**Preliminary observations of necrophagous dipteran activities and
a new host record of parasitoids on a high-rise building**

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ABSTRACT. A preliminary study on the ecological activities of necrophagous Diptera on a high-rise building in Malaysia was conducted from April to August 2010. Fresh chicken livers (approximately 500 g) serving as bait were placed on the rooftop (101.6 m from the ground). Three replicates were carried out, each replicate lasting 30 days with daily observations and fly sampling. Maggots found in the bait were collected at random: some were preserved in 70% ethanol while the others were reared to the adult stage. Results showed that the flies arrived on the bait between 8 hours and 3 days after placement. The oriental latrine blow fly *Chrysomya megacephala* (Diptera: Calliphoridae) was the first to reach this altitude, followed by the scuttle fly *Megaselia scalaris* (Diptera: Phoridae) and the flesh fly *Liopygia ruficornis* (Diptera: Sarcophagidae). The larvae of *Chrysomya megacephala* were first recovered from the bait. However, they were succeeded by sarcophagid larvae. The predominant sarcophagid larvae were then raised to the adult stage and the resulting adults were identified as *Parasarcophaga dux*. The other sarcophagid pupae were either

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parasitized by hymenopteran parasitoids or dead. We recorded two species of parasitoids emerging from *Liopygia ruficornis* pupae: *Exoristobia philippinensis* (Hymenoptera: Encyrtidae) and *Dirhinus himalayanus* (Hymenoptera: Chalcididae). *Liopygia ruficornis* is recorded as a new host for these parasitoids for the first time.

KEY WORDS: carrion flies, high-rise building, *Liopygia ruficornis*, *Exoristobia philippinensis*, *Dirhinus himalayanus*, new host record, Malaysia.

INTRODUCTION

Oviposition by flies is elicited mainly by the presence of ammonia-rich compounds, but also by moisture, pheromones and tactile stimuli (ASHWORTH & WALL 1994). Oviposition on an animal usually occurs immediately after its death (ANDERSON & VANLAERHOVEN 1996). Blow flies can be attracted to carrion over great distances by specific odours (BRAACK 1981). The timing of oviposition can be affected by several factors such as accessibility to the cadaver (ANDERSON 2001, POHJOISMÄKI et al. 2010), atmospheric temperature (CRAGG 1956, PAYNE 1965), precipitation (MAHAT et al. 2009), humidity (BROWNE 1958), insecticides (MAHAT et al. 2009, RUMIZA et al. 2010), seasons (INTRONA 1991), presence of sunlight (MACGREGOR 1999a, 1999b), habitat characteristics (HOBISCHAK 1997, SILAHUDDIN et al. 2015), burial (RODRIGUEZ & BASS 1985, VANLAERHOVEN & ANDERSON 1999), presence of semiochemicals (WALL & FISHER 2001, LEBLANC & LOGAN 2010), pheromones (ELNAIEM & WARD 1990, 1991), and can be facilitated through interkingdom signalling between microbes and blow flies (MA et al. 2012, DAVIS et al. 2013).

Several decomposition studies using animal carcasses in Malaysia have also indicated that oviposition was initiated immediately after death (OMAR et al. 1994, HEO et al. 2007) and that the insect succession patterns were basically similar in those cases (OMAR et al. 1992, 1994). The pioneering colonizers on cadavers are calliphorids such as *Chrysomya megacephala* (FABRICIUS, 1794) and *Chrysomya rufifacies* (MACQUART, 1842) and are succeeded by flesh flies (Sarcophagidae) and muscid flies (Muscidae) (LEE & MARZUKI 1993, OMAR et al. 1994, HEO et al. 2007, 2008). LEE et al. (2004) and AHMAD et al. (2007) concluded that the predominant forensically important blow fly species were those from the genus *Chrysomya* ROBINEAU-DESVOIDY, 1830.

Synthesiomyia nudiseta VAN DER WULP, 1883 (Diptera: Muscidae) was reported to colonize a dead body found on a thirteen-storey building in Malaysia (SYAMSA et al. 2012). However, little information is known regarding the arrival time and the community composition of necrophagous Diptera on buildings more than 20 floors high. The present paper provides some preliminary observations on ecological activities such as succession,

oviposition and parasitism of necrophagous insects on a high-rise building. We also report a new host record for two parasitoid species in Malaysia.

