MEDICAL AND VETERINARY ENTOMOLOGY

Characterization of necrophagus entomofauna in a typical agricultural area in Emilia-Romagna region (Northern Italy)

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Abstract

This study presents a checklist of Dipterans and Coleopterans accountable for carrion decay in the Po Valley (Italy), a contribution to the Forensic Entomology knowledge in Italy. Insects colonizing two pig carcasses in an agricultural area in Mezzani municipality (Parma, Northern Italy) were sampled by pitfall traps and original Malaise-like traps, which allowed the sampling of a very relevant number of flying insects. A checklist of 57 taxa was obtained, of which 26 were considered of forensic importance. For the latter the arrival time of adult specimens on the carcasses was recorded, as an important parameter in minimum *post mortem* interval estimation. Dipterans (6141 specimens) were the most common insects; the fastest specimens to detect and colonize the carcass belonged to the Calliphoridae family, while Fanniidae and Muscidae infested the carrion until completion of the skeletal stage. Coleopterans appeared later (308 adult specimens and 114 larvae were captured). Staphylinidae, Dermestidae, and Histeridae were the most common coleopterans sampled in this study.

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Introduction

Forensic entomology is a subject that combines arthropod science and legal system (Amendt *et al*., 2007). It applies the identification and knowledge of insect and arthropod biology to prosecutions, investigations, and civil lawsuits (Byrd & Castner, 2001). One of the most important tasks of forensic entomology is the assessment of the minimum post-mortem interval (PMI).

Usually forensic entomologist estimates the PMI aging the immature insects sampled from a corpse, considering the species development time; in that case the use of insects colonizing carcasses shortly after death (particularly blowflies), allow a good approximation of PMI. This method is clearly inaccurate for insects that start breeding late in decomposition (like coleopterans); but the estimation of a pre-appearance interval (PAI) can enable the use of data concerning these insects (Matuszewski, 2012).

The PMI could be also estimated considering the whole carrion-arthropod community by a succession-based approach, that considers the presence of the different taxa with a *recognizable and predictable patterns of successional activity* on the remains (Schoenly, 1992).

Italy lacks consistent records of necrophagous insects and their connection with carrions. Environmental and microclimatic diversity of Italy prevents the use of the data obtained in the rest of Europe (Turchetto & Vanin, 2004). This study describes the insect colonization of carrion, supplying data on when and how colonization takes place, and provides valuable information to determine PMI in a rural area of Pianura Padana during summertime, providing also useful data in the dating of arthropod community and in the PAI estimation.

Materials and Methods

The experiment was carried out at Mezzani (Parma, Northern Italy), a village on the Po River near the mouths of Enza and Parma rivers, in a typical agricultural area in the Pianura Padana.

Two pig carcasses (*Sus scrofa domestica* L.), weighing 35 kg (M1) and 45 kg (M2) were used; they were laid at 7.30 pm on May 11 and

May 14, 2006, respectively (M1, lat: 44.913329°, long: 10.432047°; M2, lat: 44.912744°, long: 10.432180°). The animals had died of natural causes in a piggery on the same day. They did not have any drug administration for 3 days before their death. The two carcasses were laid in a alfalfa field (*Medicago sativa* L.), of about 3000 m², characterized by the presence of a row of vines, at east side, and a shrub hedge with hornbeam (*Carpinus betulus* L.), willows (*Salix* spp.) and maples (*Acer campestre* L.) on the south and west side (Figure 1). The M1 carcass was laid in more sunny condition than the M2, which was put near the hedge (orientation: north-south), under conditions of persistent shade from noon. Observations and sampling ended on June 12, 2006.

The weather station in Gainago (lat: 4.891827°, long: 10.399729°), managed by the Regional Environmental Agency (ARPA), supplied the authors with climatic data for the period. The environmental average temperatures varied from 11.2°C to 22.7°C (Figure 1), while the inner temperature was measured with an electronic max-min thermometer endowed with a probe. To protect the two carcasses from macrovertebrates, an electro welded iron cage (mesh 5×10 cm) with a 1.5×0.8 m base and 0.6 m of height was used. The cages had a self made Malaise-like trap on the top to capture adult flying insect, especially dipterans. The Malaise-like traps consisted of a 0.8 m high pyramid, formed by four wooden sticks (1.2 m long) carrying a mosquito net, with a funnel trap on the top (Figure 2).

