

Accepted author version posted online: 25 July 2016

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

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Running head: Scuttle Flies from Carcasses in Waste Bin

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 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 *M. 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: Sarcophagidae) yang terawal tiba pada bangkai dan paling lama mendiami bangkai (hari 2-40). Larva *Hermetia illucens* Linnaeus (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.

Kata kunci: Lalat Mencalai, Phoridae, Pereputan, Entomologi Forensik, Diversiti

Abstract: Scuttle flies (Diptera: Phoridae) are small-sized insects of forensic importance. They are notorious for diversified species and habitats but in the context of forensic entomology, scuttle flies inhabiting corpses remain inadequately explored. With recent reports indicating there are more scuttle fly species that possibly could be found from those 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. Study was conducted in two replicates in Bangi, Selangor. Samplings were 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. Larvae and pupae were reared until 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* are new records in Malaysia whilst the *Spiniphora* sp. is hitherto an unknown species until it is linked to its males. Sealed waste bins were found to be accessible for the scuttle flies with delayed arrival (day 4-5). *Megaselia scalaris* was the major scuttle fly species attracted to the carcass and the occurrence could be

observed from day 4-7 (Replicate 1) and day 5-33 (Replicate 2). This study also reveals *Sarcophaga* spp. (Diptera: Sarcophagidae) as the earliest species to colonize the remains and the longest to inhabit carcasses (day 2-40). Larvae of *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae) and *Fannia* sp. (Diptera: Fanniidae) were found on 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.

Keywords: Scuttle Flies, Phoridae, Decomposition, Forensic Entomology, Diversity

INTRODUCTION

Scuttle flies (Diptera: Phoridae) are considered as one of the most diversified group of insects with wide range of ecological background and morphological features (Disney 1994). In forensic entomology, they are an important group of Diptera, commonly found indoors and in enclosed environments. Because of 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 better reference for minimum post mortem interval (PMI_{min}) estimation (Campobasso *et al.* 2004; Goff 1991).

The role of scuttle flies on animal carcasses as sacrosaprophagous insects is shown by their appearance on decomposing animal carcasses during succession studies and their feeding activity on decaying animal tissues. In natural environment, many scuttle fly larvae and their adults feed on various forms 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 *M. scalaris* (Disney 2008) and the “coffin fly”, *Conicera tibialis* Schmitz (Martin-Vega *et al.* 2011). Recent findings show there are more scuttle fly species that are likely to appear in forensic cases including those recorded from succession studies using animal carcasses (Disney *et al.* 2014; Kumara *et al.* 2012; Thevan *et al.* 2010; Zuha *et*

a/. 2014a). Furthermore, information on decomposition and succession patterns in confined spaces is still lacking. Hence our research on scuttle flies from concealed environments using rabbit carcasses placed inside plastic waste bins was conducted.

METHODOLOGY

Two male white rabbit carcasses (*Oryctolagus cuniculus*), each weighing 2.20 and 2.07 kg were used. Rabbits were euthanized by administering 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 Forensic Science Simulation Site, Faculty of Health Sciences UKM Bangi, Selangor (2.91°N, 101.79°E). It is an outdoor research facility adjacent to a secondary forest. The plastic waste bins used in this research are black Century® Model 5012-B, with 42.6 cm diameter of openings, 33.7 cm diameter of ground base and 46.0 cm of height. To secure the condition inside the waste bins from disturbances and contaminations, twist and lock lids were used.

There were two replicates of 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 from 13 December 2010 to 22 January 2011. In each replicate, a rabbit was euthanized at 1000hrs on the first day of study. The carcasses were placed on plastic baskets as platforms to facilitate observation and weight measurement. A temperature data logger attached with a probe, EL-USB-TC-LCD (Lascar Electronics) was inserted into the carcass anus to measure its hourly internal body temperature. Another data logger, EL-USB-2 (Lascar Electronics), was also placed above the garbage bin to record hourly microclimatic temperature and relative humidity.

Samplings were carried out daily during the first 11 days, every alternate day from 11th day until 27th day and every two days from 27th day until 40th day. To avoid contamination by insects that attempted to get into the waste bin, sampling was conducted inside a modified mosquito net located inside a portable cabin (mesh size approximately 1mm²) and layered with cotton fabric. During sampling, trapped adult scuttle flies were aspirated and preserved in 70% ethanol. Larvae and pupae, presumably of scuttle flies, were reared until adult to determine their species. Adult scuttle flies were

mounted on slides using Euparal and a few sample replicates were brought by the first author to the third author at Department of Zoology, University of Cambridge, UK, in November 2014 to validate identification of 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 rainfalls throughout research period were recorded as 156.4 mm (replicate 1) and 164.4 mm (replicate 2). Temperature data, for the ambient, waste bin, carcass and larval mass temperatures are summarized in Figure 1. Due to high humidity conditions inside the waste bins, data loggers intended to record carcasses' internal temperature were found malfunctioning from day 10 (replicate 1) and day 11 (replicate 2).