MATERIAL AND METHODS

Fresh chicken liver (weight approximately 500 g) was used as bait and placed on the rooftop of a tower located at the Universiti Teknologi MARA (UiTM) (3°04'21"N 101°29'77"E, 24 m amsl), which is at a height of 101.6 m above the ground (on the 21st floor). A total of three replicates were carried out in sequence between April and August 2010. The first day of the experiment was designated as Day 1. Each replicate lasted for 30 days and observations were recorded daily during two visits per day to the rooftop: in the morning (~10:00 hrs) and in the afternoon (~16:00 hrs). Permission from the building authority was obtained to utilize the study site. The rooftop is open, uncovered, with a water pump and a water storage tank located in the centre.

The bait was placed below the water storage tank, which acted as a shelter to prevent desiccation due to direct sunlight. Water was added *ad libitum* to maintain bait moisture. Meteorological data such as daily temperatures, humidity and precipitation throughout the study period (27th April – 4th August 2010) were retrieved from the nearest weather station (Kuala Lumpur International Airport; 2°44'51"N; 101°42'49"E). Adult flies attracted to the bait were collected by sweeping and transferred to a glass jar containing chloroform. All adult specimens were pinned, labelled, dried and kept in insect storage boxes. The adult specimens were identified to the lowest possible taxonomic rank using the keys by KURAHASHI et al. (1997), GREENBERG & KUNICH (2002) and TRIPLEHORN & JOHNSON (2005).

Maggots found on the bait were collected randomly: some were preserved in 70% ethanol, while the others were allowed to complete their life cycle. The preserved larvae were mounted on slides following the procedures suggested by LEE et al. (2004). The larval morphology was studied and identified using the keys by ISHIJIMA (1967), GREENBERG & KUNICH (2002) and SZPILA (2010). Pupae collected from the site were placed in a bijou bottle (diameter 25 mm; height 85 mm) with a screw cap. Emergent adult insects were pinned, labelled and identified. A digital stereomicroscope (Olympus SZX7, Japan) and a compound microscope (Olympus BX5, Japan) were employed for imaging and measuring purposes.

The dead and parasitized pupae were identified using a molecular technique. The DNA of each pupa was extracted using the QIAamp® DNA Mini Tissue Kit (Qiagen, Germany) following the manufacturer's protocols. PCR amplification of cytochrome oxidase subunit I and II (COI+II) prior to DNA sequencing was carried out according to TAN et al. (2010).

The DNA sequences obtained were then aligned using Molecular Evolutionary Genetics Analysis (MEGA) version 4.0 (TAMURA et al. 2007) and compared with the local fly DNA databank.

RESULTS

Climatological data

The average maximum and minimum temperatures recorded by the nearest weather station were 33.93 ± 1.33 °C and 24.61 ± 0.90 °C, respectively. The mean temperature was 29.28 ± 0.90 °C while the mean relative humidity was 83.85 ± 3.12 %; the total precipitation during the study period was 776.25 mm.

Dipteran succession and oviposition/larviposition activities

The time of fly arrival, varying between replicates, ranged from 8 hours to 3 days. The oriental latrine blow fly *Chrysomya megacephala* was the first taxon to reach the study site; it was followed by the scuttle fly *Megaselia scalaris* (LOEW, 1866) (Diptera: Phoridae) and the flesh fly *Liopygia ruficornis* (FABRICIUS, 1794) (Diptera: Sarcophagidae). Other than Diptera, ants (Hymenoptera: Formicidae) were *de facto* the first insects to arrive on the bait. However, the population of ants was not abundant and had no obvious deterrent effect on fly oviposition/larviposition. It was observed that the adult flies were most active during the daytime and that their activities ceased in the late evening (~19:00 hrs). 30 adult flies that had been attracted to the bait from April-May were collected and identified. Adults of *Chrysomya megacephala* were the earliest dipterans to arrive and were seen around the bait for a week. Adults of both *Liopygia ruficornis* and *Megaselia scalaris* arrived on the bait on the second day. These species were observed to be present on the bait for more than three weeks. Predatory insects such as spiders and wasps *Vespa* sp. (Hymenoptera: Vespidae) were also observed in the mid or later stage of liver decomposition (from Day 9 onwards).

