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# Decomposition Stages and Carrion Insect Succession on Dressed Hanging Pig Carcasses in Nan Province, Northern Thailand

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ABSTRACT.– The principal colonizing insect species and their succession pattern on six dressed, hanging pig carcasses (*Sus scrofa domestica*) in Nan province, Northern Thailand, was evaluated along with the cadaver decomposition stages in the monsoon wet, winter and summer seasons during May 2010 – August 2011 at two different habitat types, mixed deciduous forest and suburban plantation area. At each site, a dressed pig carcass was suspended by its neck from a tree branch 1.2 m of pig's feet above the ground. Carcasses were protected from carnivorous vertebrates, but not the ambient weather (wind, rain or sunlight), by ground fencing (enclosure). The ambient and internal carcass temperature, relative humidity, insect succession and rate of carcass decomposition were monitored in both habitats. From this preliminary study, 43–45 and 39–40 different cadaver-associated (non-accidental) taxa were collected in the mixed deciduous forest and suburban plantation area, respectively. The observed insect succession pattern broadly conformed to those reported from other (mostly temperate) regions. The primary and dominant necrophagous colonizers were the calliphorid dipterans, *Chrysomya megacephala* (F.) and *Achoetandrus rufifacies* (Macquart), in both habitats. Unconfirmed (insufficient sample sizes) trends of interest for further confirmation included that: (i) the decomposition rate was potentially faster in the monsoon wet and summer seasons in both habitats than in winter; and (ii) that whilst the patterns of insect secession occurred in a predictable sequence, they varied in timing and species composition between the habitats.

KEY WORDS: **Forensic entomology, insect succession, stage of decomposition, *Chrysomya megacephala* (F.)**

## INTRODUCTION

Decomposing animal carcasses provide a patchy and ephemeral resource as a changing habitat that can support, and are exploited by, a large number of micro-organisms and arthropod communities (Avila & Goff 1998; Wells & Greenberg 1992). Indeed, in the absence of any significant vertebrate scavengers, they are the main decomposers. Invasion of the cadaver by arthropods (mainly insects) occurs soon after death when the primary necrophagous colonizers, such as blowflies, are semiochemically attracted to animal remains within hours (or less) of death in one or more successive waves (Archer & Elgar 2003). They are then followed by other arthropod species that either utilize the decomposing remains in competition with other necrophagous species or are predators of the earlier colonizers (or both).

The arthropod composition and succession is influenced by the locally available arthropod pool to colonize the cadaver, and their respective interactions within and between the necrophagous and predatory species, as well as by many biotic and abiotic factors, including the biogeographical location (Galloway et al.

1989; Campobasso et al. 2001), habitat (Goff et al. 1988; Davis & Goff 2000; Eberhardt & Elliot 2008), season (Johnson 1975; Tabor et al. 2004), local climatic conditions (Shean et al. 1993; Archer 2004), physical state of the remains (Avila & Goff 1998) and the decomposition environment (Goff 1992; Payne & King 1972; Centeno et al. 2002; Voss et al. 2008). Nevertheless, the broad pattern of arthropod succession on a cadaver generally follows a somewhat predictable sequence (Bornemissza 1956; Anderson & VanLaerhoven 1996; Price et al. 1997; Shalaby et al. 2000) that has been well documented for the temperate regions of the northern and southern hemispheres (Early & Goff 1986; Schoenly 1992; Amendt et al. 2000; Archer & Elgar 2003).

Insect succession patterns are also closely linked to the progression of the decomposition of the carcass. Although cadaver decomposition is a continuous process, it can be defined into broad but visually discernible stages that are linked to the succession of specific insect groups and therefore can be used as markers for the estimation of the post-mortem interval (PMI) (Rodriguez & Bass 1983; Goff 1993; Campobasso et al. 2001). Thus, when the pattern of arthropod succession and the decomposition stage of a cadaver are known, analysis of the arthropod fauna can be used to estimate the PMI. This aspect (forensic entomology) is regularly used by police services where the time of death is unknown (Catts 1992; Dadour et al. 2001; Morris & Dadour 2005).

To enable entomological estimates of the PMI requires a comprehensive baseline reference dataset of the expected pattern of insect succession on a decomposing cadaver for a given set of parameters. Given the variation in insect species and community, their biology and life cycles and the stage of decomposition colonized by each species (community succession), in each country and biogeographic region/habitat (Wolff et al. 2001; Tabor et al. 2005; Sharanowski et al. 2008; Bonacci et al. 2011; Voss et al. 2011), it is essential to first evaluate this basic information in order to assess the potential of forensic entomology in any region. In particular, it is essential to establish the key arthropod species that will potentially be used in allowing any future accurate PMI evaluation. However, such information within the tropics, including Thailand, with its widely different insect fauna and biogeographical regions/ environmental conditions, is limited.

Within the Indo-Southeast Asia region, only a few studies relating to forensic entomology are available and are restricted to within Thailand, India and Malaysia (Sukontason et al. 2001, 2007; Meenakshi & Davinder 2003; Chin et al. 2010). Within Thailand, information pertaining to the morphology of the immature stages of potentially forensically important flies has been published for identification purposes (Sukontason et al. 2003, 2007), while the larval developmental rate of two of the potentially forensically important and common flies, *C. megacephala* and *A. rufifacies* under natural ambient temperature for estimating the PMI has been reported (Sukontason et al. 2008). However, the current knowledge of insect succession on cadavers in this region is very limited, let alone the importance and relevance of the variation in these patterns across the different biogeographical regions and seasons.

Preliminary information on insect succession, albeit based on low replications (cadaver numbers, seasons and sites), have been reported from Nakorn Pathom province (Champathet, unpublished data, 2005), Phitsanulok (Vitta et al. 2007) and Pathum Thani province (Sukchit, unpublished data, 2008), using the domestic pig (*Sus scrofa domestica* L.) as the animal model. However, the study in Phitsanulok was carried out over only the initial decomposition stages (a 30-d post-mortality period; mid-February to mid-March, 2005) (Vitta et al. 2007), while although Sukchit's study (Unpublished data, 2008) compared the insect succession and decomposition rates in two habitats (field and shaded areas), it was in a single urban locality. Therefore, investigations of insect succession throughout the whole year and covering the three different seasons should be studied. However, prior to such extensive studies, a baseline database of the arthropods associated with cadavers and insect succession patterns in the region is still required. Accordingly, the objective of this study was to investigate the succession of carrion arthropods in the northern region of Thailand, whilst building a baseline database of the species and succession patterns that are involved in cadaver decay and that may be applied for forensic entomology purposes in the region in the future.

