On carrion-associated beetles in the Sonian Forest (Belgium): observations on five deer carcasses

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On carrion-associated beetles in the Sonian Forest (Belgium):
observations on five deer carcasses

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Abstract

Carrion is an important element of temperate ecosystems, although far less studied than dead and decaying wood. In order to avoid competition, insects need to detect and colonize ephemeral resources like carrion quickly. The knowledge of this colonization is an important tool in forensic entomology. Hence, species identification is a most crucial factor. Among insects, most studies have concentrated on flies (Diptera), whereas beetles (Coleoptera) were neglected for a long time. Beetles, however, have forensic applications – they are part of the entomofaunal colonization of a carcass – and therefore recently more extensive research on forensic application of beetle data has been done. Beetle families with significance to forensic entomology are Silphidae, Staphylinidae, Histeridae, Trogidae, Dermestidae, Cleridae, Nitidulidae and Carabidae. This study documents the presence of beetles associated with decaying carcasses of deer in the Sonian Forest, Belgium. A total of 271 specimens of Coleoptera belonging to twenty-five species in six families was recorded. The number of species and specimens increased from the fresh stage to the bloated stage and active decay, to drop back down at the dry/remains stage. A discussion is given about some interesting collected species and families.

Keywords: Capreolus capreolus, cadaver ecosystem, carrion-associated insects, Coleoptera, decomposition, forensic entomology, Sonian Forest.

Introduction

Forensic science is a multidisciplinary field (MILDENHALL et al., 2006). One domain is that of forensic entomology, applying the study of insects and other arthropods to criminal investigation. So far, only recently more extensive research on forensic application of beetle data has started (DEKEIRSSCHIETER et al., 2011b; MATUSZEWSKI, 2012, MATUSZEWSKI et al., 2008, 2010, 2011; MATUSZEWSKI & SZAFALOWICZ, 2013). Carrion (whether human or animal) is often referred to as a functional ecosystem (BARTON et al., 2013; VILLET, 2011; COLLIN, 2014). It is protein-rich, resulting in a variety of organisms consuming and fragmenting it (VILLET, 2011). The actual decomposition of a carcass starts with the actions of microorganisms such as bacteria and fungi, followed by a succession of arthropods. The time of disintegration has been positively correlated to the presence of insects (PAYNE, 1965). The so-called "microclimatic patch" is characterized by increasing temperatures, which are generated in the early stages of decomposition primarily through autolysis, the activities of (anaerobic) bacteria, and insect activity (HEWADIKARAM & GOFF, 1991; MELIS et al., 2004). Depending on the temperature, humidity, body volume and activity of scavengers, the stages of the decomposition – fresh, bloated, active decay, advanced decay, dry/remains (ANDERSON et al., 1996; HORENSTEIN & LINHARES, 2011; MATUSZEWSKI et al., 2008; PAYNE, 1965; SCHILTHUIZEN & VALLENDUUK, 1998) – can take hours to many months. Of course the range of species diversity on
decomposing carcasses depends on many factors, of which biotic and abiotic conditions of the habitat (humidity of soil, tree cover, etc.), the weight of carcasses, and the presence or absence of large vertebrate scavengers can provoke differences (SELVA et al., 2005; MATUSZEWSKI et al., 2008; GU, 2014). The many interacting factors make it difficult to reliably assess the different decomposition stages (SHATTUCK, 2009). During this process each stage is more or less attractive to different insect species as a result of both physical and chemical changes in the carrion, providing the ideal habitat for certain small-bodied organisms to lay eggs and/or feed (HORENSTEIN & LINHARES, 2011). MELIS et al. (2004) refer to carcass plots as unstable ecological communities. The carrion-associated community is moderately species-rich and may contain a few dozen species at any one time (VILLET, 2011). The knowledge of this colonization is an important tool in forensic entomology (CENTENO et al., 2002), the major use of which is to estimate the time of death or the minimal post mortem interval (PMI) of a corpse, based on the insects colonizing the dead body. When estimating a (minimum) PMI, we need to take into consideration the time it has taken for the insects to reach the body for laying eggs as well as the rate of development.

