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Survey of indoor sarcosaphagous insects

Ren, L.P.[1], Deng, H.X.[1], Dong, S.Z.[1], Li, J.B.[2], Hu, X.H.[2], Cai, J.F.[1] and Guo, Y.D.[1*]
[1]Department of Forensic Science, School of Basic Medical Sciences, Central South University, Changsha 410013, Hunan, China
[2]The Changsha Public Security Bureau in Hunan, Changsha 410008, Hunan, China
[*]Corresponding author e-mail: gdy82@126.com
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Abstract. Entomological evidence provides valuable information for estimating postmortem interval and location of death in criminal or legal investigations. The colonization of sarcosaphagous insects are commonly discovered in the decomposed corpses in most indoor cases. Therefore, by analyzing the growth patterns and behavioral rhythms of these insects, the application of indoor sarcosaphagous insects in actual cases can be investigated. This study classifies the common species of indoor sarcosaphagous insects and analyzes the characteristics of these insects (such as foraging, oviposition, and growth). It further discusses the effect of micro-environment on their behavior. In addition, the research status of the application of indoor sarcosaphagous insects in forensic investigations is summarized.

INTRODUCTION

The recovery of entomological fauna from human cadavers is one of the important features of forensic investigations. Through identifying the insect specimens collected from cadavers and determining insect species and developmental stages, the estimation of postmortem interval (PMI) can be conducted in combination with entomological succession of ecological communities on the cadavers. Forensic entomology possesses a distinct advantage in estimating PMI in cases of decomposed corpses (Wang *et al*., 2008; Johnson *et al*., 2012; Zhang *et al*., 2015a). The estimation of location of death, cause of death, and other forensic medical issues can also be solved on the basis of the regional and behavioral characteristics of these insects (Catts & Haskell, 1990). Amend *et al*. (2011) indicated that the insect community succession was affected by many factors, which led to inaccurate estimation of the PMI. Therefore, the practicality of this approach is still restricted. Tomberlin *et al*. (2011) emphasized that PMI based on insect succession data determined the period of insect activity rather than PMI and did not cover the time of corpse exposition to the first wave of colonizing and ovipositing insects. This process can be called pre-colonization interval (Pre-CI) estimation.

Studies on the various influencing factors of the application of sarcosaphagous insects in forensic investigations have focused on the growth status and community succession pattern of these insects in the indoor micro-environment (Goff, 1991; Reibe & Madea, 2010a; Anderson, 2011; Nazni *et al*., 2011; Mahat & Jayaprakash, 2013; Farrell, 2015). However, the present systematic investigations on species, distributions, and morphological characteristics of indoor sarcosaphagous insects hinder the application in the estimation of PMI. Such insufficiency is explained mainly by that, data on species and behavior of sarcosaphagous insects are still mainly derived from the collection and analysis of fragments of information, rather than from systematic and dynamic research. Moreover, the present

data collection on succession pattern and growth status of indoor sarcosaphagous insects depends mainly on wild animal experiments (Reibe & Madea, 2010a; Anderson, 2011). This paper reviews the pertinent literatures relating to the application of indoor sarcosaphagous insects in forensic investigations, especially in the estimation of PMI.

Common species of indoor sarcosaphagous insects

Reviews on sarcosaphagous insects in indoor micro-environment suggest that Calliphoridae, Sarcophagidae, Muscidae, and Phortdae of Diptera, and a few species of Coleoptera are the dominant sarcosaphagous insects collected in indoor cases. However, the distribution of these species markedly varies with regional differences, diversity, and complexity of the indoor micro-environment. The current study summarizes the common species of indoor sarcosaphagous insects identified by the morphological method. The specific species are presented in Table 1.

