Zoociências ORIGINAL ARTICLE ISSN 1517-6770

EFFECT OF ANTS ON *Mus musculus* LINNAEUS, 1758 (RODENTIA: MURIDAE) CARCASSES DECOMPOSITION: A PRELIMINARY STUDY IN AN ATLANTIC FOREST FRAGMENT

LAILA FIETO RIBEIRO[1*], TÉRCIA VARGAS[2] & JULIANE FLORIANO SANTOS LOPES[3]

*[1]Programa de PósGraduação em Entomologia, Universidade Federal de Viçosa, Viçosa, MG, Brazil. *Corresponding author: lailafribeiro@gmail.com*
[2]Programa de PósGraduação em Entomologia, Universidade Federal de Viçosa, Viçosa, MG, Brazil. E-mail: terciavargas@gmail.com
[3]Programa de Pós-graduação em Ciências Biológicas, Comportamento e Biologia Animal, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil. E-mail: julianeflopes@yahoo.com.br

Abstract: Different ant species are frequently associated with carcasses during decomposition and they are present from the early until the late *postmortem* phase. Despite the common presence of Formicidae on carcasses, their role in the decomposition process is generally neglected. Here, we evaluate the effect of ants on *Mus musculus* decomposition pattern and abundance of associated dipteran fauna in the Neotropical region. We also discussed the role of different ant species in the decomposition process, emphasizing their predatory or necrophagous behaviour. Six carcasses of *M. musculus* were placed in the field and distributed equally in two treatments: "with ants" and "without ants". Only in the "without ants" treatment, ants were not allowed to access the carcasses. A total of six ant species were registered on carcasses. The most abundant ant species were *Linepithema neotropicum* and *Pheidole* sp., which exhibited an intense predatory behaviour on dipteran larvae, resulting in a delay in the decomposition process. In contrast, *Camponotus rufipes* workers were observed acting exclusively as necrophagous and their behaviour clearly accelerated the decomposition process on one carcass. Considering the entire decomposition process, the average abundance of adult flies was not different between the two treatments. However, we found a significant difference in the abundance of dipteran larvae between them. Our findings provide a preliminary study about the effect of Neotropical ant species presence on the decomposition pattern. We highlight the contradictory role that different ant species may exhibit on carcasses and how these presented findings could be applied in medico legal reports.

Key Words: carrion, forensic entomology, Formicidae, medico legal reports.

Resumo. Efeito das formigas na decomposição de carcaças de *Mus musculus* Linnaeus, 1758 (Rodentia: Muridae): um estudo preliminar em um fragmento de Floresta Atlântica. Diferentes espécies de formigas são frequentemente associadas a carcaças durante o processo de decomposição, sendo encontradas desde o começo do processo, até nos estágios finais da fase de *post-mortem*. Apesar da presença frequente de Formicidae em carcaças, o papel da presença destes insetos no processo de decomposição é marcadamente pouco explorado. No presente estudo, nós avaliamos o efeito das formigas no padrão de decomposição de carcaças de *Mus musculus* e na abundância da fauna associada de dípteros na região Neotropical. Nós também discutimos o papel de diferentes espécies de formigas no processo de decomposição, enfatizando o comportamento predador

ou necrófago das espécies. Seis carcaças de *M*. *musculus* foram expostas, sendo distribuídas igualmente em dois tratamentos: "com formigas" e "sem formigas". Apenas no tratamento "sem formigas", formigas tiveram o acesso impedido até às carcaças. Um total de seis espécies de formigas foi registrado nas carcaças. Considerando todo o período de decomposição, a abundância média de moscas adultas não diferiu entre os dois tratamentos. Entretanto, nós encontramos uma diferença significativa na abundância média de larvas de Diptera entre os tratamentos. As espécies de formigas mais abundantes foram *Linepithema neotropicum* e *Pheidole* sp., as quais exibiram intenso comportamento de predação sobre as larvas de dípteros, resultando em um atraso no processo de decomposição nas carcaças onde foram presentes. Em contraste, operárias de *Camponotus rufipes* foram observadas agindo exclusivamente como necrófagas, sendo que este comportamento da espécie claramente acelerou o processo de decomposição. Nossos resultados representam um estudo preliminar a respeito do efeito da presença de espécies de formigas Neotropicais no padrão de decomposição de carcaças animais. Nós ressaltamos diferentes papéis que diferentes espécies de formigas podem exibir em carcaças e ainda destacamos como estes resultados podem ser aplicados em relatórios médico-legais.