Species identification is a key element in forensic entomology. Species that appear almost the same to the naked eye may have different growth rates, behaviors and habitat preferences. Among the insects, especially Diptera have been given the greatest value in the process of decomposition and therefore are vital in the study of forensic entomology. Flies arrive very soon after death; in optimal conditions they can deposit eggs in the few minutes following death (GOFF, 2010). Beetles, however, exhibit huge variations in speed of arrival on a corpse (WANG et al., 2008). Only recently extensive studies were undertaken with beetles and promising results were obtained by data modeling (MATUSZEWSKI, 2011, 2012; MATUSZEWSKI & SZAFALOWICZ, 2013). The beetle community is an important part in the carrion-associated fauna (ANDERSEN & VANLAERHOVEN, 1996; HORENSTEIN & LINHARES, 2011; MIDGLEY, 2007); according to GENNARD (2012), beetle families with significance to forensic entomology are Silphidae (carrion beetles), Staphylinidae (rove beetles), Histeridae (clown beetles), Trogidae (trogid beetles), Dermentidae (hide and skin beetles), Cleridae (checkered or bone beetles), Nitidulidae (sap-feeding beetles), and Carabidae (ground beetles).

Our survey was conducted in the Sonian Forest, Belgium and focused on this beetle community associated to carcasses. To our knowledge, in Belgium, so far only one study has been published, identifying carrion-associated Coleoptera (DEKEIRSSCHIETER et al., 2011a). The latter focused solely on the Silphidae, which promote breakdown and recycling of organic matter into terrestrial ecosystems and therefore perform vital ecosystem functions (WOLF & GIBBS, 2004). Seven species were reported on six decaying pig carcasses (Sus domesticus L.) during spring: Necrophorus humator, Necrophorus vespilloides, and Necrophorus vespilloides [subfamily Nicrophorinae]; Necrodes liitoralis, Oiceoptoma thoracicum, Thanatophilus rugosus, and Thanatophilus sinuatus [subfamily Silphinae].

**Material and methods**

**Site description**

The present study was done at the Sonian Forest (Zoniënwoud, Forêt de Soignes, Soniaca Silva), located at N50°46'17" E4°25'22", a 4,421 ha forest situated southeast of Brussels. It is part of the scattered remains of the ancient Silva Carbonaria (DUVIVIER, 1862). The forest is situated in the Flemish municipalities Sint-Genesius-Rode, Hoeilaart, Overijse and Tervuren, the Brussels-capital region municipalities Ukkel, Watermaal-Bosvoorde, Oudergem and Sint-Pieters-Woluwe, and the Walloon municipalities La Hulpe and Waterloo. The Sonian Forest is an acidophilic European beech (Fagus sylvatica) and oak (Quercus sp.) forest (GRYESELS, 2003). Most of the trees are over 200 years old. The mammalian fauna formerly comprised 49 native species, seven of which have gone extinct since 1000 (VANWIJNSBERGHE, 2003; G. REINBOLD, pers. comm.).

**Material examined**

Five carcasses of roe deer, Capreolus capreolus (L.), all road-killed, were placed in fresh stage at different sites: an open beech forest (ZO1), a half-open oak forest (ZO2), a half-open mixed forest (ZO3), and a closed beech forest (ZO4) (Table 1; Fig. 1 A, B, C). Site ZO2 served for two carcasses. Depending on weather conditions, carcasses were sometimes collected and put in the refrigerator
before exposure; this explains the different time of death and date of carcass exposure in ZO4.
The carcasses were attached to the ground with one or more metal pins to prevent being dragged away by scavengers. Previous experience had learned that large vertebrate scavengers sometimes interfere and drag away carcasses/remains. As a result, the carcass at ZO4 was placed in a metal cage preventing being dragged away. Eight simple pitfall traps [10 cm (diameter) x 8 cm (height)] were positioned at the moment of carcass placement, always in a circle up to 50 cm around each carcass. Pitfall traps were without liquid because the beetles had to be caught alive (specimens recognized as rare and/or protected were identified in the field and released, as collection happened under jurisdiction of the Flemish Government).
First the presence of insects was noted, while the carcass remained untouched. After some ten minutes, the legs and head were lifted and finally the whole carcass was moved (and afterwards placed back) to collect Coleoptera underneath the carcass. Decomposition stage was assessed visually, after the body was turned. Pitfall traps were emptied on four occasions between 19.V.2010 and 13.VIII.2010 (Table 1), while inspecting the carcass. We did not make a distinction between species hand-sampled under the carcasses and found in pitfall traps. Only adult stages were included in this study.
All insects – except for species immediately recognized as rare and protected (e.g. Carabus auronitens Fabricius, 1792) – were put into 90% ethanol for storage and identified to species level or at least genus level using appropriate keys: BOEKEN et al. (2002), FREUDE et al. (1979), JANSSSENS (1960), LOHSE (1964, 1974), SCHILTHUIZEN & VALLENDUUK (1998). Observations and identification happened under a Leica MZ16 stereomicroscope at 10-115x. Classification and taxonomy are consistent with VORST (2010).
The collected material is deposited at the National Institute for Criminalistics and Criminology (NICC), Brussels.

