

## Insects on decomposing carcasses of small rodents in a secondary forest in Southeastern Brazil

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**Abstract.** The decomposition of small carcasses in the open is frequently neglected although it may provide information of forensic importance. This paper describes an experimental study of arthropod species associated with carcasses of mouse, *Mus musculus* (Linnaeus, 1758) and rat, *Rattus norvegicus* (Berkenhout, 1769) (Rodentia: Muridae). Four carcasses were left inside iron cages in sunlit and shady areas in a secondary forest in Southeastern Brazil twice a season for four seasons (n = 16 carcasses of each rodent). The carcasses were removed when arthropods ceased to visit them. The visiting and colonizing invertebrates were collected daily and identified. Immatures were also collected and reared in a laboratory for identification. We collected 6,514 arthropods (820 adults and 5,694 juvenile forms) belonging to 53 species from the families Sarcophagidae, Calliphoridae, Muscidae, Fanniidae, Syrphidae, Richardiidae, Sepsidae, Micropezidae, Otitidae, Drosophilidae, Phoridae, Dolichopodidae, Anthomyiidae, Asilidae and Lauxaniidae (Diptera), Formicidae, Ichneumonidae, Encyrtidae and Apidae (Hymenoptera), Staphylinidae (Coleoptera) and Gonyleptidae (Opiliones). *Lucilia eximia* (Wiedemann, 1819) (Diptera: Calliphoridae) and *Peckia (Pattonella) intermutans* (Walker, 1861) (Diptera: Sarcophagidae) deserve special attention because both adult and immature forms were collected in all seasons and in both areas. Our results indicate that the frequency of occurrence of these arthropods was positively associated with carcass size (mouse or rat); no marked insect succession on the carcasses occurred; and the diversity of Calliphoridae and Sarcophagidae was high, irrespective of season.

### INTRODUCTION

Determining the time of death might be of crucial importance in a forensic investigation. For this, careful analysis of the invertebrates living on/in a carcass may provide useful information. For instance, the type of organism and the developmental stage may be important in estimating how long a carcass has been in a given location. Only rapidly-colonizing species develop on carcasses because physical, chemical and biological conditions in such a microhabitat undergo rapid changes (Hanski, 1987). Although scattered and ephemeral, carrion is highly nutritional and attracts arthropods. However, the species living on/in carcasses immediately after death and the factors acting upon these species are poorly studied.

It is estimated that Diptera and Coleoptera account for 60% of the fauna associated with decomposing carcasses and, because of that, they are indeed very important in forensic science. For instance, some calliphorid Diptera of the genera *Lucilia*, *Chrysomya*, *Cochliomyia* and *Calliphora*, and various other genera of Sarcophagidae are abundant and undergo a successional change during decomposition (Bornemissza, 1957; Payne, 1972). However, other orders (Lepidoptera, Hymenoptera, Blattodea, Hemiptera, Isoptera and Dermaptera) and even other arthropods groups such as spiders, harvestmen, centipedes, millipedes, isopods and mites are frequently found

associated with decomposing remains, but are usually not included in the cadaveric fauna, possibly because nothing is known of their role in decomposition.

In the present study we collected and identified the adult arthropods visiting and immature arthropods developing in decomposing carcasses of small rodents in different seasons, in sunlit and shady areas, and on rodents of different sizes. We analyzed the relative abundance of the arthropods; distinguished the visiting fauna from the locally-developing species; and determined the pattern of insect succession on the decomposing carcasses.

### MATERIAL AND METHODS

#### Study area

The study was conducted in a secondary forest (ca. 3,500 m<sup>2</sup>) in the city of Campinas, São Paulo State, Brazil (22°49'15"S, 47°04'08"W), from August 2003 to June 2004. Both native and exotic plants occur in this area, such as *Typha* sp. (cattail), *Prunus sphaerocarpa* Michx., 1803 (myrtle-cherry), *Psidium guajava* L., 1753 (guava tree), *Croton zehntneri* Pax. & K. Hoffm., 1923 ("canelinha"), *Banisteriopsis caapi* (Spruce ex Griseb.) C.V. Morton, 1931 (caapi plant), *Persea americana* Mill., 1768 (avocado), *Prunus domestica* L., 1753 (plum tree) and *Senna multijuga* (Rich.) H.S. Irwin & Barneby, 1982 ("pau-cigarra"). The climate is markedly seasonal, consisting of a dry season with mild temperatures (winter; mid-June to August) and a warm rainy season (summer; mid-November to March). The two intermediate seasons, autumn (April–May) and spring (Sep-

tember–November), are characterized by fluctuations in temperature and rainfall (Souza & Linhares, 1997).

