Review of forensically important entomological specimens in the period of 1972 – 2002

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Abstract. Forensic entomological specimens received by the Unit of Medical Entomology, IMR., from hospitals and the police in Malaysia in the last 3 decades (1972 – 2002) are reviewed. A total of 448 specimens were received. From these, 538 identifications were made with the following results: Eighteen species of cyclorrphaga flies were identified consisting of *Chrysomya megacephala* (Fabricius) 215 cases (47.99%), *Ch. rufifacies* (Masquart) 132 (29.46%), *Ch. villeneuvi* Patton 10 (2.23%), *Ch. nigripes* Aubertin 7 (1.56%), *Ch. bezziana* Villeneuve 4 (0.89%), *Ch. pinguis* (Walker) 1 (0.22%), *Chrysomya* sp. 47 (10.49%), *Sarcophaga* sp. 28 (6.25%), *Lucilia* sp. 21 (4.69%), *Hermetia* sp. 15 (3.35%), *He. illucens* (Linnaeus) 1 (0.22%), *Hemipyrellia ligurriens* (Wiedemann) 3 (0.67%), *Hemipyrellia* sp. 2 (0.45%), *Ophyra spinigera* 1 (0.22%), *Ophyra* sp. 6 (1.34%), *Calliphora* sp. 24 (5.36%), *Synthesiomyia nudiseta* (Wulp) 1 (0.22%) and *Eristalis* sp. 1 (0.22%). Other non – fly insect specimens are *Pthirus pubis* (Linnaeus) (Pubic louse) 2 (0.45%) and Coleoptera (Beetles) 1 (0.22%). *Ch. megacephala* and *Ch. rufifacies* were the commonest species found in cadavers from different ecological habitats. *Sy. nudiseta* is an uncommon species, thus far found only on cadavers from indoors. *Sy. nudiseta* is reported for the second time in Peninsular Malaysia. A total of 329 cases (73.44%) had a single fly infestation, 109 cases (24.33%) had double fly infestation and 10 cases (2.23%) had triple fly infestation. Five cases (1.12%) had eggs and 3 cases (0.67%) had larval stages that were not identifiable. No arthropods were retrieved from cadavers in 8 cases (1.79%). In conclusion, although large number of fly species were found on human cadavers, the predominant species are still those of *Chrysomya*.

INTRODUCTION

The recovery of entomological fauna from human cadavers is one of the important features of forensic investigation. Forensic entomology plays an important role in criminal investigation. Generally, these fauna are used to determine the post - mortem interval (PMI) i.e. time of death. The first published account of the application of forensic entomology to legal medicine was reported by Yovanovitch (1888).

In Malaysia, Reid (1953) summarized the first forensic entomology case of Nevin (1950) in Penang, who found *Chrysomya megacephala* larvae in a dead woman. Reid (1953) also published notes on flies of forensic importance. Later Lee *et al.* (1984) and Lee 1989, 1996), reviewed and updated some of the IMR collections. Recently Hamid *et al*. (2003) also reviewed the Universiti Kebangsaan Malaysia collection.

In another report, Lee (1996a) studied the forensically important entomological specimens received from hospitals, police and other agencies throughout Malaysia. Lee & Marzuki (1993) studied arthropods succession on monkey carcasses either fully exposed or partially buried near the fringes of tropical forest. To add to this, Omar *et al.* (1994) made observation on arthropods on monkey carrion at a rubber tree plantation in Malaysia.

The main objective of this study is to analyse the large number of forensic entomological specimens received by the Unit of Medical Entomology, Institute for Medical Research from hospitals, police and other agencies throughout Malaysia in the 30-year period of 1972-2002.

MATERIALS & METHODS

A total of 448 specimens were received from 1972 – 2002 and studied (Table 1). From these specimens, a total of 538 identifications were reported.

Specimens were generally received in two separate containers (e.g. universal bottle). One container contained preserved specimens in 70% alcohol and the other container with live specimens for study. Each bottle, together with medical forms, was labeled with hospital identification number, police report number and short notes of the case, name of deceased (if known), date and time of collection and name of collector. A request form describing the case signed by the attending forensic pathologist was also received along with the specimens. Samples received were immediately registered in a log book for record purposes.

Various stages of flies (eggs, larvae and pupae) from the decomposing bodies were generally collected by the attending pathologist. As soon as arthropod specimens were received, they were processed immediately by established standard procedures.

Preparation of live larval specimens

In the laboratory, the larvae were transferred to a dry container with a small piece of cow liver and a layer of sawdust. Subsequently, adults emerging from the sawdust were pinned and identified using the keys provided by Kurahashi *et al*. (1997).