Palavras-chave: carcaça, entomologia forense, Formicidae, relatório médico-legal.

Introduction

Insects are usually the first animals to explore a dead body, using it for oviposition or feeding (Amendt, 2004). They are present in all processes of carcass decomposition (Payne, 1965; Jirón & Cartín, 1981; Byrd & Castner, 2010) and their life cycle pattern and behavioural ecology are considered powerful predictors for estimating the minimum *postmortem* interval (PMImin) and interpreting the events that occurred during the commission of a crime (Wells & Sperling, 2001; Bonacci *et al.,* 2011).

In forensic entomology, insects classified as necrophagous include species that feed upon the tissues of decomposing bodies during at least one phase of their lives, and as predatory, those which feed on the fauna associated in carcass decomposition. When insects feed both on decaying carrion and also on other insects, they are considered omnivorous (Smith, 1986; Oliveira-Costa, 2011).

Most studies of carcass decomposition focus on flies and beetles (Byrd & Castner, 2010). Despite the fact of ants are one of the most abundant taxa in the sarcosaprophagous community, their roles in the decomposition process are generally neglected (Arnaldos *et al.*, 2005; Ramón & Danoso, 2015). Ants can exhibit the necrophagous, predatory or omnivorous behavior (Mckinnerney, 1978; Moretti & Ribeiro, 2006; Mise *et al.*, 2010; Sales *et al.,* 2016). Necrophagous ants accelerate the process of putrefaction and cause mutilation on the decaying bodies, which can sometimes be confused with *antemortem* injuries (Campobasso *et al*., 2009). On the other hand, when acting as predators, they substan-

tially feed on other colonizers, especially on eggs and fly larvae (Moretti & Ribeiro, 2006), and consequently, delay the decomposition process (Early & Goff, 1986). An understanding of the roles of different ant species on carcasses is crucial to interpret crime circumstances and avoid wrong conclusions in forensic studies.

The intrinsic local conditions and specific entomological diversity determine the pattern of carcasses decomposition. It is therefore important develop regional forensic entomological studies to provide a useful and applicable source of information (Carvalho *et al.*, 2017). Despite many forensic entomological studies have been published in Brazil, there are important regional gaps regard how the behaviour of ant species can affect decomposition processes (see Cruz & Vasconcelos, 2000; Andrade-Silva *et al*., 2015; Maciel *et al*., 2015; Paula *et al*., 2016; Sales *et al*., 2016). Here, we evaluated the role of different ant species on carcass decomposition patterns in an Atlantic Forest fragment, in southeast Brazil. We emphasized the predatory or necrophagous behaviour of ant species and also reported the effect of ants on dipteran abundance associated with carcasses.

Methods

This study was carried out in a disturbed fragment of Atlantic Forest (21°46'48"S, 43°22'24"W), located in the municipality of Juiz de Fora, Minas Gerais state, Brazil. The climate is characterized as warm subtropical – Cwa, according to Köppen (Alvares *et al.*, 2013), with two defined seasons: a hot and rainy summer and a cooler and dryer winter. The average annual rainfall is 1.536 mm and average annual temperature is 19.4°C. The experiments were conducted during the hot season. The temperature ranged between 30.8°C and 37.1°C during the 11 days of experiment, (T_{mean} = 34.3 ± 1.83°C) and the relative humidity between 48.1% and 69.0%, (mean = 56.5 ± 7.20%).

Six individuals of *Mus musculus* (Linnaeus, 1758) (Rodentia: Muridae) ($weight_{mean}$ = 31.1 ± 1.3 g) were killed by cervical dislocation and their carcasses immediately were placed in the field. All carcasses were obtained from the vivarium of the Center for Reproductive Biology of Universidade Federal de Juiz de Fora. The procedure was approved by the Ethics Committee on Animal Experimentation of Universidade Federal de Juiz de Fora (Protocol No. 013/2009).