Moreover, 8 pitfall traps, 5 cm wide and 8 cm deep, were set around one of the cages to capture the insects of forensic interest, coleopterans above all.

The carcasses were examined twice a day to gather samples, measure internal temperature, and note the degradation stage. The insects captured were killed by freezing at –20°C for 40 min, then kept under alcohol at 70°C, and catalogued in the laboratory according morphological keys (Vienna, 1980; Smith, 1986; Peacock, 1993; Rozkolosny *et al*., 1997; Hastir & Gaspar, 2001; Frantisek *et al*., 2002; Oosterbroek *et al*., 2006).

All samples were stored at the Istituto Zooprofilattico della Lombardia e dell'Emilia-Romagna, Reggio Emilia Section.

Results

The use of Malaise-like traps allowed capturing a total of 6141 adult dipterans (Table 1).

From the first moments of deposition until the end of the experiment, both carcasses attracted blow flies (Calliphoridae), especially *Lucilia caesar* (L.), the most abundant species of this family on carcasses, but also the species *Lucilia sericata* (Meigen), *Phormia regina* (Meigen), *Calliphora vomitoria* L. and *Protophormia terraenovae* (Robineau-Desvoidy) (Table 1). Successively a relevant amount of flesh flies (Sarcophagidae), muscid flies (Muscidae) and other flies (Fanniidae), especially the species *Ophyra leucostoma* (Wiedemann) and *Phaonia errans* (Meigen), were sampled.

Both black scavenger flies (Sepsidae) and syrphes (Syrphidae) visited carcasses in the first days of exposure and their presence remained abundant. Many specimens of longlegged flies (Dolichopodidae) and moth flies (Psychodidae) were also captured (Table 1).

Some groups of adult Diptera showed different occurrence on the two carcasses: humpbacked flies (Phoridae), skipper flies (Piophilidae) and *Lucilia sericata*, were reported only on M2 while *Phormia regina* was reported only on M1. Other groups showed different abundance such as flesh flies (Sarcophagidae), *Ophyra leucostoma*, *Sepsis punctum* (F.) (Table 1). Significant differences were also noted among the total number of adult dipterans present on the two carcasses (Table 1), with a higher number sampled on M1 than on M2.

The first coleopterans sampled on M2, were the clown beetles (Histeridae), *Saprinus subnitescens* Bickhardt, and the carrion beetle (Silphidae), *Thanatophilus dispar* (Herbst). Next came the rove beetles (Staphylinidae), particularly *Creophilus maxillosus* (L.) and *Aleochara curtula* (Goeze); the other species *Ontholestes tessellatus* (Geoffroy), *Othius punctulatus* (Goeze), *Philonthus* spp, and *Padeuros* spp. were also present, but never reached a huge number (Table 2).