This baseline forensic entomological database can be used for further studies on biogeographical variation and to compare with the far more extensive databases from temperate regions, in order to evaluate its potential use in local forensic science, including estimation of PMI.

## MATERIALS AND METHODS

### Study sites

The experiments were performed during May 2010 – August 2011, encompassing the monsoon wet, winter and summer seasons, at Nan province, located in the north of Thailand. Nan still has several complex ecosystems with a high species biodiversity, whilst there is a consistently high homicide rate in the area and therefore the potential need for forensic entomology (Warangrath Ek-anankul, per. com). The two selected sites were situated in different types of habitat, located 46 km (direct distance) apart from each other.

The first site was situated in a mixed deciduous forest (18° 33' 33.431" N, 100° 47' 51.950" E) in which Dipterocapaceae, especially *Shorea obtusa* Wall. ex Blume and *Dipterocarpus tuberculatus* Roxb, plus an admixture of two species of Hypericaceae (*Cratoxylum cochinchinense* (Lour.) Blume and *Cratoxylum formosum* (Jack) Dyer), were the dominant plants. The second site was situated in a more suburban area (18° 47' 28.806" N, 100° 43' 55.135" E) and was a rubber plantation farm in which young rubber trees (*Hevea brasiliensis* Müll.Arg.) were the dominant plant species.

### Animal model

The domestic pig (*S. scrofa domestica* Erxleben.) was used as the animal model because it has been established and characterized as a reasonably good surrogate model for human cadavers (at least in temperate regions) (Goff 1992; Schoenly et al. 2007; Catts & Goff 1992). All animal models in this experiment, six small freshly killed pigs, weighing from 20 – 21 kg, were obtained from a local farm. The pigs were killed by a blow to the head (Wang et al. 2008) and were immediately transported in insect-proof plastic bags to the research sites (Schoenly et al. 2006), taking about 30 and 60 min from the time of death to their study location in the mixed deciduous forest and suburban site, respectively.

The pig carcasses were dressed in suitably sized loose fitting cotton shirts and shorts, held together with plastic rope waistbands (Joy et al. 2006) to mimic the principal local clothing of human cadavers. The carcasses were then suspended by their necks from a tree branch using a nylon rope to a height such that the lowest portion of the carcass, the hind legs, were approximately 1.20 m above the ground. The choice of carcasses hung in this manner reflects that most human cadavers found in this region are hung (Warangrath Ek-anankul, per. com). Cadavers were fenced by wooden enclosures of 2 × 2 × 2.5m, which allowed access of insects and other arthropods to the cadavers, but prevented the cadavers from being disturbed by carnivorous vertebrates without causing any significant wind, rain or sun shelter (Fig. 1). All carcasses were examined for arthropod activity immediately after positioning.

**Field protocols**

Each research site had a data logger (DT-171) to record the ambient temperature and relative humidity every 30 min throughout the experiment. Hygro-thermometer probes were inserted into the mouth, abdomen and anus of the cadaver, plus any larval masses, to record the internal carcass temperature. Physical features of carcasses in both areas were observed at the time of collecting the specimens.

Carcasses were visited between 9.00 a.m. and 2.00 p.m. every day for the first two weeks after placement, then every other day for the third week, and once a week until the carcasses were in the skeletal stage of decomposition (dry skin, cartilage and bones). At each examination period, the ambient temperature, relative humidity and the carcass's internal temperatures were recorded. The physical conditions of each carcass and visible arthropod activities were recorded whilst photographs were taken to record the physical changes. After such observations, representative arthropod specimens were collected. Adult insects on the cadaver were collected by sweep netting. Eggs, larvae, pupae and ground crawling arthropods were collected by hand using forceps from on, in, under and around the carcass (head, abdomen and anus).

Larvae were found on or near the carcasses and, especially for the prevalent dipteran larvae, may be in large masses. The masses generate heat, which speeds up their development (Anderson 1995) and so the site and temperature of each larval mass was recorded. For the predominant dipteran species, large specimens were usually older instars but, because small larvae may belong to a different species, care was taken to also collect a range of sizes. Sampled larvae were killed by immersion for 10–15 s in water heated to just below boiling point and then transferred to 95% (v/v) ethanol. Since third instar larvae leave the carcasses to pupate, the soil below the carcasses was also carefully examined to a depth of about 5–10 cm to collect pupae and empty pupal cases.

Specimens were labeled with the date and time of collection, carcass ID number and site location on/ around the body and then placed in vials. They were then transferred to the Integrative Ecology Laboratory, Chulalongkorn University (Bangkok), for identification.

Blowfly (Calliphoridae) specimens (larvae, puparia and adults) were identified using the key of Thai dipteran species (Sukontason 2010) and compared with the insect collection of the Queen Sirikit Department of Sericulture. Other arthropod specimens were identified using the insects of Australia volumes I and II (CSIRO 2000) and compared with the arthropod collection of the Museum of Natural History, Chula-longkorn University.

