## **Research Note**

## A preliminary report on ants (Hymenoptera: Formicidae) recovered from forensic entomological studies conducted in different ecological habitats in Malaysia

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**Abstract.** This study reported the ant species that were recovered from monkey carcasses in three different ecological habitats in Malaysia. The study was conducted from 9 May – 10 October 2007, 6 May – 6 August 2008 and 26 May – 14 July 2009 in forested area (Gombak, Selangor), coastal area (Tanjong Sepat, Selangor) and highland area (Bukit Cincin, Pahang), respectively. Monkey carcass was used as a model for human decomposition in this study. A total of 4 replicates were used in each of the study sites. Ants were observed to prey on eggs, larvae, pupae and newly emerged flies. This study found that ant species could be found at all stages of decomposition, indicating that ants were not a significant indicator for faunal succession. However, different species of ants were obtained from monkey carcasses placed in different ecological habitats. *Cardiocondyla* sp. was only found on carcasses placed in the coastal area; while *Pheidole longipes*, *Hypoponera* sp. and *Pachycondyla* sp. were solely found on carcasses placed in the highland area. On the other hand, *Pheidologeton diversus* and *Paratrechina longicornis* were found in several ecological habitats. These data suggests that specific ant species can act as geographic indicators for different ecological habitats in forensic entomology cases in Malaysia.

The feeding habits of insects may range from being predatory, feeding on larvae and adults of other insects to necrophagous when it consumes exudates or decomposing tissues. Generally, ants feeding on carcasses are categorised as omnivorous species (Tabor *et al.*, 2005) in forensic entomological study. They can feed on both the corpse and associated fauna. Ants feeding on corpses can cause small punctate or scratch type lesions over the skin surface, which can be easily misinterpreted as ante-mortem abrasions or resulting from strong acids (Campobasso *et al.*, 2009). However, ants are seldom reported as forensic indicators by crime scene investigators and forensic pathologists. The present study documented the presence of ants in different ecological habitat of primate carcass experiments in Malaysia.

The study was conducted in 3 different ecological habitats: forested area (Field Study Centre, University of Malaya, 16<sup>th</sup> Mile off Gombak District, Selangor), coastal area (Tanjong Sepat, Selangor) and highland area (Bukit Cincin, Genting Highland, Pahang). The ecological description of each study site is presented in Table 1. The study was

Study Site	Geographical Information	<ul> <li>Ecological Description</li> <li>A secondary forested area with very low human populations and activities.</li> <li>The nearest human dwelling was approximate 300 meters from the study site.</li> </ul>			
Field Study Center, University of Malaya, Selangor	<ul> <li>3°17'57.86"N, 101°47'00.78"E</li> <li>Elevation: 200m asl</li> </ul>				
Tanjong Sepat, Selangor	<ul> <li>2°39'12.29"N, 101°34'27.66"E</li> <li>Elevation: 6m asl</li> </ul>	<ul> <li>A coastal area with high human populations and activities.</li> <li>The beach was not clean, garbage was found around the study site.</li> <li>Human dwelling was found nearby the coastal area.</li> </ul>			
Bukit Cincin, Genting Highland, Pahang	<ul> <li>3°26'21.39"N, 101°46'59.36"E</li> <li>Elevation: 1,748m asl</li> </ul>	<ul> <li>A highland area with very minimum human activities.</li> <li>No human dwelling was found nearby the study site. The nearest human dwelling was approximate 4km from the study site.</li> <li>The highland was surrounded by primary forested area.</li> </ul>			

conducted from 9 May – 10 October 2007, 6 May – 6 August 2008 and 26 May – 14 July 2009 in forested, coastal and highland, respectively. All studies were conducted in the dry season.

The monkeys (Macaca fascicularis, Raffles) were used as a model for human Each monkey was decomposition. approximately 3 year olds and weighed 3.0 kg - 5.6 kg. At the beginning of field study, each monkey was euthanized by a single shot to the forehead from a handgun at point blank range to simulate a human murder situation. Euthanization was administered by Department of Wildlife and National Parks (PERHILITAN), Peninsular Malaysia, Ministry of Natural Resources and Environment, Malaysia. The study protocol was approved by Institute for Medical Research's Unit of Animal Care and Use Committee [Permit No.: ACUC/KKM/ 02(2/2008)].