Later the colonization by other Histeridae species – *Saprinus semi-*

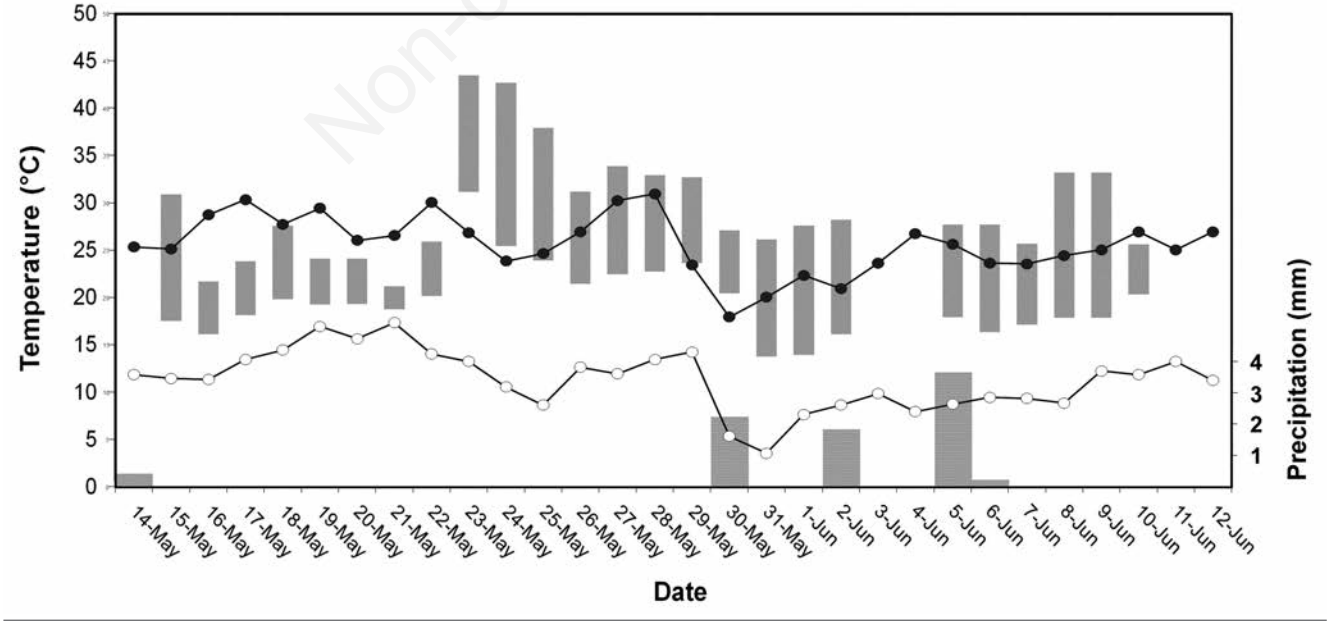

Figure 1. Weather conditions during the experiment (minimum and maximum temperatures plus precipitations) and internal temperature of carrion under the shade, M2 (gray bars).

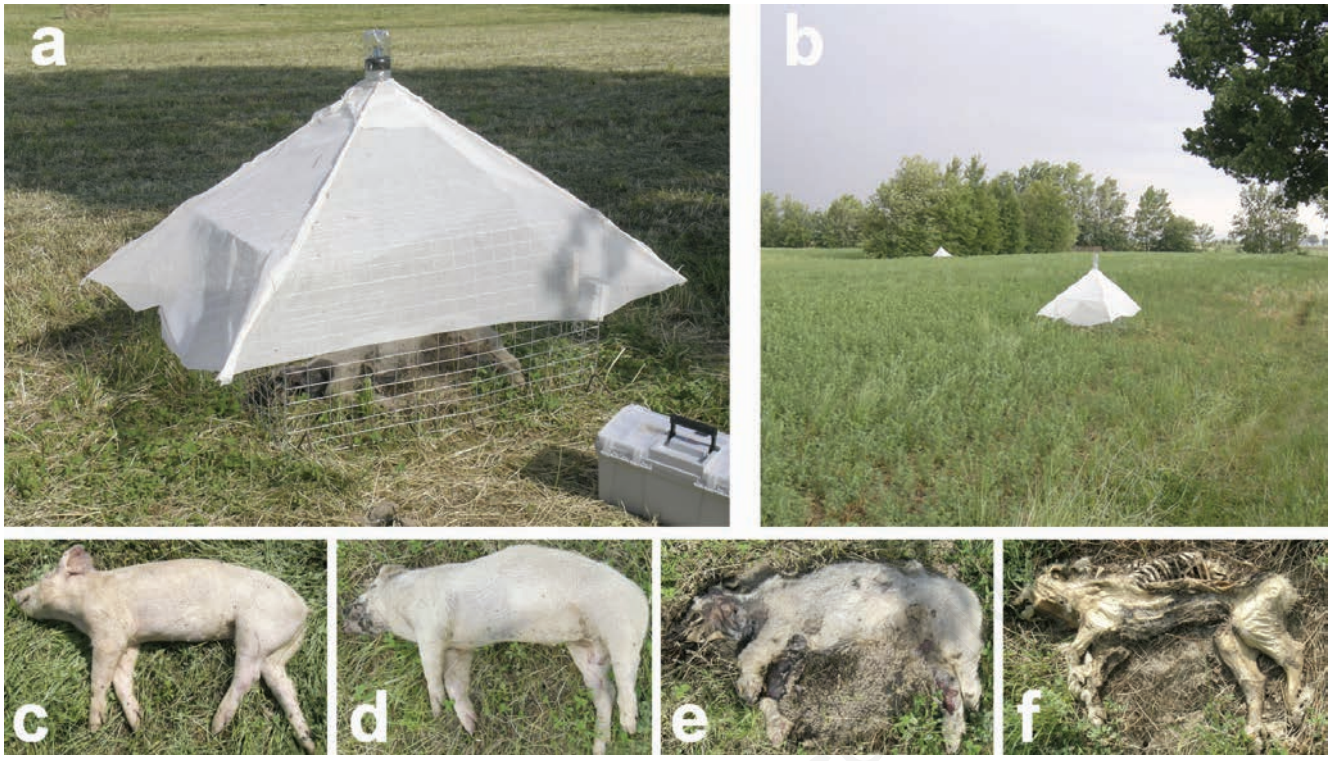

Figure 2. Malaise-like trap utilized in the experiment (a) and trap position in the study area (b). Carcass M1 at the day of exposure (c) and after 5 days (emphysematous stage) (d), 7 days (liquefactive stage) (e), 29 days (skeletal stage) (f).