Indoor blow flies (Diptera: Calliphoridae)

Blow flies, which are commonly found in human settlements, can distinctly detect carcasses inside buildings and enter dwellings to oviposit . Study on blow flies in human corpses of indoor cases has been mainly reported in Europe, America, West and Southeast Asia. (Goff, 1991; Greenberg & Kunich, 2002; Pohjoismäki *et al*., 2010; Anderson, 2011). Reaserches showed that *Chrysomya megacephala* and *Chrysomya rufifacies* were commonly discovered in indoor cases in Malaysia, Thailand and China (Sukontason *et al*., 2005; Syamsa *et al*., 2010; Bunchu *et al*., 2012; Kumara *et al*., 2012; Kavitha *et al*., 2012; Kumara *et al*., 2013; Wang *et al*., 2014; Syamsa *et al*., 2015). While *Calliphora vicina* was frequently found in indoor corpses in Columbia and New Zealand (Smeeton *et al*., 1984; Anderson, 1995). Later, in Germany, *C. vicina* and *Lucilia sericata* were the only two species of blow flies found indoors, oviposition occurred with a 24h delay after exposure (Reibe *et al*., 2010a). Until now, *C. vicina* was also reported to be a typical synantropic species in colder seasons, particularly in spring and autumn in Europe (Bugelli *et al*., 2015). Meanwhile, *L. sericata* and *Chrysomya albiceps* were collected in almost 50% of the indoor cases in Italy (Bugelli *et al*., 2015). In addition, *Cynomya cadaverina* was also discovered indoors at early spring (Hall, 1948). Afterwards, Wang *et al*. (2014) showed the existence of adult *Chrysomya chain* on the fourth day indoor after exposure in China.

Basically, the stable indoor environment is conducive to the activities of insects, but the process of decomposition outdoors is generally faster than that observed indoors. Bugelliet *et al*. (2015) investigated that the delayed arrival of *L. sericata* and *C. albiceps* in the indoor corpses uncertainty of 1 to 2 days in the estimation of PMI. Additionally, drugs also affected larval development, and brought about difference between real instar and estimated mean instar (Carvahlo, 2009; Syamsa *et al*., 2010). Wang *et al*. (2012) reported the utilization of *C. megacephala* for the estimation of PMI in an indoor case in Guangdong. The estimated 5 days was 2 days longer than the real PMI concluded by forensic investigation because larval development was delayed by the excessive amount of clozapine taken by the decedent. Moreover, Reibe and Madea (2010a) observed that *L. sericata* presented at places with strong light, while *C. vicina* was usually observed in shady environments and urban habitats (Schumann, 1971; Goff, 1991; Battan *et al*., 2007). Whether *L. sericata* and *C. vicina* are related to this phenomenon remains to be verified.

Indoor flesh flies (Diptera: Sarcophagidae)

Flesh flies have special activity behavior indoors or in relatively concealed environments (Pohjoismäki *et al*., 2010; Anderson, 2011; Kumara *et al*., 2012; Guo *et al*., 2014; Bugelli *et al*., 2015), and are frequently found to infest corpses in indoor cases (Byrd & Castner, 2009; Bonacci *et al*., 2011; Syamsa *et al*., 2012). In indoor corpses, *Sarcophaga argyrostoma* and *Sarcophaga crassipalpis* were considered as the