### Study model

We used 16 carcasses of mice (*Mus musculus*, Swiss lineage) (Linnaeus, 1758) and 16 of albino rats (*Rattus norvegicus*, Wistar lineage) (Berkenhout, 1769). The animals were killed by cervical dislocation and weighed (Table 1).

### Field and laboratory procedures

Two carcasses of each species were placed in the forest on two occasions in each season, totalling 16 animals of each species and 8 experiments over the 4 seasons (Table 1).

Each experiment was finished when the carcasses were no longer visited by adult arthropods. The carcasses were placed inside white plastic boxes (15 × 10 × 10 cm) and arranged 20 m apart in either sunlit or shady areas in the forest (a mouse and a rat in both the sunlit and shady areas). A thin metal grid at the bottom of the box allowed water to drain away and prevented the escape of insects and maggots. A 4-cm thick layer of vermiculite was placed between the metal grid and the carcass to absorb rainwater. A square steel cage (30 × 30 × 30 cm; 2 cm grid), fixed to the ground with four iron hooks, covered the plastic box (Fig. 1). This cage prevented large scavengers (e.g. lizards, dogs and vultures) from reaching the carcass.

The carcasses were observed daily for 30 min from 10:00 to 14:00 h, the peak time of insect activity on carcasses in South America (Baumgartner & Greenberg, 1985). We collected the flying insects with a hand net and the crawling arthropods directly from the carcasses with ordinary tweezers. The insects collected were brought to the laboratory, killed by freezing at –20°C and identified. Place and time of collection were recorded. Weather conditions in the field were measured daily during insect collection with a Celsius thermometer (model MM 5202-Incotermin<sup>TM</sup>) and a humidity sensor (model 4463, Stäcker & Olms<sup>TM</sup>). Further meteorological data were obtained from CEPAGRI -UNICAMP.

When the carcasses were no longer visited by arthropods, immature specimens (larvae and pupae) were collected from the vermiculite layer and reared under controlled laboratory conditions (25 ± 1°C, 60 ± 10% relative humidity) in glass containers containing a layer of vermiculite and covered with organza. The arthropods were identified at emergence.

### Statistical analyses

Numbers of species collected and their abundance were evaluated by ANOVA (analysis of variance) using homogeneity of the abundance values as the null hypothesis; means were compared using Duncan's multiple range test. ANOVA was also used to compare the number of individuals and numbers of insect families on carcasses that differed in size (mouse or rat), season when exposed (winter, autumn, spring or summer) and exposure to sun (sunlight or shade) (Table 2). We tested for interactions between carcass size × exposure to sun, carcass size × season, carcass size × insect family, season × exposure to sun, season × insect family and exposure to sun × insect family. All the analyses were performed using SAS GLM version 6.12 (SAS Institute Inc., 1988). The significance level was set at  $\alpha = 0.05$ .

### Species diversity index

To determine the diversity and seasonal variation in arthropods, the Simpson-Yule index ( $\lambda$ ) was used (Ludwig & Reynolds, 1988). This diversity index was calculated only for the families Calliphoridae and Sarcophagidae because they contain the most abundant species involved in the decomposition process.

TABLE 1. Mean climate (temperature, rainfall and relative humidity ± SD) and carcass features (weight in g and time taken to decay in parenthesis) in the different seasons.

