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Factors Affecting the Composition and Succession of Beetles in Exposed Pig Carcasses in Southern Brazil

Ana Carolina R. Ries,[1,4,] **Vinícius Costa-Silva,**[2,3] **Charles F. dos Santos,**[1] **Betina Blochtein,**[1] **and Patrícia J. Thyssen**[2,]

[1]School of Health and Life Sciences, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Rio Grande do Sul, Av. Ipiranga, 6681, Porto Alegre, 90619–900, Brazil, [2]Laboratory of Integrative Entomology, Department of Animal Biology, University of Campinas (UNICAMP), São Paulo, Brazil, [3]Laboratory of Scarabaeoidologia, Institute of Biology, Federal University of Mato Grosso (UFMT), Cuiabá, Mato Grosso, Brazil, and [4]Corresponding author, e-mail: anacarolinaries@hotmail.com

Abstract

Coleoptera is one of the largest taxon among animals and exhibits diverse eating habits. When associated with decaying corpses, beetles can be of great value in estimating the postmortem interval. In order to consolidate a useful database for the forensic field, it is necessary to study the entomological fauna associated with the carcasses in different geographical regions since the diversity of insects varies according to the biogeoclimate zone. Thus, this study aimed to assess the influence of environmental and ecological factors on the composition and succession of beetles associated with pig carcasses exposed in southern Brazil. Collections were carried out during the hot/dry and cold/wet seasons. A total of 415 specimens belonging to 18 Coleoptera families were sampled. The highest total abundance ($n = 329$) and diversity ($n = 44$ taxa) were recorded in the cold/wet season, corresponding to approximately 80% of the total sampled from the two seasons. Dermestidae (26.7%) was the family most abundantly sampled. Regarding eating habits, in an increasing order of importance were necrophagous (43.3%), predator (31.6%), and omnivorous (0.05%). In the hot/dry season, there was no faunal succession. In the cold/wet season, the succession was more associated with differences in abundance than to the presence or absence of a specific taxon by decomposition stage. Considering all the factors analyzed in the current study, three species of beetles, *Dermestes maculatus* DeGeer (Dermestidae), *Euspilotus azureus* (Sahlberg, 1823) (Histeridae), and *Oxelytrum discicolle* Brullé, 1840 (Silphidae), could be identified as being of the greatest forensic relevance in this biogeoclimatic zone.

Key words: necrophagous, Coleoptera, regional database, postmortem interval, forensic entomology

As soon as the death occurs, insects can use a carcass or a corpse as a source of food, reproduction, and foraging (Catts and Goff 1992). Colonization follows a predictable sequence and is related to the natural changes that occur in the dead body (Carvalho and Linhares 2001). The resulting distribution, called faunal succession, can be very useful for estimating the postmortem interval or PMI (Goff 1992, Byrd and Castner 2010). Patterns of colonization or succession of insects mediated by decomposition can be influenced by a number of factors, among which, probably the most important, is the geographic region or biogeoclimatic zone (Anderson 2010). This is because habitat, soil, and climate have a major impact on the types and species present at a given location (Moura et al. 1997, Turchetto and Vanin 2004, Anderson 2010).

Coleoptera is one of the largest and most diverse orders of the animal kingdom, with approximately 400,000 species cataloged worldwide (Bouchard et al. 2011). In addition to their evident diversity of shapes and colors, they also exhibit various types of eating habits (Marinoni 2001). When associated with decaying corpses, beetles can be of great value in estimating the PMI (Schroeder et al. 2002, Souza et al. 2014, Matuszewski and Mądra-Bielewicz 2016, Lira et al. 2020) or investigating any displacement of a corpse (Ururahy-Rodrigues et al. 2008).

In South America, the few studies with beetles of forensic importance have focused on surveying local species, on investigating the feeding and behavioral habits of species associated with carcasses or corpses, on the effects of habitat, climate or type of carcass on the species colonization rate and in the elaboration of taxonomic keys (e.g., Pessôa and Lane 1941; Moura et al. 1997; Carvalho et al. 2000; Centeno et al. 2002; Mise et al. 2007, 2008, 2013; Almeida and Mise 2009; Aballay et al. 2012; Silva and dos Santos et al. 2012; Costa-Silva et al. 2017). Usually, information about the dipteran life cycle has been more abundantly used to estimate the time for which

a corpse was colonized, in particular, because dipterans are among the first colonizers of this resource (Greenberg 1991). Due to adaptive changes in the oral masticatory apparatus (Crowson 1981), necrophagous beetles are able to feed on rigid and dry tissues (e.g., cartilage, tendons, skin, nails) and are therefore frequently seen in the later stages of decomposition (Mise et al. 2007, 2008; Costa-Silva et al. 2017). Beetles could also be used as a biological clock to measure the time of death, for 2 or more weeks (Matuszewski and Mądra-Bielewicz 2016), an interval higher than that observed for dipterans. However, certain deficiencies in studies of taxonomy and the collection of biological and ecological information have hindered the efficient use of this group of organisms in the forensic field (Thyssen *unpublished data*).

As the diversity of insects, including Coleoptera, varies according to the biogeoclimate zone, in order to consolidate a useful database for the forensic field, it is necessary to study the entomological fauna associated with the carcasses in different geographical regions. Thus, this study aimed to assess the influence of environmental (season, temperature, humidity, and rainfall) and ecological (eating habits) factors on the composition (abundance and diversity) and succession of beetles associated with pig carcasses exposed in southern Brazil.

Materials and Methods

Ethics Approval and Consent to Participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures were performed in accordance with protocols approved by the CEEA (Ethics Committee on Animal Experimentation) of the Pontifical Catholic University of Rio Grande do Sul (#13/00369).