Table 1. Common species of indoor sarcosaphagous insects

Order	Family	Subfamily	Genus	Species
Diptera	*Calliphoridae*	*Calliphorinae*	*Calliphora*	*Calliphora vomitoria* *Calliphora vicina*
			Lucilia	*Lucilia sericata* *Lucilia cuprina* *Lucilia illustris*
			Cynomya	*Cynomya cadaverina*
		Chrysomyinae	*Chrysomya*	*Chrysomya rufifacies* *Chrysomya chain* *Chrysomya albiceps* *Chrysomya megacephala*
		Chrysomya chain	*Protophormia* *Phormia*	*Protophormia terraenovae* *Phormia regina*
	Sarcophagidae	*Sarcophaginae*	*Parasarcophaga*	*Parasarcophaga crassipalpis* *Parasarcophaga albiceps* *Parasarcophaga ruficornis* *Parasarcophaga similis* *Parasarcophaga argyrostoma* *Sarcophaga impatiens* *Sarcophaga tibialis* *Sarcophaga caerulescens*
			Bercaea	*Bercaea cruentata*
			Helicophagella	*Helicophagella melanura*
			Boettcherisca	*Boettcherisca peregrina*
	Muscidae	*Reinwardtiinae*	*Synthesiomyia*	*Synthesiomyia nudiseta*
		Azeliinae	*Hydrotaea*	*Hydrotaea spinigera*
	Phortdae	–	*Megaselia*	*Megaselia scalaris* *Megaselia spiracularis* *Megaselia curtineura*
		–	*Puliciphora*	*Puliciphora borinquenensis* *Puliciphora obtecta* *Puliciphora beckeri*
		–	*Diplonevra*	*Diplonevra peregrina*
	Fanniidae	*Fanniinae*	*Fannia*	*Fannia canicularis* *Fannia trimaculata*
Coleoptera	*Cleridae*	–	*Necrobia*	*Necrobia rufipes*
	Staphylinidae	–	*Creophilus*	*Creophilus maxillosus*
	Histeridae	–	*Saprinus*	*Saprinus splendens*
	Dermstidae	–	*Dermestes*	*Dermestes maculatu* *Dermestes undulatu* *Dermestes peruvianus* *Dermestes lardarius* *Dermestes haemorrhoidalis* *Dermestes ater* *Drmestes tessellatocollis*

dominant population (Kumara *et al.*, 2012; Cherix *et al.*, 2012; Bonacci *et al.*, 2014; Baz *et al.*, 2015). And in Italy and Switzerland, *Bercaea cruentata* was frequently found in indoor cases (Leccese, 2004; Cherix *et al.*, 2012). Recently, in Switzerland, *Sarcophaga caerulescens* and *Parasarcophaga similis* have been reported to be the dominant species colonizing corpses in indoor cases, *S. argyrostoma* was commonly found indoors during summer (Cherix *et al.*, 2012). Interestingly, the involvement of *S. argyrostoma* in indoor cases has also been reported in Poland (Draber-Monko *et al.*, 2009). Moreover, *S. caerulescens* was dominant species found in indoor corpses in Finland and Spain (Pohjoismäki *et al.*, 2010; Baz *et al.*, 2015). In comparision, *Parasarcophaga albiceps*, *Boettcherisca peregrina*, *Parasarcophaga ruficornis* and *Sarcophaga tibialis* were discovered in indoor cases in China, Thailand and Spain (Chen, 2000; Chen, 2010; Yin *et al.*, 2014; Sukontason *et al.*, 2007; Baz *et al.*, 2015). *Sarcophaga impatiens* and *S. crassipalpis* were also found to colonize corpses at the earliest stage in Australia (Reibe & Madea, 2010a; Farrell *et al.*, 2015).

At present, we also summarize the decedent's personal details from the indoor cases discovered in Hunan, mainly including the species of indoor flesh flies. The specific details are presented in Table 2. Furthermore, Syamsa *et al.* (2015) currently analyzed three indoor cases in high-rise buildings and indicated that a large number of flesh flies colonized the corpses. Unfortunately, the authors failed to identify the species level because of insufficient taxonomical studies concerning the larvae of this taxon.

In summary, more than 10 common species of flesh flies exhibit indoor behavior. The specific details are attached in Figure 1. Flesh flies are obviously dominant population in indoor cadavers and have significant potential in forensic application (Guo *et al.*, 2016; Fu *et al.*, 2016). However, the insufficient taxonomical, growth, and development data of flesh flies poses various obstacles to forensic experts in applying these flies to the estimation of PMI (Battan *et al.*, 2007; Zhang *et al.*, 2015b).