Phase	Climate			Carcass weight in g (time taken to decay)			
	Temp. (°C)	Rainfall (mm)	R.H. (%)	mouse		rat	
				sunlit	shady	sunlit	shady
Winter I (Aug 2003)	21.01 ± 3.64	0.78 ± 1.56	58.82 ± 13.47	27.23 (17)	29.45 (16)	206.53 (13)	164.50 (14)
Winter II (Sept 2003)	22.44 ± 2.79	1.03 ± 3.10	51.67 ± 2.52	20.14 (8)	35.23 (8)	215.80 (9)	200.20 (8)
Spring I (Oct 2003)	27.43 ± 2.64	0.00 ± 0.00	48.86 ± 7.99	42.23 (6)	45.31 (7)	239.70 (6)	178.55 (6)
Spring II (Dec 2003)	26.50 ± 2.35	6.18 ± 4.55	78.0 ± 4.90	35.90 (4)	40.69 (5)	251.75 (6)	179.25 (6)
Summer I (Feb 2004)	31.25 ± 2.75	2.25 ± 4.50	64.25 ± 9.64	41.25 (4)	40.65 (4)	156.11 (4)	180.40 (4)
Summer II (Mar 2004)	28.33 ± 2.66	3.92 ± 9.50	75.17 ± 11.14	44.42 (5)	42.16 (6)	187.52 (5)	185.95 (5)
Autumn I (Apr 2004)	22.22 ± 1.92	4.54 ± 11.21	73.33 ± 8.19	28.26 (8)	25.63 (8)	183.07 (9)	166.10 (9)
Autumn II (May 2004)	16.94 ± 1.81	5.28 ± 10.52	79.69 ± 6.70	30.74 (14)	28.05 (16)	167.90 (8)	184.29 (13)

## RESULTS

### Weather conditions and decaying process

Mean temperatures, relative humidities and rainfall in each season are shown in Table 1. The period of decay varied in different seasons and for different sizes of carcass. The decay sequence was: initial decay, putrefaction, black putrefaction, fermentation and dry decay (Borne-missza, 1957) (Fig. 1).

During the experiment, a mouse carcass placed in the sunlit area underwent mummification. This carcass was removed after 17 days and there was no trace of maggots.

### Insect fauna and statistical analyses

6,514 arthropods (820 adults and 5,694 immatures) were collected from the 32 carcasses. Even though it was primarily intended to collect only flying insects (e.g. flies, wasps and bees), we collected specimens belonging to 21 families in four orders: Diptera (95.39%), Hymenoptera (4.28%), Opiliones (0.26%) and Coleoptera (0.06%) (Table 2).

More adult insects were collected from mouse than rat carcasses ( $F = 5.59$ ;  $P < 0.0221$ ). More were also collected in spring than in the other seasons ( $F = 2.91$ ;  $P < 0.0439$ ). Adult insects were more numerous on carcasses placed in sunlit areas ( $F = 8.48$ ;  $P < 0.0054$ ) and the most abundant insect family was the Formicidae ( $F = 4.58$ ;  $P < 0.0001$ ). There is a significant association between the number of insect families and the size of the carcass ( $F = 8.82$ ;  $P < 0.0001$ ). However, there were no interactions between the parameters carcass size and exposure to sun ( $F = 0.10$ ;  $P < 0.7565$ ), carcass size and season ( $F = 0.56$ ;  $P < 0.6404$ ), season and exposure to sun ( $F = 1.21$ ;  $P < 0.3143$ ), season and insect family ( $F = 1.15$ ;  $P < 0.3330$ ) and exposure to sun and insect family ( $F = 0.80$ ;  $P <$

TABLE 2. Insects collected from the 32 carcasses in sunlit (SU) or shady (SH) areas in the four seasons.