Preparation of preserved larval samples for identification

Preserved larva samples were prepared according to the method described by Lee *et al* (1984). Larvae were first washed in distilled water, then cleared in 10% KOH solution for 2-6 hours. The larvae were washed again in distilled water, transferred to 10% acetic acid for ½ hour and washed again in distilled water. At this stage, all the internal organs of the maggots were removed and the posterior spiracles were cut transversely. The specimens were then dehydrated in ascending series of ethanol (30%, 50%, 70%, and 90%) for ½ hour each. The larvae were then soaked in absolute alcohol for at least one hour and then transferred into clove oil. The specimens were mounted onto a glass slide using Canada Balsam and left in incubator for 1-2 days. The slide was examined under a light microscope for taxonomy studies and identification in order to determine the post-mortem interval (PMI).

RESULTS

The total number of fly samples from different ethnic groups from 1972 – 2002 are presented in Table 1. A total of 448 samples were received. From these, 538 identifications were made. The majority of the fly species was recorded from Chinese cadavers (122 cases), 69 cases were from Malays and from Indian, 60 cases while 8 cases were recorded from the local aborigines. One case came from Sabah. There were 5 infant cases, 3 males and 2 females. Other races accounted for 41 cases while the ethnicity of 142 cases was unknown due to unavailability of information. Out of 448 cadavers, only 185 samples contained sufficient information for statistical analysis of the distribution of age groups in terms of race and sex of the subjects (Table 2). In most cases, the male cadavers outnumbered the females. Most of the victims were aged between 15-54. Table 3 shows the total number of fly

larvae species identified. A total of 17 species were identified comprising 7 species of *Chrysomya* (92.86%), 1 species of *Calliphora* (5.36%), 2 species of *Hemetia* (3.57%), 2 species of *Hemipyrellia* (1.12%), 2 species of *Ophyra* (1.56%), 1 species of *Lucilia* (4.69%), 1 species of *Sarcophaga* (6.25%) and *Synthesiomysia nudiseta* (0.22%). *S. nudiseta* is an uncommon species, found only on cadavers from indoors. This is the first case reported thus far from the collection of the Institute for Medical Research. The majority of infestation is single species accounting for 61.15% (Table 4). The ecological habitats where the cadavers were found are shown in Table 5. For ease of reference, these habitats were arbitrarily divided into rural, residential & aquatic. *Chrysomya* larvae were predominantly found in cadavers in the rural areas, fairly common in aquatic areas but seldom in the residential areas (Table 6). Overall, *Chrysomya* species alone were the most commonly found larvae in all the 3 habitats (56.5%).

DISCUSSION

IMR has the largest collection of forensic entomology cases in Malaysia due to its long history of utilizing this method for PMI determination since 1950. Despite a wealth of information, however, there has been no known comprehensive review of these collections. This report, though only reviewing the specimens received in the period of 1972-2002, however, provided the first insight of the specimens collected and analysed.

The analysis of the collection revealed several important observations: (i) a large number of flies were attracted to human cadavers under tropical conditions. A total of 18 cyclorrphagic fly species were recorded. This reflected the diversity of carrion associated flies in Malaysia, (ii) the predominant fly species were those of

Chrysomya species in which a total of 7 species were recorded. *Ch. megacephala* was the commonest fly seen. This has also been reported by previous studies (Lee, 1989; Omar *et al*., 1994; Hamid *et al*., 2003). Their ability to survive and compete successfully in the carrion environment accounted for the predominance of *Chrysomya* species, (iii) other fly maggots were also found, albeit in much lower frequency; probably showing that they may not be as successful as *Chrysomya* larvae in the carrion habitat, (iv) The presence of a particular fly species always revealed information on the ecology of the crime scene. For example, the presence of *Eristalis* species indicated that the corpse found in an environment associated with water, as the larvae of this fly are aquatic. Such knowledge is important in determining if the corpse has been moved from the crime scene to other sites and (v) most of the fly infestation in corpses constituted only single species (61.15%), which further provided proof of inter-fly competition in the carrion environment.

The collection also contained several fly maggot specimens that could not be identified. This was attributed to poor killing and preservation techniques, as reported by Lee (1989). Killing the maggots in boiling water, for example, caused charring of the larvae and blackened the skin surfaces, thus masking important taxonomic features and rendering identification difficult or impossible. To avoid this, warm water at about 60-70^{o}C should be used to kill the maggots. Preservation of killed maggots in solutions other than 70% ethanol would damage them, making subsequent mounting difficult, hence affecting important taxonomic profiles.

The study of the cadaver fauna constitutes the most important forensic application of entomology. In forensic entomology the fly maggots are the major source of investigation. Forensic investigation using fly maggots is an important tool in determining time of death or the PMI. However, the pre-requisites for conducting a

proper and precise age-dating are: (i) collection of representative samples in the crime scene, (ii) proper preservation of entomological samples, (iii) careful mounting of the specimens, (iv) ability to identify taxonomically and (v) knowledge of the life history of carrion-associated flies.

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