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Scuttle Flies (Diptera: Phoridae) Inhabiting Rabbit Carcasses Confined to Plastic Waste Bins in Malaysia Include New Records and an Undescribed Species

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Abstrak: Lalat mencalai (Diptera: Phoridae) ialah serangga kecil berkepentingan forensik. Ia amat terkenal dengan kepelbagaian spesies di habitat yang berlainan. Namun, dalam konteks entomologi forensik, kajian mengenai lalat mencalai yang dijumpai pada mayat masih kurang diberikan perhatian. Memandangkan terdapat pelbagai laporan yang menunjukkan kemungkinan terdapat kepelbagaian spesies lalat mencalai pada persekitaran mayat atau bangkai mereput, satu kajian pereputan bangkai haiwan di lokasi tertutup telah dijalankan. Kajian ini bertujuan merekodkan kehadiran lalat mencalai pada bangkai arnab yang diletakkan di dalam tong sampah selama 40 hari. Kajian ini dijalankan sebanyak dua replikasi di Tapak Simulasi Sains Forensik, Universiti Kebangsaan Malaysia, Bangi, Selangor. Persampelan lalat mencalai dilakukan mengikut selang masa berlainan di dalam kelambu yang diubahsuai. Di dalam kelambu tersebut, lalat yang terperangkap ditangkap dan diawet di dalam larutan etanol 70%. Larva dan pupa dipelihara sehingga peringkat dewasa untuk pengenalpastian spesies. Hasil kajian mendapati terdapat enam spesies lalat mencalai daripada bangkai, iaitu *Dahliphora sigmoides* Schmitz ♂, *Gymnoptera simplex* (Brues) ♀, *Megaselia scalaris* (Loew) ♂♀, *Puliciphora borinquenensis* (Wheeler) ♂, *Puliciphora obtecta* Meijere ♀ and *Spiniphora* sp. ♀. Lalat *D. sigmoides* dan *P. obtecta* adalah rekod baharu di Malaysia manakala *Spiniphora* sp. adalah spesies yang belum dapat dikenal pasti sehingga ia dihubungkan dengan lalat jantannya. Lalat mencalai didapati berupaya memasuki tong sampah yang ditutup rapat (hari 4–5) dengan *Megaselia scalaris* sebagai lalat mencalai yang paling banyak ditemui pada bangkai pada hari 4–7 (replikasi pertama) dan hari 5–33 (replikasi kedua). Turut ditemui ialah *Sarcophaga* sp. (Diptera: Sacrophagidae) yang terawal tiba pada bangkai dan paling lama mendiami bangkai (hari 2–40). Larva *Hermetia illucens* Linneaus (Diptera: Stratiomyidae) dan *Fannia* sp. (Diptera: Fanniidae) pula dijumpai pada bangkai ketika tempoh pertengahan peringkat pereputan lanjut. Keputusan ini memperluaskan pengetahuan mengenai kepelbagaian spesies lalat mencalai berkepentingan forensik serta lain-lain lalat di persekitaran bangkai yang dilitupi di Malaysia.

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Kata kunci: Lalat mencalai, Phoridae, Pereputan, Entomologi forensik, Diversiti

Abstract: Scuttle flies (Diptera: Phoridae) are small-sized insects of forensic importance. They are well known for diversified species and habitats, but in the context of forensic entomology, scuttle flies' inhabitance of corpses remains inadequately explored. With recent reports indicating the existence of more scuttle fly species possibly inhabiting these environments, a decomposition study using animal carcasses in enclosed environments was conducted. The aim was to record the occurrence of scuttle flies on rabbit carcasses placed in sealed plastic waste bins for a 40-day period. The study was conducted as two replicates in Bangi, Selangor. Sampling was carried out at different time intervals inside a modified mosquito net as a trap. Inside the trap, adult scuttle flies were aspirated and preserved in 70% ethanol. The fly larvae and pupae were reared until their adult stage to facilitate identification. From this study, six scuttle fly species were collected, i.e., *Dahliphora sigmoides* (Schmitz) ♂, *Gymnoptera simplex* (Brues) ♀, *Megaselia scalaris* (Loew) ♂♀, *Puliciphora borinquenensis* (Wheeler) ♂, *Puliciphora obtecta* Meijere ♀ and *Spiniphora* sp. ♀. Both *D. sigmoides* and *P. obtecta* were newly recorded in Malaysia, whilst the *Spiniphora* sp. was considered an unknown species until it was linked to its male counterpart. The sealed waste bins were found to be accessible for the scuttle flies with delayed arrival (day 4–5). *Megaselia scalaris* was the primary scuttle fly species attracted to the carcass, and its occurrence could be observed between days 4–7 (replicate 1) and days 5–33 (replicate 2). This study also revealed *Sarcophaga* spp. (Diptera: Sarcophagidae) as the earliest species to colonize the remains and the longest to inhabit them (days 2–40). The larvae of *Hermetia illucens* (Linneaus) (Diptera: Stratiomyidae) and *Fannia* sp. (Diptera: Fanniidae) were found on the carcasses during the mid-advanced decay period. These findings expand the knowledge on the diversity of forensically important scuttle flies and coexisting dipterans in enclosed environments in Malaysia.