Indoor scuttle flies (Diptera: Phoridae)

PMI is estimated mostly on the basis of the identification of larval instar of blow and flesh flies. However, the arrival of these flies at the corpse may be delayed in relatively sealed indoor (Reibe & Madea, 2010b; Farrell, 2015). Scuttle flies reach the body earlier than other insects due to their unique biological behavior and presence in nearly closed environments (such as sealed plastic bags, indoors, and graves) and low temperature spaces where activities of other flies are limited. Therefore, scuttle flies are

Table 2. We summarize the decedent's personal details from the indoor cases in Hunan, mainly involving the species of sarcosaprophagous flies indoors. Sex, age, cause of death, stage of decay are also specified in the table

No	PMI (days)	Gender	Age (years)	Manner of death	Decomp	Species
1	4	♀	25	alcoholism	early	*P. crassipalpis; P. similis*
2	15	♂	37	drug overdose	advanced	*P. albiceps*
3	5	♂	32	suicide-CO poisoning	early	*B. peregrina*
4	28	♂	68	illness	advanced	*B. cruentata*
5	37	♂	70	illness	advanced	*H. melanura*
6	14	♀	44	homicide	moderate	*B. peregrina*
7	78	♀	50	undetermined	advanced	*H. melanura; B. cruentata*
8	17	♂	18	sexual Asphyxia	advanced	*B. peregrina*
9	10	♂	45	drug overdose	moderate	*P. crassipalpis*
10	7	♀	37	homicide	early	*P. albiceps; P. similis*

Figure 1. The species and distribution of indoor flesh flies (Diptera: Sarcophagidae).
In the figure, I: Bercaea cruentata, II: Parasarcophaga argyrostoma, III: Sarcophaga caerulescens, IV: Parasarcophaga similis, V: Parasarcophaga crassipalpis, VI: Sarcophaga tibialis, VII: Sarcophaga impatiens, VIII: Parasarcophaga ruficornis, IX: Parasarcophaga albiceps, X: Boettcherisca peregrina, XI: Helicophagella melanura. The name of every country is abbreviated. ESP: Spain, ITA: Italy, CH: Switzerland, PL: Poland, FI: Finland, CHN: China, THA: Thailand, MAS: Malaysia, AU: Australia. It shows that the species and distribution of indoor flesh flies are obviously different in each country.

considered to be the first colonizers to reach the corpses in indoor cases occurred at ground level or high altitudes (Manlove & Disney, 2008; Reibe & Madea, 2010b; Kumara *et al*., 2010; Zuha *et al*., 2012; Syamsa *et al*., 2015).

The species of scuttle flies commonly found in indoor cases mainly included *Megaselia scalaris* (Kumara *et al*., 2010; Anderson, 2011; Bugelli *et al*., 2015). Reibe & Madea (2010b) indicated that blow flies were delayed to reach indoor corpses, while *M. scalaris* could infest corpses and oviposit within a short time after exposure. In an indoor case of Pisa, *M. Scalaris*, *C. vicina* and *C. albiceps* were mainly collected, using the available data of *C. vicina* and *C. albiceps*, PMI of 13–27 days was estimated, while using those of *M. scalaris* indicated PMI of 21 (18–23) days (Bugelli *et al*., 2015). Thus, *M. scalaris* was considered to be accurate in the estimation of PMI in indoor cases. Moreover, *Megaselia curtineura*, *Megaselia spiracularis*, *Puliciphora borinquenensis*, *Puliciphora obt*ecta and *Puliciphora beckeri* have also been first reported in indoor cases in Malaysia (Kumara *et al*., 2010; Zuha *et al*., 2014).

In June 2015, we put a pig carcass in the basement with all windows closed, the means and standard deviations of the ambient temperatures and relative humidities in study sites were 16.0±0.5°C, 88.0±3.0%. Four hours later, *Diplonevra peregrina* (Diptera: phoridae) was commonly found on the legs of the carcass. The specific description is presented in Figure 2.