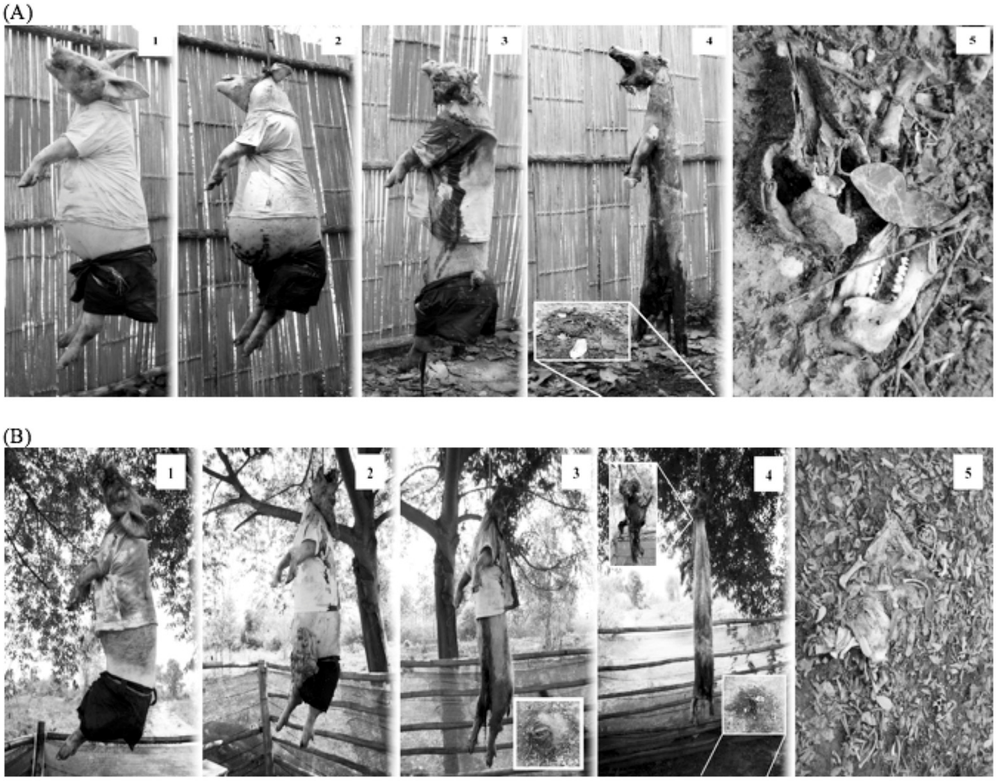

**FIGURE 1.** Five defined stages of pig decomposition: (1) fresh, (2) bloated, (3) active decay, (4) post-decay and (5) skeletonization stages. Shown are representative images of the (A) mixed deciduous forest and (B) suburban sites in Nan province, northern Thailand, during May 2010 – August 2011.

## RESULTS AND DISCUSSION

This study examined the species diversity of colonizing insects with respect to their succession pattern and cadaver decomposition stages for six pig carcasses, three in each of a suburban and mixed deciduous forest habitat. Focus was centered on the succession of Diptera, Coleoptera and Hymenoptera, as representatives of these orders were considered the primary indicator species for estimating the time of death (Champathet, unpublished data, 2005; Sukchit, unpublished data, 2008).

### Diversity of insects associated with pig carcasses.

The diversity of insects found on the six carcasses are summarized in Table 1 (mixed deciduous forest) and Table 2 (suburban plantation area). Fifty one taxa were recorded (33 species, 4 morphospecies and 14 unidentified beyond order). These could be further partially (tentatively) classified as 21 likely carrion feeders, 8 scavenger, 6 predatory, 6 accidental (plus 2 accidental/ saprophytes), 2 scavenger-predators, a parasitoid and 5 carrion feeders/predatory species in the mixed deciduous forest and the same in the suburban plantation area except for only 17 carrion and five accidental (plus one accidental/saprophyte)

**TABLE 1.** Carrion insects and the number of individuals found associated with hung-above ground pig carcasses at mixed deciduous forest in Wieng Sa district, Nan province, northern Thailand during May 2010 – August 2011: Monsoon wet (May - October), winter (November - February), Summer (March – April).

**MIXED DECIDUOUS FOREST**

| Order | Family / Likely role | Species | Monsoon wet | Winter | Summer |
|---|---|---|---|---|---|
| Diptera | Calliphoridae/ Carrion feeders | *Chrysomya megacephala* | 713 | 1209 | 2189 |
| | | *Chrysomya bezziana* | 3 | 9 | 2 |
| | | *Chrysomya nigripes* | 72 | 34 | 29 |
| | | *Chrysomya thanomthini* | 1 | 0 | 0 |
| | | *Chrysomya pinguis* | 0 | 0 | 1 |
| | | *Chrysomya chani* | 0 | 0 | 2 |
| | | *Achoetandrus rufifacies* | 781 | 1974 | 1932 |
| | | *Achoetandrus villeneuvi* | 13 | 3 | 0 |
| | | *Lucilia cuprina* | 1 | 4 | 0 |
| | | *Hypopygiopsis infumata* | 0 | 0 | 1 |
| | | *Hemipyrellia ligurriens* | 23 | 129 | 0 |
| | Muscidae / Carrion feeders | *Hydrotaea spinigera* | 28 | 76 | 88 |
| | | *Musca domestica* | 29 | 2 | 14 |
| | | *Musca sorben* | 11 | 0 | 0 |
| | | *Atherigona* sp.1 | 17 | 43 | 29 |
| | Sarcophagidae / Carrion feeders | *Sarcophaga dux* | 12 | 29 | 4 |
| | | *Sarcophaga peregrina* | 8 | 4 | 8 |
| | | *Sarcophaga ruficornis* | 9 | 0 | 3 |
| | Drosophilidae | *Drosophila melanogaster* | 0 | 2 | 0 |
| | Phoridae / Carrion feeder | Unidentified | 2 | 5 | 1 |
| | Piophilidae / Scavenger | *Piophila casei* | 1 | 0 | 0 |
| | Sepsidae / Scavenger | Unidentified | 7 | 49 | 48 |
| | Stratiomyidae / Predatory | *Hermetia illucens* | 4 | 0 | 0 |
| | Asilidae / Predatory | Unidentified | 4 | 1 | 6 |
| Coleoptera | Cleridae / Scavenger-Predatory | *Necrobia ruficollis* | 39 | 149 | 138 |
| | | *Necrobia rufipes* | 167 | 44 | 149 |
| | Dermestidae / Scavenger | *Dermestes maculatus* | 210 | 171 | 196 |
| | Hybosoridae / Scavenger | *Phaeochrous emarginatus* | 2 | 2 | 13 |
| | Scarabaeidae / Scavenger | *Coprophanaeus* sp. | 1 | 1 | 0 |
| | | *Onthophagus tricornis* | 24 | 13 | 1 |
| | Trogidae / Scavenger | *Polynonchus* sp. | 1 | 0 | 1 |
| | | *Afromorgus chinensis* | 0 | 1 | 0 |
| | Staphylinidae / Predator | Unidentified 1 | 13 | 34 | 12 |
| | | Unidentified 2 | 23 | 13 | 19 |
| | | Unidentified 3 | 35 | 28 | 12 |
| | Silphidae / Carrion feeder | *Necrophila (Deutosilpha) luciae* | 0 | 1 | 0 |
| | Cicindelidae / Predator | Unidentified | 0 | 1 | 0 |
| Hymenoptera | Formicidae / Predator-fresh carrion feeder | *Oecophylla smaragdina* | 13 | 48 | 19 |
| | | Pheidologeton diversus | 21 | 23 | 14 |
| | | *Crematogaster physocrema* | 12 | 11 | 18 |
| | | *Camponotus rufoglaucus* | 49 | 8 | 23 |
| | | *Monomorium destructor* | 1 | 31 | 1 |
| | Braconidae / Parasitoid | Unidentified | 1 | 6 | 2 |
| | Apidae / Accidental-Saprophyte | *Melipona* sp. | 1 | 1 | 5 |
| | Acrididae/ Accidental | Unidentified | 1 | 1 | 0 |
| Orthoptera | Coreidae / Accidental | Unidentified | 9 | 7 | 6 |
| Hemiptera | Blattidae / Accidental | Unidentified | 9 | 8 | 1 |
| Blattodea | Unidentified / Accidental | Unidentified | 1 | 0 | 8 |
| Isoptera | Unidentified / Scavenger | Unidentified | 58 | 58 | 66 |
| | **Total insect samples** | | 2430 | 4233 | 5061 |