Within the first 8 hours of bait exposure, a batch of yellowish eggs was deposited by *Chrysomya megacephala*. The first instars hatched from the eggs on the second day and then developed into 2nd instars on the third day. On the fourth and subsequent days, 3rd instars were present. Larval stages of three fly species, namely *C. megacephala*, *Liopygia ruficornis* and *Parasarcophaga dux* (THOMSON, 1869) (Diptera: Sarcophagidae), were recorded on the bait from the second day onwards. In less than a week, *Chrysomya megacephala* larvae were replaced by sarcophagid larvae, which eventually dominated the bait. The underlying reason for this larval succession was not clear, i.e. it could not be determined whether *C. megacephala* larvae were predated by sarcophagids. Although some

post-feeding sarcophagid larvae migrated from the bait to a drier place for pupation, many others remained on the chicken livers. The first pupa was found on the seventh day. Throughout the decomposition process, the adult phorid flies were seen scuttling around the bait, but no oviposition was initiated. We failed to collect adult *Parasarcophaga dux* from the field, although its larvae and pupae were recovered from the baits. All adult sarcophagids collected were identified morphologically as *Liopygia ruficornis*.

Parasitism

Molecular analysis of all parasitized pupae revealed the species of *Liopygia ruficornis*. In general, the survival rate of Sarcophagidae in this replicate was 25% (all emerged as *Parasarcophaga dux*), while the percentage of parasitism was 58%. In contrast, there was 100% mortality in *Liopygia ruficornis* pupae. The complete life cycle of *Parasarcophaga dux* from eggs to adults was 15 to 22 days ($n = 3$, mean temperature 29.95 ± 0.75 °C). The developmental duration of the larval stage was 4 to 9 days. The first pupae formed on Day 7 and the pupal stage lasted for 11 – 13 days (Table 1).

Table 1. Developmental duration of *Parasarcophaga dux* (Diptera: Sarcophagidae) ($n = 3$; mean temperature 29.95 ± 0.75 °C).

Developmental stages	Day	Cumulative day
1 st instar larva (through larviposition)	1	1
2 nd instar larva	1	2
3 rd instar larva (including post-feeding stage)	2 – 7	4 – 9
Pupa	11 – 13	15 – 22
Adult	Emerged between Day 15 and 22	

We reported two species of parasitoids emerged from *Liopygia ruficornis* pupae, namely *Exoristobia philippinensis* ASHMEAD, 1904 (Hymenoptera: Encyrtidae) (Fig. 1) and *Dirhinus himalayanus* WESTWOOD, 1836 (Hymenoptera: Chalcididae) (Fig. 2).

The total “pupation period” for fly pupae ($n = 2$) parasitized by *Exoristobia philippinensis* ranged from 20 to 23 days at a mean temperature 29.83 ± 0.84 °C, which was prolonged for another 8 – 12 days (in comparison with the normal pupation period for *Liopygia ruficornis*, reported as 11.7 ± 0.14 days at 27 ± 4 °C (SUKHAPANTH et al. 1988). The parasitoids emerged from the fly pupae through several self-chewed circular holes on the puparium. *Exoristobia philippinensis* emerged in a swarm of 20 – 50 individuals from a single pupa. Table 2 summarizes the developmental period for *E. philippinensis*.

Fig. 1. A. *Exoristobia philippinensis* (Hymenoptera: Encyrtidae) emerged in a group from a *Liopygia ruficornis* pupa. B. Close-up view of *Exoristobia philippinensis*. C. Dead *Liopygia ruficornis* pupa parasitized by *Exoristobia philippinensis* with four tiny circular holes (average hole diameter 0.53 mm).

Fig. 2. A. Newly emerged *Dirhinus himalayanus* (Hymenoptera: Chalcididae) from a *Liopygia ruficornis* pupa. Note the swollen hind femur (arrow). B. Lateral view of head. C. Dorsal view of head.