Each carcass was placed inside a cage (15 cm x 26 cm x 10 cm) protected by 1.6 cm wire mesh to prevent access by vertebrate scavengers. Carcasses were distributed equally in two treatments: "with ants" and "without ants". The carcasses were distributed in transect, interspersing the treatments at 10 m intervals. In both treatments, a tray containing water and detergent (9:1) was attached below to prevent access by ants and to allow their colonization by flying insects. In the treatment "with ants", cages were connected to the soil surface by four plastic bridges (7 cm x 10 cm) that allowed ants access the carcass without contact with the solution (Figure 1).

Figure 1. Cage used in "with ants" treatment. Trays were filled with water and detergent and plastic bridges were installed to allow ant access to carcass after cage placement.

The observations started one day after the death of the mice, which corresponded to the first day of decomposition and was performed daily until the carcasses reached the final stage of decomposition. Each carcass was inspected for 15 min, always in the interval between 2 p.m. and 6 p.m. The observations were distributed in 3 repetitions of 5 min each with an interval of 3 min between them. At this time, we registered the abundance of ant workers on carcasses and also ant species feeding behaviour by *ad libitum* observation. We registered if ant species fed directly on carrion or on larvae over the carcasses, categorizing them as necrophagous or predatory, respectively. In each repetition, a scan sampling was performed to record the abundance of adult dipterans. After observations, ants were sampled through manual collection and transferred to a vial filled with ethanol (70%). In laboratory, ants were sorted to species and identified to the lowest taxonomic level possible using available taxonomic keys (Fernandez, 2003) and with the additional assistance of taxonomists (see Acknowledgments). Adult flies were not collected to avoid interference in their oviposition and consequently, in the process of colonization and decomposition. In order to measure the abundance of dipteran larvae present during the decomposition process, we quantified the larvae found in the carcass, cage area and fallen in the

tray at the end of each carcass observation. We classified the stages of carcass decomposition following Payne (1965) as: fresh, bloated, active decay, advanced decay, dry stages.

To compare the abundance of adult flies between the two treatments, we pooled the three abundance registered data and conducted generalized linear mixed models (GLMMs) with quasi-Poisson error distribution. We treated individual carcasses as a random effect in the analysis to control for pseudo-replication stemming from our repeated measures design. Further, the Student t-test was used to compare the abundance of dipterans larvae between the two treatments. All analyses were conducted using the software R (R Core Team, 2016) with model checked for the distribution of errors and over-dispersion in the data. We carried out the GLMM using the lme4 v 1.1-11 package (Bates *et al*., 2015).

Results

Decomposition time at the treatment "with ants" varied between 4 and 11 days among carcasses, while the three carcasses of the treatment "without ants" reached the dry stage in the fifth day of exposure (Table 1). In total, six ant species were observed on the carcasses (Table 1).

Table 1. The decomposition time until dry stage, ant species with their respective abundance and feeding behaviour, and total dipteran larvae abundance observed in each carcass.

Carcass	Ant access	Decomposition time	Ant species	Total ant abundance	Feeding behaviour	Total larvae abundance
1	Without	5 days	-	-	-	1931
2	With	10 days	*Camponotus rufipes* (Fabricius, 1775)	3	Necrophagous	1018
			Camponotus scipio Forel, 1908	17	Predatory	
			Camponotus sp. Mayr, 1861	1	Necrophagous	
			Linepithema neotropicum Wild, 2007	296	Predatory	
			Pheidole sp. Westwood, 1839	308	Predatory	
3	Without	5 days	-	-	-	1881

Table 1. Continuation.

4	With	4 days	*Acromyrmex subterraneus* (Forel, 1893)	1	Necrophagous	733
			Camponotus rufipes (Fabricius, 1775)	76	Necrophagous	
			Linepithema neotropicum Wild, 2007	45	Predatory	
			Pheidole sp. Westwood, 1839	1	Predatory	
5	Without	5 days	-	-	-	1694
6	With	11 days	*Camponotus rufipes* (Fabricius, 1775)	12	Necrophagous	1182
			Camponotus scipio Forel, 1908	15	Predatory	
			Linepithema neotropicum Wild, 2007	277	Predatory	
			Pheidole sp. Westwood, 1839	433	Predatory	

Workers of *Camponotus rufipes* (Fabricius) were registered from the 1st until the 5th day of decomposition (Figure 2), feeding on mice carrion (see Table 1) and thus acting strictly as necrophagous ants. These ants were especially abundant on the faster decomposed carcass (C4), at the fresh and bloated stage. This carcass had the skull totally exposed with limbs and tail dilacerated at the 2nd day of observation (Figure 3), reaching the dry stage at the 4th day of decomposition.