species. The most dominant insect species collected from six pig carcasses are *A. rufifacies*, *C. megacephala*, *Chrysomya nigripes* Aubertin, *Hemipyrellia ligurriens*

**TABLE 2.** Carrion insects and the number of individuals found associated with hung-above ground pig carcasses at a suburban area in Muang Nan district, Nan province, northern Thailand during May 2010 – August 2011: Monsoon wet (May - October), winter (November - February), Summer (March – April).

**SUB URBAN AREA**

| Order | Family / Likely role | Species | Monsoon wet | Winter | Summer |
|---|---|---|---|---|---|
| Diptera | Calliphoridae/ Carrion feeders | *Chrysomya megacephala* | 686 | 1023 | 1804 |
| | | *Chrysomya bezziana* | 3 | 9 | 0 |
| | | *Chrysomya nigripes* | 62 | 27 | 25 |
| | | *Chrysomya thanomthini* | 1 | 0 | 0 |
| | | *Achoetandrus rufifacies* | 723 | 1896 | 1683 |
| | | *Achoetandrus villeneuvi* | 7 | 1 | 0 |
| | | *Lucilia cuprina* | 2 | 4 | 1 |
| | | *Hemipyrellia ligurriens* | 14 | 87 | 0 |
| | Muscidae / Carrion feeders | *Hydrotaea spinigera* | 24 | 67 | 55 |
| | | *Musca domestica* | 27 | 1 | 16 |
| | | *Musca sorben* | 8 | 0 | 0 |
| | | *Atherigona* sp.1 | 13 | 43 | 19 |
| | Sarcophagidae / Carrion feeders | *Sarcophaga dux* | 10 | 20 | 3 |
| | | *Sarcophaga peregrina* | 7 | 3 | 6 |
| | | *Sarcophaga ruficornis* | 8 | 0 | 2 |
| | Phoridae / Carrion feeder | Unidentified | 1 | 3 | 3 |
| | Piophilidae / Scavenger | *Piophila casei* | 1 | 0 | 0 |
| | Sepsidae / Scavenger | Unidentified | 5 | 43 | 41 |
| | Asilidae / Predatory | Unidentified | 2 | 2 | 4 |
| Coleoptera | Cleridae / Scavenger-Predatory | *Necrobia ruficollis* | 19 | 130 | 112 |
| | | *Necrobia rufipes* | 184 | 60 | 138 |
| | Dermestidae / Scavenger | *Dermestes maculatus* | 187 | 149 | 166 |
| | Hybosoridae / Scavenger | *Phaeochrous emarginatus* | 3 | 5 | 17 |
| | Scarabaeidae / Scavenger | *Coprophanaeus* sp. | 0 | 1 | 1 |
| | | *Onthophagus tricornis* | 32 | 19 | 13 |
| | Trogidae / Scavenger | *Polynonchus* sp. | 0 | 0 | 1 |
| | | *Afromorgus chinensis* | 0 | 1 | 0 |
| | Staphylinidae / Predator | Unidentified 1 | 48 | 49 | 59 |
| | | Unidentified 2 | 28 | 21 | 16 |
| | | Unidentified 3 | 31 | 29 | 21 |
| | Silphidae / Carrion feeder | *Necrophila (Deutosilpha) luciae* | 0 | 1 | 0 |
| Hymenoptera | Formicidae / Predator-fresh carrion feeder | *Oecophylla smaragdina* | 0 | 43 | 18 |
| | | Pheidologeton diversus | 18 | 14 | 13 |
| | | *Crematogaster physocrema* | 14 | 10 | 11 |
| | | *Camponotus rufoglaucus* | 57 | 1 | 25 |
| | | *Monomorium destructor* | 2 | 0 | 0 |
| | Braconidae / Parasitoid | Unidentified | 2 | 1 | 9 |
| | Apidae / Accidental-Saprophyte | *Melipona* sp. | 1 | 1 | 2 |
| Orthoptera | Acrididae/ Accidental | Unidentified | 12 | 1 | 10 |
| Hemiptera | Coreidae / Accidental | Unidentified | 9 | 6 | 8 |
| Blattodea | Blattidae / Accidental | Unidentified | 1 | 1 | 7 |
| Isoptera | Unidentified / Accidental | Unidentified | 78 | 84 | 79 |
| | **Total insect samples** | | 2330 | 3856 | 4388 |

Wiedemann, *Hydrotaea spinigera* Stein, *Atherigona* sp., *Musca domestica* L., *Necrobia ruficollis* Fabricius, *Necrobia rufipes* DeGeer, *Dermestes maculatus*

DeGeer and *Pheidologeton diversus*. In contrast to the report on the comparative insect fauna succession on indoor and outdoor monkey carrion in a semi-forested area in Malaysia (Ahmad et al. 2011), *H. ligurriens, Atherigona* sp. *M. domestica*, *N. ruficollis, N. rufipes, D. maculatus* and *Pheidologeton diversus* were not found in this study. Meanwhile, the predominant Diptera (Calliphoridae: *A. rufifacies* and *C. megacephala*) colonized all the carcasses in both habitats in the early stages of decomposition but were rarely found in the skeletal stage. This finding agreed with the study of monkey carrion in Malaysia (Ahmad et al. 2011).

