

## Short Communication

### First report of *Histiostoma* sp. (Astigmata: Histiostomatidae) phoretic on a forensically important blowfly, *Chrysomya villeneuvei* (Diptera: Calliphoridae)

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**Abstract.** This is the first record of phoretic histiostomatid mites found on a forensically important blow fly species, *Chrysomya villeneuvei* (Diptera: Calliphoridae), collected from decomposing rabbit carcasses placed in Bukit Lagong Forest Reserve, Sungai Buloh and MARDI Cameron Highlands, Malaysia. The blow flies frequenting around the carcasses were first captured using an insect net. After pinning, they were examined under a stereomicroscope and mites phoretic on their body were carefully removed and preserved in 70% ethanol. Mites were cleared in lactic acid before mounting on slides using Hoyer's medium and identified under a compound microscope. The flies and their mites were identified as *C. villeneuvei* and deutonymphs of *Histiostoma* spp. (Astigmata: Histiostomatidae), respectively. This insect-mite association may be useful to provide insights regarding the minimum post-mortem interval and the location of death in forensic entomological investigations.

The identification of insect species allows forensic entomologists to link the duration of the developmental stages of insects for minimum post-mortem interval (mPMI) determination (Turchetto & Vanin, 2004). Necrophagous insects such as flies and beetles are commonly utilized for this purpose in forensic investigations (Byrd, 2000; Kulshrestha & Satpathy, 2001). Flies, especially Calliphoridae or blow flies, helped providing valuable mPMI estimation as they are usually the primary colonisers to decomposing bodies (Eberhardt & Elliot, 2008; Martin-Vega *et al.*, 2017). In Malaysia, flies that are frequently associated with forensic cases are Calliphoridae such as *Chrysomya megacephala*, *Chrysomya*

*rufifacies*, *Chrysomya nigripes* and *Chyrosmya villeneuvei* (Kavitha *et al.*, 2013).

Phoresy is the process where mites attached themselves to animals or other arthropod hosts while restricting their own development and feeding behaviour for dispersal (Farish & Axtel, 1971). A survey in Brazil reported few findings of phoretic mesostigmatid mites on necrophagous flies (Sato *et al.*, 2018). As for astigmatid mites, phoresy is commonly found in the families Acaridae and Histiostomatidae (OConnor, 2009). Within Histiostomatidae, *Myianoetus muscarum* was found in abundance on muscid flies, *Synthesiomyia nudiseta*, which were collected from human remains (Pimsler *et al.*, 2016). Another member of

the same family, which was often reported on carcasses (Goff *et al.*, 1986; OConnor, 2009), beetles (e.g., Trogidae) and flies (e.g., Sciariidae and Phoridae), was from genus *Histiostoma* (Perotti *et al.*, 2010). To date, there is no report of these mites on Calliphoridae in Malaysia.

The specificity of mites to their microhabitats and their life cycles has been used as supporting data in a few crime investigations (Hani *et al.*, 2018; Kwak & Schubert, 2019). As for death investigations, mites assisted by confirming location of crime scenes and the possible environment in which the victim might have been previously exposed (Szelecz *et al.*, 2018). Mites, especially the phoretic ones, have been beneficial to determine the estimated mPMI in various cases (Gonzalez-Medina *et al.*, 2013; Kamaruzaman *et al.*, 2018). In Malaysia, the studies of Acari for forensic purposes are still scarce (Azima *et al.*, 2011; Silahuddin *et al.*, 2015). Therefore, it is imperative to report any related finding of phoretic mites and their respective insect hosts collected from carrion or their surrounding in this country for further forensic evaluation and applications. This paper reports the first finding of *Histiostoma* spp. on a forensically important blow fly, *C. villeneuvei* from decomposing rabbit carcasses placed in Bukit Lagong Forest Reserve, Selangor, and the Malaysian Agricultural Research and Development Institute (MARDI) at Cameron Highlands, Pahang, Peninsular Malaysia.

The blow flies surrounding rabbit (*Oryctolagus cuniculus*) carcasses were captured using an insect net from Bukit

Lagong Forest Reserve, Sungai Buloh, Selangor, (3.25°N 101.616667°E, 368 m above sea level (a.s.l)) and MARDI Cameron Highlands (4.468029°N 101.384754°E, 1450 m a.s.l.). The study was conducted for 4 months, from July 2018 to October 2018. The blowflies were captured daily at 0900 (1 hour per day) from fresh stage of decomposition until advanced decay stage of rabbit carcasses. A killing jar containing cotton balls soaked in absolute ethyl acetate was used to kill the collected adult flies. The dead flies were subsequently pinned on the right side of the upper thorax region before placing them in the oven at 40°C for 4–5 days for dehydration purposes. Blow fly specimens were then identified at genus and species levels by using Greenberg & Kunich (2002) prior to their labelling. They were examined under a stereomicroscope (Olympus SZ51, Japan) and mites that phoretic on the body regions of the flies were carefully removed using a fine brush and preserved in 70% ethanol. A total of 20 phoretic mites (10 random mites from all infested blow flies collected in both study locations) were cleared in lactic acid, mounted on slides using Hoyer's medium and observed under a compound microscope (Olympus BX53, Japan) for their identification based on common mites' morphological keys (Krantz & Walter, 2009; Negm & Alatawi, 2011). The host blow flies were identified as *C. villeneuvei* and their phoretic mites were *Histiostoma* spp.