After death was confirmed, the monkey carcasses were immediately clothed with a white cotton short sleeve t-shirt to simulate human cadavers. The monkey carcasses were immediately placed outdoors in shaded areas that were near bushes. Each carcass was then covered with metal wire mesh  $(2.5 \text{ cm}^2)$  cage measuring 90 cm (length) x 60 cm (wide) x 45 cm (height). The cage was bottomless which allowed the carcass to be in direct contact with the ground. To avoid activity by scavengers the cage was fixed to the ground using 15 cm metal hooks at each side. Another monkey carcass was placed indoor on a cemented ground of a wooden hut [300 cm (length) x 300 cm (wide) x 300 cm (height)], that had 4 windows [120 cm (height) x 45 cm (wide)] on each side of the wall. The windows of the wooden hut were open throughout the study period to allow insects access into the hut. A total of 2 replicates were used in outdoor and indoor, respectively.

Each carcass was monitored hourly (10 minutes for each hour) for the first three days; this was reduced to daily on the fourth day of decomposition. The carcasses were monitored in this fashion until fly larvae ceased to be observed or bones began to be observed. Ants that were visible on the surface and under the carcasses were collected. Representative samples of ants were taken from different parts of the monkey carcass; every attempt was made to disturb the carcass and its invertebrate inhabitant minimally. Ants were sampled from the carcass using forceps and preserved in 70% alcohol. The specimens were then identified by using the taxonomy key of Bolton (1994), Eguchi (2001) and Holldobler & Wilson (1990).

Figure 1 shows ant species presence with relation to decomposition stage and ecological habitat. Each carcass passed through the five stages of decomposition: fresh, bloating, active decay, advanced decay and dry/remains. Within 15 minutes of death, ants were attracted to the blood of the freshly killed monkeys. Heo et al. (2007) reported that flies in the family Muscidae were the first to be attracted to pig carcasses, followed by ants and spiders; this study occurred inside of an oil palm plantation located in Tanjong Sepat, Selangor, Malaysia. Vitta et al. (2007) also reported ants as the first arthropod to be attracted to the wounds around the necks of the pig carcasses. However, Heo et al. (2007) and Vitta et al. (2007) did not identify the ant specimens visiting the pig carcasses in their studies.

Table 2 presents a summary of ants recovered from different ecological habitats in forensic entomology studies. Only 3 species of ants belonging to 2 subfamilies were obtained from carcasses placed in forested area, namely Pheidologeton diversus (Jerdon, 1851) (Formicidae: Myrmicicnae), Tetramorium sp. (Formicidae: Myrmicicnae) and Odontoponera transversa (Smith, 1857) (Formicidae: Ponerinae). On the other hand, 4 species of ants belonging to 2 and 3 subfamilies were found on carcasses placed in coastal area and highland area, respectively. The coastal area ant species were: Oecophylla smaragdina (Fabricius, 1775) (Formicidae: Formicinae), Paratrechina longicornis (Latreille, 1802) (Formicidae: Formicinae), Cardiocondyla sp. (Formicidae: Myrmicicnae) and *Pheidologeton diversus* (Formicidae: Myrmicicnae), while those found in the highland were: Paratrechina longicornis (Formicidae: Formicinae), Pheidole longipes (Latreille, 1802) (Formicidae: Myrmicicnae), *Pachycondyla* sp. (Formicidae: Ponerinae) and *Hypoponera* sp. (Formicidae: Ponerinae).