Study Area and Experimental Design

The study was performed in a rural environment, with at least three nearby farms keeping livestock in the surroundings, especially cattle and sheep, to the south of Porto Alegre (30°14′18″S, 51°07′19″W), in the state of Rio Grande do Sul, Brazil. According to the Köppen-Geiger climatic classification (Alvares et al. 2013), the state of Rio Grande do Sul experiences predominantly a humid subtropical climate, with the average annual temperature ranging between 12 and 23°C and significant rainfall throughout the year (approximately 1,300 mm per year). Two types of vegetation cover predominate in the state: plains (also called 'pampas') dominated by grasses, which cover almost 60% of the territory, and forests consisting of both mixed broadleaved and coniferous forests. Coastal vegetation or sandbanks comprise only 5% of the total area (IBGE 2020).

Carcasses of six domestic male pigs (*Sus scrofa* Linnaeus), weighing approximately 12 kg each, were exposed in an open area, directly to sunlight and 30 m apart. Animals were euthanized with a single shot from a .38 cal. firearm on day 1 and at the site of the study; the bullet went through the head laterally, ear to ear. Anesthetics and sedatives were not used to prevent any changes in the decomposition or attractiveness of insects to corpses (Campobasso et al. 2001). After that, the carcasses were immediately placed inside metal frame cages (1.0 × 1.0 × 1.0 m) to exclude large vertebrate scavengers. Underneath the cages, metal trays with sawdust were placed to collect adult or, if it were the case, immature insects leaving the carcasses to pupate. Two types of traps were used to collect adult insects, leaving the carcasses. A Shannon trap with 100 cm³ and an opening at 30 cm from the ground, consisting of a PVC frame in the form of inverted funnel and covered with transparent fabric, was placed over each cage (Carvalho et al. 2000). Six pitfall traps, consisting of 500 ml plastic containers, were placed 30 cm apart from each cage at ground level; each trap received approximately 300 ml of water and 1 ml of liquid detergent, which were replaced daily after collection.

Assessment of Weather Conditions and Stages of Decomposition

Weather conditions (temperature, relative humidity, and rainfall) in the field were measured daily during insect collection with a Celsius thermometer (model MM 5202-Incoterm), a humidity sensor (model 4463, Stäcker & Olms), and a rain gauge with 203 mm capacity. Further meteorological data were obtained from the Brazilian National Institute for Space Research (INPE 2020).

All carcasses were photographed daily to identify the different stages of decomposition. These stages were characterized by physical parameters and defined a posteriori, by criteria available in the literature.

Collection and Identification of Adult Insects

Collections were carried out in January and September 2014 during two seasons characterized by fluctuations in temperature and rainfall, hot/dry (mid-October to March), and cold/wet (mid-April to September). Each experiment was finished when the carcasses were no longer visited by adult arthropods.

The carcasses were observed daily for 30 min between 1100 and 1300 hours, the peak time of insect activity in South America (Baumgartner and Greenberg 1985). Adult insects were collected actively, with the help of tweezers (under or directly from the exposed pig carcasses and inside Shannon traps), and passively, using pitfall traps disposed around each carcass. All specimens collected were preserved in 70% ethanol, and then were pinned at the laboratory. Taxonomic keys (Almeida and Mise 2009, Vaz-de-Mello et al. 2011, Casari and Ide 2012) and comparison with reference collections were used for species identification. When necessary, expert taxonomists were consulted. Most of pinned specimens were deposited at the scientific collection of the Entomological Forensic section of the Insect Collection of the Museum of Science and Technology (MCT) of the Pontifical Catholic University of Rio Grande do Sul (PUCRS).

Ecological Categories

As noted by Smith (1986), insects associated to the carcasses could be classified into one of four ecological categories: 1) necrophagous species, that feed directly on the carcass, 2) predators and parasites of the necrophagous species, 3) omnivorous, that fed on both carcass and associated arthropods, and 4) incidental species, having no direct relationship to the carcass. This information was used to classify the collected beetles in order to assess which species, from their ecological categories, may have forensic relevance.

Data Analysis

A priori, a discriminant analysis (DA) was performed by using the function *lda* from the 'MASS' package (Venables and Ripley 2002) to assess the similarity (within-group) among carcasses exposed simultaneously at the same season. After that, a multivariate analysis of variance (MANOVA) and a pairwise permutational MANOVA (999 permutations) were performed by using the function *pairwise.perm.manova*() from the 'RVAideMemoire' package (Hervé 2015) to evaluate possible differences between groups. In addition, a confusion matrix was created using a Jackknife resampling method ('leave-one-out'), followed by the

determination of misclassification numbers using a significance test obtained by the function MVA.test (2,000 permutations, method '*fdr*') from the 'RVAideMemoire' package (Hervé 2015) (Supp Material [online only]).

To assess how the abundance and diversity (discrete variables) of the beetles were affected by the factors measured in this longitudinal study, two generalized linear mixed models (GLMMs) with a Poisson family (*link*=log) were fitted. Both models were run by using the function *glmer* from the 'lme4' package (Bates et al. 2015). In both *abundance*-GLMM and *diversity*-GLMM, the following variables (= fixed factors) were assessed: decomposition stages, season, weather conditions (temperature, humidity, and rainfall) and some interactions between some variables (pairwise comparisons). 'Carcasses' were designated as random factors. Pairwise comparisons were done by using the function *glht*, and then the differences tested with Tukey in 'multcomp' package (Hothorn et al. 2008).

An additional exploratory analysis, multivariate correspondence analysis (MCA), was performed to assess the degree of correspondence between beetles of different ecological categories and the decomposition stages of exposed carcasses. For this, the function CA from the 'FactoMineR' package (Lê et al. 2008) was used. All statistical analyses were carried out with the help of R (R Core Team 2016).

