

Larvae of Forensically Important Flies associated with Rabbit Carcasses Placed in a Closed Room in Kuching, Sarawak

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ABSTRACT

Introduction: This is the first report on insects associated with rabbit carcasses in indoor environment in Kuching, Sarawak. **Methods:** This study was conducted on June till August of 2016. Rabbit carcasses (*Oryctolagus cuniculus*) which were used as the animal model, were placed inside a dark room in a building at Forensic Medicine Department in Sarawak General Hospital. The fly larvae infesting on the carcasses were collected until the decomposition process completed. Throughout the 15 days of experiment, the fly larvae were sampled on the carcasses indoor at 28.9 ± 0.3 °C and $69.6 \pm 1.0\%$ humidity. **Results:** The fly larvae activity was observed during the active decay stage and was identified as *Synthesiomyia nudiseta* (van der Wulp, 1883) (Diptera: Muscidae) and *Sarcophaga* spp. (Diptera: Sarcophagidae). Only these two species were found co-existing on the carcasses. **Conclusions:** *S. nudiseta* and *Sarcophaga* spp. could be used as an entomological evidence involving indoor cases in Kuching, Sarawak because both species were dominant and active carcass decomposers in indoor environment.

KEYWORDS: *Synthesiomyia nudiseta*, *Sarcophaga* spp., indoor environment, forensic entomology.

INTRODUCTION

The post mortem interval (PMI) is important in investigations of homicides and other untimely deaths. The postmortem changes in physical or biological of the dead body can provide useful and most reliable PMI indicators. However, as the time since death progress, that information became less useful and more accurate results are often obtained using ecological information. It has long been discovered that the minimum PMI (mPMI) can be estimated by calculating the most developed larvae on the dead body [1].

There are several factors that can affect the body decomposition and insects' composition on carrion, such as scene context (indoor, outdoor) [2], body position (hanging, burial depth) [3-4], body condition (burned, cause of death, etc.) [5], and individual characteristics of the cadaver (age, sex, body weight, and microbiome) [6]. All these factors can

change the rate of decomposition, insect succession, and insect composition.

It was known that insects can colonized dead bodies in several different locations [7] including in poorly accessible environments [8], though insects have different abilities to access corpses indoors. This is based on a comparative study conducted in Hawaii where it was reported that there are markedly different species composition found between in- and outdoor cases. From the study, a total of 22 species found in outdoor cases and only five species found in indoor cases. These five species also found in outdoor cases [8]. Corpses in houses or apartments are frequently found in late stages of decay infested by larvae of family Calliphoridae, Sarcophagidae, Muscidae and Phoridae [9]. In this scenario, PMI calculations can be complicated as it is unclear when the flies found the body and start laying eggs [10].



In Malaysia, the entomological data on insect colonisation of indoor cases are lacking even though statistics from peninsular Malaysia from year 2010 to 2013 had shown that, about 85.3% of cases were associated with body found indoors and only 14.7% involved outdoor locations such as river, monsoon drain, disused mine and bushes [11].

In this paper, we report the decomposition process and the associated fly larvae infesting on the carcasses in indoor environment, Kuching, Sarawak from June – August of 2016. Data collected can be used as a reference for future research studies or investigations involving indoor cases in Sarawak.

MATERIALS AND METHODS

Study site and preparation of animal model

This study was conducted in a closed room located at

Forensic Medicine Department of Sarawak General Hospital in Kuching (1.544316, 110.340710) (Figure 1). The room size was 3.35 m x 5.48 m and consisted of only two lightings and an exit door. There was no air conditioner, fan, window or furniture in the room.

Animal model used in this study was a male rabbit (*Oryctolagus cuniculus*). The rabbit was purchased locally and weighed approximately 1.3 kg (8 cm, H x 5 cm, W x 30 cm L). The rabbit carcass was prepared by a trained animal handling personnel from Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak (UNIMAS) (1.462804, 110.431832) (Figure 2). A cervical dislocation was conducted to avoid open wound. Then, the rabbit carcass was brought to the study site immediately. During transportation, the carcass was wrapped in a plastic bag and put in an air-tight box to avoid the carcass' odour to attract the insects in open environment.