Table 2. Developmental duration for *Exoristobia philippinensis* and total number of parasitoids emerged from *Liopygia ruficornis* pupae (n = 2; mean temperature 29.83 ± 0.84 °C). Table headers: ID – pupa ID, DL – date of larviposition, DP – date of pupariation, DE – date of parasitoid emergence, PS – pupal stage (day), N – number of parasitoids emerged, PP – “prolonged pupal stage” (day) as compared to unparasitized *L. ruficornis* pupa.

ID	DL	DP	DE	PS	N	PP*
1	28 th April	4 th May	27 th May	23	20	8 – 12
2	28 th April	7 th May	27 th May	20	47	
3	Unknown	Unknown	27 th May	Indeterminable	50	Indeterminable
4	Unknown	Unknown	27 th May	Indeterminable	24	

* the normal pupal stage in *Liopygia ruficornis* lasts 11 – 12 days at 27 ± 4 °C (SUKHAPANTH et al. 1988)

An adult of *Dirhinus himalayanus* was first sighted as it landed on a pupa on Day 15. On the next day, we collected four sarcophagid pupae from the site; three of them had been parasitized, the parasitoid (*D. himalayanus*) emerging 30 – 32 days later. The unparasitized pupa developed into an adult *Parasarcophaga dux*. On Day 17, another five pupae were collected. Three of them were parasitized by *Dirhinus himalayanus* and one was parasitized by *Exoristobia philippinensis*; no parasitoid or adult fly emerged from the last pupa, so it was presumed dead. The percentage of pupal parasitism was 67 % by *Dirhinus himalayanus* and 11 % by *Exoristobia philippinensis*. Only one adult *Parasarcophaga dux* emerged from the nine pupae collected; the survival rate was thus very low (11%). The developmental period for *Dirhinus himalayanus* (n = 6; mean temperature 26.32 ± 1.99 °C) is summarized in Table 3. Survival rate, percentage of parasitism and mortality rate between the replicates are compared in Table 4. All the specimens from the parasitized pupae belonged to *Liopygia ruficornis* (species identity confirmed by molecular technique), and the emerged adults were identified morphologically as *Parasarcophaga dux*.

Table 3. Developmental duration for *Dirhinus himalayanus* in *Liopygia ruficornis* pupae (n = 6; mean temperature 26.32 ± 1.99 °C). Table headers as in the Table 2.

ID	DL	DP	DE	PS	N	PP*
5	20 th July	3 rd August	2 nd September	30	1	18 – 23
6	20 th July	3 rd August	3 rd September	31	1	
7	20 th July	3 rd August	4 th September	32	1	
8	20 th July	4 th August	5 th September	32	1	
9	20 th July	4 th August	5 th September	32	1	
10	20 th July	4 th August	7 th September	34	1	

* the normal pupation period in *Liopygia ruficornis* lasts 11 – 12 days at 27 ± 4 °C (SUKHAPANTH et al. 1988)

Table 4. Percentage of survival, parasitism and mortality of sarcophagid pupae during the study period.

Month	April – May	July – August
Percentage (%)		
Survival	25 ^a	11 ^b
Parasitism	58 ^c	78 ^d
Mortality	17	11
Total	100 (n = 12 pupae)	100 (n = 9 pupae)

a. All emerged adults were *Parasarcophaga dux* (n = 3).

b. The emerged adult was *P. dux* (n = 1).

c. 58 % of sarcophagid pupae were parasitized by *Exoristobia philippinensis*. All parasitized pupae were *Liopygia ruficornis* (n = 7).

d. 67% of sarcophagid pupae were parasitized by *Dirhinus himalayanus* and 11 % by *Exoristobia philippinensis*. (Total percentage of parasitism = 78 %). All parasitized pupae were *Liopygia ruficornis* (n = 7).

DISCUSSION

Temperature, rainfall and humidity are the main factors influencing dipteran oviposition, especially in an open area. MAHAT et al. (2009) concluded that rain, depending on its intensity, delayed oviposition by 1 – 2 days and prolonged the pupation period to 1 – 3 days. However, in the present study, no rain was recorded on the first day of the experiment.

