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Research Note

Multiple species of scuttle flies (Diptera: Phoridae) as contaminants in forensic entomology laboratory insect colony

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Abstract. In forensic entomology, larval rearing usually includes the presence of biological contaminants including scuttle flies (Diptera: Phoridae). Scuttle flies are recognized as forensically important insects and have been reported causing nuisance and contamination in laboratory environments. This paper reports for the first time the finding of multiple scuttle fly species affecting colonies of third instar larvae of the Oriental latrine blowfly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae), reared indoors at the Forensic Science Simulation Site, Universiti Kebangsaan Malaysia. Adult scuttle flies were discovered inside a rearing container after the emergence of adult *C. megacephala*., The scuttle fly species are *Megaselia scalaris* (Loew), *M. spiracularis* Schmitz and *Puliciphora borinquenensis* (Wheeler). Notes on the life history and biology of these species are discussed herein.

Forensic entomology deals mainly with the collection of live and preserved sarcosaprophagous insects to investigate minimum post mortem interval (mPMI) (Tomberlin *et al.*, 2011). In forensic entomology laboratories, live larvae are collected and reared to facilitate species identification during the adult stage. In order to develop growth models as reference to estimate mPMI, larvae are reared at constant temperatures in a controlled laboratory setting, and this procedure usually requires the utilization of animal tissues, such as beef or porcine liver, as a larval substrates (Byrd, 2001). Without proper rearing precautions, rearing media may be colonized by other carrion feeding insects capable of entering enclosed environments, such as scuttle flies (Diptera: Phoridae).

Due to their occurrence in concealed environments such as coffins or in clandestine shallow graves of buried remains, scuttle flies are considered to be important dipteran species of forensic significance (Benecke, 2008; Martin-Vega *et al.*, 2011). They can also easily penetrate narrow openings in laboratories and contaminate insect cultures (Costa *et al.*, 2007; Mongiardino Koch *et al.*, 2013).

This report is an incidental finding during a preliminary research on carrion-visiting scuttle fly in aquatic and non-aquatic environment at the Forensic Science Simulation Site, Universiti Kebangsaan Malaysia, Bangi, Selangor (2.91°N, 101.79°E). It is intended to elaborate the presence of multiple species of scuttle flies in the larval

culture of the Oriental latrine blow fly, *C. megacephala*.

In this study, two rabbits, weighing between 2.5 - 2.9 kg were sacrificed by lethal dose of phenobarbital drug via intravenous injection. Carcasses were placed in 60 (*length*) × 30 (*width*) × 35 (*height*) cm transparent water tanks from 3 March until 2 April 2014. One carcass was drowned in 10 *l* of water (aquatic) while another was placed in a dry environment (non-aquatic). Both water tank openings were covered with two layers of metal wire mesh, the upper layer with 1 cm holes to avoid scavenging by animals and the lower layer with approximately 2 mm holes to permit the entrance of scuttle flies and block the presence of common forensic flies such as blow flies and flesh flies. The water tanks were placed side by side on plastic benches (approximately 0.5 m from the ground) and left exposed in outdoor environment.

Throughout the duration of this study, it was found that blow flies from different developmental stages (i.e. egg, larvae, pupae and adults), colonized the carcass in the aquatic tank. In the non-aquatic tank, there was no presence of blowfly. It was possible that blow flies managed to gain entry into the tank through small gaps at the edge of the water tank and oviposited on the carcass. On 6 and 7 March 2014, four batches of larvae consisting of 20 individuals per batch (presumably blow fly larvae of approximately similar length), were sampled from the surface of the carcass in the aquatic environment to facilitate identification of their species. The four larval batches were placed in separate 500 ml cylindrical plastic containers, supplied with approximately 100 g of cow liver as food source. Container lids were fitted with nylon mesh (<1.0 mm openings) for ventilation and to prevent larvae from escaping. Rearing took place in a portable cabin with uncontrolled room temperature (21-25ºC) and relative humidity (≈65-80%). During this stage, the larvae were presumed to be a single blowfly species based on the gross morphology of the larvae and the abundance of similar-looking adult blowflies on carcass. The containers and larvae were checked every alternate day to ensure adequate food supply until eclosion occurred on 15 March. Adults from all replicates were identified as *C. megacephala* and allowed to die naturally in the rearing container to record their longevity.