Table 1. Overview of study sites, sampling scheme, and decomposition stages during sampling.

<table>
<thead>
<tr>
<th>Site</th>
<th>Vegetation</th>
<th>Death of animal</th>
<th>Date of carcass exposure</th>
<th>Sex</th>
<th>Weight [kg]</th>
<th>Metal cage</th>
<th>Sampling Date [days since exposure]</th>
<th>Decomposition stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZO3</td>
<td>half-open mixed forest</td>
<td>7.V.2010</td>
<td>7.V.2010</td>
<td>male</td>
<td>24.30</td>
<td>absent</td>
<td>16.VI.2010 [40]</td>
<td>dry/remains</td>
</tr>
</tbody>
</table>

Results

The sites were inspected at 19.V.2010 (ZO1 and ZO2), 16.VI.2010 (ZO2, ZO3), 6.VIII.2010 (ZO4), and 13.VIII. 2010 (ZO4); the carcasses were found in different stages of the decomposition process (Table 1).
A total of 271 Coleoptera were collected, belonging to six families: Carabidae, Cleridae, Geotrupidae, Histeridae, Silphidae, and Staphilinidae. Twenty-five species were recorded; all specimens but one (Oxypoda sp.) were identified to species level. An overview of the number of specimens and the number of species per family is given in Table 2. The number of species as well as the number of collected specimens increased from the fresh stage to the bloated stage and active decay, and dramatically declined toward the dry/remains stage (Fig. 2).
The most abundant family was Geotrupidae (n = 140), but none were collected at 16.VI.2010 (ZO2, ZO3; both dry/remains). All individuals found during other samplings belong to the same species, Geotrupes stercorarius. The Cleridae family was the least abundant, with one specimen collected, Necrobia violacea, only observed during the dry/remains stage (ZO2, 16.VI.2010). Silphidae was the most diverse family, with nine representing species: Nicrophorus humator, N. investigator, N. vespillo, and N. vespilloides [subfamily Nicrophorinae]; Necrodes littoralis (Fig. 1D), Oiceoptoma thoracicum, Phosphuga atrata, Thanatophilus rugosus, and T. sinuatus [subfamily Silphinae].
Fig. 1. A. Carcass ZO2 in fresh stage (sampled on 19.V.2010), showing one pitfall trap filled with four Geotrupes stercorarius beetles. B. Carcass ZO4 in active decay (sampled on 13.VIII.2010), with a high number of maggots feeding on the corpse. C. Carcass ZO3 in dry stage (sampled on 16.VI.2010); all that remains was hard, dry skin, cartilage, and bones. D. A specimen of Necrodes littoralis, preying on a maggot. This species was only found in this study during active decay = on carcasses ZO1 (19.V.2010) and ZO4 (13.VIII.2010). Photos by Danny Haelewaters (A, B, C) and René Krawczynski (D).

Fig. 2. Number of species and specimens, given for each sampling.
Table 2. Complete list of Coleoptera species sampled during the study of five carcasses of deer in the Sonian Forest (Zoniënwoud), Belgium. Families and species names are placed in alphabetical order. For each species the feeding strategy is given (DEKEIRSSCHIETER et al., 2011b; KOČÁREK, 2003).