Results

Weather Conditions and Decomposition Stages

During the hot/dry season, temperature averages of 31.3 ± 4.4°C and relative humidity of 55.3 ± 14.7% were recorded, in addition to an accumulated rain of 13.1 mm. In the cold/wet season, temperature averages of 19.3 ± 1.9°C and relative humidity of 78.4 ± 5.9% were recorded, in addition to an accumulated rain of 161.4 mm.

According to the criteria set forth by Reed (1958) and Monteiro-Filho and Penereiro (1987), four decomposition stages were recognized in this study, then defined as fresh, bloated, decay, and dry. In both seasons, the number of stages was the same, but the total decomposition time varied from 10 d for the hot/dry season, up to 34 d for cold/wet season.

Abundance and Diversity of Coleoptera

A total of 415 individuals belonging to 18 Coleoptera families were identified (Table 1). The highest total abundance and diversity were recorded in the cold/wet season (Fig. 1), corresponding to approximately 80% of the total sampled from the two seasons.

Among the most abundant Coleoptera families ($n > 30$) were Dermestidae ($n = 111$, 26.7%), Histeridae ($n = 62$, 14.9%), Chrysomelidae ($n = 58$, 13.9%), Carabidae ($n = 43$, 10.3%), and Silphidae ($n = 31$, 7.4%) (Table 1). Except for Silphidae (represented by one species), the taxa diversity within each family was inversely proportional to the total abundance as observed for Chrysomelidae (16 taxa), Carabidae (nine taxa), Histeridae (three taxa), and Dermestidae (one species) (Table 1).

By season, it was observed that Dermestidae + Carabidae and Chrysomelidae were more prevalent in the cold/wet and hot/dry seasons, respectively, while Histeridae and Silphidae were found exclusively in the cold/wet season (Table 1). Regarding possible preferences by season, it is worth noting that less abundant families like Cleridae were registered only in the hot/dry season and others such as Cantharidae, Cerambycidae, Hybosoridae, Lampyridae, Tenebrionidae, and Trogidae were seen exclusively in the cold/wet

season (Table 1). As shown by the GLMM, the abundance of species associated with the carcasses was not significantly affected by the season ($\chi^2 = 1.02$; $P = 0.31$) (Table 2). Although the total abundance was much higher in one of the seasons (cold/wet), it must also be considered that this season had the largest number of sampling days in view of the carcass exposure period ($n = 34$). Thus, when comparing the average of specimens collected per day between seasons, it is possible to observe very similar results between cold/wet $\bar{x} = 8.6$ and hot/dry ($\bar{x} = 9.7$ seasons. Abundance was also not significantly affected by different environmental conditions (Table 2). Beetle diversity was significantly affected by the season ($\chi^2 = 20.9$; $P < 0.001$), particularly associated with temperature ($\chi^2 = 11.4$; $P < 0.001$) and rainfall ($\chi^2 = 14.7$; $P < 0.001$) (Table 3), main factors that characterize each season.

By decomposition stages: abundance ($\chi^2 = 10.7$; $P < 0.01$) and diversity ($\chi^2 = 24.9$; $P < 0.001$) were significantly different in each of the carcass decomposition stages (Tables 2 and 3) and for both the total values were higher in the decay stage (Fig. 1). The pairwise comparisons showed that for both abundance and diversity, the bloated/decay interaction was the most relevant when compared to the others, in agreement with the trend observed in the raw data (Fig. 1).

By species, *Dermestes maculatus* (DeGeer, 1774) (Dermestidae) ($n = 111$, 26.7%) was the most abundant, followed by *Euspilotus azureus* (Sahlberg, 1823) (Histeridae) ($n = 60$, 14.4%) and *Oxelytrum discicolle* (Brullé, 1840) (Silphidae) ($n = 31$, 7.5%), with the three species representing almost 50% of all specimens sampled (Table 1). In addition, these species were predominant (*D. maculatus*) or exclusively found (*E. azureus* and *O. discicolle*) in the cold/wet season. Some species, such as *Galerita melanartha* Chaudoir, 1869 (Carabidae) and *Ataenius picinus* Harold, 1867 (Scarabaeidae) were observed exclusively in the cold/wet season, while *Necrobia ruficollis* (Fabricius, 1775), *Necrobia rufipes* De Geer, 1775 (Cleridae) and *Dyscinetus dubius* (Olivier, 1789) (Scarabaeidae) were exclusively recorded in the hot/dry season, and even so in low numbers. Individuals without specific identification or species classified as incidental were not considered here.

Ecological Categories

Taking into account the eating habits and the type of relationship of the beetles with their food resource (in this case, the carcasses), all four ecological categories were represented in this study. Of the 56 sampled taxa, 7 were classified as necrophagous, 7 as omnivorous, 16 as predators, and 26 as incidental (Table 1). The most abundant species by ecological category were: *D. maculatus* (necrophagous), *E. azureus* (predator), *A. picinus* (omnivorous), and *Chalepus sanguinicollis* (L.) (Chrysomelidae) (incidental) (Table 1).

By season, the abundance of necrophagous beetles was always greater ($n = 157$ and $n = 23$ in cold/wet and hot/dry seasons, respectively), than that observed for beetles classified in other ecological categories such as predators ($n = 129$ and $n = 2$) and omnivorous ($n = 12$ and $n = 8$) in cold/wet and hot/dry seasons, respectively (Table 1). However, there is a striking contrast between diversity and abundance by season: at the cold/wet season, 16, 5, and 4 taxa of predators, necrophagous and omnivorous were recorded, respectively, while at the hot/dry season 4, 4, and 2 taxa of necrophagous, omnivorous and predators were recorded, respectively (Table 1).