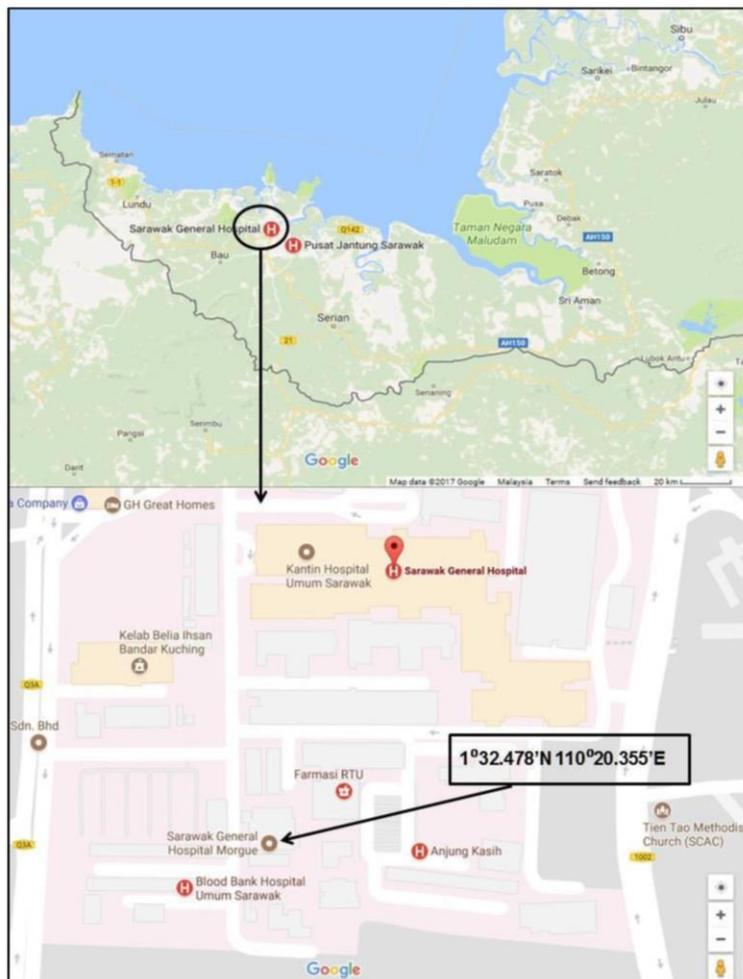


Figure 1 Map showing the location of the study site, Forensic Medicine Department of Sarawak General Hospital, Kuching, Sarawak (Courtesy of Google Maps 2021)