Specimens	Season							
	Winter		Spring		Summer		Autumn	
	SH	SU	SH	SU	SH	SU	SH	SU
ORDER COLEOPTERA								
Staphylinidae (n = 04) (C)								
<i>Eulissus chalybaeus</i> *				04				
ORDER DIPTERA								
Anthomyiidae (n = 01)* (C)								01
Asilidae (n = 01)* (C)					01			
Calliphoridae (n = 5,284) (B, C)								
<i>Chrysomya albiceps</i> (Wiedemann, 1819)	13	14	12	83	01	77		02
<i>Chrysomya megacephala</i> (Fabricius, 1794)*	09	11	04	32	02			
<i>Lucilia eximia</i> (Wiedemann, 1819)	297	13	1,234	1,257	198	1,341	22	17
<i>Hemilucilia segmentaria</i> (Fabricius, 1805)	02		16	01	18		404	203
<i>Cochliomyia macellaria</i> (Fabricius, 1775)*				01				
Dolichopodidae (n = 14)* (C)			01		02	01	09	01
Drosophilidae (n = 25) (B, C)								
<i>Drosophila</i> sp.*							09	16
Fanniidae (n = 258) (C)								
<i>Fannia</i> sp.	10		102	35	63	42		06
Lauxaniidae (n = 01)* (C)								01
Micropezidae (n = 16)* (C)	03	01	09	01	01		01	
Muscidae (n = 22) (C)								
<i>Musca domestica</i> (Linnaeus, 1758)*				01				
<i>Sarcopromusca pruna</i> (Shannon & Del Ponte, 1926)**			20					
<i>Ophyra solitaria</i> (Albuquerque, 1958)*					01			
Otitidae (n = 122) (B, A)								
<i>Euxesta</i> sp.*	01					84	01	36
Phoridae (n = 03) (C)								
<i>Megaselia scalaris</i> (Loew, 1866)				01			02	
Richardiidae (n = 02)* (C)	01				01			
Sarcophagidae (n = 380) (C)								
<i>Peckia (Squamotodes) ingens</i> (Walker, 1849)		16	03	01				
<i>Titanogrypa (Cucullomyia) larvicida</i> (Lopes, 1935)*								01
<i>Peckia (Euboettcheria) anguilla</i> (Curran & Walley, 1934)*								01
<i>Peckia (Euboettcheria) collusor</i> (Curran & Walley, 1934)**		05						
<i>Helicobia pilifera</i> Lopes, 1939*		01						
<i>Helicobia pilipleura</i> Lopes, 1939*	01						01	06
<i>Sarcophaga (Lipoptilocnema) crispina</i> Lopes, 1938*								02
<i>Sarcophaga (Lipoptilocnema) crispula</i> Lopes, 1938*	01	01						
<i>Oxysarcodexia angrensis</i> Lopes, 1933*		01			02		01	
<i>Oxysarcodexia carvalhoi</i> Lopes, 1946			04		02		06	
<i>Oxysarcodexia riograndensis</i> Lopes, 1946*		03						
<i>Oxysarcodexia thornax</i> (Walker, 1849)*	02	02			03			
<i>Parasarcophaga</i> sp.*						01		
<i>Peckia (Pattonella) intermutans</i> (Walker, 1861)	37	03	08	02	17	03	39	12
<i>Peckia (Peckia) chrysostoma</i> (Wiedemann, 1830)	02	02	02	02	01			
<i>Peckia (Peckia) pexata</i> (Wulp, 1895)*				01				01
<i>Ravinia belforti</i> (Prado & Fonseca 1932)*					02			01
<i>Sarcodexia lambens</i> (Wiedemann, 1830)*	04	09		03	01			05
<i>Sarcophaga (Liopygia) ruficornis</i> (Fabricius, 1794)		146	02				01	
<i>Sarcophagula</i> sp.		02				05		
<i>Microcerella halli</i> (Engel, 1931)*		01						
Sepsidae (n = 09)* (C)	01				01	06		01
Syrphidae (n = 75) (B, C)								
<i>Ornidia obesa</i> (Fabricius, 1775)*		01	03	36		03		
<i>Copestylum</i> sp.*							01	
<i>Allograpta obliqua</i> (Say, 1823)*				30				
<i>Baccha</i> sp.*				01				
ORDER HYMENOPTERA								
Encyrtidae (n = 67)								
<i>Tachinaephagus zealandicus</i> (Ashmead, 1904)**				67				
Ichneumonidae (n = 04)* (C)				01			02	01
Formicidae (n = 179) (A)								
<i>Cephalotes clypeatus</i> (Fabricius, 1804)*				82				
<i>Camponotus</i> sp.*				04				
<i>Atta sexdens</i> (Linnaeus, 1758)*				02				41
<i>Camponotus abdominalis</i> (Fabricius, 1804)*								50
Apidae (n = 29) (B, A)								
<i>Tetragonisca angustula</i> (Latreille, 1811)*								29
ORDER OPILIONES								
Gonyleptidae (n = 17) (B, A, C)								
<i>Mischocyx cuspidatus</i> (Roewer, 1913)*				17				

( ) In Duncan's multiple range test performed only for the parameter family, the variables followed by the same letter in the column are statistically similar. Taxon without \*: adults and immatures were collected; \*: only adults collected; \*\*: only immatures collected.