Regarding the decomposition stage, the abundance and composition of ecological categories varied according to the season in which the carcasses were exposed. In hot/dry season, there was basically no

Table 1. Abundance of Coleoptera associated with pig carcasses exposed in southern Brazil by ecological category, season and stage of decomposition, where: I: fresh, II: bloated, III: decay, and IV: dry

Taxa	Ecological categories	Season								Total
		Hot/dry				Cold/wet				
		I	II	III	IV	I	II	III	IV	
Cantharidae										4
Cantharidae sp.	Predator	0	0	0	0	0	0	4	0	4
Carabidae										43
Bembidion sp.	Predator	0	0	0	0	0	2	6	0	8
Brachinus sp.	Predator	0	0	0	0	0	0	1	0	1
Carabidae sp.	Predator	0	0	0	1	0	1	1	0	3
Galerita melanarthra	Predator	0	0	0	0	0	1	8	3	12
Galerita sp.	Predator	0	0	0	0	0	0	0	1	1
Mesus sp.	Predator	0	0	0	0	0	0	3	0	3
Notiobia sp.	Predator	0	0	0	0	0	0	1	0	1
Pheropsophus sp.	Predator	0	0	0	0	0	2	10	0	12
Scarites sp.	Predator	0	0	0	0	0	0	2	0	2
Cerambycidae										3
Compsocerus violaceus	Incidental	0	0	0	0	0	0	2	1	3
Chrysomelidae										58
Alticini sp. 1	Incidental	0	0	1	1	0	0	0	0	2
Alticini sp. 2	Incidental	0	0	0	3	0	0	0	0	3
Alticini sp. 3	Incidental	0	0	0	4	0	0	0	0	4
Alticini sp. 4	Incidental	0	0	1	0	0	0	0	0	1
Cassidini sp.	Incidental	0	0	0	2	0	0	0	0	2
Chaetocnema spp.	Incidental	0	0	12	20	0	1	0	0	33
Chalepus sanguinicollis	Incidental	0	0	0	0	0	0	2	0	2
Chrysomelidae sp.	Incidental	0	0	0	0	0	0	1	0	1
Colaspis sp.	Incidental	0	0	0	0	0	0	2	0	2
Cornulactica sp.	Incidental	0	0	0	0	1	0	0	0	1
Desmogramma bivia	Incidental	0	0	0	0	1	0	0	0	1
Diabrotica sp.	Incidental	0	0	0	0	0	0	1	0	1
Diabrotica speciosa	Incidental	0	0	0	0	0	0	1	1	2
Iucetima minor	Incidental	0	0	0	0	0	1	0	0	1
Kuschelina vigintinitata	Incidental	0	0	0	0	0	1	0	0	1
Omophoita octoguttata	Incidental	0	0	0	1	0	0	0	0	1
Cleridae										18
Necrobia ruficollis	Necrophagous	0	0	0	1	0	0	0	0	1
Necrobia rufipes	Necrophagous	0	0	0	17	0	0	0	0	17
Coccinellidae										7
Coccinellidae sp.	Incidental	0	1	0	0	0	0	0	0	1
Cycloneda sanguinea	Incidental	0	0	0	0	1	2	1	0	4
Epilachna cacica	Incidental	0	0	0	0	0	1	0	0	1
Eriopis connexa	Incidental	0	0	0	0	0	1	0	0	1
Curculionidae										5
Curculionidae spp.	Incidental	1	0	0	1	0	1	1	0	4
Naupactini sp.	Incidental	0	0	0	0	0	1	0	0	1
Dermestidae										111
Dermestes maculatus	Necrophagous	0	0	0	4	0	7	99	1	111
Elateridae										6
Elateridae spp.	Incidental	0	0	3	2	0	0	1	0	6
Histeridae										62
Euspilotus azureus	Predator	0	0	0	0	0	14	29	17	60
Hister sp.	Predator	0	0	0	0	0	0	0	1	1
Saprinus sp.	Predator	0	0	0	0	0	0	1	0	1
Hybosoridae										1
Hybosoridae sp.	Omnivorous	0	0	0	0	1	0	0	0	1
Lampyridae										5
Lampyridae sp.	Incidental	0	0	0	0	0	0	1	0	1
Lucidota spp.	Incidental	0	0	0	0	2	1	1	0	4
Nitidulidae										8
Nitidulidae spp.	Necrophagous	0	0	1	0	1	2	4	0	8
Scarabaeidae										18
Aphodiinae sp.	Omnivorous	0	0	1	1	0	0	0	0	2
Ataenius picinus	Omnivorous	0	0	0	0	0	4	4	0	8