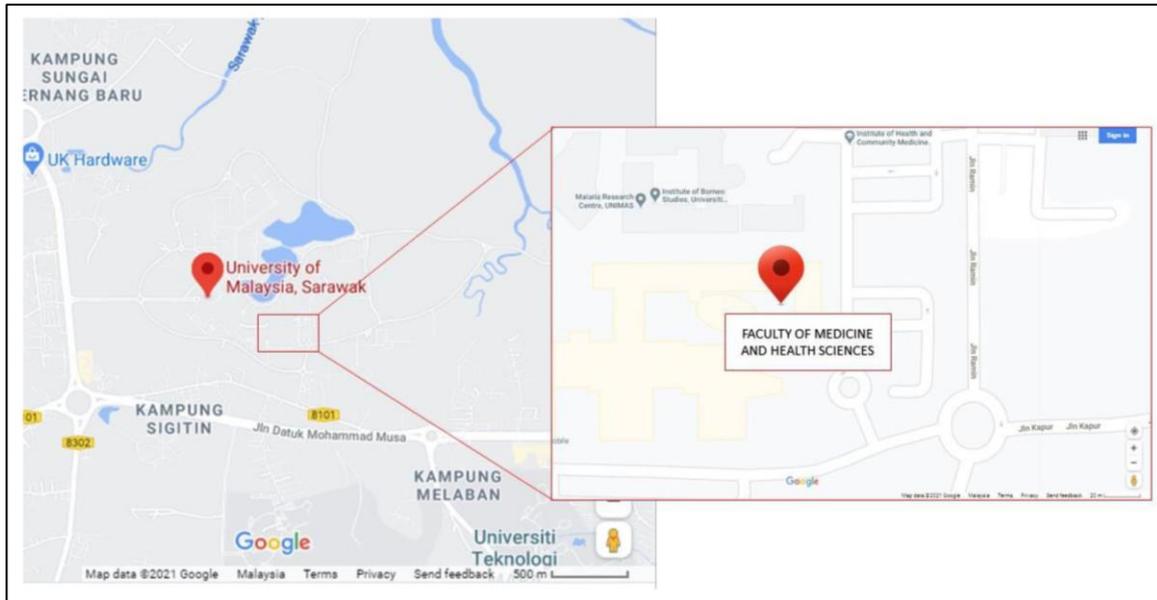


Figure 2 Map showing Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, Kota Samarahan Sarawak. The location where the animal preparation procedure was conducted (Courtesy of Google Maps 2021)

At the study site, the plastic covering the carcass was removed, and the carcass was placed in the center of the room. During the experiment period, the site was visited twice daily, at 8 am and 5 pm to observe the decomposition process and for insects' collection. During the experiment, the lights in the room were turned off and only one person was allowed to enter the room during site visit to minimize in and out movement in the room. All the temperatures and humidity indoor and outdoor were recorded for comparison, as well as the entomological data and decomposition period. The experiment was conducted using only one carcass per trial, and the trial was repeated three times. The first, second and third trial was conducted in June, July and August 2016, respectively. Each trial lasted for 15 days, and two-week gap was allowed between each trial.

The fly larvae collection

Approximately 20 to 30 third instar larvae were collected during active decay stage. In this experiment, only third instar larvae were proceeded with species identification. The larvae were collected from the head area, rear area (near the anus) and around the abdomen of the carcass. One-third of the larvae collected were reared into adults. Unfortunately, the adult specimens were damaged and were not included in this paper.

Third instar larvae found at the study site were collected using forceps and spatula. The larvae were then washed with distilled water and killed with warm water around 80 °C for 30 seconds and placed in 70% ethanol for at least three days to allow for absorption of alcohol into the tissues and rendering them adequately preserved for the next preparation steps. The larvae processing procedure for identification followed method by Omar et al. [12].

Posterior parts of each larva were excised and all the internal organs were removed by forceps. Larvae were first cleared by soaking in 10% potassium hydroxide (KOH) solution for 24 hours and this depending on the size of the larvae to clear all of the soft tissues inside the larvae. The larvae were then placed in glacial acetic acid for seven minutes to neutralize the KOH solution, and subsequently passed through ascending concentrations of alcohol; 80%, 90%, 95% and 100% respectively for 30 minutes each. The larvae were soaked in clove oil for 30 minutes and rinse with a few drops of xylene. It was then placed on a glass slide and mounted with Canada balsam and dried at 30°C overnight. The specimens were examined under Nikon Eclipse E20 digital camera attached to Kyowa medilux-12 microscope for taxonomic identification. The fly larvae were identified by referring to larvae characteristics from Syamsa et al. [13] and Afravi et al. [24].

Environmental parameters

The indoor and outdoor relative humidity and ambient temperatures were recorded using data logger (Extech Instrument Rht20, USA). The data was compared for their differences using Independent sample T-test at significant level $p < 0.05$.

RESULTS

The indoor and outdoor environmental parameters

During the study period of 15 days, for three trials of experiment, the mean ambient temperatures recorded was $28.9 \pm 0.3^\circ\text{C}$ and $31.5 \pm 0.4^\circ\text{C}$, for indoor and outdoor environment, respectively (Figure 3). Meanwhile, the mean relative humidity (RH) recorded was at $69.6\% \pm 1.0\%$, and $78.2\% \pm 2.0\%$ for indoor and outdoor, respectively (Figure 4).