However, *M. scalaris* myiasis on humans and animals has been reported and summarized by several authors, and can lead

Figure 2. The species of scuttle flies (Diptera: Phoridae) commonly discovered in concealed environment.
A pig corpse was in a basement with all windows closed, four hours later, *Diplonevra peregrina* (Diptera: phoridae) was commonly found on the legs of the carcass.

to inaccuracy in PMI estimation (Syamsa *et al.*, 2012). Bugelli *et al.* (2015) reported an Italian indoor case in which *Sarcophaga* larvae and *M. scalaris* pupae were present in the corpse. The developmental stage of *Sarcophaga* larvae indicated a minimum colonization time of 1–2 days, whereas the presence of *M. scalaris* pupae suggested a colonization time of approximately 1 week (6–8 days). In fact, the decedent has been alive for 2 days prior to the discovery of the body. These data were in agreement with the estimated PMI based on the developmental stage of *Sarcophaga* larvae, while data concerning *M. scalaris* pupae were consistent with a myiasis occurring several days (nearly 5 days) before death. Moreover, *M. scalaris* is also an omnivorous species because they can consume a wide spectra of food, including decomposing organic matter and artificial media (Disney, 2008).

In indoor cases, sealed environment may result in the delay in insect arrival. However, messy and dirty houses may create a certain micro-environment that is conducive to the breeding of sarcosaphagous insects prior to the occurrence of death. Therefore, lighting, temperature, humidity, hygienic condition, possible passage of insects, and activity and predation of other insects must be comprehensively considered to correctly apply the entomological approach in indoor cases. Notably, the myiasis and medication history receive inadequent concern.

Indoor house flies (Diptera: Muscidae)

House flies were the common species to infest and colonize corpses in indoor cases mainly in Malaysia and Thailand (Schumann, 1971; Nazni *et al.*, 2007; Sukontason *et al.*, 2007; Syamsa *et al.*, 2012; Syamsa *et al.*, 2015), while *Synthesiomyia nudiseta* was a rare species in Malaysia found only on indoor cadavers (Lee *et al.*, 2004; Nazni *et al.*, 2007; Kavitha *et al.*, 2013b). In 2012, a decomposing body of an adult female was found on the top floor of a 13-story building in Kuala Lumpur, Malaysia. The body was colonized by the larvae of *S. nudiseta* for the first time. The PMI was estimated at approximately 5 days to 9 days (Syamsa *et al.*, 2012). Syamsa *et al.* (2015) also reported that *S. nudiseta* has colonized a corpse in a high-rise building in Malaysia. However, whether the presence of *S. nudiseta* in a high-rise building was due to the unique behavior of this species or accidental factors still requires further investigation by forensic entomologists.

Omar *et al.* (1994) investigated the developmental cycle of *S. nudiseta*. Their experiment confirmed that the first oviposition of *S. nudiseta* occurred 4 days after exposure in indoor cases. In 2009, Kumara *et al.* (2009b) reported the growth the larve of *S. nudiseta* in the indoor fluctuating temperatures of Malaysia. The total developmental time was 13.4 ± 0.8 days. Later, Abu Hassan *et al.* (2015) studied the immature life cycle of *S. nudiseta* from the egg stage until adult emergence was 14.0 ±1.0 days under fluctuating temperature of indoor environments. Recently, Syamsa *et al.* (2015) indicated that the time of the first oviposition of this species was inconsistent with that reported by Omar *et al.* (1994). The reasons for this divergence may be that the oviposition process can be affected by many biotic (such as inter-specific and intraspecific competition and ecological habits) and abiotic factors (such as weather condition, temperature, humidity, degree of decay, and accessibility to insects), which may ultimately change the ecology of carrion flies (George *et al.*, 2013; Tomberlin *et al.*, 2011).

Indoor Coleoptera insects

Coleoptera (beetles) usually appears during the middle and later stages of decomposition of corpse. Data of the species, quantity, occurrence time, and succession pattern of beetles in the corpses are important in the estimation of PMI for dry human skeletal remains (Wang *et al.*, 2008).