Decomposition Stages

Out of five decomposition stages commonly observed on animal decomposition, four stages of decomposition were observed during study period, i.e. fresh, bloated, active decay and advanced decay stage. The dry remain stage or skeleton stage was not observed in both replicates. In the first replicate, bloating stage was noticeable on day 2-3 while active decay began on day 4. Active decay stage was recognized from the onset of body weight reduction, caused by the decomposition gases escaping from the abdomen. Soft body tissues around eyes and genitals were decayed and ruptured. On day 7, the skins were darkened, bodily fluid started to accumulate at the base of the waste bin and there were strong odors of decomposition, indicating the beginning of advanced decay stage. In this confined environment, the decomposition process of rabbit carcasses occurred rather slowly as weight gradually decreased until day 40. There were also no apparent changes of the physical appearance of the carcass from day 7 to day 40 except superficial parts of the skins and extremities became dark and viscous. During second replicate, similar observations were recorded except the bloating stage could not be properly substantiated with its swollen physical form as the weight showed

gradual decrement from day 1. Larvae aggregations were also recorded in replicate 2, in contrast to replicate 1, where larvae were more dispersed on the carcass.

Insect Activity

The occurrence of scuttle flies and coexisting dipterans according to decomposition stages is summarized in Figure 3. In the first replicate, *M. scalaris* larvae were collected on day 4-7 on the carcass. There were limited occurrence of female adult *M. scalaris* on day 13 and 21. On day 5-40, we observed water droplets formed on the internal side of the waste bin prevented the phorid larvae to reach the carcass.

Larvae of *Sarcophaga* spp. were found to thrive in this environment but in limited amount (approximately <30). The larvae were collected from carcass from day 3 until day 40 but no pupa was present. Larvae of *H. illucens* were also found on day 19-40. There was an occasion when the first dipteran eggs discovered on day 3 at the edge of the waste bin opening but our attempt to determine the species was unsuccessful. The entire cluster of eggs were damaged due to the opening mechanism of the waste bin lid. Majority of Calliphoridae such as *Chrysomya megacephala* (Fabricius), *Chrysomya nigripes* Aubertin and *Chrysomya rufifacies* (Macquart) were found restricted to outside of the waste bin. There was only one occurrence *Chrysomya chani* Kurahashi eggs collected at the narrow gap between the waste bin lids on day 5 in replicate 1.

In the second replicate, the scuttle flies were more diversified although the condition inside the waste bin was almost similar to the first replicate. *Gymnoptera simplex*, *M. scalaris*, *P. borinquenensis*, *P. obtecta* and *Spiniphora* sp. were discovered inside the waste bin. Larvae of *M. scalaris* were collected from day 5 and its pupae were found on day 7, along with *Spiniphora* sp. larvae. Pupae of *P. borinquenensis* was recovered on day 36. In the case of *M. scalaris*, moribund female adults were found on the carcass on day 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.

Similar to replicate 1, *Sarcophaga* spp. were found inside the bin from day 3 until day 40. But this time, 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 mostly concentrated during day 17-40. On day 25 and 27, adult *Fannia* sp. emerged from the pupa were found inside the waste bin.

DISCUSSIONS

A total of six scuttle fly species were recorded in this study from both replicates, i.e. *D. sigmoides* ♂, *G. simplex* ♀, *M. scalaris* ♂♀, *P. borinquenensis* ♂, *P. obtecta* ♀ and *Spiniphora* sp. ♀. *Megaselia scalaris* has been featured in forensic and medical cases worldwide (Disney, 2008) including Malaysia (Thevan *et al.*, 2010). Previous studies indicate this species as dominant indoor species (Kumara *et al.* 2012; Zuha *et al.* 2015). In indoor forensic cases, it can be a potential indicator to 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 sequential pattern. Although the adults could gain entry into the sealed waste bin, they were not the earliest to inhabit carcass in both replicates.

In this study, *D. sigmoides* (Figure 2A) was recorded for the first time found near animal carcass environment in Malaysia. This species is small, approximately 0.8 mm in length with generally dark brown body. The taxonomic features of this species have been described with distinctive venation of the wings (Zuha *et al.* 2014b). However the interaction between *D. sigmoides* and animal carcass could not be confirmed because it is 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 *Dahliphora zaitzevi* Mikhailovskaya from the far east of Russia (Mikhailovskaya, 2002).