<table>
<thead>
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</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>active decay</td>
<td>fresh</td>
<td>dry/remains</td>
<td>dry/remains</td>
<td>bloated</td>
<td>active decay</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>1</td>
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</tr>
<tr>
<td></td>
<td>Carabus nemoralis Müller, 1764</td>
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<td>1</td>
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<td>1</td>
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<tr>
<td></td>
<td>Carabus problematicus Herbst, 1786</td>
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<td>5</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>2</td>
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<tr>
<td></td>
<td>Carabus violaceus Linnaeus, 1758</td>
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<td>2</td>
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<td></td>
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<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
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<tr>
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<td>Necrobia violacea (Linnaeus, 1758)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Geotrupidae</td>
<td>Geotrupes stercorarius (Linnaeus, 1758)</td>
<td>1</td>
<td>12</td>
<td>75</td>
<td>52</td>
<td>140</td>
<td></td>
<td></td>
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<td>Hypocaccus rugifrons (Paykull, 1798)</td>
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<td>Margarinus striola (Sahlberg, 1819)</td>
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<td>6</td>
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<td></td>
<td>Saprinus semistriatus (Scriba, 1790)</td>
<td>25</td>
<td>1</td>
<td>26</td>
<td>26</td>
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<tr>
<td>Silphidae</td>
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<td>6</td>
<td>11</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td>predator, necrophagous</td>
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<tr>
<td></td>
<td>Nicrophorus humator (Gleditsch, 1824)</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>8</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Nicrophorus investigator Zetterstedt, 1824</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td></td>
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<td>1</td>
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<td>13</td>
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<td></td>
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<tr>
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<td></td>
<td>1</td>
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<td></td>
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<td></td>
<td>Oxypoda sp.</td>
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<td></td>
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<td></td>
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<tr>
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<td>Philonthus politus (Linnaeus, 1758)</td>
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<td>predator</td>
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<tr>
<td></td>
<td>Philonthus succicola Thomson, 1860</td>
<td>1</td>
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<td>Philonthus tenacicornis Mulsant &amp; Rey, 1853</td>
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<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Discussion

General findings

It is worth studying carcasses, at least if no artificial interventions occur: the body should remain as intact as possible, free from any human disturbance. This way large numbers of insects and scavengers can be observed and an idea of the natural colonization process can be attained.

In this study 271 specimens were collected. In comparison, DEKEIRSSCHIETER et al. (2011a) on pig carcasses found a much higher number of silphid specimens (n = 1579). This difference can be attributed to a number of reasons: 1) the sampling protocol, 2) the frequency of sampling and 3) larger carcasses provide resources for more insects (GU et al., 2014). During our study we did not fill the pitfall traps with soapy water or alcohol. The use of such “living traps” probably contributed significantly to the low number of collected specimens, since larger Carabus spp. and Staphylinidae will eat many of the smaller beetles (W. DEKONINCK, pers. comm.). This also means...
that the contents of the traps probably only accounted for the 1-2 days before emptying, not the entire period in between collections.

Four beetle species stand out because of their higher number of collected individuals during this survey. These species are Geotrupes stercorarius (Geotrupidae, n = 140), Saprinus semistriatus (Histeridae, n = 26), Carabus violaceaeus (Carabidae, n = 23), and Nicrophorus vespilloides (Silphidae, n = 23). This does not necessarily imply that the other species reported in this study are scarcer, but they were in any case much less attracted by the carrion bait. It is also possible that the period of activity for some species is prior to May-June. As to C. violaceaeus, it is proposed that it is not a very selective species, since significant numbers are also found in pitfall traps filled with peanut butter (W. Troukens, pers. comm.).

Melis et al. (2004) found that both staphylinid Creophilus maxillosus and silphid Nicrophorus humator are carrion-dependent, i.e. their activities concentrated on the carcass itself and reaching the carcass by flying directly to it. Further research is needed to confirm this. Tantawi et al. (1996) observed Creophilus maxillosus only in the dry/remains phase, but Horenstein & Linhares (2011) found both adults and larvae throughout the decay process. We can confirm with the latter study, i.e. C. maxillosus is not restricted to the dry/remains phase, but most often found as soon as maggots have hatched.