The predominant species collected from the pig cadavers in this study were *A. rufifacies* and *C. megacephala*, which were also the main species found from the early to mid decomposition stages on all cadavers (seasons and habitats). They have previously been used in the estimation of the minimum PMI of a cadaver found floating in a reservoir in Lumpang province, northern Thailand (Sukontason et al. 2005), and they are therefore likely to be a forensically important flies in the north of Thailand. A variety of factors, such as the climate, season, sun exposure, urban or suburban scenarios, frequency of collection and the number of animal models, can affect the species diversity of insects associated with a corpse in different regions of the world (Campobasso et al. 2001).

**Insect succession**

In total, approximately 2,500 adults and 2,200 larvae were collected from each pig carcass over the study period. In the mixed deciduous forest the samples were comprised of 51 arthropod taxa (33 species / 4 morphotypes and 14 unidentified beyond order) from 26 families in 7 orders (Table 1), including 21 and 6 taxa of carrion feeders and predacious insects, respectively. In the suburban plantation area, the collected samples were comprised of 45 arthropod taxa (28 species/ 4 morphotypes and 14 unidentified beyond order) from 24 families in 7 orders (Table 2), including 17 and 6 taxa of carrion feeders and predacious insects, respectively.

The sampled larval forms were principally comprised of Diptera (> 8,000 specimens), Coleoptera (approximately 1,500 specimens) and Hymenoptera (approximately 300 specimens) on all cadavers (and in all three seasons and in both habitats), with unidentified representatives from 7 other orders, although at least four of these are likely to be accidentals rather than true cadaver associated arthropods. The analysis of the insect succession pattern on the pig carcasses is summarized in Fig. 2 and discussed as follows.

*(i) Fresh stage:* Diptera were the primary initial colonizers of all six carcasses. More than 20 species of flies attended the carcasses at one stage or another of the carcass decomposition at both habitats (Tables 1, 2). Early colonizers (primary colonizing necrophagous species) that arrived and oviposited during the initial stage of decomposition were principally the calliphorid flies *C. megachephala* and *A. rufifacies*. Their eggs were especially prevalent in the ears, nose and eyes of the carcass. However, the formicid ant (Hymenoptera) *Oecophylla smaragdina* Fabricius was the first species observed to arrive at the carcassess in both areas. Ants can be present at all stages of carrion decomposition as they are opportunistic feeders on fly eggs and larvae. They are typically observed on the carcass shortly after death and during the early postmortem period up to the late decomposition stages