Table 1. Continued

| Taxa	Ecological categories	Season								Total
		Hot/dry				Cold/wet				
		I	II	III	IV	I	II	III	IV	
Dyscinetus dubius	Omnivorous	0	0	0	4	0	0	0	0	4
Ontherus sulcator	Omnivorous	0	0	1	0	0	0	1	1	3
Scarabaeinae sp.	Omnivorous	0	0	0	1	0	0	0	0	1
Silphidae										31
Oxelytrum discicolle	Necrophagous	0	0	0	0	0	10	20	1	31
Staphylinidae										22
Aleochara sp.	Predator	0	0	0	0	0	1	11	0	12
Philonthus sp.	Predator	0	0	0	0	0	1	0	0	1
Staphylinidae spp.	Predator	0	0	0	1	2	4	2	0	9
Tenebrionidae										1
Epitragus sp.	Omnivorous	0	0	0	0	0	0	1	0	1
Trogidae										12
Omorgus suberosus	Necrophagous	0	0	0	0	0	1	3	4	8
Polynoncus brasiliensis	Necrophagous	0	0	0	0	0	0	2	2	4
Total abundance		86				329				415
Total relative abundance (%)		20.7				79.3				100
Total diversity (taxa)		20				44				56
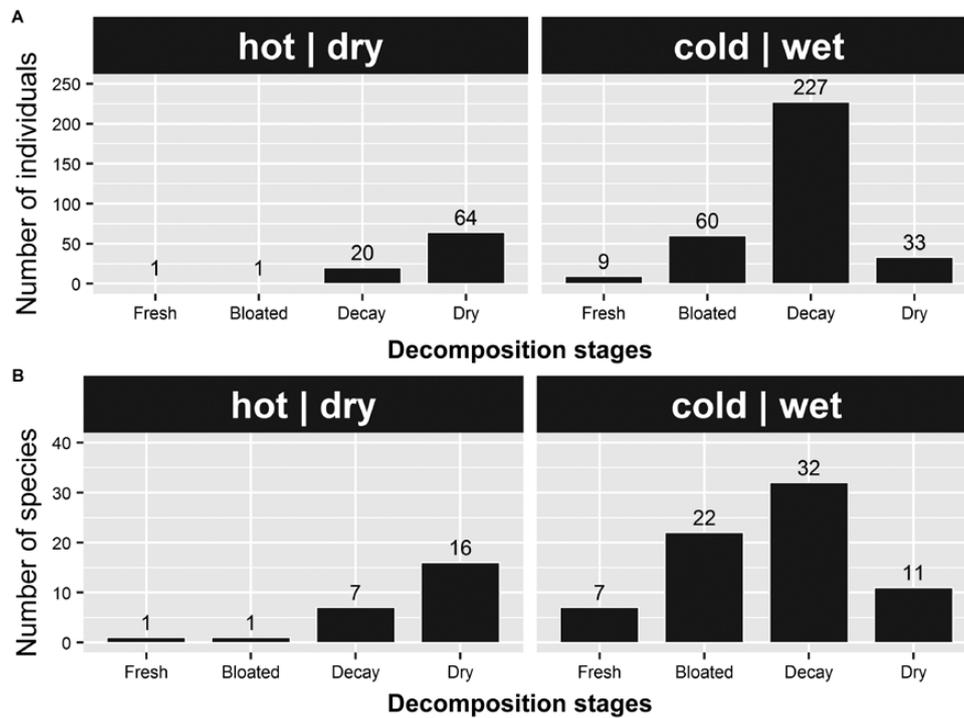
Fig. 1. Coleoptera associated with pig carcasses exposed in southern Brazil by season and stage of decomposition, where: A: abundance and B: diversity.

variation, but a strong predominance of necrophagous and omnivorous in the dry stage was observed. In cold/wet season, though with only 4 individuals sampled in the fresh stage, all ecological categories were present; in the other decomposition stages, it was recorded: in bloated, predators ($n = 26$) and necrophagous ($n = 20$), in decay, necrophagous ($n = 128$), predators ($n = 79$) and omnivorous ($n = 6$), and lastly in the dry, predators ($n = 22$), necrophagous ($n = 6$) and omnivorous ($n = 1$) (Table 1). Niche overlap between families and species of beetles, taking into account the most abundant and those

classified as necrophagous, predators or omnivorous, was often observed within each stage of decomposition (Table 1).

The MCA showed that there is a high degree of correspondence between beetles of different ecological categories and the decomposition stages of the exposed carcasses ($\chi^2 = 48.49$, df = 9, $P < 0.001$). Thus, as seen in Fig. 2, necrophagous beetles were more strongly related to the decay stage, while omnivorous to the bloated and dry stages, predators to the bloated and decay stages, and incidental to the fresh and dry stages.

Table 2. Effect of the decomposition stages, season, and weather conditions (temperature, humidity, and rainfall) variables on the Coleoptera abundance associated with pig carcasses exposed in southern Brazil, according to the generalized linear mixed model (GLMM) (pairwise comparisons are shown in the 'variables' column)

Variables	χ^2	df		P
Decomposition stages	10.7	3		**<0.01**
Season	1.02	1		0.31
Temperature	1.84	1		0.17
Humidity	1.76	1		0.18
Rainfall	3.59	1		0.05
Temperature: humidity	2.66	1		0.10
Temperature: rainfall	1.92	1		0.16
Humidity: rainfall	0.30	1		0.58
Temperature: humidity: rainfall	1.48	1		0.22
Pairwise comparisons (seasons)				
	Estimate	SE	z-value	P
Cold/wet | hot/dry == 0	0.33	0.32	1.01	0.31
Pairwise comparisons (decomposition stages)				
	Estimate	SE	z-value	P
Bloated | dry == 0	0.06	0.22	0.28	0.77
Fresh | bloated == 0	−0.46	0.76	−0.61	0.54
Bloated | decay == 0	0.45	0.15	2.97	**0.002**
Fresh | dry == 0	−0.53	0.76	−0.69	0.48
Decay | dry == 0	0.38	0.20	1.88	0.06
Fresh | decay == 0	0.91	0.77	1.18	0.23
Random effects	Variance	SD	N	
Carcasses	0.0	0.0	6	

Bold values indicate significant differences. df: Degrees of freedom. *z-value*: standard score, i.e., standard deviations from their means. Negative values, when raw score is below the mean; positive, when above. P: probability to find z-scores by chance. N: number of carcasses.