The total number of collected blow flies were 262 and 116 in Bukit Lagong Forest Reserve and MARDI Cameron Highlands, respectively (Table 1). There were approxi-

Table 1. The total number of blow flies according to species collected from the study sites in Bukit Lagong Forest Reserve and MARDI Cameron Highlands

| Adult Diptera                 | Abundance of adult Diptera collected |                         |
|-------------------------------|--------------------------------------|-------------------------|
|                               | Bukit Lagong Forest Reserve          | MARDI Cameron Highlands |
| <i>Chrysomya megacephala</i>  | 40                                   | 58                      |
| <i>Chrysomya rufifacies</i>   | 112                                  | 26                      |
| <i>Chrysomya villeneuvei</i>  | 45                                   | 16                      |
| <i>Hypopygiopsis violacea</i> | 5                                    | 0                       |
| <i>Lucillia cuprina</i>       | 60                                   | 0                       |
| Unidentified species          | 0                                    | 16                      |
| Total                         | 262                                  | 116                     |

mately 150 deutonymphs of *Histiostoma* spp. found phoretic on several adults *C. villeneuvei*. The finding of phoretic mites could be related to the unique behaviour of *C. villeneuvei* where the males eclosed from its pupa earlier than the females and waited for the females in order to secure a successful copulation (Omar *et al.*, 1994). The time taken by the male flies while waiting for the females provides ample time for the mites to attach themselves on the body of the flies.

The host flies were captured in both study locations; on Day 16 postmortem in Bukit Lagong Forest Reserve and on Day 18 post-mortem at MARDI, Cameron Highlands when the rabbit carcasses were already at the advanced decay stage. In Bukit Lagong Forest Reserve, it took 7 days for the rabbits to reach the advanced decay stage at the average temperature of 25.5° and mean precipitation 2.9 mm. Meanwhile in MARDI, Cameron Highlands, the rabbit carcasses took longer time to reach the advanced decay stage which was at Day 11 with the average temperature of 22.1° and the mean precipitation of 0.8 mm. A similar decomposition study showed that rabbit carcasses reached the advanced decay stage at Day 17 in Cameron Highlands (Silahuddin *et al.*, 2015). The finding of phoretic *Histiostoma* spp. at this advanced decay stage coincided with previous forensic reports of Acari collection from animal carcasses (Perotti & Braig, 2009; Perotti *et al.*, 2010). It is assumed when the carcasses reached the advanced decay stage of

decomposition, the carrion resource became limited for the existing arthropods. Therefore, for survival purposes, the astigmatid mites, *Histiostoma* spp. rode on possibly newly emerged adults, *C. villeneuvei* from the carcasses, in order to move to a more resourceful environment (e.g., new carrion). The evidence of finding *Histiostoma* spp. on the thorax and abdomen of *C. villeneuvei* at both study locations indicated that one of the common insect hosts for *Histiostoma* spp. is *C. villeneuvei*.

The histiostomatid mites may be useful as an indicator in cases where the dead body was not at the crime scene or has been removed from its original location. The mites might indicate the body has been moved from the original crime/death scene after it has been decomposed at the advanced decay stage. The specificity of Acari to their microhabitat has proven that it can be used as the geographical indicator in forensic investigations. The finding of synanthropic mites, *Sancassania berlesei* (Astigmata: Acaridae) and *Macrocheles matrius* (Mesostigmata: Macrochelidae) on the human bones found in Swiss forest indicated that the victims were first murdered elsewhere before being buried there (Szelecz *et al.*, 2018). The detection of mites specific to the Australasia region has helped to solve a robbery case by narrowing down the location of the robbed money (Hani *et al.*, 2018). Besides our study, other researches have also reported *C. villeneuvei* as among the early colonisers for corpses and