punctatus (F.), *Margarinotus brunneus* (F.), and *Carcinops pumilio* (Erichson) – occurred. The first specimens of dermestid beetles (Dermestidae), *Dermestes frischii* Kugelann, appeared on the 7th day until the end of the experiment (Table 2, Figure 3).

The specimens belonging to *Dermestes undulatus* Brahm were not sampled through pitfall traps, but rather through direct inspection of the carcass at the end of the experiment. The PAI of the coleopteran species, with 20 or more specimens collected, was 3 days for *Thanatophilus dispar* and *Saprinus subnitescens*, with a ground-level temperature (T_g) average of 19.2°C, 5 days for *Creophilus maxillosus* (T_g average of 20.5°C), 6 days for *Dermestes frischii* (T_g average of 21.1°C), and 7 days for *Aleochara curtula* (T_g average of 21.1°C). The ground-level temperatures were obtained increasing daily ambient temperatures of 0.5°C (Matuszewski, 2012).

Other typical cadaverous insect were sampled with a few specimens: checkered beetles (Cleridae), *Necrobia violacea* (L.) and *Necrobia rufipes* (De Geer), and scarab beetles (Scarabaeidae) *Aphodius* spp. and *Onthophagus* spp.

Discussion and Conclusions

The study was focused on obtaining a check list of saprofagous insects, particularly those of forensic interest, and on describing the time of their first visit on the carcasses. A checklist of 56 taxa was obtained, of which 26 were considered of forensic importance.

More than an half of dipterans sampled during the experiment (Calliphoridae, Muscidae, Fanniidae, and Sarcophagidae) are reviewed in literature as common forensic key indicators to determine minimum PMI. Blow flies (*Lucilia caesar, Lucilia sericata, Phormia regina*) were the first to colonize of the carcasses (Table 1). The low number of

Figure 3. *Dermestes frischii* specimens mating on one of the carrions.

Protophormia terraenovae specimens collected could be due to the preference of this species for the urban areas. A relevant number of *Phormia regina* specimens was sampled, this species, attracted by large carcasses, showed a peak of appearance later than other Calliphoridae. *Lucilia caesar* was the most abundant collected species, demonstrating its adaptability to a typical cultivated area, scantily covered by trees, although literature (Baz *et al*., 2007; Vanin *et al*., 2008) usually reviews this species as typical woodland insect.

Muscidae were, among Diptera, the group with the largest number of specimens sampled. The species whose larvae become predators of maggots during the last stages of development were abundant (*Ophyra* spp., *Hydrotaea* spp., and *Phaonia* spp.), and *Ophyra leucostoma* was detected earlier in the experiment respect to literature data (Merz, 2005) (Table 1).

Other flies of forensic interest belonging to the Piophilidae and the Phoridae families (Smith, 1986) were captured only on M2 (Merz, 2005).

Table 1. Adult dipterans sampled on carrions M1 and M2.