Gymnoptera simplex has been previously recorded as a sarcosaprophagous species and reared on dead molluscs (Bohart & Gressitt, 1951), dead beetles and a dead rat (Disney & Sinclair 2008). This genus is currently represented by three species, where two are Palaearctic and *G. simplex* is a tropical species (Disney & Sinclair 2008). The first record of *G. simplex* in Malaysia was found bred from molluscs but previously referred as *Gymnoptera orientalis* (Meijere) (Beaver, 1987). *Gymnoptera orientalis*, together with *Gymnoptera molluscovora* (Bohart) and *Gymnoptera neotropica*

(Borgmeier) was later synonymized as *G. simplex* (Disney, 2003a). Similar to many other phorids, this species displays striking dimorphism between male and female with its larvae and pupae that resemble those of the *Fannia* sp. (Colyer, 1957; Zuha & Disney, 2014). The presence of *G. simplex* on rabbit carcass in this study suggests its potential occurrence on medium size carrion of mammals.

Other species with remarkable sexually dimorphic features are *P. borinquenensis* and *P. oblecta*, which can be differentiated by their abdominal tergites. In *P. borinquenensis*, the tergites are usually restricted to dorsal face of the abdomen, while tergites in *P. oblecta* are commonly very wide and the lateral margins extended onto flanks (Disney 1999). They are a carrion-breeding species and females that were collected in this research are flightless (Zuha *et al.* 2014a). The first report of *P. borinquenensis* referred to specimens from a decomposing beetle (Wheeler, 1906). This is a warm-climate species and distributed worldwide (Disney 2003b). Its synonym, *Puliciphora wymani* Bohart was previously found in dark places, suggesting the preference of these species in similar condition to the waste bins (Bohart & Gressitt, 1951). The finding of *P. oblecta* on animal carcass is new for Malaysia (Figure 2C). It has been recorded to exploit dead arthropods and associated with termites (Disney 1994). Previously, this species has been recorded from Tokyo (Mitsui & Nakayama, 2012) while its synonym, *Puliciphora togata* Schmitz, is widespread in Sumatra, Phillipines, Thailand and China (Borgmeier 1966; Disney 2005). This finding further expands the knowledge of its geographical distribution and its habits.

Since identification of Phoridae mostly relies on males, female *Spiniphora* could not be named until they are linked to their males. *Spiniphora* sp. was found inhabiting the carcass on day 7-8. Current taxonomic descriptions of from Nearctic, Palearctic and Oriental regions cover 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 from Russian Far East (Michailovskaya, 1998). In the Oriental Region, *Spiniphora* is represented by seven species (Disney & Banziger 2009). Genus *Spiniphora* can be distinguished by having a pair of bristles in basal half of mid tibia and bristle at tip of each fifth tarsal segment is dorso-ventrally flattened (Disney 1994). Female *Spiniphora* sp. from this study is 2.0 mm long with dark brown tergites 1-6 and yellowish venter (Figure 2B). Surface of ocellar region with a distinct dark triangular patch while the last tergites are covered by a well-defined flap. It remains an undescribed species until linked to its male.

It is noteworthy to mention that the waste bin restricted the access of Calliphoridae to the carcass with the only attempt made by the oviposition of *C. chani* on day 5 in replicate 1. *Chrysomya chani* has appeared in succession studies in Malaysia (Nazni *et al.* 2011; Omar *et al.* 1994) including indoor (Nazni *et al.*, 2011) and has been featured in forensic cases (Nazni *et al.* 2015; Sukontason *et al.* 2007). Compared to Phorids and Calliphorids, the Sarcophagids seem to be the most successful species to gain entry and feed on the carcass. Since almost 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 underneath 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 could not ascertain their access strategy. It has been recorded in many forensic cases worldwide (Dunn, 1916; Lee & Cheong, 1982; Lee, 1989) and reported as a cause of enteric pseudomyiasis in Malaysia (Sanniah *et al.* 1994). Due to this current limited knowledge, we propose further research to understand interactions of Sarcophagidae and Stratiomyidae in concealed environments.

CONCLUSION

PMI_{min} estimation of decomposing cadavers in concealed environment can be more difficult when such condition limits or delays the access of sarcosaprophagous insects. However, this study shows that the phorids thrived in this environment and they are likely to be used as reference to estimate PMI_{min}. The results also further extend our knowledge on the diversity of forensically important species in this region. Another aspect to examine further is the bionomics of scuttle flies with respective decomposition stages, including their coexistence with other forensic dipterans in concealed environments such as Sarcophagidae, Muscidae and Stratiomyidae.