Table 3. Effect of the decomposition stages, season, and weather conditions (temperature, humidity, and rainfall) variables on the Coleoptera diversity associated with pig carcasses exposed in southern Brazil, according to the generalized linear mixed model (GLMM) (pairwise comparisons are shown in the 'variables' column)

Variables	χ^2	df		P
Decomposition stages	24.9	3		**<0.001**
Season	20.9	1		**<0.001**
Temperature	11.4	1		**<0.001**
Humidity	0.53	1		0.46
Rainfall	14.7	1		**<0.001**
Temperature: humidity	0.63	1		0.42
Temperature: rainfall	2.49	1		0.11
Humidity: rainfall	0.41	1		0.51
Temperature: humidity: rainfall	3.20	1		0.07
Pairwise comparisons (seasons)				
	Estimate	SE	z-value	P
Cold/wet | hot/dry == 0	0.51	0.11	4.57	**<0.001**
Pairwise comparisons (decomposition stages)				
	Estimate	SE	z-value	P
Bloated | dry == 0	0.04	0.05	0.90	0.36
Fresh | bloated == 0	−0.24	0.15	−1.61	0.10
Bloated | decay == 0	−0.13	0.03	−3.79	**<0.001**
Fresh | dry == 0	−0.29	0.15	−1.91	0.05
Decay | dry == 0	−0.18	0.04	−3.79	**<0.001**
Fresh | decay == 0	0.10	0.15	0.71	0.47
Random effects	Variance	SD	N	
Carcasses	0.005	0.07	6	

Bold values indicate significant differences. df: Degrees of freedom. *z-value*: standard score, i.e., standard deviations from their means. Negative values, when raw score is below the mean; positive, when above. P: probability to find z-scores by chance. N: number of carcasses.

Discussion

Environmental Factors and Decomposition Stages

In general, the environmental conditions and the decomposition stages of the carcasses exposed to the open environment did

not differ from those that had been observed in previous works conducted in biogeoclimatic zones close or similar to those of this study (Centeno et al. 2002; Mise et al. 2007, 2008; Battán-Horenstein and Linhares 2011; Zanetti et al. 2015a,b). As expected, the carrion decomposition time was directly affected by temperature, humidity, and rainfall (Reed 1958), factors closely associated with the season. The fauna associated with this particular type of resource also tends to be affected by the set of environmental factors and the decomposition process (Campobasso et al. 2001). This is because when the carcass decomposes more slowly, it will remain in the environment and be explored for a longer time (Moura et al. 2005), both by a greater number (abundance) and by a more diverse group (diversity) of individuals, which coincides with what was observed in this study.

An unusual decomposition stage, called adipocere-like, has been documented in some studies that have been conducted in southern Brazil (e.g., Moura et al. 1997, Souza et al. 2008). This stage can be characterized, mainly, by the abrupt halt of the usual march of decomposition, caused by the combination of low temperatures (< 10°C) and high relative humidity (>90%). This results in the production of a viscous putrefying material that is not very attractive to insects, in addition to becoming an inappropriate substrate for the development of their immature. None of the carcasses exposed in the cold/wet season reached the adipocere-like stage because the environmental conditions were not suitable for this. However, it is important to be aware of this possible occurrence in this biogeoclimate zone, since it can affect the abundance and diversity of the colonizing fauna.

Abundance and Diversity of Coleoptera

Usually, information on Coleoptera of forensic importance is scarce and lacks standardization as to the type of animal model used, the environment where the experiments are conducted (considering the rural, urban, and wild classifications) and the techniques of sample collection. There is even precariousness in the identification of species and their eating habits. Depending on the question to be answered, pig carcasses still appear to provide the most comprehensive

experimental basis for empirical studies in forensic entomology (Matuszewski et al. 2020). However, the animal model must not be dissociated from the other points that may allow the reproducibility of results.

In the biogeoclimatic zone in which this study is inserted, approximately 40% of the surveys on local fauna were carried out on the carcasses of rats or rabbits (Moura et al. 1997, 2005; Souza et al. 2008; Silva and dos Santos 2012; Costa-Silva et al. 2017). In some cases, only the abundance data may be useful, due to the fact that the ratio between the amount of insects and the body mass of the animal can be easily extrapolated. Fauna diversity, on the other hand, seems more difficult to compare between different animal models and among distinct environments due to the degree of interference that this can exert on adult insects (Campobasso et al. 2001). Regarding the total abundance of Coleoptera, our findings did not differ proportionally from those observed by Mise et al. (2007, 2008), Battán-Horenstein and Linhares (2011), Silva and dos Santos (2012) and Costa-Silva et al. (2017). Silva and dos Santos (2012) observed that the abundance also did not differ significantly between seasons, in addition, both the climatic conditions and carcass decomposition intervals proved to be very similar to those found in this study. In contrast to our observations, Battán-Horenstein and Linhares (2011) and Costa-Silva et al. (2017) recorded higher abundances in the summer, which corresponds to season hot/dry. Other studies (Centeno et al. 2002; Souza et al. 2008; Zanetti et al. 2015a,b) did not provide abundant data.