The life cycle of *Parasarcophaga dux*, based on the emerged adults available, ranged between 15 – 22 days, with a larval stage of 4 – 9 days and a pupal stage lasting 11 – 13 days. In Guam, BOHART & GRESSITT (1951) reported that larval development in *P. dux* was seven days at a temperature of 29.5 °C. ALWAR & SESHIAH (1958) carried out a study in India at temperatures of 23 – 34 °C and found that the larval stage lasted 4-8 days. This finding is very similar to our result. In South Africa, the larval stage was longer at 25 °C, i.e. 8.2 – 10.2 days (ASPOAS 1991). In Malaysia, THEVAN et al. (2013) studied the larval growth of *P. dux* at temperatures fluctuating around 28.9 ± 1.2 °C and a relative humidity of 64 ± 10 %; they found that the larval and pupal stages of *P. dux* lasted for 5.5 days and 3.8 days, respectively. A total of 12.8 days elapsed from the first instar to adult emergence. In Thailand, SUKONTASON et al. (2010) reported that there were differences in developmental duration according to season. They found that prepupae began to form 72 hours, 84 ± 12 hours and 96 hours after the first instar's appearance in summer, the rainy season, and winter, respectively. In Saudi Arabia, development of *P. dux* varied according to temperature and was faster at higher temperatures: developmental duration from first instar to adult emergence required 51.8, 33.0, 25.0, 16.4, and 15.1 days when reared at 16, 20, 24, 28, 32 and 36 °C, respectively (AL-MISNED 2004). Developmental studies have shown variability in the life cycle of *P. dux* according to seasons, temperatures and

geographical locations across the globe. This phenomenon could be due to differences in genotypes as well as phenotypic plasticity (TARONE & FORAN 2006, HWANG & TURNER 2009).

Various parasitoid species from different dipteran hosts have been reported in Malaysia by OMAR et al. (1988, 1991, 2008), SULAIMAN et al. (1990, 1991, 1993) and HEO et al. (2009). BOUCHEK & NARENDHAN (1981) recorded *Dirhinus himalayanus* from *Sarcophaga* sp. while SULAIMAN et al. (1990) found *Dirhinus himalayanus* to parasitize only pupae of *Musca domestica* LINNAEUS, 1758 collected in a poultry farm in Malaysia. KADARSAN & JEFFERY (1981) found that a colony of *Sarcophaga* sp. in the laboratory was parasitized by *Exoristobia philippinensis*. OMAR et al. (2008) and HEO et al. (2009) documented the natural hosts of *E. philippinensis* as *Musca domestica* and *Chrysomya rufifacies*, respectively.

In this study, we determined that *Liopygia ruficornis* is a natural host of *Exoristobia philippinensis* and *Dirhinus himalayanus*. Moreover, the ability of these parasitoids to search for and locate host pupae in a high-rise building is remarkable. We noticed that both species of parasitoids parasitized fly pupae at different intervals, and we hypothesized that this could be a behaviour to avoid interspecific competition. For instance, *Exoristobia philippinensis* arrived earlier than *Dirhinus himalayanus*: whether there is a successional wave of parasitoids cannot be ruled out. It is not clear why *D. himalayanus* was absent during April-May but present in the later months (July – August): perhaps this was due to differences in the bionomics or temporal dynamics of that particular species. The high percentage of pupal parasitism by *D. himalayanus* implies that it might be the dominant species or perhaps a superior competitor to *Exoristobia philippinensis*. Other than interspecific competition, facultative hyperparasitism or intraguild predation could occur (FISCHER 1991, CUSUMANO et al. 2013). According to this hypothesis, *Dirhinus himalayanus* might inject its eggs into fly pupae that have already been parasitized by *Exoristobia philippinensis*. However, further investigations on multiple parasitism or hyperparasitism should be carried out on these parasitoids. GADEN et al. (2006) studied the attack rate of parasitoids and found that *Dirhinus himalayanus* had the highest rates of host attack and progeny production from *Sarcophaga bullata* (PARKER, 1916) (Diptera: Sarcophagidae) and *Hydrotaea aenescens* (WIEDEMANN, 1830) (Diptera: Muscidae), followed by *Stomoxys calcitrans* (LINNAEUS, 1758) and *Musca domestica*. Besides, the differences in life histories demonstrated by *Dirhinus himalayanus* and *philippinensis* may play a role in their successful parasitism. In this case, *Dirhinus himalayanus* seems to possess a K-selection strategy, as it produces only one offspring per individual host, and has a larger body size and a longer life span. In contrast, *Exoristobia philippinensis* exhibited properties of an r-strategist, such as a shorter developmental time, a smaller body size, the production of multiple offspring from one individual host, as well as the lower

probability of surviving to adulthood (MACARTHUR & WILSON 1967).