On the other hand, *Linepithema neotropicum* Wild and *Pheidole* sp. were especially abundant on the other two carcasses, which took a longer time to reach the dry stage (Table 1). Both ant species were observed on almost all days of decomposition time (Figure 2), strictly preying on dipteran larvae (Figure 4; Figure 5).

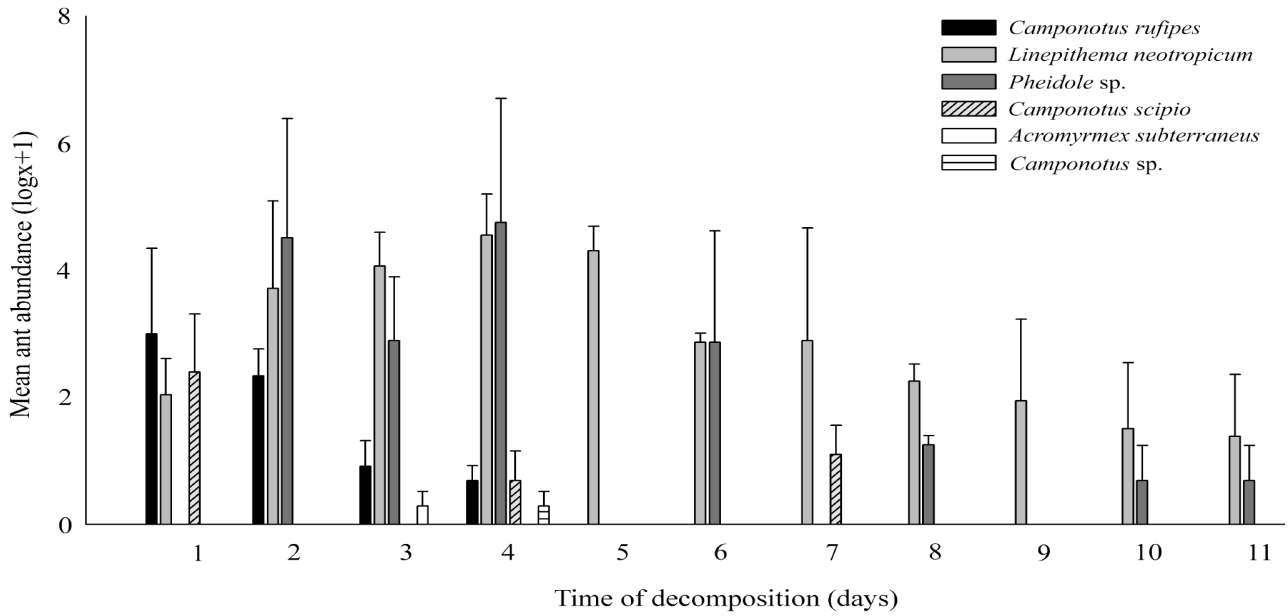

Figure 2. Mean abundance log-transformed of ant species that colonized *M. musculus* carcasses over the 11 days of exposure. Ant abundance data were log-transformed for plotting and provide a better visualization. Bars represent standard errors.

