



Original paper

Ultrastructure of male genitalia of blow flies (Diptera: Calliphoridae) of forensic importance

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ABSTRACT

Male genitalia of blow flies (Diptera: Calliphoridae) are distinctive in their morphological features and are often used for species identification. The aim of this work was to investigate the male genitalia of blow flies of medical and forensic importance from Thailand at the ultrastructural level, using scanning electron microscopy (SEM). Flies in two subfamilies were examined: Chrysomyinae [*Chrysomya bezziana* Villeneuve, *Chrysomya chani* Kurahashi, *Chrysomya nigripes* Aubertin, *Chrysomya pinguis* (Walker), *Chrysomya rufifacies* (Macquart), *Chrysomya thanomthini* Kurahashi & Tumrasvin, and *Chrysomya villeneuvei* Patton] and Luciliinae [*Hemipyrellia ligurriens* (Wiedemann), *Hypopygiopsis infumata* (Bigot), *Hypopygiopsis tumrasvini* Kurahashi, *Lucilia cuprina* (Wiedemann), *Lucilia papuensis* Macquart, *Lucilia porphyria* (Walker), and *Lucilia sinensis* Aubertin]. Particular attention was paid to the main distinguishing features such as the shapes of the cercus and the surstylus, and the complex structure of the distiphallus. The differentiation of the male genitalia of these species at the SEM level is discussed and compared to the conditions in closely related species such as *Chrysomya megacephala* (Fabricius). A key for the identification of 14 blow fly species based on male genitalia is provided.

1. Introduction

Blow flies are insects of medical, veterinary, and forensic importance on a global scale because of the fact that many species use breeding places that are close to human and animal dwellings. Adult blow flies are not only a nuisance as a pest but also mechanical vectors of numerous pathogens (namely viruses, bacteria, protozoan cysts, helminth eggs, and fungi) that may cause sickness in humans (Greenberg, 1971; Getachew et al., 2007; Chaiwong et al., 2012a, 2014). Their larvae can cause myiasis in living humans and animals, especially the genera *Cochliomyia* Townsend, *Chrysomya* Robineau-Desvoidy, *Lucilia* Robineau-Desvoidy, and *Calliphora* Robineau-Desvoidy (Zumpt, 1965; Hall et al., 2016). Besides, immature stages of blow fly found in human corpses are important in forensic entomology because they can be used for estimating the minimum time since death (PMI_{min}) (Lee et al., 2004; Sukontason et al., 2007; Amendt et al., 2011). In Thailand, blow flies of the subfamilies Chrysomyinae and Luciliinae have been recognized as significant insects of medical and forensic importance, including the blow fly species in the genera *Chrysomya*, *Lucilia*, *Hemipyrellia*

Townsend, and *Hypopygiopsis* Townsend (Sukontason et al., 2007; Bunchu, 2012; Chaiwong et al., 2012b).

Species identification of adult blow flies based on morphology relies on the external morphological characteristics of the body and the genitalia. However, such external characteristics of the body can be highly variable, which may lead to incorrect species identification (Holloway, 1991; Williams and Villet, 2014; Grella et al., 2015). The male genitalia is one of the most important morphological characteristics for species identification due to its highly species-specific and extremely divergent form (Rognes and Paterson, 2005; Briceño et al., 2016). Moreover, the male genitalia are often used for determining evolutionary relationships among taxa (Giroux et al., 2010; Whitmore et al., 2013; Buenaventura and Pape, 2015). Male genitalia of blow flies have been studied for species recognition under light microscopy (LM) in several species of forensic importance, for example, *Chrysomya bezziana* Villeneuve (Senior-White et al., 1940), *Chrysomya megacephala* (Fabricius) (Senior-White et al., 1940; Park, 1977), *Chrysomya pinguis* (Walker) (Senior-White et al., 1940; Park, 1977), *Chrysomya rufifacies* (Macquart) (Senior-White et al., 1940; Silva et al., 2012), *Chrysomya*

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nigripes Aubertin (Senior-White et al., 1940), *Lucilia cuprina* (Wiedemann) (Senior-White et al., 1940; Park, 1977), *Lucilia papuensis* Macquart (Senior-White et al., 1940; Park, 1977; Tumrasvin et al., 1977), *Lucilia porphyryna* (Walker) (Senior-White et al., 1940; Park, 1977), *Lucilia sinensis* Aubertin (Senior-White et al., 1940; Tumrasvin et al., 1977), *Hemipyrellia ligurriens* (Wiedemann) (Senior-White et al., 1940; Park, 1977), *Hemipyrellia pulchra* (Wiedemann) (Senior-White et al., 1940), *Hypopygiopsis infumata* (Bigot) (Kurahashi, 1977), and *Hypopygiopsis tumrasvini* Kurahashi (Kurahashi, 1977; Moophayak et al., 2011), while a few species have been studied using scanning electron microscopy (SEM), namely *C. megacephala* (Chaiwong et al., 2008), *Cochliomyia hominivorax* (Coquerel) (Leite, 1995), and *Cochliomyia macellaria* (Fabricius) (Leite, 1995).

Based on male genitalia, the important structures that can be used for species identification consist of the cercus, surstylus, and phallus (Senior-White et al., 1940; Kurahashi, 1977; Park, 1977). The last one, especially, is formed highly species-specific, but its structure is very complex and difficult to study under light microscopy. Consequently, SEM is the most suitable method for studying its structure. Taking into account the limited information on previous SEM studies of adult blow flies, in particular the male genitalia, this study was undertaken to illustrate the morphological structures of the male genitalia of the medically and forensically important blow fly species of Thailand. A detailed ultrastructure of this distinctive characteristic is given since it is very useful for species recognition and comparative studies between closely related species.