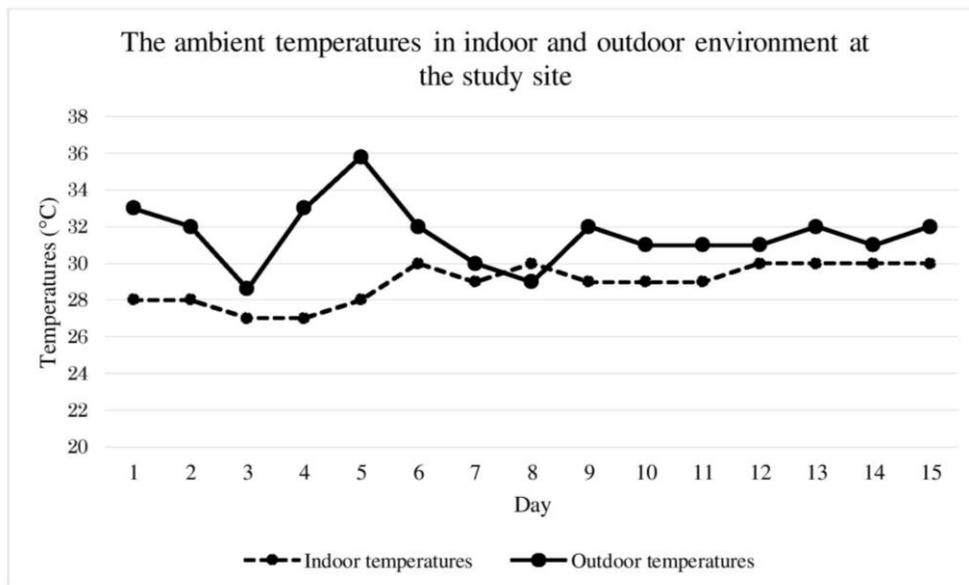


Figure 3 Daily ambient temperature in relation to the decomposition stages of rabbit carcass in indoor and outdoor environment at Forensic Medicine Department, Sarawak General Hospital, Kuching, Sarawak

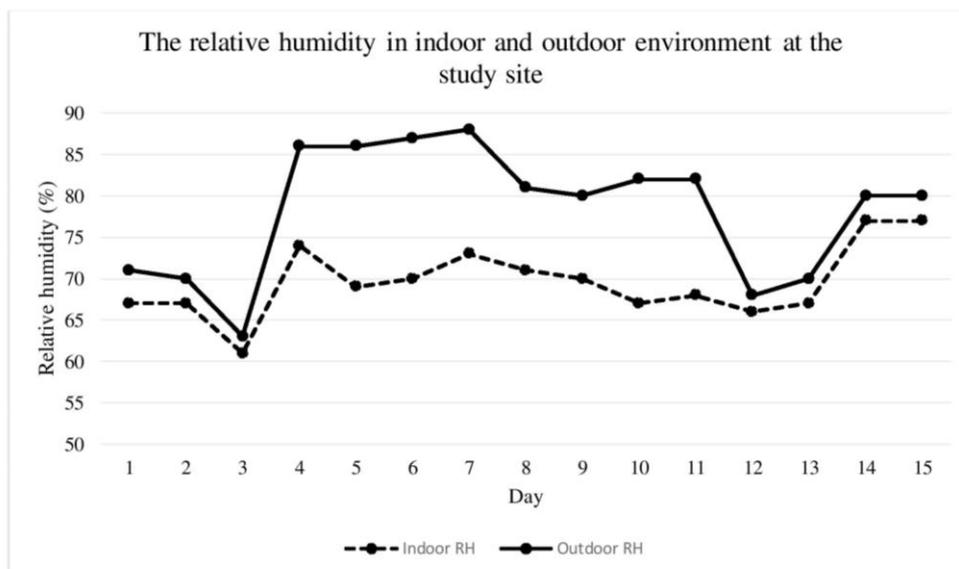


Figure 4 Daily relative humidity in relation to the decomposition stages of rabbit carcass in indoor and outdoor environment at Forensic Medicine Department, Sarawak General Hospital, Kuching, Sarawak

From the line graph plotted for daily temperature in indoor and outdoor environment, it can be observed that there was a difference between the temperatures in both environments. Generally, the temperatures at outdoor environment were higher in most days except for the 8th day due to rain. For relative humidity, the humidity inside the room was found to be less humid than the outside environment. From the sample t-test analysis, it was found that, there was a significant difference in the temperatures for indoor environment ($28.9\text{ }^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$) and outdoor environment ($31.5\text{ }^{\circ}\text{C} \pm 0.4^{\circ}\text{C}$) [$t(28) = 4.028$, $p = 0.000$]. While for RH, there was also a significant difference between indoor RH ($69.6\% \pm 1.0\%$) and outdoor RH ($69.6\% \pm 1.0\%$) [$t(28) = 3.266$, $p = 0.015$].