On 18 March, researchers noticed adult scuttle flies on the surface of the remaining decomposed cow 's liver in one of the rearing containers. There were also a few scuttle fly puparia identical to *M. scalaris*, near the liver, implying development of the flies took place in the rearing container. By comparing with known developmental period of *M. scalaris* at similar room temperature (Zuha & Omar, 2014), the age of pupae was estimated between 4–4.6 days from oviposition. This suggests development of scuttle flies began 8–9 days from the first day rearing of *C. megacephala* larvae.

Although the nylon mesh layer remained intact, we found that the size of the holes on the mesh permitted the entry of adult scuttle flies. There was also no evidence to suggest parasitoidism or parasitism of scuttle flies on *C. megacephala*, as all specimens reached eclosion. However, we do not rule out the possibility that adult scuttle flies were attracted to a few moribund or dead adult *C. megacephala* inside the rearing container. Scuttle flies were aspirated from the container and preserved in 70% ethanol. Using stereomicroscopic analysis, those specimens were all subsequently identified as male and female *M. scalaris* (♂=6, ♀=7), *M. spiracularis* (♂=2, ♀=3) and *P. borinquenensis* (♂=9, ♀=48). Identification of *M. scalaris* and *M. spiracularis* was confirmed via taxonomic keys based on Indo-Australian phorid flies (Borgmeier, 1966, 1967), while *P. borinquenensis* was referred to previous consultation with the third author and his publications (Disney, 1999; Disney & Sinclair, 2008).

In the absence of other species, *M. scalaris* has been reported as a subject of interest in forensic entomology as the best reference for PMI estimation in concealed environment such as graves and in coffins (Campobasso *et al.*, 2004; Reibe & Madea, 2010). An exhaustive background

information and natural life history of this species has been described (Disney, 2008). It is a causative agent of urinary myiasis (Wakid, 2008) and contaminant of food products (Nickolls & Disney, 2001; Brown & Oliver, 2007). In a laboratory environment, it has been found infesting triatomine bugs (Costa *et al.*, 2007) and mantids (Mongiardino Koch *et al.*, 2013). In nature, this species is also a parasitoid of ticks (Andreotti *et al.*, 2003). Due to its widespread occurrence in laboratory insect colonies, some authors suggest preventive measures such as reducing the density of reared specimens, as well as rearing in drier conditions and a cleaner environment (Costa *et al.*, 2007; Mongiardino Koch *et al.*, 2013).

Although the occurrence of *M. spiracularis* in forensic cases is limited, it has been detected on human corpses indoors in Malaysia (Thevan *et al.*, 2010). This species is considered medically important, causing lung (Komori *et al.*, 1978) and intestinal myiasis (Ogawa *et al.*, 1959). Currently known distributions of *M. spiracularis* are Malaysia, Japan, Taiwan (Borgmeier, 1966), China (Feng & Liu, 2012) and New Zealand (Brown & Oliver, 2007). Less is known about the bionomics of this species but it has recently been extensively studied due to its importance in forensic and medical entomology (Feng & Liu, 2012, 2013).

Puliciphora borinquenensis is possibly the most interesting species in this report, due to its significant sexually dimorphic features. Females are flightless and have an egg-shaped abdomen (Wheeler, 1906). Males airlift the flightless females to oviposition sites (Miller, 1984). The larvae consume varying types of decaying organic materials (Disney, 1994). While females of several species of this genus are found in termite or ant nests, (Disney, 1999) they have yet to be detected in forensic cases (Boehme *et al.*, 2010). Apart from this finding, *P. borinquenensis* was reported infesting a laboratory culture of cockroaches (Miller, 1979). The authors noticed the occurrence of this species on a rabbit carcass placed inside a sealed luggage (Zuha *et al.*, 2014). Current work to systematically identify *Puliciphora* species and other scuttle flies of forensic importance is still in progress.

Due to the natural ability of scuttle flies to exploit small openings to reach the food source, we suggest proper measures to be taken into consideration when rearing insects for forensic entomological procedures. Researchers are advised to ensure the laboratory is clean, well ventilated and rearing containers to be kept in sealed incubators or using cloth to modify rearing containers. It is also important to conduct further research to better understand the effect of such contamination on laboratory insect colonies including its implications in forensic entomology.

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