Species	Day after placement (M1/M2)													
	1	2	3	4	5	6	7-9	10-12	13-15	16-18	19-21	22-25	25-28	Tot
Calliphoridae														
Lucilia caesar	20/5	17/7	16/25	1/3	35/24	19/11	2/5	2/9	0/3	7/6	5/0	0/2		124/102
Lucilia sericata		0/1	0/6	0/3	0/4	0/8		0/2	0/10	0/2	0/14	0/5	0/5	0/60
Phormia regia		1/0	1/0		13/0	32/0					3/0		1/0	51/0
Protophormia terranovae					0/3				1/3					1/6
Calliphora vomitoria				1/1			0/1							1/2
Sarcophagidae														
Sarcophaga spp.	4	6/1	4/3	1/0	8/0	17/2	4/2	5/11	2/4	2/4	4/4	1/0	0/1	58/32
Muscidae														
Phaonia spp.	3/12	31/11	21/5	20/0	3/0	7/11	11/17	19/14	0/4	0/16	5/9	0/2	0/2	122/101
Ophyra leucostoma		13/0	22/24	4	172/41	1324/0	49/2	21/11	0/10		5/1	0/4	7/0	1617/93
Hydrotaea ignava				1/5		27/4		0/7	3/0	12/11	3/2	0/3	0/1	46/33
Other species*		9/1	18/3	1/15	24/5	15/0	11/1	37/1	1/2	0/9	13/7	4/1	10/4	143/49
Fannidae														
Fannia spp.		3/0	8/9	2/3	27/6	72/10	40/3	24/106	6/22	13/59	20/174	7/12	11/27	233/431
Sepsidae														
Sepsis punctum	0/2	59/0	87/1	41/4	135/13	401/6	131/0	98/26		0/10	8/6		57/6	1017/74
Other species		1/0	1/0		3/0	3/0								8/0
Otitidae														
Seioptera vibrans		2/0	3/0		13/2	39/0	2/0							59/2
Physiphora alceae			1/0		4/0	9/0		2/0						16/0
Dolichopodidae														
Ortochile spp.	0/149	68/16	56/20	7/9	29/16	28/15	19/0	21/19	0/4	5/11	4/6	0/4	3/4	240/273
Milichiidae														
Meoneura spp.		1/0			7/0	11/0	10/0	3/0			1/0			33/0
Other species					2/0	5/0	2/0	4/0						13/0
Psycodididae														
Psycoda alternata			3/0					12/0	585/0	1/0	2/0			603/0
Syrphidae														
Sphaerophoria sp.			2/0	0/1	4/2	2/4	4/0	38/6	13/19	8/6	36/17	10/1	11/4	128/49
Other species			0/1		0/4	0/1	0/1	0/2					0/2	0/11
Piophilidae							0/4		0/3	0/12		0/6	0/28	0/53
Chyronomidae			7/0		1/0	5/0	2/0	22/0	41/0	5/0	20/0		13/0	116/0
Phoridae										0/1			0/2	0/3

Others collected family (specimens): Pipunculidae (5), Tephiridae (8), Ragionidae (3), Sciaridae (2), Drosophilidae (9), Empididae (12), Lauxaniidae (12), Lonchopteridae (1), Tachinidae (1), Sphaeroceridae (16), Stratiomyidae (2), Tabanidae (1), Tipulidae (6), Ceratopogonidae (12), Scatopsidae (3), Cecidomyiidae (30). *Among sampled Muscidae spp.: *Musca domestica, Musca autunnalis, Muscina assimilis, Muscina stabulans, Muscina pabulorum, Stomoxiys calcitrans, Graphomya maculata, Thricops separ*.

Between other sampled dipterans *Fannia* spp., *Sepsis punctum*, *Seioptera vibrans*, *Physiphora alceae*, *Sphaerophoria* sp. are reviewed as forensic indicator in same extent (Smith, 1986). Also Psychodidae was observed on decaying carcasses, but forensic entomology does not consider this family useful in PMI estimation (Erzinçlio lu, 1996). While forensic role are is not recognised to other sampled insects as chironomids or *Ortochile* and *Meoneura* specimens.

The present study highlighted a difference in abundance and visiting time of adult dipterans on the two pig carcasses, probably in large part due to the differences in shading conditions (Table 1). These differences could also explicated by the different length of putrefactive stages, largely influenced by the sun exposition of the two carcasses. Sarcophagidae together with *Hydrotaea ignavia* and *Phormia regina* species, with a known preference for sunlit conditions (Sharanowski *et al*., 2008; Smith, 1986), were more abundant on the M1, which was more exposed to the sun than M2 (Table 1). Conversely *Lucilia sericata* was sampled in shadow condition, suggesting a preference of this species for this condition, while heliophilia of blow flies was already described (Smith, 1986). Such observations suggest the need to consider all environmental conditions when estimating PMI for forensic purposes; even shade conditions and season (Sharanowski *et al*., 2008).