Decomposition stages

The rabbit carcasses decomposition process took 15 days to complete (Table 1). Five stages of decomposition were identified, which were fresh, bloated, active decay, advanced decay and dry remains stage (Figure 5). The fresh stage begins following death. The rabbit became rigor mortis and no odour yet release from the carcass (Figure 5A). There were no changes in the physical appearance of the carcass.

On the next day, the carcass' abdomen was inflated, and the body began to purge decomposition fluids and bad odour was released (Figure 5B). However, the odour released does not attract any insects at this stage. On the 4th day, the carcass abdomen deflated and more decomposition liquid oozes out from the carcass natural orifices and from the abdomen. During this time, strong odour was released, few adult flies from family Muscidae and Sarcophagidae found on the carcass. It is possible that the adult flies were entering the room through the door when researchers' visiting the study site.

On the next day, first instar larvae can be observed around the head and rear area which indicating the decomposition process was in active decay stage (Figure 5C). The larvae around the head, rear and a few on abdomen area were collected and processed for

species identification. Following the active decay stage was the advance decay, there were fewer adult flies observed compared to the stage before. Larvae activities were also less compared to that in the active decay stage. The odour started to fade when the carcass reached this stage (Figure 5D). The final stage was the dry remain stage. At this stage, there were no adult flies present. Most of the soft tissue and flesh were gone but the fur was still visible on the rabbit carcass (Figure 5E).

Flies' species collected and identified at study site

There were only two fly species identified infesting on the carcasses in this experiment namely *Synthesiomyia nudiseta* (van der Wulp, 1883) (Diptera: Muscidae) and *Sarcophaga* spp. (Diptera: Sarcophagidae). In this paper, we only discussed the characteristics of the 3rd instar of both flies.

Description of *Synthesiomyia nudiseta* larvae

Observation under compound microscope has shown that, the larva has a pair of posterior spiracle (Figure 6A) and each spiracle had a complete peritreme and slits in the form of "S" shape with a prominent button. Meanwhile, at the anterior end, cephalopharyngeal skeleton with a strong hook was observed (Figure 6B). Anterior spiracle (Figure 6C) was also observed, which consists of five papillae. On the surface of the abdominal segment of the larva, inter-segmental spines can be seen in a row, each in triangular shape with pointed tip (Figure 6D).

Description of *Sarcophaga* spp. larvae

The posterior spiracle of 3rd instar larvae of *Sarcophaga* spp. consist of distinct inner projections between the spiracular slit. The peritreme has no button (Figure 7A). The cephalopharyngeal skeleton was observed with a strong hook part, equipped with an accessory sclerite placed beneath the hook (Figure 7B). Its' anterior spiracles consist of 12 papillae arranged in a single row (Figure 7C). The spines were arranged singly with base of pigmented part of each spine were concave in shape (Figure 7D).