In Brazil, between 10 and 19 families and between 15 and 88 species of Coleoptera have been associated with decaying corpses and carcasses (Almeida et al. 2015). The number of families and taxa sampled in this study were within the expected limits and exceeded what was observed in other surveys carried out in the same region such as Moura et al. (1997) (eight taxa), Centeno et al. (2002) (nine taxa), Souza et al. (2008) (two taxa), Battán-Horenstein and Linhares (2011) (11 taxa), Silva and dos Santos (2012) (25 taxa), and Zanetti et al. (2015a, b) (38 taxa). These variations in diversity among several studies may be associated with the non-pig animal
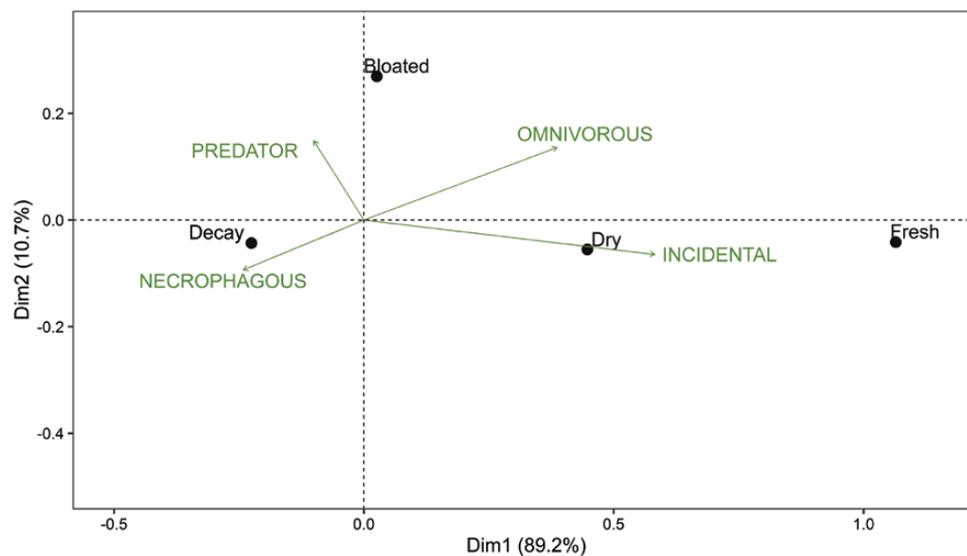
Fig. 2. Multivariate correspondence analysis (MCA) showing the degree of correspondence between beetles of different ecological categories (in green) and the decomposition stages (in black) of exposed carcasses in southern Brazil. Note: species of beetles present in the plot represent each of the ecological categories observed in this study, where: necrophagous = *Dermestes maculatus*, omnivorous = *Ataenius picinus*, predator = *Euspilotus azureus*, and incidental = *Cycloneda sanguinea*. The square cosine and the contributions are shown in SuppTable S1 (online only).

model (Moura et al. 1997, Souza et al. 2008, Silva and dos Santos 2012) and the type of environment in which the carcasses or corpses were exposed, by combining both factors or by another variable (e.g., sampling methodology). Our study was conducted in a predominantly rural environment, as well as those by Battán-Horenstein and Linhares (2011) and Zanetti et al. (2015a,b), while the others were performed in urban (Silva and dos Santos 2012) or in the wild (Moura et al. 1997; Centeno et al. 2002; Moura et al. 2005; Mise et al. 2007, 2008; Souza et al. 2008; Costa-Silva et al. 2017) areas.

The choice of techniques that will be used for the collection of organisms must take into account, which is the most appropriate way to sample the target fauna and, if applicable, even choose a combination of methodologies. For example, Mise et al. (2007) pointed out sampling differences taking into account the collection method used. The authors, who sampled 112 taxa on pig carcasses using different collection methodologies, reported that Staphylinidae was more abundant in the modified Shannon trap, while Silphidae was more abundant in pitfall traps. Studies that used a single collection strategy (Centeno et al. 2002, Battán-Horenstein and Linhares 2011, Zanetti et al. 2015a,b), which was not always the most appropriate for beetles (Souza et al. 2008), showed diversity well below expected. Therefore, in this study, we chose to use three different sampling methodologies.

As for the identification of specimens, a considerable number of studies has been restricted most of the time or only up to the family level (Moura et al. 1997; Mise et al. 2008; Battán-Horenstein and Linhares 2011; Silva and dos Santos 2012; Zanetti et al. 2015a,b). Thus, what has now been called by some authors as a 'species' is understood as a 'taxa' and this may not adequately reflect the ecological category in which it fits, since within the family taxon species with different eating or behavioral habits can be found. Incomplete or inaccurate identification undoubtedly damages the understanding and comparison of diversity among different studies, which in themselves can also present their regional differences. Of course, the difficulty in identifying more specifically one of the most diverse insect orders like Coleoptera is understandable. However, it also draws attention to the need to expand knowledge about taxonomy, the number of specialists, and alternative tools, such as molecular (Malewski et al. 2019), to overcome the taxonomic impediment of insects of forensic interest.

As observed in the other studies (Battán-Horenstein and Linhares 2011), abundance and diversity differed significantly among the decomposition stages, with the beetles predominantly distributed in the later stages (decay and dry). The fauna composition, in particular among the most abundant species and families, was quite similar to what has been seen in other studies. Intriguingly, *O. discicolle* (Silphidae) and any species from the families Silphidae, Carabidae, and Dermestidae were not collected in the carcasses exposed by Centeno et al. (2002), Battán-Horenstein and Linhares (2011), and Silva and dos Santos (2012).

Ecological Categories and Succession

At least 30 taxa, which represents 79.7% of the Coleoptera sampled in this study, may have some forensic relevance in estimating the PMI. It is possible to support this statement because, according to Smith (1986), some ecological categories are directly associated with the corpse (necrophagous and omnivorous), or with the fauna that consumes this food resource (predators and parasites). Incidental taxa were excluded, of course, due to the lack of predictability with which they reach a corpse. In contrast to what was observed in other research conducted under similar climatic conditions (Centeno et al. 2002; Mise et al. 2007, 2008; Battán-Horenstein and Linhares 2011; Zanetti et al. 2015a,b), in this study a high number of incidental species were collected, particularly from the family Chrysomelidae. This was due to the favorable conditions for the permanent presence of the species considered, in most cases, as agricultural pests, in the environment in which the carcasses were exposed (Milléo et al. 2013).