Key words: Scuttle Flies, Phoridae, Decomposition, Forensic Entomology, Diversity

INTRODUCTION

Scuttle flies (Diptera: Phoridae) are considered to be one of the most diversified groups of insects with a wide range of ecological backgrounds and morphological features (Disney 1994). In forensic entomology, they constitute an important group of Diptera and are commonly found indoors and in enclosed environments. With their small size, they can penetrate narrow gaps and reach corpses faster than other common groups of forensically important flies such as Calliphoridae and Sarcophagidae (Bugelli *et al.* 2015; Zuha *et al.* 2015). In the absence of other flies on a carcass in an enclosed environment, scuttle flies can be utilized as a helpful reference for minimum post-mortem interval (PMI_{min}) estimation (Campobasso *et al.* 2004; Goff 1991).

The role of scuttle flies on animal carcasses as sarcosaprophagous insects is demonstrated by their appearance on decomposing animal carcasses during succession studies and their feeding activity on decaying animal tissues. In a natural environment, numerous scuttle fly larvae and their adults feed on various types of decaying animal tissues (Beaver 1987; Disney 1994; Walker 1957; Zaidi & Chen 2011).

However, current information on forensically important scuttle flies from succession studies is limited to a few common species, such as the cosmopolitan

Megaselia scalaris (Disney 2008) and the "coffin fly", *Conicera tibialis* Schmitz (Martin-Vega *et al.* 2011). Recent findings indicate that more scuttle fly species are likely to appear in forensic cases, including those recorded in succession studies using animal carcasses (Disney *et al.* 2014; Kumara *et al.* 2012; Thevan *et al.* 2010; Zuha *et al.* 2014a). Furthermore, information on decomposition and succession patterns in confined spaces is still lacking. Hence, our research was conducted on scuttle flies in enclosed environments using rabbit carcasses placed inside plastic waste bins.

METHODOLOGY

Two male white rabbit carcasses (*Oryctolagus cuniculus*), each weighing 2.20 kg and 2.07 kg were used. The rabbits were euthanized by administering a lethal injection of pentobarbital drug (0.1 ml/g) (UKM Animal Ethics Committee Reference: FSK/FRSIC/2011/NOOR&ZUHA/16-NOVEMBER/404-NOVEMBER-2011-NOVEMBER-2012-AR-CAT2). This study was conducted at the Forensic Science Simulation Site of the Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Bangi, Selangor (2.91˚N, 101.79˚E), an outdoor research facility adjacent to a secondary forest. The plastic waste bins used in this research were black Century® Model 5012-B (Malaysia), with openings 42.6 cm in diameter, a base 33.7 cm in diameter and a height of 46.0 cm. Twist and lock lids were used to secure the conditions inside the waste bins from disturbances and contamination.

There were two replicates of the study, each with a duration of 40 days. The first replicate was conducted from 4 October 2010 to 13 November 2010, and the second replicate was conducted from 13 December 2010 to 22 January 2011. In each replicate, one rabbit was euthanised at 1000 hrs on the first day of the study. The carcasses were individually placed in plastic baskets as platforms to facilitate observation and weight measurement. A temperature data logger attached with a probe, EL-USB-TC-LCD (Lascar Electronics, Salisbury Wiltshire, UK), was inserted into the carcass anus to measure its hourly internal body temperature. Another data logger, EL-USB-2 (Lascar Electronics, Salisbury Wiltshire, UK), was placed on top of the garbage bin to record hourly microclimatic temperature and relative humidity.