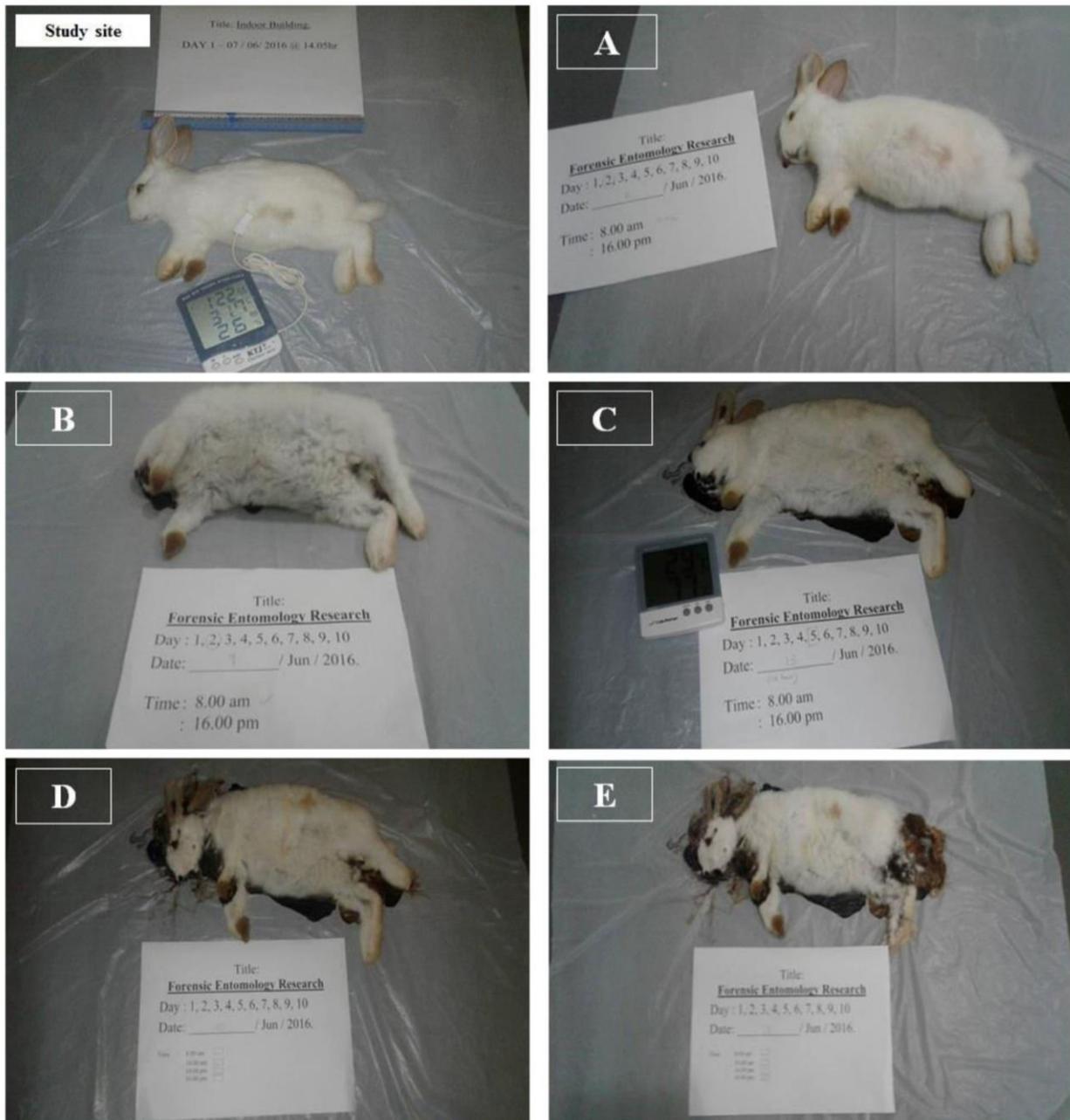


Figure 5 Decomposition stages of a rabbit carcass in closed room, Forensic Medicine Department, Sarawak General Hospital. (A) fresh. (B) bloated. (C) active decay (D) advanced decay (E) dry remains