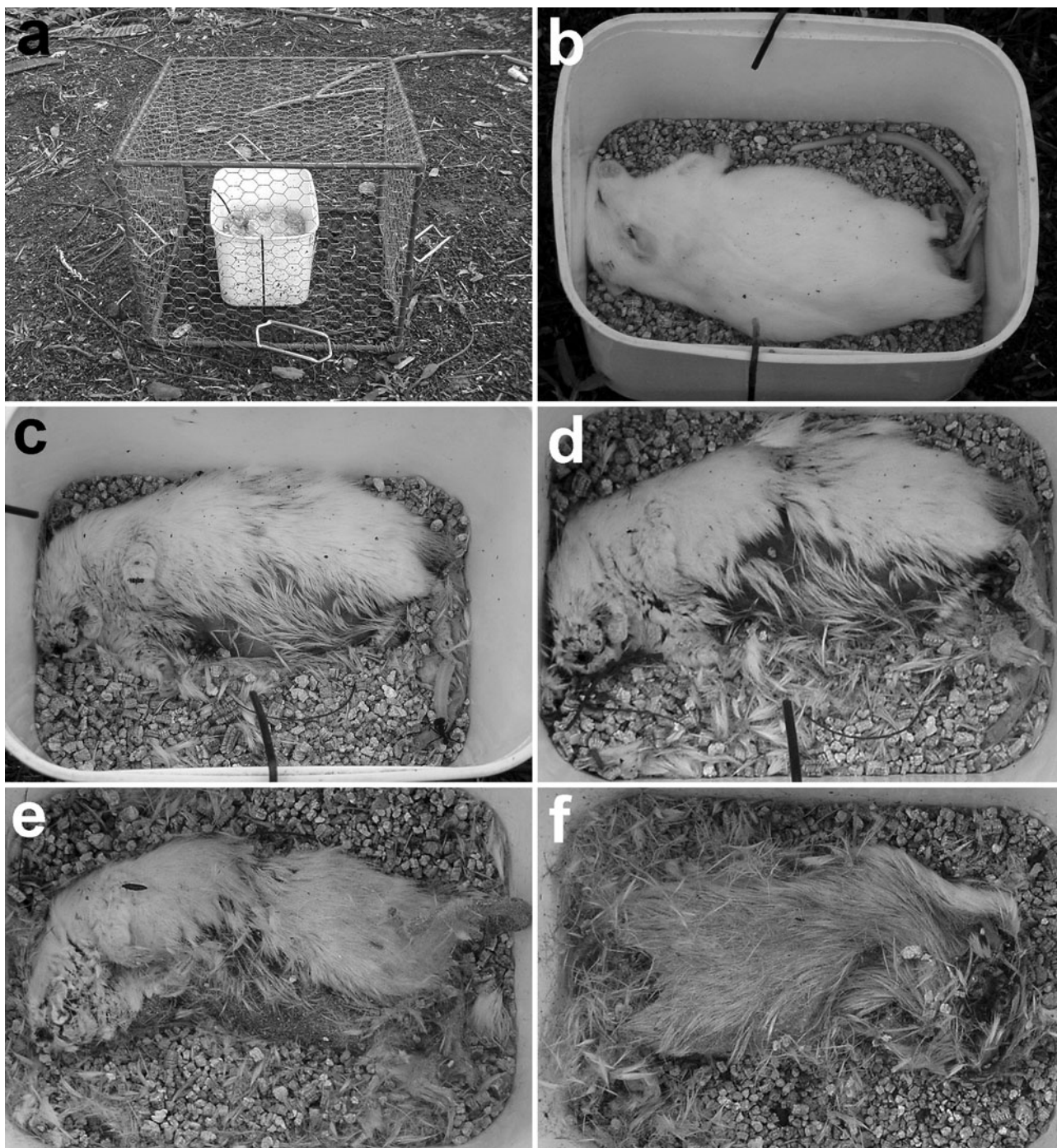


Fig. 1. Experimental cage (a) and decomposition stages of a rat carcass over the course of the experiment: (b) initial decay, (c) putrefaction, (d) black putrefaction, (e) fermentation and (f) dry decay.