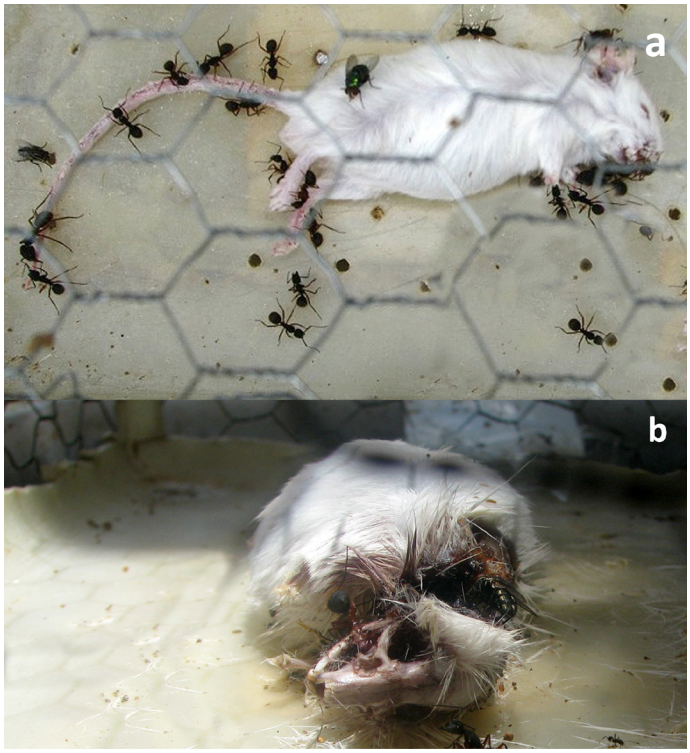

Figure 3. Colonization of *M. musculus* carcass (C4): a) First day of decomposition, with presence of *Camponotus rufipes* workers; b) Second day of decomposition, with the evidence of the skull totally exposed.

Figure 4. *Linepithema neotropicum* worker carrying a dipteran larvae from "with ants" carcass.

Figure 5. *Pheidole* sp. workers carrying a dipteran larvae from "with ants" carcass.

Similar to *C. rufipes*, *Camponotus scipio* Forel was abundant only during the fresh and bloated stages (Figure 2) and only removed dipteran larvae from the carcass, thus not exhibiting the necrophagous behaviour. Only one individual of *Acromyrmex subterraneus* (Forel) and *Camponotus* sp. were observed transporting a small piece of mice carrion with their mandibles.

In all six exposed carcasses, the decay can be considered accelerated. Dipteran eggs were registered at the first day of observation and larvae, at the second day. Considering the whole decomposition time, the average abundance of adult flies was not different ($\chi^2 = 1.272$; $p = 0.54$) between the treatments "with ants" (977 ± 227) and "without ants" (1835 ± 181; see Figure 6). However, we found a significant difference in abundance of dipteran larvae between the two treatments ($t = -5.7294$; $df = 4$; $p = 0.004$; Figure 7).

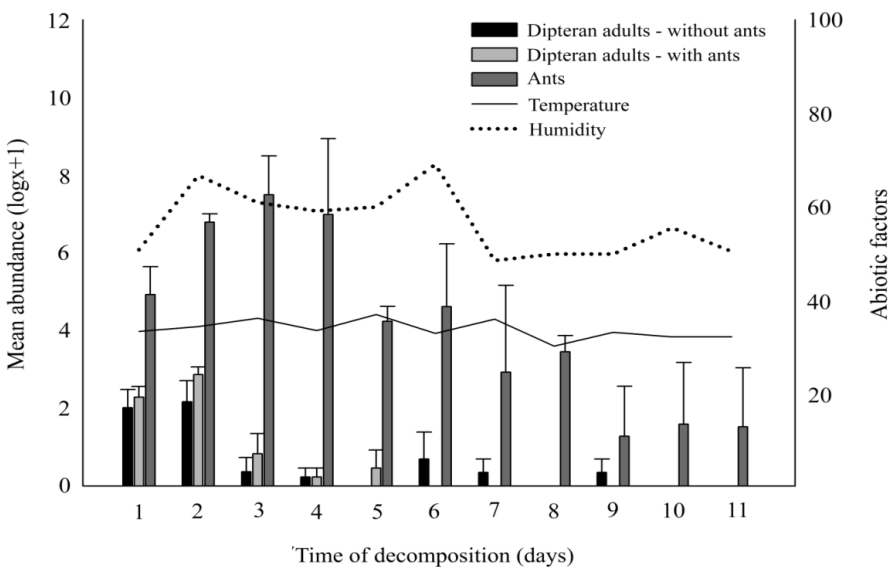

Figure 6. Mean abundance log-transformed of ants and dipteran adults that colonized *M. musculus* carcasses in the treatments "with ants" and "without ants". Air temperature and humidity over the 11 days of carcasses exposure are also presented. Mean abundance data were log-transformed for plotting and provide a better visualization. Bars represent standard errors.