Regarding total abundance and eating habits, in increasing order of importance were necrophagous (43.3%), predators (31.6%), and omnivorous (0.05%). With slightly different percentages, this same trend was observed within each season, in agreement with the observations made by Zanetti et al. (2015b), but differently from what was recorded by Mise et al. (2007) and Costa-Silva et al. (2017), in whose studies predators were the most dominant fauna (>50%). Except for Zanetti et al. (2015b), among the necrophagous and at the family level diversity was low, as has been observed in other studies (Centeno et al. 2002; Mise et al. 2007, 2008; Battán-Horenstein and Linhares 2011; Costa-Silva et al. 2017). On the other hand, the predators, although less abundant, were evidently the most diverse. Predators of necrophagous insects are more generalist (Souza and Linhares 1997) and competition for exploiting available resources more efficiently, probably favors diversification in this micro-ecosystem. Factors such as temperature, humidity, and rainfall are known to alter the physical state of corpses (Catts and Goff 1992). In our study, we evidenced that the climatic conditions and the physical state of the carcasses, in both seasons, were not favorable to colonization by dipterans, which are, in general, the most abundant fauna in the carcasses (Carvalho et al. 2000). This decreases the supply of food for predatory beetles and favors the exploitation of the resource by the necrophagous.

The succession differed from other studies at the family level (Centeno et al. 2002; Battán-Horenstein and Linhares 2011; Zanetti et al. 2015a,b). As recorded by Mise et al. (2008), we did not observe a species substitution along the carcass decomposition, but a species addition process, where most species did not show a clear occupation limit between the different decomposition stages. In the hot/dry season, there was no faunal succession; in the cold/wet season, the succession was more linked to differences in abundance than to the presence/absence of a specific taxon by stage of decomposition. Furthermore, as reinforced by the MCA, the colonization order followed the patterns usually observed in terms of the ability to exploit the food resource: predators/ necrophagous > necrophagous/ predators/ omnivorous > predator/ necrophagous/ omnivorous. As is well documented in the literature, it is this set of observations that can support the PMI estimate (Byrd and Castner 2010).

The Value of Species for the Forensic Field

Dermestes maculatus is one of the most widely reported necrophagous species in the Neotropical region, particularly in the locations with biogeoclimatic characteristics similar to those reported in this study (Carvalho et al. 2000, Centeno et al. 2002, Mise et al 2007, Segura et al. 2009, Battán-Horenstein and Linhares 2011, Aballay et al. 2012, Costa-Silva et al. 2017). This species usually consumes muscles, tendons and the dry integument, so it is more commonly associated with the later decomposition stages; it can also be found in burned remains (Zanetti et al. 2019). Zanetti et al. (2016) studied the effect of temperature on the number of larval instars of *D. maculatus* and found a wide variation among populations of different origins, which demonstrates that biological studies on this species need to be further expanded, investigating its use in the PMI estimate. Another necrophagous species, *N. rufipes*, is commonly seen in decaying corpses concomitant with the presence

of *D. maculatus* (Carvalho et al. 2004), showing preferences for warmer months (>25°C) (Battán-Horenstein and Linhares 2011). Both species, *D. maculatus* and *N. rufipes*, have been reported colonizing and feeding on decaying human remains (Carvalho et al. 2000).

Euspilotus azureus, like other species of Histeridae, is a voracious predator of Diptera eggs and larvae (Mise et al. 2007). It has a wide distribution throughout South America and is present in urban, rural, and wild environments (Aballay et al. 2012). It can be found alongside other predatory beetles such as *Galerita* spp., *Pheropsophus* spp. (Carabidae) and *Aleochara* spp. (Staphylinidae), and is among the most abundant species collected in carcasses, throughout the year and at all the distinct decomposition stages (Mise et al. 2007). Caneparo et al. (2017) evaluated the influence of different temperatures on the life cycle of *E. azureus* and concluded that its optimal development range is between 20 and 25°C. This probably explains why no specimens were found in the season hot/dry season, in our study.

Oxelytrum discicolle has a distribution very similar to that recorded for *E. azureus* in South America (Lira and Vasconcelos 2016). The species can be found in urban and rural environments, but the greater abundance associated with carcasses exposed in forests has shown a certain preference for this environment (Carvalho et al. 2000). Adults feed mainly on the decomposing substrate, but they can also be seen preying on dipteran larvae, while their immature ones are exclusively necrophagous (Oliva and Di-Iorio 2008). In several locations in Brazil, it has been seen since the early stages of decomposition (Carvalho et al. 2004, Mise et al. 2007, Costa-Silva et al. 2017), but Lira et al. (2019) demonstrated that the species has a marked preference for the most advanced stages, as well as for the rainiest periods. In our study, we confirmed the preference of this species for the more advanced stages (decay and dry) and for the rainiest season (cold/wet). The report of this species in human corpse (Lira et al. 2020) reinforces its importance in the forensic area.

Conclusions

The family and the taxa sampled here were within the expected limit for beetles associated with carcasses in this biogeoclimate zone, which encompasses Brazil. The use of multiple collection strategies probably contributed to this successful sampling. The highest abundance and diversity of beetles were recorded in the cold/wet season. However, the abundance was not significantly different between different seasons; the average number of beetles collected per day was quite similar in both. Carcasses exposed at the cold/wet season took three times longer to decompose than those exposed at the hot/dry season.

In contrast to what was found in some studies, the necrophagous fauna was more representative than those classified as predators and omnivorous. In the hot/dry season, there was no faunal succession. In the cold/wet season, the succession was more associated with differences in abundance than to the presence or absence of a specific taxon by the decomposition stage. Considering all the factors analyzed in the current study, three species of beetles, *Dermestes maculatus* (Dermestidae), *Euspilotus azureus* (Histeridae), and *Oxelytrum discicolle* (Silphidae), could be identified as being of the greatest forensic relevance in this biogeoclimatic zone.

Based on what we have seen, future studies to expand a database should consider: 1) standardizing the use of a unique and widely accepted animal model among those working with forensic entomology; 2) using more appropriate collection methods to sample the target organisms of the study; 3) evaluating possible interference of the type of environment in faunal diversity; 4) the need to invest in taxonomic efforts to identify species.

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