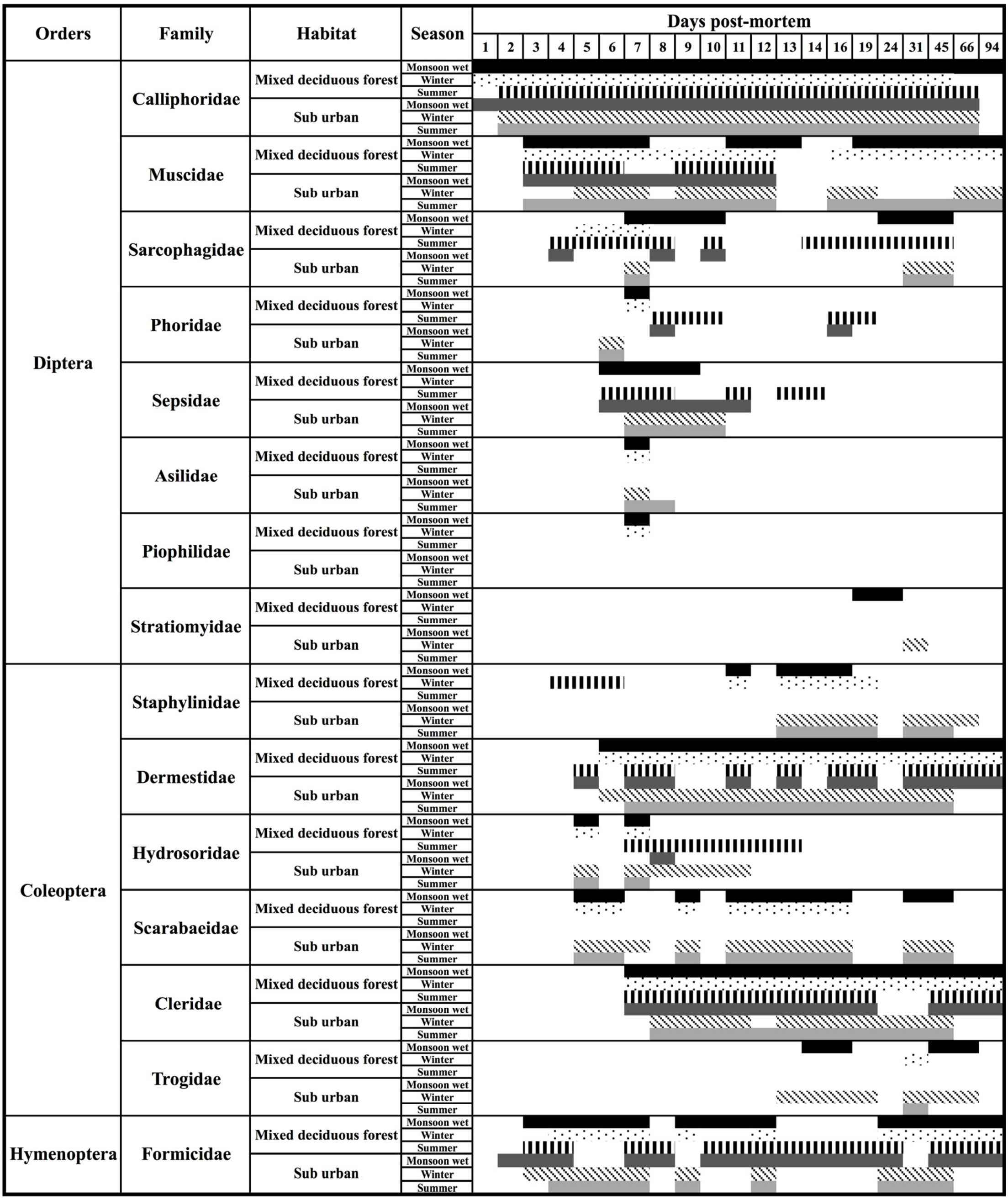


**FIGURE 2.** Insect succession patterns on pig carcasses in the mixed deciduous forest and the suburban habitat. Numerals indicate the time since death in days in Nan province, northern Thailand, during May 2010 – August 2011: Monsoon wet (May - October), winter (November - February), Summer (March – April).

even after all the fly larvae have left the carcasses. In contrast to the report on insect succession on a pig carcass in a Malaysian oil palm (Chin et al. 2010), where several adults of *H. spinigera* Stein (Diptera: Muscidae) were seen at the cadaver within 10 min of being hung, in this study at Nan province no blowflies were observed at the cadavers during the first 3 h after death and *in situ* positioning of the pig cadavers.

*(ii) Bloat stage:* In the bloated stage, *C. megacephala* and *A. rufifacies* were the most numerous fly larvae collected in both habitats, and accounted for 40% and 60% of the collected samples in this period, respectively, which is similar to that reported previously (Shalaby et al. 2000; Chin et al. 2010). Adult *M. domestica* (Linnaeus) regularly visited the carcasses in common with several other previous studies. *Chrysomya nigripes* adults and larvae made their first appearance during the bloat stage, but this blowfly species was predominantly absent.

*(iii) Active decay stage:* In the active decay stage, the head of each carcass was entirely infested with calliphorid larvae in various stages of development. Larvae feeding on the carcasses formed dense larval feeding masses and larvae falling from hanging carcasses were restricted to the ground substrate for the remainder of their development, feeding on decomposition fluids beneath the hanging cadavers, called the "drip zone" (Shalaby et al. 2000). Carcasses stretched towards the ground and the fluids fell from the carcasses towards the ground from day 5 at both sites. These large larval masses were still mainly comprised of *C. megacephala* and *A. rufifacies* in all six carcasses (i.e. in all three seasons at both habitats). However, subject to the caveat of insufficient sample replicates, *Chrysomya pinguis* Walker*, Chrysomya chani* Kurahashi*, Hypopygiopsis infumata* Bigot and *Synthesiomyta nudiseta* VanDerWulp were observed only on the carcass in the summer season in the mixed deciduous forest and *Drosophila melanogaster* was found only in the winter season in the mixed deciduous forest.

*(iv) Advanced stage:* The carcasses slowly reached the post-decay stage, when they began to fall apart and therefore provided a food resource for the few remaining Diptera larvae on the ground under the carcasses. The clothing had fallen off and become fluid engulfed (Fig. 1). Adult braconid parasitic wasps of Diptera were commonly observed in both habitats and were collected from around the carcasses at the late advanced stage. However, dipteran larvae and pupae were not collected for live rearing or dissection to check for parasitoids, and so the timing of parasitism and of what hosts and to what parasitism level remains unknown. Adult Cleridae and Dermestidae were observed on carcasses from the active decay stage until the dry phase, with representatives of the Cleridae, Dermestidae, Hybosoridae, Scarabaeidae, Trogidae, Staphylinidae, Siliphidae, Cicindelidae and Chrysomelidae being observed on all six carcasses. Coleopteran larvae were observed in association with the carcasses in the late advanced stage until the skeletal stage of decomposition, although larvae of the Cleridae species *Necrobia ruficollis* and *Necrobia rufipes* along with *Dermestes maculates* (Dermestidae) were commonly found on the cadaver during skeletonization.

*(vi) Skeletal stage:* Coleoptera were present on the carcasses in the last decomposition stage, and their abundance increased considerably during the advanced and skeletal stage in all carcasses becoming the dominant taxa during the late dry decomposition stage. Dominant coleopteran species included Cleridae, Dermestidae, Scarabaeidae and Staphylinidae.

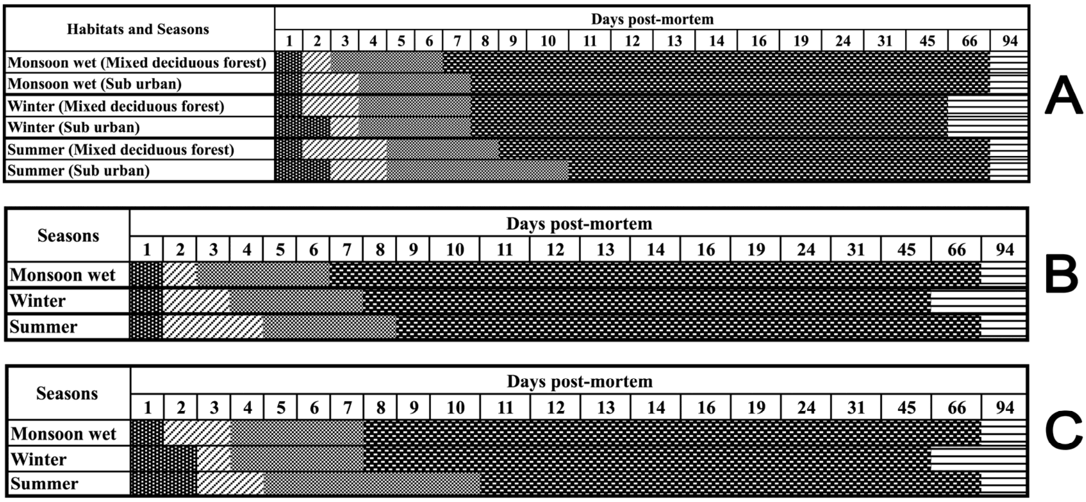


**FIGURE 3.** Stages of decomposition for (A) all pig carcasses, and those in the (B) mixed deciduous forest site and the (C) suburban site; fresh stage ( ), bloated stage ( ), active decay stage ( ), post-decay stage ( ) and skeletonization ( ) in Nan province, northern Thailand, during May 2010 – August 2011: Monsoon wet (May - October), winter (November - February), Summer (March – April).