Figure 1 presents the ant species collected during different stages of decay on different monkey carcasses. Ants were observed in all the decomposition stages of the carcasses. These ants were observed

		Decomposition stages					
Subfamilies	Species	Fresh	Bloating	Decay	Advanced Decay	Remains	
	Forested Area	: Ulu Gomb	ak, Selangor				
	(3°17'57.86"	N, 101°47'00	).78"E)				
Myrmicicnae	Pheidologeton diversus						
	Tetramorium sp.						
Ponerinae	Odontoponera transversa						
	Coastal Area (2°39'12.29"						
Formicinae	Oecophylla smaragdina						
	Paratrechina longicornis						
Myrmicicnae	Cardiocondyla sp.						
	Pheidologeton diversus						
	Highland Area (3°26'21.39"				· · · ·		
Formicinae	Paratrechina longicornis		1 1				
Myrmicicnae	Pheidole longipes						
Ponerinae	Hypoponera sp.						
	Pachycondyla sp.						
Outdo	oor Indoor						

Figure 1. Species of ants obtained from different decomposition stages of monkey carcasses in forested area, coastal area and highland in Malaysia

Table 2. Ants recovered from forensic entomological studies in forested area, coastal area, highland and oil palm plantation in Malaysia

Subfamilies		Study Site					
			Heo <i>et al.</i> (2009)				
	Species	Ulu Gombak, Selangor (Forested) <sup>a</sup>	Tg. Sepat, Selangor (Coastal) <sup>a</sup>	Bukit Cincin, Pahang (Highland)ª	Tg. Sepat, Selangor (Oil Palm Plantation) <sup>b</sup>		
Formicinae	Anoplolepis gracilipes (Smith) Oecophylla smaragdina (Fabricius)		+		+ +		
	Paratrechina longicornis (Latreilla)		+	+			
Myrmicicnae	Cardiocondyla sp. Pheidole longipes (Latreille)		+	+			
	Pheidologeton diversus (Jerdon)	+	+		+		
	<i>Tetramorium</i> sp.	+			+		
Ponerinae	Diacamma sp.				+		
	Hypoponera sp. Odontoponera transversa (Smith)	+		+	+		
	Pachycondyla sp.			+			

<sup>a</sup>4 Replicates (2 monkey carrions placed indoor and 2 monkey carrions placed outdoor)

<sup>b</sup>2 Replicates (2 pig carrions placed outdoor)

+Present of ant

actively preying upon the eggs, maggots, pupae and newly emerged flies throughout the study period. Our findings were similar to that reported by Campobasso et al. (2009) in which ants were present at all stages of decomposition as they are typically observed shortly after death or during the early postmortem period, but even later once the fly maggots had left the carrions. Both studies observed ant activity shortly after death and during the early stages of decay; this ant activity continued even after all fly and maggot activities ceased. Additionally, ants also have been reported as predators in the context of forensic entomology by Smith (1986). Our results revealed that *Tetramorium* sp. was only found visiting carcasses placed indoors; while Oecophylla smaragdina, Cardiocondyla sp., Hypoponera sp. and Pachycondyla sp. were only found on carcasses placed outdoors. However, no specific references were reported on the foraging behaviour and localities preferences of ants obtained from forensic entomological studies conducted in indoor and outdoor.

Campobasso *et al.* (2009) reported that the main role of ants in faunal succession varied from predators on the eggs and larvae of other insects (mainly Diptera), thus reducing significantly the rate of decomposition; to scavengers on the flesh or exudates from the corpse itself. Ants have been shown to negatively impact the occurrence and development of forensically significant flies on the corpse, the presence or absence of ants should be taken into account in every case involving postmortem interval estimates based on entomological evidence. Furthermore, ants can feed on both the corpse and associated fauna. Thus, ants fed on the carcasses also could hasten the loss of carcass mass (Campobasso et al., 2009). However, we did not study the relationship between decomposition rates of the carcasses and the presence of ants in this study.

Ants were also reported to play an important role in inflicting postmortem injuries to the carcasses (Moura *et al.*, 1997). In this study, ants were found to be attracted to the blood stains and fly eggs on the fresh carcasses, but no lesions were observed to be caused by ants. According to Campobasso *et al.* (2009), the feeding action of ants could

cause many irregular, serpiginous, scalloped areas of superficial skin loss, and small punctate and scratch-type lesions may be observed on the body, which were results of postmortem ant bites. These injuries consisted of small and rather shallow gnawed holes could be easily misinterpreted as antemortem abrasions or resulting from strong acids.