0.5715), having as response variable the abundance of insects.

Regarding the immature forms, five families of Diptera were collected, all which utilize carrion for breeding: Calliphoridae (89.25%), Sarcophagidae (4.98%), Fanniidae (4.21%), Muscidae (0.35%) and Phoridae (0.01%). Insects of the species *Tachinaephagus zealandicus* (Ashmead, 1904) (1.17%) (Hymenoptera: Encyrtidae) were found parasitizing the pupae of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) and completed their development in the laboratory.

The number of immature specimens was higher in rat than in mouse carcasses ( $F = 9.45$ ;  $P < 0.0106$ ). No significant difference in the abundance of immatures was found among the seasons ( $F = 0.57$ ;  $P < 0.6439$ ), in the two exposure treatments ( $F = 0.00$ ;  $P < 0.9912$ ) and among insect families ( $F = 1.64$ ;  $P < 0.2292$ ). The same was observed for the interactions between carcass size and exposure to sunlight ( $F = 0.17$ ;  $P < 0.6862$ ), carcass size and season ( $F = 0.22$ ;  $P < 0.8784$ ), season and exposure to sunlight ( $F = 0.65$ ;  $P < 0.5981$ ), season and insect family ( $F = 0.47$ ;  $P < 0.7926$ ) and exposure to sunlight and insect family ( $F = 0.33$ ;  $P < 0.7202$ ).

TABLE 3. Simpson-Yule ( $\lambda$ ) diversity index for adult flies of the families Calliphoridae and Sarcophagidae collected from either rat or mouse carcasses, in the four seasons.

Season	Rat		Mouse	
	Sarco-phagidae	Calli-phoridae	Sarco-phagidae	Calli-phoridae
Winter	0.24	0.36	0.02	NC
Spring	NC	0.50	0.24	0.38
Summer	0.66	0.40	0.11	NC
Autumn	0.14	0.64	0.66	NC

NC = not calculated.

### Diversity index

The diversity of both Calliphoridae and Sarcophagidae was high for rat carcasses, as indicated by the Simpson-Yule index ( $\lambda$ ), which was below 0.50 in most cases, except for calliphorids in autumn and spring and sarcophagids in summer. For mouse carcasses,  $\lambda$  was mostly below 0.50 except for sarcophagids in autumn (Table 3).

### DISCUSSION

The calliphorid *L. eximia* and the sarcophagid *Peckia* (*Pattonella*) *intermutans* were the only species for which both adult and immature forms were found in all seasons and in both areas. Our results indicate that *L. eximia* is especially adapted for colonizing small carcasses. This strategy possibly avoids competition for food with other necrophagous diptera that colonize carcasses of bigger animals. Another important and unexpected finding was the juvenile forms of *S. pruna*, which is not recorded as necrophagous. Therefore, this species can now be considered to be of potential forensic interest.

The number of Sarcophagidae species collected ( $n = 21$ ) and that developed in the carcasses ( $n = 7$ ) (Table 2) increases the list of species of potential forensic utility in the Neotropical region and is substantial compared to that recorded in other studies carried out in Brazil – Carvalho et al. (2000) (collected:  $n = 1$ ; developed:  $n = 1$ ), Carvalho & Linhares (2001) (collected:  $n = 21$ ; developed:  $n = 1$ ), Oliveira-Costa et al. (2001) (collected:  $n = 9$ ; developed:  $n = 3$ ), Monteiro-Filho & Penereiro (1987) (collected  $n = 10$ ; developed: not recorded) and Souza & Linhares (1997) (collected:  $n = 17$ ; developed:  $n = 3$ ). There are three possible explanations for this. First, difficulties in precisely identifying the species of this family may lead to the inclusion of similar specimens in the same species. Second, the studies were carried out in different areas, such as the state of Rio de Janeiro (Oliveira-Costa et al., 2001), which have particular seasonal and/or environmental features. Finally, different carcasses were used: pig carrion was utilized by Carvalho et al. (2000), Carvalho & Linhares (2001) and Souza & Linhares (1997), while albino rats (*Rattus rattus*) and human corpses found in crime scenes were used by Monteiro-Filho & Penereiro (1987) and Oliveira-Costa et al. (2001), respectively.

