, demonstrating its great potential for use during the search for forensic evidence (Carvalho *et al.* 2004). However, their aggressive nature interferes with the colonization of other species, such as *Cochliomyia macellaria* (Fabricius) from the Calliphoridae family (Faria *et al.* 2004; Rosa *et al.* 2011). In addition, *C. albiceps* can be considered a keystone species in the determination of PMI, as this species shows no preference for station or geographical area (Souza & Linhares 1997), and it has been found to be associated with several decaying resources, including human bodies (Rodriguez & Bass 1983; Oliveira & Vasconcelos 2010). In this study, *C. albiceps* was the most abundant species of blowfly in the samples and was present during all stages of decomposition. Rosa *et al.* (2011) also noted that this species was the most observed in decomposing carcasses in Cerrado. In another study also conducted in southern Brazil, Vianna *et al.* (2004) reported a higher occurrence of the species during the warmer months, where temperatures ranged from 18.5°C to 23.5°C. The findings from the Vianna *et al.* (2004) study are in accordance with what was observed in the present study. One factor that likely contributes to the high abundance of this species is the fact that it intensively consumes food over a short period of time (Prado & Guimarães 1982) and their presence in subsequent stages may result from the production of new individuals from the carcass investigated. Among the families belonging to Coleoptera, Scarabaeidae was represented more than any of the others; Histeridae and Silphidae were the second and third most abundant families in this group. Such families are commonly associated with decaying carcasses, mainly as predators (Mise *et al.* 2007). Members of the Scarabaeidae family are also consumers of decaying matter (Marinoni *et al.* 2001). Aphodiinae sp. 1 was the most abundant within the Scarabaeinae subfamily, and this particular species was associated with the black putrefaction stage and is usually associated with manure. However, some species can feed on different types of carcass material from decaying plants and animals (Mise *et al.* 2007). The large number of individuals from this subfamily may be related to the sampling method used, the ability of this genus to dig into the carcass or the diversity of the species with respect habitat and food preferences (Scholtz & Grebennikov 2005).

The Formicidae family was the taxon with the largest number of morphospecies in the decomposing carcasses (10 spp.), in agreement with other research studies (Monteiro-Filho & Penereiro 1987; Cruz & Vasconcelos 2006). Although few forensic studies have highlighted this taxonomic group, Cruz & Vasconcelos (2006) emphasized the importance of ants associated with decomposing bodies in a region of the Atlantic Forest in Pernambuco; ants occupy different ecological niches and may accelerate decomposition. *Camponotus* spp. was the most abundant, supporting the results published by Moura *et*

al. (1997) in Curitiba; this study recorded gender as important medico legal.

Regarding the stages of carcass decomposition, the largest number of insects was collected during Black Putrefaction, which can be attributed to a longer duration of this stage (six days). Additionally, this stage provides an increased availability of developmental resources for the insects, a fact that was also observed by Carvalho & Linhares (2001) in São Paulo. The odor from the putrefied carcasses resulted in the greatest attraction of colonizers, both in terms of abundance and the diversity of insects. However, there was a low Equitability of Pielou index value, indicating an unequal distribution of this insect fauna on carcasses during this stage. This observation was related to high diversity and low dominance, and the Black Putrefaction stage became the most distinct stage in the entire decomposition process. The initial stages (I and II) were similar to the final stage of decomposition (V) with respect to the diversity index. The low diversity observed during the Fresh stage, as determined by the Margalef index, and the high distribution ratio diversity, as assessed by the Equitability of Pielou index, suggest a more uniform distribution of the species as is observed during the Skeletonization stage. This may be related to decreased biomass and subsequent reduced food availability, reducing the diversity of insects that colonized the decomposing carcasses and resulting in increased dominance (Berger-Parker = 0.389).

The accumulation curve for insect fauna species collected from the three carcasses did not reach an asymptote, a situation often observed in other similar studies (Lopes *et al.* 2007; Sousa 2008). The curve suggests the need for a greater sampling effort to observe rare species and to access the real wealth of the taxa in the study area. This result is consistent with findings in the Neotropical region where there are highly diverse insect species (Sousa 2008). Furthermore, there was a limited abundance of most species due to the low consistency of most species.

Knowledge of the standard succession of insects that colonize carcasses in specific geographic areas is relevant for determining the time of death. The information presented in this case study revealed the insect fauna observed on cadaverous carcasses and the pattern of colonization in southern Brazil. The results from this study indicate key species colonizing the carcasses during each decomposition stage that was considered. Based on indices of species dominance, the following species were prevalent in the various stages indicated: (I) Fresh - *L. eximia*; (II) Chromatic and (III) Bloat - *C. albiceps*; (IV) Black Putrefaction and (V) Skeletonization - Aphodiinae sp. 1. However, this pattern may vary under other environmental conditions, emphasizing the need for similar studies in this geographical area.

The use of forensic entomology as a routine tool in criminal forensic cases in the state is still facing many challenges. However, significant improvements can be made with respect to standardizing the methods used based on the knowledge of systems, taxonomy and insect ecology. The study of insect fauna associated with decaying carcasses, the definition of decomposition stages for a given geographic area and the relationship with abiotic factors are key elements for establishing a database for future use in criminal investigations.

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