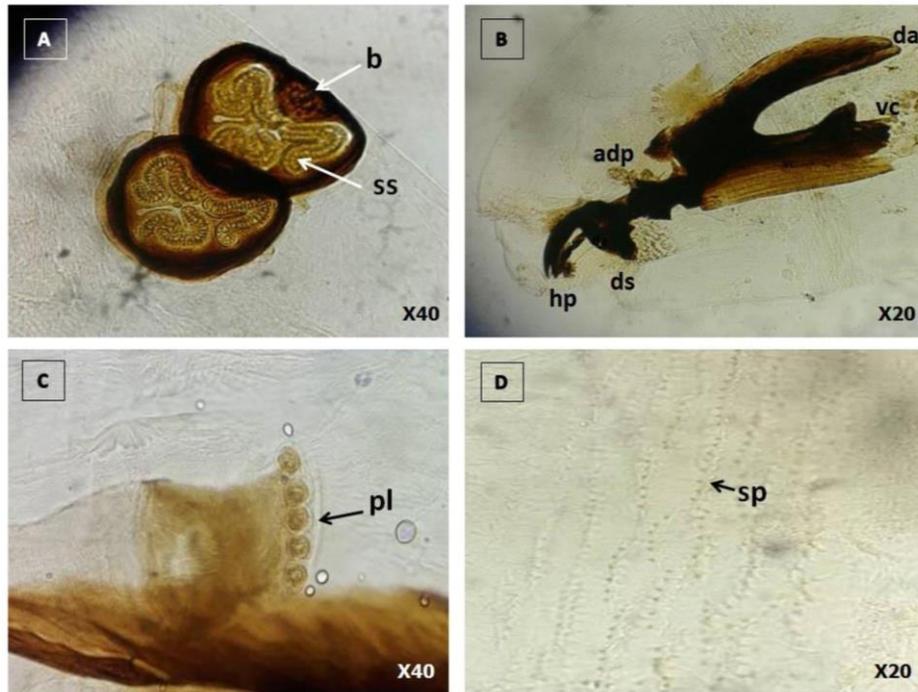


Figure 6 Third instar of *Synthesiomyia nudiseta*. (A) posterior spiracle showing three ‘s-shaped’ slits (ss) with prominent button (b). (B) cephalopharyngeal skeleton with dorsal arm (da), hook part (hp), anterior dorsal process (adp), dental sclerite (ds) and ventral cornua (vc). (C) anterior spiracle with papillae (pl). (D) spine (sp)

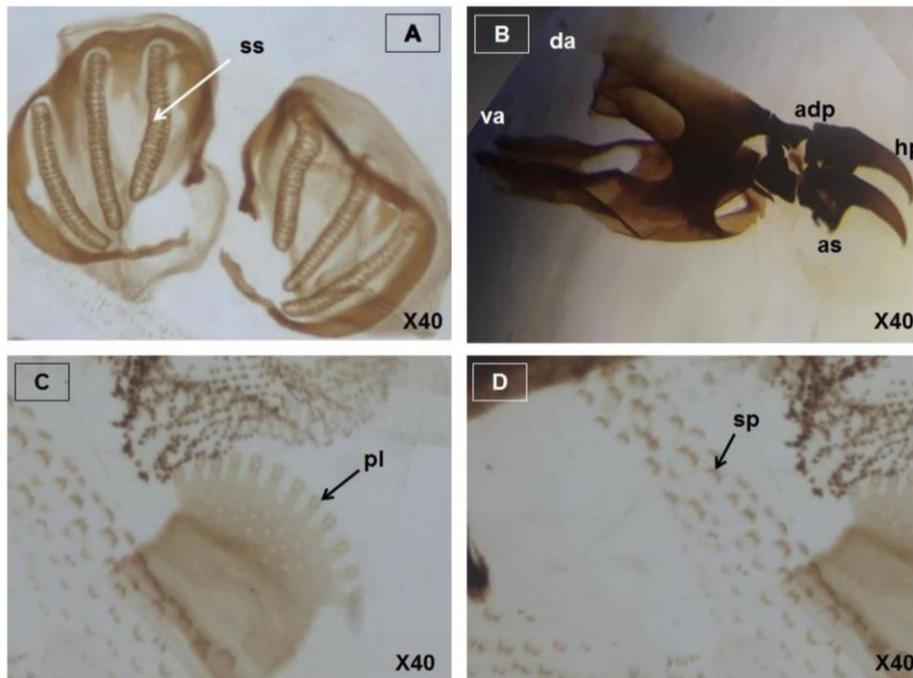


Figure 7 Third instar of *Sarcophaga* spp. (A) posterior spiracle with peritreme (p) and slits (ss). (B) cephalopharyngeal skeleton with dorsal arm (da), ventral arm (va), hook part (hp), anterior dorsal process (adp) and accessory sclerite (as). (C) anterior spiracle with papillae (pl). (D) spine (sp).

DISCUSSION

Decomposition of rabbit carcasses in indoor environment

Decomposition occurs to all organisms that has died. The process starts at the cellular level and progress to macroscopic, which form the post mortem changes [14]. Generally, the decomposition process consists of five stages which are fresh, bloated, active decay, advanced decay and dry remains [15]. The period of each stages vary depends on certain factors such as, the size of the corpse/carrion, the environment such as temperatures or humidity, the condition of the body, whether it is exposed to outside environment or trapped in an indoor environment [16].

In this study, the five stages of decomposition process were observed (Table 1) and time period for the decomposition process to end was longer than previous study that was carried out in open environment using the same animal model. In the previous study, it took only nine days for the rabbit carcasses to decompose completely [17], while in this study, the decomposition process took 15 days to complete. Other study in peninsular Malaysia, for example a study conducted in Sungai Buloh, Selangor and Dungun Terengganu also had shown that the rabbit carcasses that were let decomposed in an open environment took nine and seven days to complete respectively [18].