Previous studies on the ecological relationship between insects and the cadavers they were found on in Malaysia (Ahmad et al. 2011) and Pakistan (Zaidi & Chen 2011) revealed them to be necrophagous, necrophilous and omnivorous species. This trend was also observed in this study, with necrophagous species that feed only on decaying tissues in fresh and bloat stage represented by *Chrysomya* spp., *M. domestica* and Sepsidae; necrophilous species that prefer to feed on decaying tissues in active decay stage and omnivorous species that feed on decaying tissues in advanced and skeletal stage being *N. rufipes* and ants, respectively. Accordingly the ecological niches of insects associated with carcassess in Thailand are broadly similar in Malaysia and Pakistan, as expected.

**Stages and rate of decomposition**

Decomposition is a continuous process that varies in its rate and characteristics depending on the environment. Nevertheless, the stages of decomposition can be defined, albeit with some overlap, by the key observable physical changes to the state of the carcass. This is of direct relevance since the pattern of insect succession depends on different carrion insects being attracted to the varying biological, chemical and physical changes the carcass undergoes throughout the process of its decay. Here, the process was divided into the five easily discernible stages of the fresh, bloat, active decay, post-decay and skeletonization stages, as defined previously (Gennard 2006; Goff 2009), and representative images are shown in Fig. 1.

The period of decomposition stages was similar in both habitats. The six carcasses in the present study all remained in the fresh stage for the first day, but in the winter and summer seasons in the suburban habitat a delayed progression through the subsequent stages of decomposition was evident (Fig. 3). The carcass in the summer season in the suburban habitat had longer bloat and active decay stages than the other carcasses. This tentative but unconfirmed observation contrasts with the report on insect succession in Hawaii (Shalaby et al. 2000), where the fresh, bloat and active decay stages were all longer than in this study. However, if correct, this likely reflects the local climate differences and especially the temperature and humidity (see Fig. 4).

(i) *The fresh stage*: (Fig. 1) was found up to the end of the first day post-mortem, with no physical appearance of decomposition being evident, although the body temperature had equilibrated to the environmental temperature by the end of this period. Three carcasses in the mixed deciduous forest site and one carcass in the suburban site remained in the fresh stage for the first day, but the carcasses in the winter and summer seasons in the suburban habitat showed a delayed progression through the subsequent stages of decomposition (Fig. 3).

*(ii) The bloated stage*: (Fig. 1), defined as when the body started to show external signs of decomposition, such as bloating, changes in the abdomen colour to green, onset of a putrid smell of the body and expulsion of fecal materials from the intestine, was noted from 2–4 d post-mortem. There was a noticeable increase in adult insect activity around each cadaver as well, but the larvae were largely restricted to areas around the wounds and natural openings, presumably due to the strong anaerobic environment inside the carcasses. The fastest decomposition in this stage (at 24 h) was in the monsoon wet season in the mixed deciduous forest site and in winter in the suburban habitat, but was longer in the other carcasses reaching 3 d for that in the summer season in the mixed deciduous forest (Fig. 3).

*(iii) Active decay stage:* The body started to turn from green to a dark colour (Fig. 1), and the external body form was broken due to the affect of the growing dipteran larvae and the gasses inside the body expanding. The greatest percentage of the carcass biomass was removed during this stage (determined by visual appearance) as a result of the larval feeding masses. The end of this stage was marked by the decomposition fluids from the carcasses and Diptera larvae falling to the ground, wandering final instar larval migration before pupation and the bones becoming visible. Most carcasses spent approximately 4 d in this stage of decomposition, but the decomposition of the cadaver in the summer season at the suburban site took longer (6 d) in this stage (Fig. 3).

*(iv) Advanced stage:* In the fourth (post-decay) decomposition stage the body started to dry and fall apart (Fig. 1). The putrid smell decreased and the last Diptera larvae left the carcass remains. The duration of this decomposition stage in winter was similar in both habitats (~14 d) but varied between the two habitats in the other seasons and was slowest in the monsoon wet season at both habitats. Overall, the net time of the first four decay stages was shortest in the winter season in both habitats at 37 d compared to 57–62 d in the other seasons (Fig. 3).

*(v) Skeletonization stage:* After all the organs and soft tissue were consumed, the skeleton remains slowly breaks down. This was the longest stage as the physical makeup of bones takes longer to decompose (Fig. 2).

In summary, the number of decomposition stages found in the present study was similar to those of previous studies in Malaysia (Chin et al. 2010), but differed in their decomposition rates.

**Temperature**

The ambient temperature in the mixed deciduous forest was higher than that in the suburban habitat in each of the three seasons, whilst at each location it was