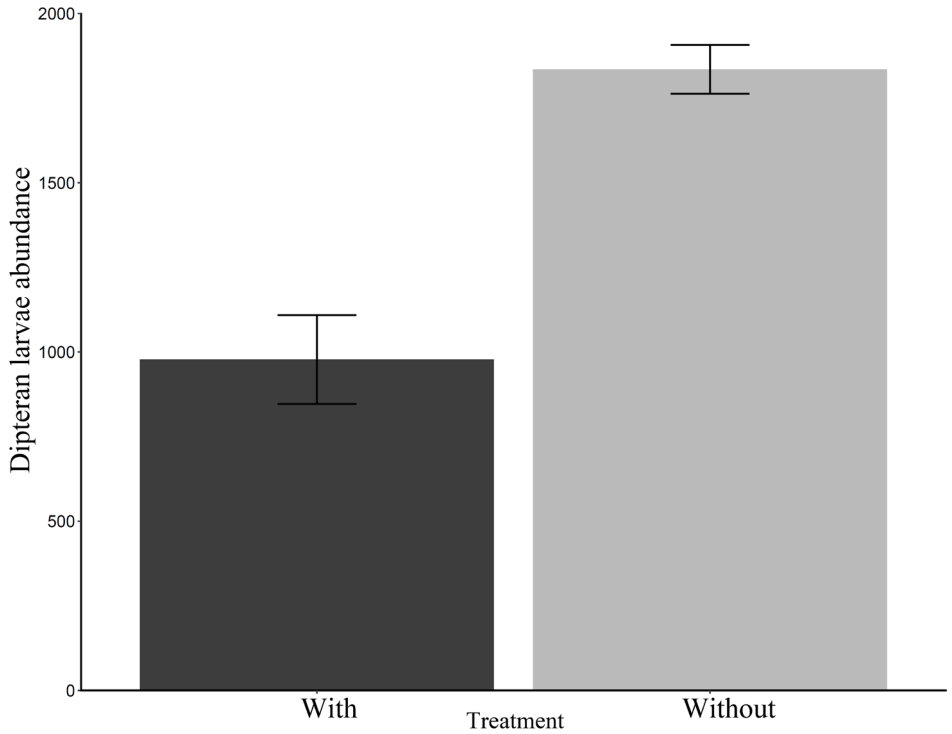

Figure 7. Variation in dipteran larvae abundance among treatments. Bars represent standard errors.

Discussion

The present study highlights the species-specific consequences of ant presence to carcasses decomposition patterns. On carcasses which ant access were allowed, we observed large differences in the time of decomposition among them: only four days when ant species fed directly on carrion, and 10 or 11 days when species predated intensively on dipteran larvae. The short decomposition time registered for C4 carcass was probably related by the high necrophagous behaviour of *C. rufipes* registered at the former decomposition stages. Exhibiting a contrasting behaviour, *L. neotropicum* and *Pheidole* sp. were probably responsible for the decomposition delay of the other carcasses with ant access. Their occurrence affected the sarcosaprophagous community due to intense removal of immature stages of dipterans from the carcasses. In contrast to what had been reported for other ant species (Wells & Greenberg, 1994; Archer & Elgar, 2003; Chen *et al.*, 2014; Sales *et al.*, 2016), we did not observe ants preying on adult flies or exhibiting aggressive behaviour against them.

A delay in the decomposition time registered for two carcasses where *L. neotropicum* and *Pheidole* sp. were highly abundant suggested an important role of these species to the decomposition pattern, a result of their predatory behaviour. Besides *L. neotropicum* is widespread in Neotropical region, the present study compris-

es the first record of this species colonizing carcasses in Brazil. *Pheidole* Westwood is one of the most hyperdiverse and prevalent ant genera in the world (Wilson, 1976; Pie & Traniello, 2007) and there is a wide range of feeding preferences and habits among species that belong to this taxon (Fowler *et al*., 1991). In the neotropics, it was reported that workers of *Pheidole radoszkowskii* Mayr mainly exhibited necrophagous behaviour but were also observed preying on dipteran larvae (Sales *et al*., 2016). Thus, the inference about the role of *Pheidole* in the rate of decomposition is applicable only when the particular species behaviour and ecology is known.