During the first 11 days, samples were taken daily. Between day 11 and day 27, samples were taken every alternate day. Between day 27 and day 40, samples were taken every two days. To avoid contamination by insects attempting to enter the waste bin, sampling was conducted inside a modified mosquito net located within a portable cabin (mesh size approximately 1 mm^2) and layered with cotton fabric. During the sampling, trapped adult scuttle flies were aspirated and preserved in 70% ethanol. The larvae and pupae, presumably of scuttle flies, were reared until adulthood to determine their species. Adult scuttle flies were mounted onto slides using Euparal (ASCO Laboratories, Manchester, UK), and a few samples were brought by the first author to the third author at the Department of Zoology, University of Cambridge, UK, in November 2014 to validate the identification of the species.

RESULTS

Environmental Condition

Outdoor ambient temperatures during each research period ranged between 26.8–32.0°C (replicate 1) and 25.2–29.8°C (replicate 2) whilst relative humidity was 59.5–75.0% (replicate 1) and 72.5–95.0% (replicate 2). Total rainfall during each research period were recorded as 156.4 mm (replicate 1) and 164.4 mm (replicate 2). Temperature data for the surroundings, the waste bins, the carcasses and larval mass are summarised in Figure 1. Due to highly humid conditions inside the waste bins, the data loggers recording the carcasses' internal temperature began to malfunction on day 10 (replicate 1) and day 11 (replicate 2).

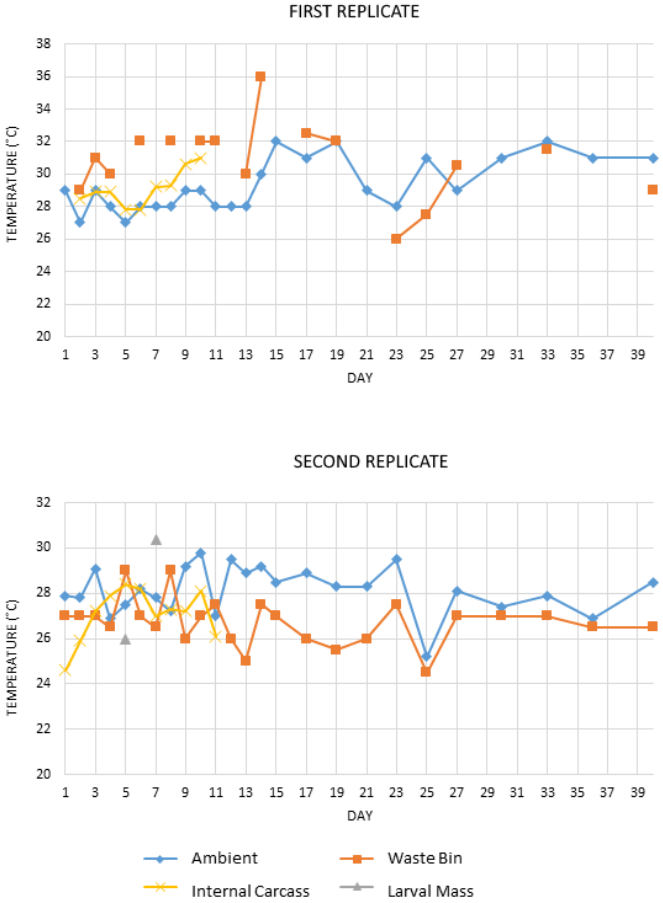

Figure 1: Temperature recordings of the research environments: the surroundings, waste bin, carcass and larval mass in replicates 1 and 2.

Decomposition Stages

Of the five commonly observed animal decomposition stages, four stages were observed during the study period, i.e., fresh, bloated, active decay and advanced decay. The remaining dry stage or skeleton stage was not observed in either replicate. In the first replicate, the bloating stage was observed on day 2–3, while active decay began on day 4. The active decay stage was recognised from the onset of body weight reduction caused by decomposition gases escaping the abdomen. Soft body tissues around the eyes and genitals had decayed and ruptured. On day 7, the skin had darkened, and bodily fluid had started to accumulate at the base of the waste bin. Strong odours of decomposition developed, indicating the beginning of the advanced decay stage. In this confined environment, the decomposition process of the rabbit carcass occurred rather slowly as its weight gradually decreased until day 40. No apparent changes of the carcass's physical appearance emerged between day 7 and day 40 except for superficial parts of the skin, and the extremities became dark and viscous. During the second replicate, similar observations were recorded except for the bloating stage, which could not be properly substantiated by the carcass's swollen physical form since its weight had gradually decreased since day 1. Larvae aggregations were also recorded in replicate 2, where in contrast to replicate 1, larvae were more dispersed throughout the carcass.