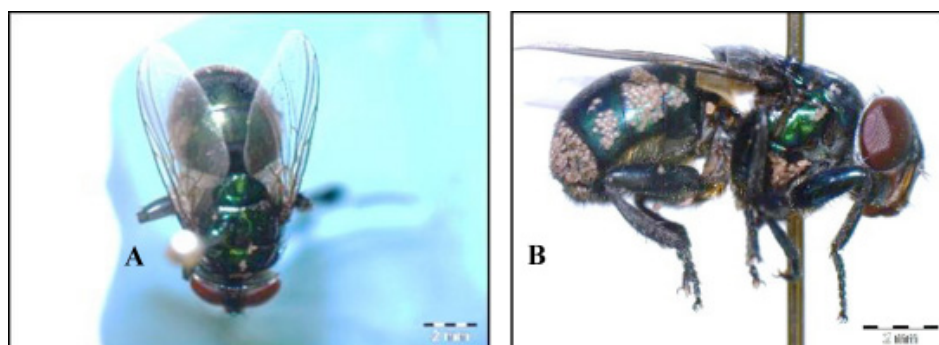


Figure 1. Adult of *C. villeneuvei*. (A) Dorsal view showing several phoretic mites on the surface of prothorax and mesothorax; (B) Lateral view showing phoretic mites on notopleuron, mesopleuron, sternopleuron, hypopleuron, coxae, trochanters, femur, and abdominal tergites 1-5.

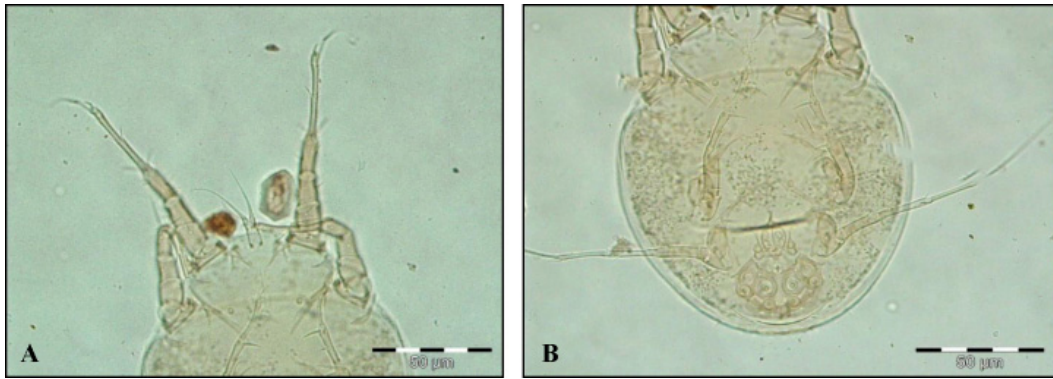


Figure 2. Microscopic morphology of the deutonymph mite, *Histiostoma* sp. in ventral view. (A) Anterior part (40x); (B) Posterior part (40x).

carcasses found in jungles and highlands (Chen *et al.*, 2010; Kumara *et al.*, 2012; Silahuddin *et al.*, 2015). Therefore, the finding of *Histiostoma* spp. on dead bodies at other localities than previously mentioned might indicate suspicious movement of bodies from original death locations.

Furthermore, phoretic mites have been known to assist in mPMI determination. Mites, *Poecilochirus austroasiaticus* (Mesostigmata: Parasitidae), collected at a crime scene helped to provide mPMI of 13 days despite the absence of their respective carrier beetles (Gonzalez-Medina *et al.*, 2013). Also, mites from the family Macrochelidae that phoretic on both flies and beetles can provide secondary mPMI estimation for death investigations (Kamaruzaman *et al.*, 2018). As for our study, the discovery of these mites on *C. villeneuvei* or on dead bodies may help in the mPMI estimation based on their presence during the advanced decay stage of decomposition as indicated in the current study (i.e., Day 7-PMI at lowland jungle and Day 11-PMI at highland). Thus, it is vital to document more association or relationship between phoretic mites and their respective insect host for further application in forensic entomological cases.

The relationship between *Histiostoma* spp. with their blowfly host, *C. villeneuvei* remains debatable. One case of parasitic association has been observed for a phoretic mite, *Macrocheles subbadius*

(Mesostigmata: Macrochelidae), that feeds off the haemolymph of its host, *Drosophila nigrospiracula* (Diptera: Drosophilidae) (Polak, 1996). Houck & Cohen (2001) observed the caudal ventral sucker of phoretic deutonymphal *Hemisarcoptes cooremani* (Astigmata: Hemisarcoptidae) is capable to pierce their host beetle, *Chilocorus cacti* (Coleoptera: Coccinellidae) despite having limited mouthparts. The same observation might apply to *Histiostoma* spp. and *C. villeneuvei* as the mites collected from the blowflies were in their deutonymphal stage. At this stage, mites in general, lacked mouthparts and did not feed, but further studies have shown that the definition of phoresy itself need to be revisited (Camerik, 2010). Thus, the finding of *Histiostoma* spp. on *C. villeneuvei* requires further research for better understanding of their ecological relationship.

In conclusion, mites are potentially useful in forensic investigations as they are ubiquitous in their distribution. The finding of *Histiostoma* spp. phoretic on *C. villeneuvei* could be applied in forensic investigations for mPMI estimation and death location determination in Malaysia. Therefore, continuous cataloguing of mites' distribution in various geographical habitats such as oil palm plantations, streams, lakes and mangrove swamps is necessary to incorporate acarology into death investigations.

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