ACKNOWLEDGEMENT

Authors would like to the funding institution, School of Diagnostic & Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, for providing technical support. We appreciate the help from Mr. Heo Chong Chin, from Universiti Teknologi Mara (UiTM), for providing assistance to identify the family Muscidae. The first author's learning visit to the Department of Zoology, University of Cambridge, UK was supported by Young Researcher Incentive Grant GGPM-2014-018. The third author's studies of Phoridae are currently supported by a grant from the Balfour-Browne Trust Fund (University of Cambridge).

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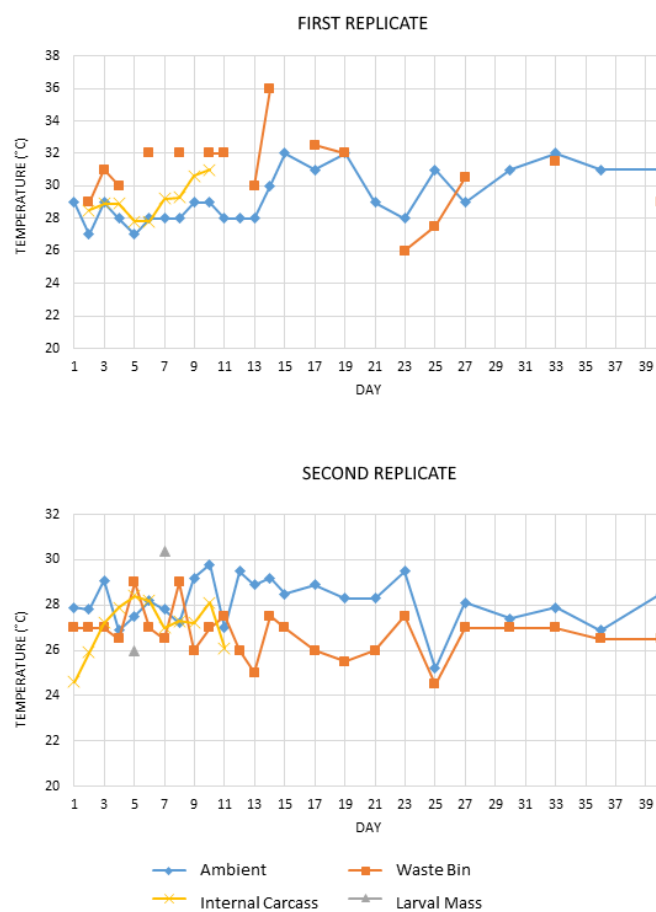


Figure 1: Temperature values of the research environments: the ambient, waste bin, carcass and larval mass temperature in replicate 1 and 2.