All emerged adults from pupae were identified as *Parasarcophaga dux* but none of the *Liopygia ruficornis* pupae survived parasitoid attack. There are two possible explanations for this: 1) preference of parasitoids, 2) temporal mismatch between the formation of *Parasarcophaga dux* pupae and the arrival of the parasitoid. Parasitoids may have preference over another host if given a choice, where this choice might include types of habitats, as well as the volatile chemicals, odours, age, size, shape and texture of the hosts (VINSON 1976). Temporal mismatch is defined here as the difference between host pupation time and the arrival time of parasitoids. As such, *P. dux* outcompeted *Liopygia ruficornis* through apparent competition by manipulating the pupation time. However, this hypothesis needs to be examined empirically in order to understand the host-parasitoid interactions.

The parasitism of fly pupae can be vitally important, especially in the determination of the minimum postmortem Interval (mPMI), as highlighted by workers from Australia (VOSS et al. 2009, 2010). Both species are likely to serve as indicators of mPMI owing to their high percentage of parasitism in this study. In our study, the “prolonged pupation period” was due to parasitism. In fact, the “prolonged pupation period” was due to the parasitoid larvae feeding on the developing pharate within the puparium and eventually killing the host (GRASSBERGER & FRANK 2003). Therefore, we recommend forensic entomologists to retain fly pupae until eclosion for a longer period in order to allow for the possible emergence of parasitoids, which might be useful for the determination of mPMI by working backwards based on the developmental time of the dipteran host (from eggs to pupal stage) and the developmental periods of parasitoids (from egg insertion to parasitoid emergence). In the case of *Exoristobia philippinensis*, the first parasitized sarcophagid pupa could have occurred on Day 6 or 7 after bait exposure and the pupation period could last for 23 days, whereas *Dirhinus himalayanus* parasitized the first pupa on Day 12 or 13 and remained in the pupa for another 34 days. In general, we noted that *D. himalayanus* had a longer development time than *Exoristobia philippinensis*. This result was almost identical to that of SRINIVASAN & AMALRAJ (2003), who found a mean generation time for *Dirhinus himalayanus* of 36 days. GRASSBERGER & FRANK (2003) studied the relationship between temperature and the development time of a parasitoid, *Nasonia vitripennis* (WALKER, 1836), on the pupae of *Protophormia terraenovae* ROBINEAU-DESVOIDY, 1830 (Diptera: Calliphoridae). They found that the development time was inversely correlated with temperature in this species and suggested the use of parasitoids as a reliable mPMI indicator. In nature, fly pupae are mostly found buried under the soil (DENLINGER 1972, OMAR et al. 1994, HEO et al. 2008), which is what may protect them from becoming parasitized. GADEN (2002) did a study on the efficacy of parasitoid attack on host pupae in different types of habitats and at different depths. He found that *Dirhinus himalayanus* confined itself to the soil surface when searching for a suitable fly host in manure, and the

number of pupae killed fell with increasing soil depth. In this study, the floor on the rooftop is made of concrete; the larvae thus have to pupate on this surface, which exposes them to the external environment. Consequently, forensic entomologists should be alert to the fact that exposed fly pupae may be parasitized and should monitor the emergence of parasitoids.

In this study, we highlight the ecological and biological activities (succession and oviposition/larviposition) of necrophagous dipterans on a high-rise building, the general developmental duration of *Parasarcophaga dux*, a new host record of a parasitoid (i.e., *Liopygia ruficornis*) and parasitoid developmental duration in the hosts. Entomological studies should be carried out in urban environments as rapid urbanization has driven changes in insect ecology, and consequently in the application of insect sciences.

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