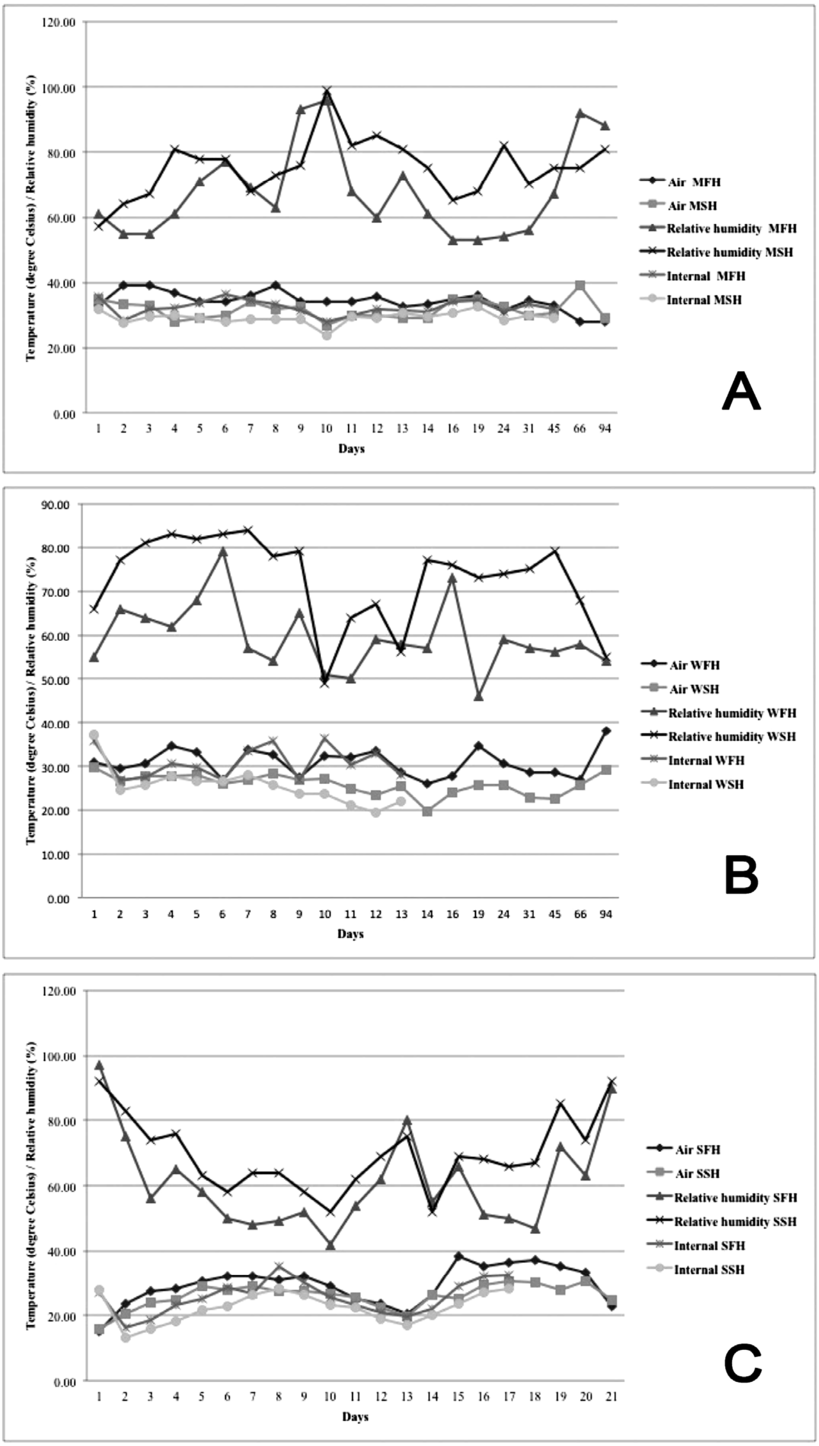


**FIGURE 4.** Temperature and relative humidity in and around the pig carcass in (A) monsoon wet, (B) winter and (C) summer seasons in the mixed deciduous forest and suburban habits. in Nan province, northern Thailand, during May 2010 – August 2011: Monsoon wet (May - October), winter (November - February), Summer (March – April). Legend: M = monsoon wet season, W = winter, S = suburban, FH = mixed deciduous habitat, and SH= suburban habitat.

**TABLE 3.** The average and range of the internal pig carcass temperature in the mixed deciduous forest (MDF) and the suburban (S) habitats in each season, along with the ambient temperature and relative humidity, in Nan provice, northern Thailand during May 2010 – August 2011: Monsoon wet (May - October), winter (November - February), Summer (March – April).

| Season | Pig carcass temperature (°C) | | Ambient temperature (°C) | | Ambient relative humidity (%) | |
|---|---|---|---|---|---|---|
| | MDF | S | MDF | S | MDF | S |
| Monsoon wet | 32.4±2.2 | 29.2±1.8 | 34.3±2.9 | 31.6±2.9 | 67.3±13.9 | 75.2±9.4 |
| Values: (Min-Max) | (28.0-36.5) | (23.8-32.5) | (28.0-39.0) | (27.0-39.0) | (53.0-96.0) | (57.0-99.0) |
| Winter | 30.8±3.6 | 25.6±4.3 | 30.8±3.2 | 25.9±2.4 | 59.4±7.7 | 72.7±10.0 |
| Values: (Min-Max) | (26.9-36.2) | (19.5-37.3) | (25.9-38.0) | (19.8-29.7) | (46.0-79.0) | (49.0-84.0) |
| Summer | 25.7±5.3 | 22.4±4.6 | 29.4±6.0 | 26.0±3.9 | 61.1±3.9 | 69.7±11.4 |
| Values: (Min-Max) | (16.3-35.3) | (13.2-28.2) | (15.2-38.5) | (15.7-30.7) | (42.0-97.0) | (52.0-92.2) |

highest in the monsoon wet season and lowest in the summer (Table 3; Fig. 4). The internal carcass temperature showed the same trend of being higher in the mixed deciduous forest and in the monsoon winter season. However, they were lower than the ambient temperature (at the same location) in the monsoon wet and summer seasons, and similar to the ambient temperature in the winter season (Table 3; Fig. 4). The internal carcass temperature higher than the ambient temperature when the heat generated by larval aggregation (larval mass) (Fig. 4; A: day 7, B and C: day 8).

In contrast, the relative humidity was numerically higher in each season in the suburban locality than in the mixed deciduous woodland, whilst it was predictably highest in the monsoon wet season.

Compared between seasons, the amount of variation in the meteorological data in the summer season was greater than in the winter and monsoon wet seasons because the summer has a greater variety of weather types including a summer monsoon.

## CONCLUSION

In total, 45 cadaver-associated arthropod taxa excluding accidentals were collected, of which 28 were identified to species and five to morphospecies. The dominant species in both habitats were *C. megacephala* and *A. rufifacies,* which were also the primary necrophagous colonizers. All species found on the cadavers in the suburban habitat were also found on the cadavers in the mixed deciduous forest. The insect succession occurred at a predictable sequence based upon that reported from other regions, although how much the differences between these two sites may reflect true biogeographical variation rather than just stochastic variations awaits further research. This is the first report of the insect succession and the decomposition stages on hanging pig carcasses in Thailand, but is clearly in need of future in depth studies for confirmation of the potential trends observed and to establish a database of the carrion insects and their succession patterns under different climatic (seasonal) and biogeographical regions. Eventually, we hope to establish a forensic entomology data base for Thailand.

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