Table 1 The period of decomposition process of rabbit carcasses in each stage

| Decomposition stages | Day |
|----------------------|-----------|
| Fresh | Day 1 |
| Bloated | Day 2-5 |
| Active decay | Day 5-8 |
| Advanced decay | Day 8-12 |
| Dry remains | Day 12-15 |

While a study in semi forested area Ulu Kelang, Selangor has reported that the decomposition process of monkey carcasses was delayed for two days if compared to the outdoor carcasses [19]. Other study on decomposition of carcasses in indoor environment, the post decay stage started at day 8-40. On the 8th day of their study, the carcass became drier, less pungent and flattened. These characteristics were observed until the last day of observation which was in day-40 [20]. Similar observation also made in other study where the carcasses were placed in a concealed environment (dustbin). The post decay started at day-7 and similar features observed until day 40 [21].

The insects associated with decomposition of rabbit carcasses in indoor environment

Qualitatively, there are two fly species co-existing in this experiment which were *S. nudiseta* and *Sarcophaga* spp. The larvae of these two species were collected during active decay stage of the experiment. In the previous study conducted in peninsular Malaysia, more insects can be found on the indoor carrion, such as flies (Order Diptera) beetles (Order Coleoptera) and ants (Order Hymenoptera) [19, 20, 21].

Ahmad et al. [19] had shown higher species richness of flies infesting the monkey carcasses in indoor environment that includes flies from family Calliphoridae, Muscidae, Stratiomyidae and Sarcophagidae. This is maybe due to their study design, where the indoor environment was not completely concealed. The hut where they put the carcasses was a wooden hut and all the windows in the hut were slightly opened to allow entrance of the flies. Other studies where the carcasses were placed in a closed cabin had shown the presence of three species of scuttle flies which were *Megaselia scalaris* (Loew, 1866) (Diptera: Phoridae), *Megaselia spiracularis* (Schmitz, 1938) (Diptera: Phoridae) and *Dohrniphora cornuta* (Bigot, 1857) (Diptera: Phoridae) [20]. None of these species were found in our study.

The main difference of their studies from ours is the location where the study was conducted. It is known that higher insect species richness can be found in forested area [22]. For example, Ahmad et al. [19] conducted their study inside a hut in a semi-forested area and Zuha et al. [20] conducted their study in a

portable cabin adjacent to a secondary forest. However, our study was conducted inside a room, in a building, located in a city. There is no secondary forest or bushes nearby our study site.

The presence of *S. nudiseta* in indoor environment has been reported in Malaysia inside a high-rise building associated with a human corpse [13]. The fly larvae were colonizing the body of adult female at the top floor of thirteen-story building. *Synthesiomyia nudiseta* are normally associated with a corpse indoors at ground level [13]. Other case report which involve human corpse in high rise building in Kuala Lumpur, have reported similar fly species as in our study which were *S. nudiseta* and *Sarcophaga* spp. [23].

It is difficult to identify the species of Sarcophagidae, but at family level adult and larvae were easily identified [25]. In this study, the flesh flies collected were identified as *Sarcophaga* spp. From previous case report in peninsular Malaysia, flesh flies are among the frequent and dominant fly species infesting on human corpse indoor, while infrequent and occasional in outdoor environment [19]. According to report by Kumara et al. [23] when comparing between the indoor and outdoor cases, there was only one species from this family found in outdoor cases, which later identified as *Sarcophaga princeps* (Wiedemann, 1830) (Diptera: Sarcophagidae). Other sarcophagid flies found during their study was *Sarcophaga* spp.

CONCLUSION

In this study, fly species associated with decomposing rabbit carcasses placed in a closed room at the Forensic Medicine Department, Sarawak General Hospital were *S. nudiseta* and *Sarcophaga* spp. They occurred at an average indoor ambient temperature 28.9°C and relative humidity 69.7%. Other forensically important insects were not present in this study.

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Authors' contributions

Robin Maramat: conduct research, identification and photography of specimen, and writing methodology.

Norliza Ibrahim: provide space for field experiment.

Marlini Othman: statistical analysis, graph preparation and result writing.

Nor Aliza Abdul Rahim: writing, editing whole manuscript and correspondence.

Conflict of Interest

Authors declare none.

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