As early as 1965, *Dermestes lardarius* and *Dermestes haemorrhoidalis* were applied to estimate the PMI as 1–2 years in the case of a skeletonized body discovered in a Copenhagen flat. This case was one of the earliest reports on PMI estimation using beetles. Later, *Dermestes maculatus* was the only species found indoors and then the PMI was estimated as no longer than 5

months in Germany. (Schroeder *et al.*, 2002). Additiontally, in Malaysia, *Dermestes ater* was reported to estimate the PMI as 14 days in an indoor case. Meanwhile, the authors emphasized that the unusually rapid arrival of dermestids was most likely attributed to the warm climate indoors (Kumara *et al.* 2009a). Nazni *et al.* (2011) also experimentally confirmed that the order Coleoptera was more diverse with 6 families recovered in indoor condition: Lampyridae, Lycidae, Scarabaeidae, Sliphidae, Staphylinidae and Tenebrionidae, unfortunately, they were not identified as a specific species. Recently, Charabidze *et al.* (2014) analyzed 81 cases involving the invasion of beetles in France and confirmed *Dermestes peruvianus* were mainly distributed in indoor cases. In China, Chen (2000) experimentally found that *D. frischii* and *Drmestes tessellatocollis* were dominant beetles in indoor environments in Guizhou. Furthermore, the time of invasion of carcasses was approximately 40 days after exposure. Wang *et al.* (2014) experimentally confirmed in Shenzhen that beetles distributed in indoor mainly including *D. maculatus*, *Creophilus maxillosus*, *Saprinus splendens*, and *Necrobia rufipes*. Adult of *C. maxillosus*, *S. splendens* and *D. maculatus* mainly appeared in 6-17 days, 7-10 days, and after 13 days, respectively. *D. maculatus* and *N. rufipes* were discovered more frequently indoors than outdoors.

As a dominant population during the middle and later stages of decomposition of carcasses, sarcosaphagous beetles play a pivotal role in estimating the PMI of dry human skeletal remains. In addition, posture changes of carcasses and the specific damage of a part of the carcass are closely related to the decomposition and depend on the unique necrophagous characteristics of the beetles. However, forensic entomologists still remain a challenge in speculating the succession pattern of beetles using various factors and correctly distinguishing antemortem events from the influence of beetles after death.

Limitations

In criminal cases, the incidence rate of indoor cases is generally higher than that of outdoor cases. Indoor cases have their own particularities and limitations. For example, relatively closed indoor micro-environment can slow down the decomposition rate of cadavers and hinder the spread of putrid odor. These phenomena can contribute to the delay in the arrival and oviposition of sarcosaphagous insects. In addition, bodies of socially isolated persons are usually difficult to be discovered indoors. Furthermore, a few sarcosaphagous insects may invade into indoor cadavers in advance under the temptation of certain baits (such as putrid food and waste). Moreover, a large number of maggots oviposited by sarcosaphagous insects can accelerate the decomposition rate of cadavers and produce considerable metabolic heat energy. Such change in temperature may remarkably change the micro-environment and influence the growth rate of maggots on cadavers.

Indoor micro-environments where corpses are usually found at the middle and later stages of decomposition are complex and have certain particularities (Archer *et al.*, 2005; Long *et al.*, 2015), thus, the application of entomological evidence is advantageous in the estimation of PMI in indoor cases (Wang *et al.*, 2008; Johnson *et al.*, 2012). However, various factors may influence the species and distribution of sarcosaphagous insects, such as the position of cadavers in the building, the height of the building, the direction and seal of the interior windows and the lighting, ventilation and temperature of the house.

Expectation

The rapid development of molecular biology technology in recent years has facilitated the application of molecular markers of sarcosaphagous insects as a supplementary measure of morphological identification. Integrating gene expression data with traditional morphological data is an important direction in the research on sarcosaphagous insects. Estimation of the time covering the entire maturity of sarcosaphagous insects can significantly improve the accuracy in the estimation of PMI. The following directions can be considered in future studies: the identification efficiency of different gene fragments,

conjoint analysis of molecular identification methods to further optimize the molecular markers of sarcosaphagous insects, collecting samples and related data of sarcosaphagous insects in indoor cases, and identifying the species in combination with molecular identification methods. Forensic experts must estimate PMI using sarcosaphagous insects in actual cases, explore and establish a compound mathematical model, verify and correct the estimation by postmortem phenomena and investigations, and provide an improved method for the estimation of PMI in forensic cases.

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