In this study, ants were reported occurring in all the decomposition stages, indicating that ants were not a significant indicator for carcasses succession or post mortem interval estimation. However, we found that different species of ants visited the monkey carcasses placed in different ecological habitats (Table 2). Cardiocondyla sp. was only found on carcasses placed in the coastal area; while Pheidole longipes, Hypoponera sp. and Pachycondyla sp. were found on carcasses placed in the highland area. Compared to study conducted by Heo et al. (2009), only Anoplolepis gracilipes (Smith, 1857) and *Diacamma* sp. (Mayr, 1862) were found on pig carcasses placed in oil palm plantations. On the other hand, Pheidologeton diversus, Tetramorium sp. and Odontoponera transversa found on carcasses placed in the forested area were also found in the oil palm plantation as reported by Heo et al. (2009). We also noticed that some of the ants were found in more than one ecological habitat, e.g. Oecophylla smaragdina was found in both coastal area and oil palm plantation, Paratrechina longicornis was found in both coastal and highland areas, Odontoponera transversa was found in both forested areas and in oil palm plantations and Pheidologeton diversus was found in all ecological habitats surveyed except highland areas. It is possible that some ant species can be used as geographical indicators while other species are too widely distributed to indicate a specific locality.

Goff and Win (1997) also reported the combined use of forensically important flies and ants to estimate the PMI. In their study, human remains were discovered in a metal box. Both ants and flies were used to estimate the PMI. The developmental time of the strationyid fly, *Hermetia illucens* (Linnaeus, 1758) was used in conjunction with the time required for ants, *Anoplolepsis gracilips* to establish a colony capable of producing alate (winged) reproductives.

Preliminary findings indicate that ants may serve as an indicator of geographic locality; this information could aid crime scene investigations in the future. More studies should be conducted in different locations to obtain more accurate information to determine the precise role of ants in forensic investigation.

Additional studies of ant behavior and distribution will further add to the body of knowledge used by forensic investigators. A comparative study on faunal succession in the presence and absence of ants should also be studied in order to investigate the role of ants in PMI estimation in this region.

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## REFERENCES

- Bolton, B. (1994). *Identification guide to the ants genera of the world*. Harvard University Press: London.
- Campobasso, C.P., Marchetti, D., Introna, F. & Colonna, M.F. (2009). Postmortem artifacts made by ants and the effect of ant activity on decompositional rates. *American Journal of Forensic and Medical Pathology* **30**: 84-87.

- Eguchi, K. (2001). A revision of the Bornean species of the ant genus *Pheidole* (Insecta: Hymenoptera: Formicidae: Myrmicinae). *Tropics Monograph Series* **2**: 1-154.
- Goff, M.L. & Win, B.H. (1997). Estimation of postmortem interval based on colony development time for Anoplolepsis longipes (Hymenoptera: Formicidae). Journal of Forensic Science 42: 1176-1179.
- Heo, C.C., Marwi, M.A., Ahmad Firdaus, M.S., Jeffery, J. & Omar, B. (2007). A preliminary study of insect succession on a pig carcass in a palm oil plantation in Malaysia. *Tropical Biomedicine* 24: 23-27.
- Heo, C.C., Marwi, M.A., Hashim, R., Abdullah, N.A., Chen, C.D., Jeffery, J., Kurahashi, H. & Omar, B. (2009). Ants (Hymenoptera: Formicidae) associated with pig carcasses in Malaysia. *Tropical Biomedicine* 26: 106-109.

- Holldobler, B. & Wilson, E.O. (1996). *The Ants*. Harvard University Press: Cambridge.
- Moura, M.O., Carvalho, C.J. & Monteiro-Filho, E.L. (1997). A preliminary analysis of insects of medico-legal importance in Curitiba, State of Parana. *Memorias do Instituto Oswaldo Cruz* **92**: 269-274.
- Smith, K.G.V. (1986). A manual of forensic entomology. Cornell University Press, Ithaca, New York.
- Tabor, K.L., Fell, R.D. & Brewster, C.C. (2005). Insect fauna visiting carrion in Southwest Virginia. *Forensic Science International* 150: 73-80.
- Vitta, A., Pumidonming, W., Tangchaisuriya, U., Poodendean, C. & Nateeworanart, S. (2007). A preliminary study on insects associated with pig (*Sus scrofa*) carcasses in Phitsanulok, northern Thailand. *Tropical Biomedicine* **24**: 1-5.