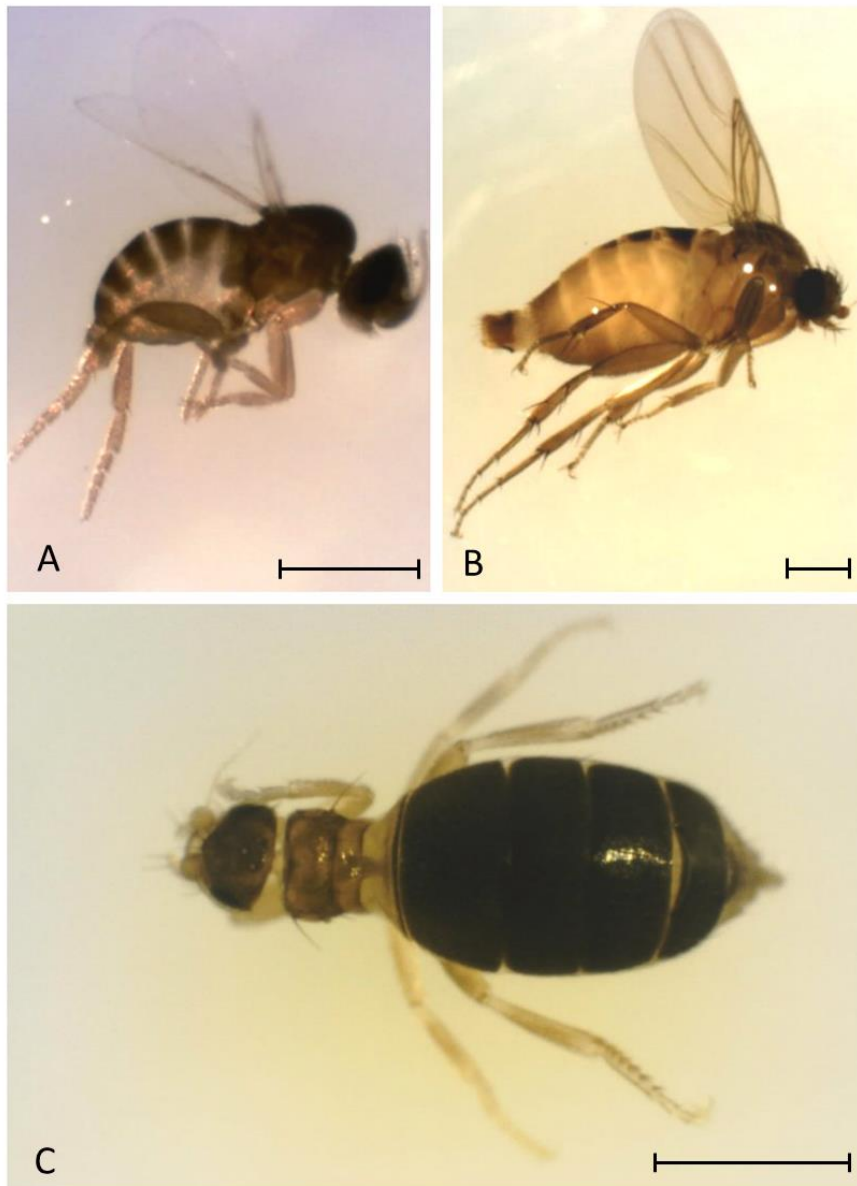
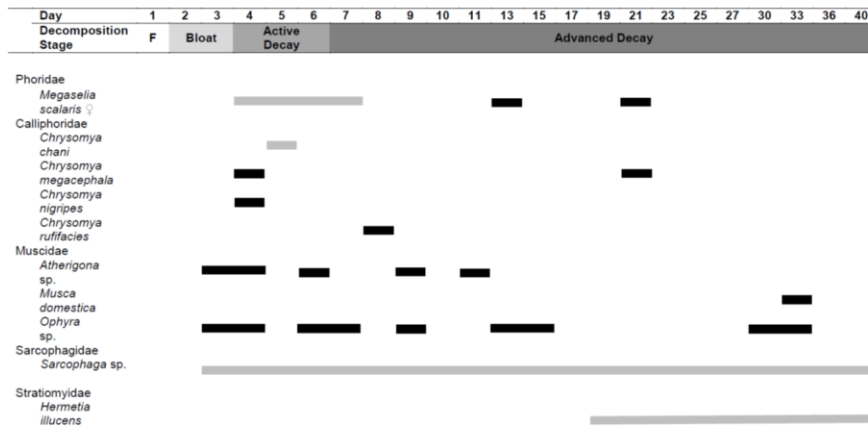


Figure 2: New records and undescribed species collected from the carcass environments, A. *Dahliphora sigmoides*, B. *Spiniphora* sp., C. *Puliciphora obtecta*. Bar = 0.5mm

A



B

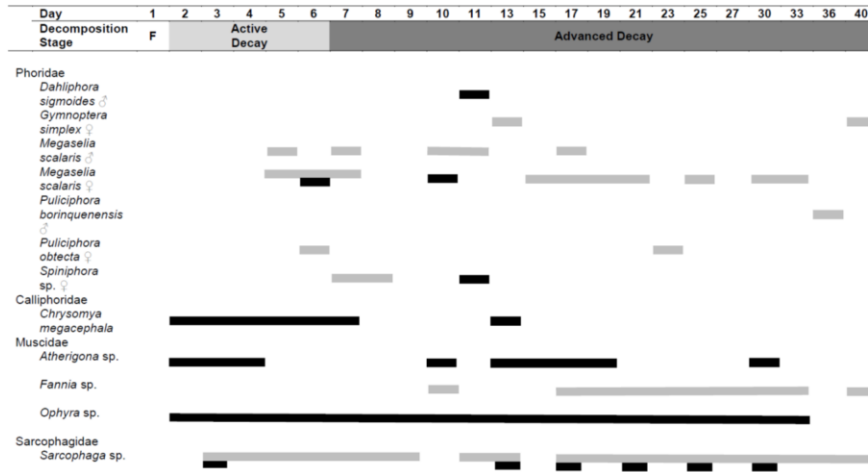


Figure 3: Faunal succession of scuttle fly and other sarcosaprophagous insect on carcass in waste bin during first (A) and second (B) replicate. Indicators: F-Fresh stage, grey lines - insect occurrence inside waste bin, black lines - insect occurrence outside waste bin.