The occurrence of *C. rufipes* in forensic studies in Neotropical region was already reported (Luederwaldt, 1911; Moura *et al*., 1997; Souza *et al.*, 2008; Andrade-Silva *et al.*, 2015) but their particular role to the decomposition process was ignored. Our study showed a clear contribution of *C. rufipes* in accelerating the decomposition time. Also, they were able to cause lacerations on carrion due their intense necrophagous behaviour, which should be taken into account in medicolegal reports for a proper distinguish between *post* and *ante-mortem* injuries.

The present study is a preliminary description about the role of Neotropical ant species on carcasses decomposition pattern. We demonstrate that carcasses where *Linepithema neotropicum* and an unidentified *Pheidole* species were abundant presented a delay in their decomposition time, probably in function of the predatory behaviour of these species. In contrast, carcass where *Camponotus rufipes* were abundant showed an accelerated decomposition time, especially due to *C. rufipes* acting as necrophage. We recommend that Formicidae should not be a neglected taxa in forensic conclusions and we point out the need to expand the knowledge about ant species-specific effect on carcasses and applying it to medicolegal reports.

Acknowledgments

We are grateful to Rodrigo Feitosa for ants identification and to Centro de Biologia da Reprodução of Universidade Federal de Juiz de Fora for supplying the carcasses. We also thank Melissa Van de Wetering for her suggestions in the first draft of this manuscript. We thank the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for supporting grants.

References

Alvares, C.A.; Stape, J.L.; Sentelhas, P.C.; gonçalves, J.L.M. & sparovek, G. 2013. Köppen's climate classification map for Brazil. **Meteorologische Zeitschrift 22**: 711-728.

Amendt, J.; Krettek, R. & Zehner, R. 2004. Forensic entomology. **Naturwissenschaften 91**: 51-65.

Andrade-silva J.; Pereira, E.K.C.; Silva, O.; Santos, C.L.C.; Delabie, J.H.C. & Rebêlo, J.M.M.

2015. Ants (Hymenoptera: Formicidae) associated with pig carcasses in an urban area. **Sociobiology 62**: 527-532.

Archer, M.S. & Elgar, M.A. 2003. Effects of decomposition on carcass attendance in a guild of carrion-breeding flies. **Medical and Veterinary Entomology 17**: 263-271.

Arnaldos, M.I.; Garcia, M.D.; Romera, E.; Presa, J.J. & Luna, A. 2005. Estimation of post-mortem interval in real cases based on experimentally obtained entomological evidence. **Forensic Science International 149**: 57-65.

Bates, D.; Maechler, M.; Bolker B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. **Journal of Statistical Software 67:** 1-48.

Bonacci, T.; Brandmayr, T.Z.; Brandmayr, P.; Vercillo, V. & Porcelli, F. 2011. Successional patterns of the insect fauna on a pig carcass in southern Italy and the role of *Crematogaster scutellaris* (Hymenoptera: Formicidae) as a carrion invader. **Entomological Science 14**: 125-132.

Byrd, J.H. & Castner, J.L. 2010. **Forensic Entomology: the utility of arthropods in legal investigations.** CRC Press, Boca Raton.

Campobasso, C.P.; Marchetti, D.F.; Intron, F. & Colonna, M.F. 2009. Postmortem artifacts made by ants and the effect of ant activity on decompositional rates. **The American Journal** of **Forensic Medicine and Pathology 30**: 84-87.

Chen, C.D.; Nazni, W.A.; Lee, H.L.; Hashim, R.; Abdullah, N.A.; Ramli, R.; Lau, K.W.; Heo, C.C.; Goh, T.G.; Izzul, A.A. & Sofan-azirun, M. 2014. A preliminary report on ants (Hymenoptera: Formicidae) recovered from forensic entomological studies conducted in different ecological habitats in Malaysia. **Tropical Biomedicine 31**: 381-386.