2. Materials and methods

2.1. Fly strains

Males of *Chrysomya chani* Kurahashi, *C. pinguis*, *Chrysomya thanomthini* Kurahashi & Tumrasvin, *C. (=Achoetandrus) rufifacies*, *Chrysomya (=Achoetandrus) villeneuvei* Patton, *C. (=Ceylonomyia) nigripes*, *L. cuprina*, *L. papuensis*, *L. porphyryna*, *L. sinensis*, *H. ligurriens*, *H. infumata*, and *H. tumrasvini* used in this study were obtained through field collection during 2014–2015 and kept as pinned specimens in the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand. For *C. bezziana*, about 50 of the late third instar larvae were obtained from a cutaneous myiasis case in dog, investigated in the Animal hospital in Chiang Mai province. They were placed in a rearing box and sawdust was provided as a place for pupation and reared to the adult stage in the laboratory. They were identified using the taxonomic keys of . The details of the species and the collection sites are given in Table 1.

Table 1
Collection data of specimens used in this study.

Subfamily	Species	Province (Location)	GPS reference		Number of specimens
			Latitude	Longitude	
Chrysomyinae	<i>Chrysomya bezziana</i>	Chiang Mai (Muang)	18°47' 46"N	98°57' 40"E	5
	<i>Chrysomya chani</i>	Lampang (Doi Khun Tan)	18°23' 35"N	99°12' 54"E	8
	<i>Chrysomya nigripes</i>	Lampang (Doi Khun Tan)	18°23' 35"N	99°12' 54"E	4
	<i>Chrysomya pinguis</i>	Chiang Mai (Doi Nang Kaew)	19°03' 53"N	99°22' 34"E	8
	<i>Chrysomya rufifacies</i>	Laboratory colony (origin Chiang Mai)	18°47' 25"N	98°58' 22"E	8
	<i>Chrysomya thanomthini</i>	Chiang Mai (Chom Thong, Doi Inthanon)	18°34' 27"N	98°28' 54"E	4
	<i>Chrysomya villeneuvei</i>	Chiang Mai (Doi Nang Kaew)	19°03' 53"N	99°22' 34"E	8
	<i>Hemipyrellia ligurriens</i>	Chiang Mai (forest area, Mae Hia)	18°46' 01"N	98°56' 08"E	7
	<i>Hypopygiopsis infumata</i>	Lampang (Doi Khun Tan)	18°23' 35"N	99°12' 54"E	6
	<i>Hypopygiopsis tumrasvini</i>	Chiang Mai (Sirindhorn observatory)	18°47' 21"N	98°55' 16"E	3
Luciliinae	<i>Lucilia cuprina</i>	Laboratory colony (origin Chiang Mai)	18°47' 25"N	98°58' 22"E	8
	<i>Lucilia papuensis</i>	Lampang (Doi Khun Tan)	18°23' 35"N	99°12' 54"E	8
	<i>Lucilia porphyryna</i>	Chiang Mai (Doi Nang Kaew)	19°03' 53"N	99°22' 34"E	8
	<i>Lucilia sinensis</i>	Chiang Mai (Doi Nang Kaew)	19°03' 53"N	99°22' 34"E	3

2.2. Scanning electron microscopy (SEM) preparation

The abdominal segment at tergite 4th was cut from pinned specimens using a sharp scalpel under a dissecting microscope (Olympus, Japan). The obtained terminal ends were boiled in 10% potassium hydroxide (KOH) at ~90 °C for 10 min; thereafter, they were cleaned by rinsing in 70% ethanol before dissection in a glass plate containing 70% ethanol to obtain the genitalia. For the SEM process, the specimens were completely dried at room temperature for a week. All the specimens were then attached to double-stick tape on an aluminum stub. Any debris remaining on the surface of the specimens was removed. The specimens were then coated with gold in a sputter-coating apparatus, and viewed under a JEOL-JSM6610LV scanning electron microscope (JEOL, Japan).

2.3. Terminology

In this paper, the terminology of the male genitalia follows Rognes (1991), except “phallus”, which is used instead of “aedeagus”.

3. Results

3.1. General characteristics of the phallus

The different characteristics of the phallus of blow flies found in Thailand between subfamilies Chrysomyinae and Luciliinae are illustrated in Fig. 1. The phallus is divided into two parts, including the short basiphallus and the elongated distiphallus. The distiphallus is the most important structure used for species identification, and it consists of the paraphallus, hypophallus, and acrophallus. The paraphallus is paired, sclerotized, and characterized by symmetrical structures, expanding from the dorsal part of the distiphallus distal to the hypophallic lobes in lateral view (Fig. 1a). It is situated between the hypophallic lobes and the acrophallus in ventral view (Fig. 1c, d) but in some species it is indistinct or even completely absent. The hypophallus is bilobed and its symmetrical structure directly expands outward from the ventral part of the distiphallus. The hypophallic lobes of Luciliinae are strongly sclerotized with a serrated ridge at the proximal part, while in Chrysomyinae it is absent (Fig. 1b, d). Therefore, the presence of the sclerotized hypophallus with its serrated ridge at the proximal part of the hypophallus lobe is the main distinguishing feature of the phallus between the blow fly species in the subfamilies Chrysomyinae and Luciliinae. The acrophallus or genital opening, allowing the sperm exit, is a semi-tubular structure, which is situated between the hypophallic lobes and is always covered with microserrations on its surface, either near to its base or near to the tip, or both (Fig. 1).

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