The beetles sampled were mainly predators; many of them feed on larvae laid by dipterans, although necrophagous and mixed-behaviour species are not infrequent. The first coleopterans that visited the carcass were Carabidae, opportunistic predators attracted by insects already present. They do not lay eggs on it and are of little interest for forensic purposes. Precocity of colonization of carcass was largely influenced by the insect diet: *Saprinus subnitescens,* attracted by dipteran eggs and larvae in the first stages of development, was one of the first species reaching the carrion. Also the Silphidae *Thanatophilus dispar* appeared precociously: it feeds on both Diptera larvae and carcass itself (Smith, 1986) and also uses the carrion to lay their eggs (Hastir & Gaspar, 2001). The same applies to the other species *Saprinus semipunctatus* (Nuorteva, 1970) and *Carcinops pumilio* (Morgan, 1983). Rove beetles were detected later, when the maggots were big enough to be predated: this was the case of *Creophilus maxil-*

Table 2. Adult and larvae of coleopterans sampled on carrions M2.

Species	Day after placement														
	1	2	3	4	5	6	7-9	10-12	13-15	16-18	19-21	22-25	25-28	I[a]	Tot
Silphidae															
Thanatophilus dispar				3	3	1		3	4	2	3	1			20
Silphidae larvae								1	1	7	6	3	2	1	21
Histeridae															
Saprinus subnitescens				1	2		11	4	6	8	9	11	14		66
Saprinus semipunctatus							6	1	1	1		2	2		13
Margarinotus brunneus							1	1	1	1		1			5
Carcinops pumilio								1	2						3
Staphylinidae															
Creophilus maxillosus						1	8	13	5	7	3		3		40
Aleochara curtula							11	11	3	13	9	1	2		50
Ontholestes tessellatus								1			1				2
Philonthus sp.										2	1				3
Othius punctulatus										1		1			2
Padeuros sp.							1								1
Staphilinidae larvae									3	9	24	22	5		63
Cleridae															
Necrobia violacea										1			1	2	4
Necrobia rufipes								1							1
Dermestidae															
Dermestes frischii							6	7	17	11	14	13	10	6	84
Dermestes undulatus														8	8
Dermestidae larvae											1		1	21	23
Scarabaeidae															
Aphodius sp.				1			1		1						3
Onthophagus sp.										1					1
Monotomidae															
Monotoma sp.							1							1	2
Lampyridae larvae										2	4		1		7

losus, like insects belonging to the *Aleochara* genus that pursue Diptera pupae to parasite them (Drea, 1966).

The last coleopterans colonizing the carcass were dermestid beetles, necrophagous insects common in advanced stages of decay. The cycle of these insects develops entirely on site and is distinguished by longer development times than the other coleopterans considered in the observations. *Dermestes undulatus* was detected only at the end of the experiment by a direct inspection on the carcass, not through the use of pitfall traps. These data clearly stress that the capture methods used on an experiment may affect the sampling.

Interestingly, Lampyridae larvae were also found on pitfall traps, perhaps attracted by maggots as a food source. Although larvae of these insects seem to limit feeding snails, there is evidence that other soft-bodied animals form part of their diet (Hess, 1920), which could possibly include dipteran larvae, by analogy with rove beetles that feed on snails and maggots.

The sampling of several coleopteran larvae confirmed that some of these insects have a development cycle progressing entirely on site (Table 2, Figure 3), and, in some cases, such features warrant their use as indicators for estimating the PMI (Arnaldos *et al*., 2005). Their use as forensic indicator may also incur some contraindications, first of all the exposure of carrion and the laying of eggs are not simultaneous, so a PAI must be considered in the PMI estimation. This article reported the PAIs of different species, this value are consistent with the formerly reported intervals, for example the 5 day PAI (obtained with a ground-level temperature average of 20.5°C) of *Creophilus maxillosus* is similar to those reported by Matuszewski (2012). Moreover, the life cycle of coleopterans may be more complex and unstable than in dipterans, but their longer development times (*Dermestes* spp. become adults in about 2 months) (Binaghi, 1989), could be useful in estimating PMI in advanced decay (Midgley *et al*., 2010), or in particular condition, when dipteran larvae are rare. However, the development times of many necrophagous dipterans are well known (Marckenko, 1988; Grassberger & Reiter, 2001), the scientific literature provides data for a few coleopterans (Midgley *et al*., 2010), focused on these insects as damaging vectors for foodstuffs (Supplementary Table), and forensic applicable data are needed.

The evaluation of environmental conditions, the identification of the different species present on the remains, not limiting to more forensically-utilized species, the knowledge of their biology, the consideration of the trophic relationships between sampled species may produce many, and often neglected, data, valuable clues for assessing the minimum PMI, bringing the eventual solution of a crime closer.

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