Early, M. & Goff, M.L. 1986. Arthropod succession patterns in exposed carrion on the island of Oahu, Hawaiian Islands, USA. **Journal of Medical Entomology 23**: 520-531.

Fernández F. 2003. **Introducción a Las Hormigas De La Región Neotropical**. Instituto de Investigacíon de Recursos Biológicos Alexander von Humboldt, Bogotá, Colombia.

Fowler, H.G.; Forti, L.C.; Brandão, C.H.R. & Vasconcelos, H.L. 1991. Ecologia nutricional de formigas, p.131–223. In: Panizzi, A.R. & Parra, J.R.P. (eds). **Ecologia nutricional de insetos e suas implicações no manejo de pragas.** Editora Manole Ltda, São Paulo.

Jirón, L.F. & Cartín, V.M. 1981. Insect succession in the decomposition of a mammal in Costa Rica. **Journal of New York Entomological Society 89**: 158-165.

Luederwaldt, G. 1911. Os insectos necrophagos

paulistas. **Revista do Museu Paulista 8**: 414-433.

Mckinnerney, M. 1978. Carrion communities in the northern Chihuahian Desert. **Southwestern Naturalist 23**: 563-576.

Mise, K.M.; Souza, A.S.B.; Campos, C.M.; Kepler, R.L.F. & Almeida, L.M. 2010. Coleoptera associated with pig carcass exposed in a forest reserve, Manaus, Amazonas, Brazil. **Biota Neotropical 10**: 321-324

Moretti, T.C. & Ribeiro, O.B. 2006. *Cephalotes clypeatus* Fabricius (Hymenoptera: Fomicidae): hábitos de nidificação e ocorrência em carcaça animal. **Neotropical Entomology 35**: 412-415.

Moura, M.O.; Carvalho, C.J.B. & Monteiro-filho, E.L.A. 1997. A preliminary analysis of insects of medical-legal importance in Curitiba, state of Paraná. **Memórias do Instituto Oswaldo Cruz 92**: 269-274.

Oliveira-costa, J. 2011. **Entomologia forense: quando os insetos são vestígios**. Millennium, Campinas.

Payne, J.A. 1965. A summer carrion study of the baby pig *Sus scrofa* Linnaeus. **Ecology 46**: 592-602.

Pie, M.R. & Traniello, J.F.A. 2007. Morphological evolution in a hyperdiverse clade: the ant genus *Pheidole*. **Journal of Zoology 271**: 99-109.

R Development Core Team. 2016. **R: A language and environment for statistical computing. R Foundation for Statistical Computing**. Vienna, Austria. Available from URL: http://www.r-project.org/

Ramón, G. & Danoso, D.A. 2015. The role of ants (Hymenoptera: Formicidae) in forensic entomology. **Revista Ecuatoriana de Medicina y Ciencias Biológicas 36**: 19-26.

Sales, T.A.; Daemon, E. & Lopes, J.F.S. 2016. Necrophagous or predators? The role of *Pheidole radoszkowskii* Mayr (Hymenoptera: Formicidae) on *Rattus norvegicus* (Berkenhout) carcasses (Rodentia: Muridae). **Journal of Natural History 50**: 971-974.

Smith, K.G.V. 1986. **A Manual of Forensic Entomology**. Cornell University Press, London.

Souza, A.S.B.; Kirst, F.D. & Krüger, R.F. 2008. Insects of forensic importance from Rio Grande do Sul state in Southern Brazil. **Revista Brasileira de Entomologia 52**: 641-646.

Wells, J.D. & Sperling, F.A.H. 2001. DNA-based identification of forensically important Chrysomyinae (Diptera: Calliphoridae). **Forensic Science International 120**: 110-115.

Wells, J.D. & Greenberg, B. 1994. Effect of the red imported fire ant (Hymenoptera: Formicidae) and carcass type on the daily oc-

currence of postfeeding carrion-fly larvae (Diptera: Calliphoridae, Sarcophagidae). **Journal of Medical Entomology 31**: 171-174.

Wilson, E.O. 1976. Which are the most prevalent ant genera? **Studia Entomologica 19**: 187-200.

Recebido: 06/01/2018

Revisado: 06/05/2018

Aceito: 22/06/2018