Insect Activity

The presence of scuttle flies and coexisting dipterans according to decomposition stage is summarised in Figure 2. In the first replicate, *M. scalaris* larvae were collected from the carcass between days 4–7. There was a limited presence of female adult *M. scalaris* on days 13 and 21. Between days 5–40, we observed that water droplets had formed on the internal side of the waste bin, preventing phorid fly larvae from reaching the carcass.

Larvae of *Sarcophaga* spp. were found to thrive in this environment but in limited quantities (approximately <30). The larvae were collected from the carcasses between day 3 and day 40, but no pupae were present. Larvae of *H. illucens* were also found between days 19–40. On one occasion on day 3, dipteran eggs were discovered on the edge of the waste bin opening, but our attempt to determine the species was unsuccessful. The entire cluster of eggs was damaged due to the opening mechanism of the waste bin lid. The majority of Calliphoridae such as *Chrysomya megacephala* (Fabricius), *Chrysomya nigripes* (Aubertin) and *Chrysomya rufifacies* (Macquart) were found confined to the outside of the waste bin. On only one occasion on day 5, *Chrysomya chani* (Kurahashi) eggs were collected in the narrow gap between the waste bin lid and the waste bin in replicate 1.

In the second replicate, the scuttle flies were more diverse despite the fact that the condition inside the waste bin was almost identical to the first replicate. *Gymnoptera simplex*, *M. scalaris*, *Puliciphora borinquenensis, Puliciphora obtecta* and *Spiniphora* sp. were discovered inside the waste bin. Larvae of *M. scalaris* were collected starting on day 5, and the pupae were found

on day 7 along with *Spiniphora* sp. larvae. Pupae of *P. borinquenensis* were recovered on day 36. In the case of *M. scalaris*, moribund female adults were found on the carcass between days 15–33, but there were no empty puparia found inside the bin. Outside the bin, a single male *D. sigmoides* was recorded on day 11.