Silphidae

In our study, a total of nine species of carrion beetles were recorded (n = 54), adding Nicrophorus investigator and Phosphuga atrata to Dekeirsschieter et al.’s (2011a) data on Silphidae. Nicrophorus vespillo was recorded only once (ZO4, 6 Aug 2010). Scott (1998) lists it as an early summer breeder (May-July), which is the period for most of our samplings. The restricted occurrence of N. vespillo in our study may be due to its almost exclusive preference for open meadow habitat (Kentner & Streit, 1990; Kočárek, 2003; Otronen, 1988) and the fact that it is now less common in comparison to N. investigator and N. vespilloides (7.5%, 15.5%, and 77%, respectively; Otronen, 1988). Phosphuga atrata is an accidental species on carrion, feeding on molluscs (Kočárek, 2003). Oiceoptoma thoracicum as well as Nicrophorus humator and N. vespilloides are typical forest-inhabiting species (Dekeirsschieter et al., 2011a; Kentner & Streit, 1990; Kočárek, 2003; Scott, 1998).

So far, little is known about possible preferences of beetles associated with decomposing cadavers of specific species (Schilthuizen & Vallenduuk, 1998). Yet, the suggestion that species of the genus Nicrophorus are depending only on rodent carrion (e.g. Meierhofer et al., 1999) or small carcasses in general (Müller, 1987; Smith & Merrick, 2001) seems unlikely since we found thirty-three individuals of Nicrophorus during our study. During a decomposition study in nature reserve De Kaaiastoop (Tilburg, the Netherlands), Van Wielink (2004) also reported Nicrophorus upon carcasses of deer and fox. Moreover, during the Necros Project in Brandenburg, East Germany (see Gu et al., 2014), N. humator was found at a 140 kg red deer carcass (R. Krawczynski, pers. obs.). The explanation of this discrepancy lies in the fact that Nicrophorus hunts on large carcasses and breeds under small ones (Dekeirsschieter et al., 2011b).

Kočárek (2003) found that Thanatophilus rugosus and T. sinuatus show exclusive preference for open field habitats, but we recorded both species in beech forest (T. rugosus: ZO1, 19 May 2010; T. sinuatus: ZO4, 13 August 2010). We also found both species during another study in a pine forest in Brandenburg (Germany). The identification of species in the genus Thanatophilus is not evident. Thanatophilus rugosus is recognizable by its wrinkled elytra. Thanatophilus dispar Herbst, 1793 and T. sinuatus, however, are difficult to distinguish. Since T. dispar is rare (since 1960 only two observations in the Netherlands, no observations found in Belgium; Schilthuizen & Vallenduuk, 1998), we may suggest our specimens (ZO4, 13 August 2010) to be T. sinuatus. Not the identification key but the species-specific shape of the seventh tergite was conclusive (i.e. with semicircular notch as in T. sinuatus).
Geotrupidae

The family Geotrupidae comprises coprophagous species; they are associated with the dung of mammals. ZAGLER (2009) sampled Geotrupes stercorarius in pitfall traps baited with pig dung and human dung; DORMONT et al. (2004) sampled the same species in traps baited with cattle dung and horse dung, finding a significant preference for cattle dung. In this study on deer carcasses we collected G. stercorarius in high abundance, although not any individual was collected in the dry/remains stage. In a study in Brandenburg, however, the species is abundant at all carcasses (LYSAKOWSKI, 2013) and even attracted to large bones and tunnels underneath them. A wide array of bait sources have been used for sampling dung beetles (SPECTOR, 2006, and references therein), including different kinds of carrion, as well as dung, fungi, millipedes, and chicken eggs. More research is needed to fully comprehend the ecological specificities of G. stercorarius.

Suggestions for future research

Looking back on this study, we realize that more specimens could have been collected using a different sampling protocol (although in any case we would not have been allowed to collect protected nor rare species). One factor to improve our results (higher number of specimens, more species diversity) would be to empty the "living traps" every 3-4 days.

Although forensic entomology is used to aid murder investigations, only recently studies of the coleopteran colonization on individual carcasses have been made. Yet, faunal inventory data are desirably linked to the ecological coherence of the system studied. The knowledge obtained from this kind of research is necessary in determining faunal colonization related to stages of decomposition, to estimate the (minimum) post mortem interval (PMI) or Time of Colonization (Period of Insect Activity), and consequently the time of death (CENTENO et al., 2002).

A thorough comparative study between carrion beetles upon cadavers of deer, foxes, pigs and other species is needed, taking certain caution into account as beetles typically run away with the least interference (VAN WIELINK, 2004). Although standardized carcasses of the same size may bring results that are more easily comparable, studying differently sized carcasses may also be helpful, e.g., for examining the bodies of children. It is suggested that larger carcasses / corpses show different insect species (GU et al., 2014).

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