We assembled a number of species in three distinct groups of arthropods, which we consider unusual: dip-

teran species not traditionally included in forensic studies (e.g. Syrphidae and Otitidae) (G1); insects from the order Hymenoptera (G2) and arthropods other than insects (G3). In G1, the presence of *Allograpta obliqua* (Say, 1823) (Diptera: Syrphidae) was particularly remarkable; its larvae usually feed on aphids that are agricultural pests. However, according to Bugg & Ditcher (1989), in the absence of aphids these larvae may feed on pollen or other food sources in order to complete their development. This syrphid larva may feed on carcasses, which contain a high protein content. However, the possibility that the white plastic boxes attracted the hoverflies must be considered (Laubertie et al., 2006). Also in G1, the saprophagous flies of the genus *Euxesta* were the most abundant adults in the summer. This genus is generally found on decaying vegetable matter and caterpillar faeces (Link et al., 1984). In G2, the presence of *Tetragonisca angustula* on animal carcasses may be related to mineral or higher humidity needs rather than to their protein requirement. They could be also attracted to fungi that grow on carcasses during the dark decay stage (Bornemissza, 1957). In fact, some mineral and protein-rich fungi that grow in microhabitats used by stingless bees play an important role in *T. angustula* nutrition (Gilliam et al., 1988). The record of *Mischonix cuspidatus* (Roewer, 1913) (Opiliones: Gonyleptidae) specimens in G3 was remarkable since Opiliones are rarely referred to in forensic science. Nevertheless, a more detailed investigation of the feeding habits of this species is necessary to clarify whether it benefits from the carcass or is a predator of the visiting fauna. Moreover, if we had used pitfall traps of the type designed by Kočárek (2000), it is likely we would have collected more specimens of harvestmen, beetles and other crawling arthropods (e.g. cockroaches, spiders, scorpions).

A large number of Diptera were collected from the carcasses in spring and summer. This is likely a seasonal effect, since high temperatures and humidities shorten insect development time and accelerate decomposition, which attracts more flies for oviposition or larviposition. Similar results are recorded by Monteiro-Filho & Penereiro (1987), who studied decomposition of albino rats in a disturbed primary forest, also in the city of Campinas.

The present study did not find clear evidence of insect succession in the carcasses. However, other studies dealing with small carcasses (e.g. Bornemissza, 1957; Cornaby, 1974; Monteiro-Filho & Penereiro, 1987) describe colonization as a uniquely patterned process, which can be invaluable in forensic investigations. There was no clear association between decomposition stage x insect fauna. Moreover, no "colonization waves" were recorded, i.e., flies being abundant during the initial decomposition stages followed by predacious Hymenoptera (e.g. ants) and, in the advanced stages, Coleoptera. We observed a simultaneous arrival of flies (from various families), ants and beetles on the carcasses, as also recorded by Moura et al. (2005). Because small carcasses are a short-lasting resource, the insects may have been

colonizing simultaneously and establishing themselves in a competitive interaction. Further studies are needed in which the adults and immatures are carefully sampled and more accurately identified, using the decay stages for temporal delimitation (Moura et al., 2005).

The diversity indices obtained for Calliphoridae and Sarcophagidae flies confirmed that there is a high diversity of species throughout the year. Carvalho & Linhares (2001), however, found a higher diversity of arthropods on pig carcasses during winter, possibly because carcasses take longer to decay at lower temperatures and relative humidities, thus permitting the arrival of a wider array of arthropods. Nonetheless, we should consider the probable existence of different strategies for dealing with carcasses of different sizes, which potentially explains the different diversity indices.

The dimensions of the carcasses (rat/mouse) affected the frequency of adult and immature insects. This finding disagrees with Norris (1965), who reported that this parameter is only different for carcasses that differ greatly in size. He also asserted that carcass dimension is relevant only in slightly-disturbed environments. However, we found carcass size to be an important parameter although the study area is located in the midst of an urbanized portion of Campinas, disturbed by human activities.

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