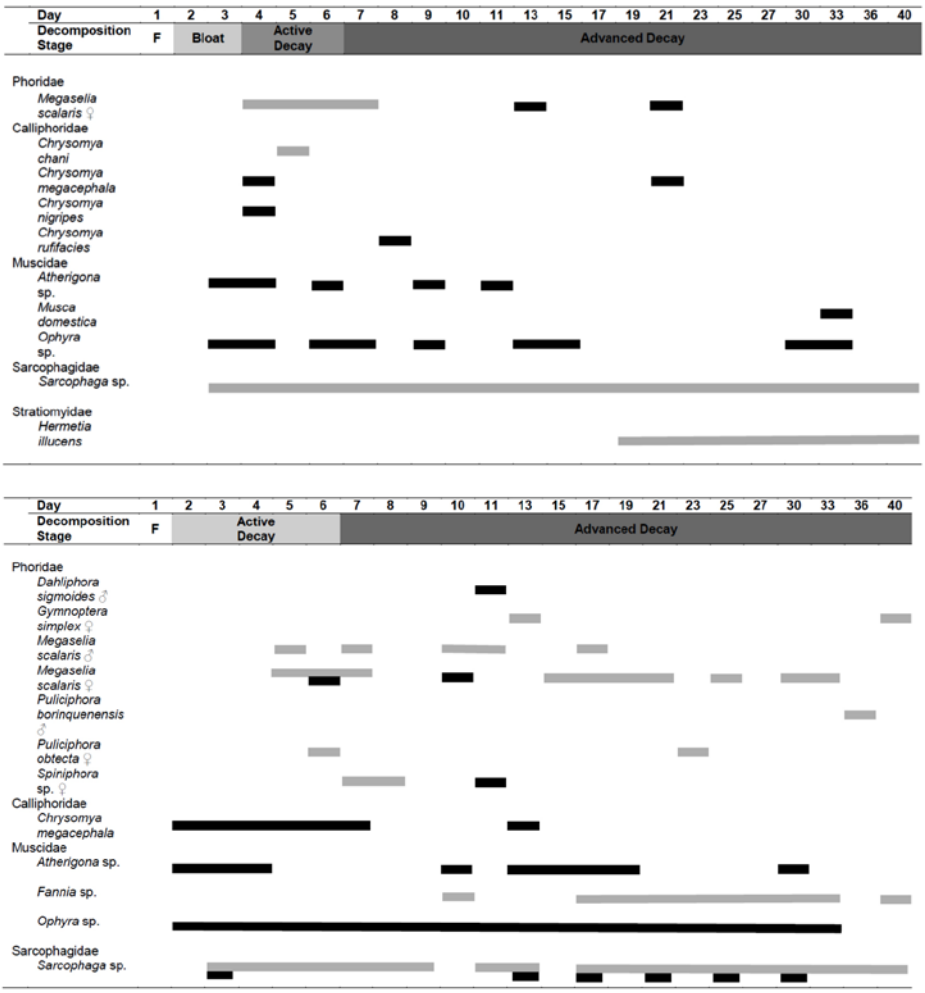

Figure 2: Faunal succession of scuttle flies and other sarcosaprophagous insects on carcass in waste bin during first (A) and second (B) replicates. Indicators: F-Fresh stage, grey lines - insect occurrence inside waste bin, black lines - insect occurrence outside waste bin.

Similar to replicate 1, *Sarcophaga* spp. were found inside the bin between days 3–40. On this occasion, however, the presence of Calliphorids was limited to adult *C. megacephala* outside the waste bin. The first occurrence of *Fannia* sp. was reported on day 10, but their presence was mostly concentrated between days 17–40. On days 25 and 27, adult *Fannia* sp. that had emerged from puparia were found inside the waste bin.

DISCUSSION

In total, six scuttle fly species were recorded in both replicates of this study, i.e., *D. sigmoides* ♂, *G. simplex* ♀, *M. scalaris* ♂♀, *P. borinquenensis* ♂, *P. obtecta* ♀ and *Spiniphora* sp. ♀ (Figure 3). *Megaselia scalaris* has been discovered in forensic and medical cases worldwide (Disney 2008) including Malaysia (Thevan *et al.* 2010), and previous studies indicate that this species is predominantly an indoor species (Kumara *et al.* 2012; Zuha *et al.* 2015). Therefore, in indoor forensic cases, this species can potentially help estimate PMI_{min} (Campobasso *et al.* 2004; Reibe & Madea 2010), especially in the absence of other sarcosaprophagous species (Bugelli *et al.* 2015; Greenberg & Wells 1998; Reibe & Madea 2010; Thevan *et al.* 2010). In this study, the successive pattern of *M. scalaris* could only be observed in replicate 2, where different morphological stages occurred in a sequential pattern. Although the adults could gain entry into the sealed waste bin, they were not the earliest to inhabit the carcass in either replicate.

This study was the first recorded event in Malaysia in which *D. sigmoides* was found near an animal carcass environment. This species is small in size, approximately 0.8 mm in length, typically with a dark brown body. The taxonomic features of this species include distinctive venation of the wings (Zuha *et al.* 2014b). However, the interaction between *D. sigmoides* and the animal carcass could not be confirmed because it was the only scuttle fly species collected outside the waste bin. Knowledge on the ecology, taxonomy and distribution of this genus is still limited. Currently, only five known species have been described, i.e., the Australian *D. sigmoides* (Schmitz 1928) from the Bismarck Archipelago and specimens from Sulawesi, Indonesia, in the University of Cambridge, Museum of Zoology, the Neotropical *Dahliphora crenaticornis* (Borgmeier) and *Dahliphora dispar* (Borgmeier) (Borgmeier & do Prado 1975; Borgmeier 1961) and *Dahliphpra zaitzevi* (Mikhailovskaya) from far eastern Russia (Michailovskaya 2002).

Gymnoptera simplex has been identified as a sarcosaprophagous species and has been researched in dead mollusks (Bohart & Gressitt 1951), dead beetles and a dead rat (Disney & Sinclair 2008). This genus is currently represented by three species; two are Palaearctic, and the third *G. simplex* is tropical (Disney & Sinclair 2008). The first recorded occurrence in Malaysia of *G. simplex,* previously referred to as *Gymnoptera orientalis* (Meijere), was in mollusks (Beaver 1987). *Gymnoptera orientalis*, along with *Gymnoptera molluscovora* (Bohart) and *Gymnoptera neotropica* (Borgmeier), were later synonymized as *G. simplex* (Disney 2003a). Similar to many other phorids, this

species displays striking dimorphism between males and females, and its larvae and pupae resemble those of the *Fannia* sp. (Colyer 1957; Zuha & Disney 2014). The presence of *G. simplex* on the rabbit carcasses in this study suggests its potential occurrence on medium-sized mammal carrion.

Figure 3: New records and undiscovered species collected from the carcass environments, A. *Dahliphora sigmoides*, B. *Spiniphora* sp., C. *Puliciphora obtecta.* Bar = 0.5 mm

Other species with remarkable sexually dimorphic features are *P. borinquenensis* and *P. obtecta*, which can be differentiated by their abdominal tergites. In *P. borinquenensis*, the tergites are usually restricted to the dorsal face of the abdomen. However, tergites in *P. obtecta* are typically very wide, and the lateral margins are extended onto the flanks (Disney 1999). They are carrion-breeding species, and females that were collected in this research were flightless (Zuha *et al.* 2014a). The first reported occurrence of *P. borinquenensis* in Malaysia was in a decomposing beetle (Wheeler 1906). While it is a warm-climate species, its distribution is worldwide (Disney 2003b). Its synonym, *Puliciphora wymani* (Bohart) was previously found in dark locations, suggesting the preference of these species for similar conditions to that of the waste bins (Bohart & Gressitt 1951). The finding of *P. obtecta* in the animal carcass was novel for Malaysia. Previously, this species had been recorded in dead arthropods and had been associated with termites (Disney 1994). Additionally, this species had been recorded in Tokyo (Mitsui & Nakayama, 2012) while its synonym *Puliciphora togata* Schmitz had been widespread in Sumatra, the Philippines, Thailand and China (Borgmeier 1966; Disney 2005). This study's findings further expand the knowledge of the geographical distribution and habits of this species.

Since the identification of Phoridae largely relies on the males, female *Spiniphora* cannot be identified until they are linked to their males. *Spiniphora* sp. was found inhabiting the carcass on days 7–8. Current taxonomic descriptions of this species include the Nearctic, Palaearctic and Oriental regions covering 20 species including the cosmopolitan and forensically important *Spiniphora bergenstammi* (Mik) (Disney 1994; Oliva 2004). Two new species, *Spiniphora dichotoma* (Michailovskaya) and *Spiniphora leleji* (Michailovskaya) were recorded in far eastern Russia (Michailovskaya 1998). In the Oriental region, *Spiniphora* is represented by seven species (Disney & Banziger 2009). The genus *Spiniphora* is distinguished by a pair of bristles in the basal half of the mid-tibia, and bristles at the tip of each fifth tarsal segment are dorso-ventrally flattened (Disney 1994). *Spiniphora* sp. females in this study were 2.0 mm long with dark brown tergites (First to sixth tergites) and yellowish venter. The surface of the ocellar region included a distinct dark triangular patch, while the last tergites were covered by a well-defined flap. Until they are linked to the males, the females remain an undescribed species.

It is noteworthy to mention that the waste bin restricted the access of Calliphoridae to the carcass, and its only attempt involved the oviposition of *C. chani* on day 5 in replicate 1. *Chrysomya chani* previously appeared in succession studies in Malaysia (Nazni *et al.* 2011; Omar *et al.* 1994) including indoor environments (Nazni *et al.* 2011), and it has been discovered in forensic cases (Nazni *et al.* 2015; Sukontason *et al.* 2007). Compared to Phorids and Calliphorids, the Sarcophagids appeared to be the most successful species to gain entry and feed on the carcasses. Since nearly all Sarcophagids are ovoviviparous (Marshall 2012), the larvae deposited near the waste bin lid could gain entry and reach the carcass by squeezing through small gaps beneath the lid. However, in this study, identification was carried out only to the genus level, as all larval and pupal specimens were preserved in 70% ethanol. There are

currently 17 Malaysian species listed as forensically important in Malaysia (Tan *et al.* 2010), but attempts to understand the bionomics of Sarcophagidae in confined environments are scarce. *Hermetia illucens* was also recorded during the final 20 days of this study in replicate 1, but we were unable to ascertain their access strategy. This species has been recorded in many forensic cases worldwide (Dunn 1916; Lee & Cheong 1982; Lee 1989) and has been reported as a cause of enteric pseudomyiasis in Malaysia (Sanniah *et al.* 1994). Due to this limited knowledge, we propose further research to understand interactions of Sarcophagidae and Stratiomyidae in confined environments.

CONCLUSION

The minimum post-mortem interval estimation of decomposing cadavers in confined environments can be more difficult when such conditions limit or delay the access of sarcosaprophagous insects. However, this study demonstrates that phorids thrive in this environment and could likely to be used as a reference to estimate PMI_{min}. The results also extend our knowledge of the diversity of forensically important species in this region. Another aspect to examine further is the bionomics of scuttle flies with respect to decomposition stages, including their coexistence with other forensic dipterans in confined environments such as Sarcophagidae, Muscidae and Stratiomyidae.

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