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Review Paper

Parasarcophaga (Liopygia) ruficornis (Diptera: Sarcophagidae): A flesh fly species of medical importance

Suwannayod, S., Sanit, S., Sukontason, K. and Sukontason, K.L.*
Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
*Corresponding author e-mail: klikitvo@med.cmu.ac.th
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Abstract. *Parasarcophaga (Liopygia) ruficornis* is a well-known flesh fly species of medical importance, both as a myiasis-producing agent and fly seen in a forensic entomology context. This study performed a comprehensive literature review of this fly species, dealing with morphology, bionomics and medical involvement. Important characteristics used to identify *P. ruficornis* have been provided for both its third instar and adult for identification purpose in the future.

INTRODUCTION

The medical importance of the flesh fly species, *Parasarcophaga (Liopygia) ruficornis*, is of particular interest in many parts of the world, either as a myiasis-producing agent or fly seen in a forensic entomology context. Geographically, this species has been recorded as an Old World fly although it has invaded the New World. In the USA, it has been recorded from Hawaii (Davis & Goff, 2000), California, Florida, Massachusetts, New York, North Carolina, Pennsylvania and Washington D.C. (Alfred, 2011). In the Oriental region, *P. ruficornis* has been found in many countries such as Thailand (Sucharit *et al*., 1976), Malaysia (Kumara *et al*., 2012), Singapore (Sugiyama *et al*., 1990) and India (Sreevatsa *et al*., 1990).

The genetic characterization of *P. ruficornis* has been compared with that of *Parasarcophaga dux* and *Parasarcophaga argyrostoma* using allozyme and RAPD-PCR markers, which indicated a very close relationship between these species (Bajpai *et al*., 2011). However, based on the sequences of mitochondrial cytochrome oxidase gene *subunits I* and *II* (*COI* and *COII)*, the *ruficornis*-group was one of the six major clades of forensically important sarcophagids in Malaysia, with the other five being the *peregrina*-group, *albiceps*-group, *dux*-group, *pattoni*-group and *princeps*-group (Tan *et al*., 2010).

Morphology

In the forensic entomology aspect, identification of larval specimens that are found to be associated with a corpse is essential before they are used further in investigation [e.g., estimation of post-mortem interval (PMI_{min}) from the larvae collected]. Although flesh flies, including *P. ruficornis*, are commonly larviparous (larviposit the first instar), they also have been documented as oviparous in a laboratory colony. Sukhapanth *et al*. (1988) provided information of *P. ruficornis* eggs measuring 1.6±0.33 mm in length. Morphologically, the *P. ruficornis* larva, which is similar to other flesh fly larvae, exhibits a distinct morphological feature in having its posterior spiracle

situated within a terminal concavity of the last abdominal segment. The larva typically has a vermiform robust body, comprising a head region, three thoracic segments and eight abdominal segments (Figure 1A). The length of the first, second and third instar is 6.8±0.45 mm, 11.8±0.07 mm and 16.9±0.08 mm, respectively (Sukhapanth *et al.*, 1988). With the aid of light microscopy, the third instar of *P. ruficornis* has been documented by focusing on the main features used to differentiate from the other forensically important flesh fly species. On each lateral side of the prothorax, a pair of anterior spiracles having 11-15 papillae arranged in a single row (Figure 1B). The posterior spiracle is located in a terminal concavity of the last abdominal segment and is characterized by (1) the distinct inner projections between the spiracular slits (Figure 1C), (2) the prominent tail of the upper end of the peritreme, and (3) position of the peritreme at the base of the middle slit (Sukontason *et al.*, 2010). All larval instars of this fly species have been described by scanning electron micrograph (SEM) (Singh *et al.*, 2012), highlighting the sensory organs (dorsal, terminal and ventral organs) located on the cephalic segment as well as strong, slightly curved mouth hooks. In this review, the sharp bladed mouth hooks of the first instar are clearly demonstrated in Figure 2. Features of the internal cephalopharyngeal skeleton of all instars were also illustrated by Singh *et al.* (2012).

Puparia of *P. ruficornis* were measured as 11.7±0.14 mm in length (Sukhapanth *et al.*, 1988). A very short pupal respiratory horn was observed dorsolaterally in the first abdominal segment (Singh *et al.*, 2012). Regarding adults, males are slightly larger than females, measuring 13.8±0.16 mm and 13.2±0.01 mm, respectively (Sukhapanth *et al.*, 1988). The prime characteristics of *P. ruficornis* differ from two other forensically important species; *P. dux* and *P. peregrina*, which have yellowish orange third antenna and palpus (Figure 3). In adult flesh flies, only males could be identified, of which each species was endowed with distinct characteristics of terminalia (Figure 4). Regarding flesh flies of forensic importance, the key to identify adult males of the South American genera was published (de Carvalho & de Mello-Patiu, 2008), while that for identifying species including *P. ruficornis* in Thailand has been updated (Chaiwong *et al.*, 2009). Informative characteristics of male genitalia of this species, particularly the distiphallus, have been displayed using SEM (Giroux *et al.*, 2010).

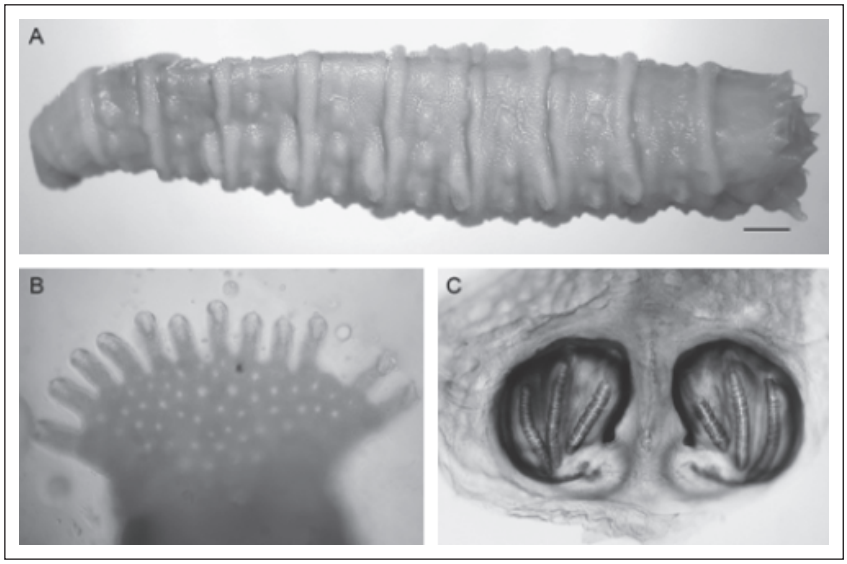

Figure 1. Third instar of *P. ruficornis*. (A) Lateral view. Bar = 1 mm. (B) Anterior spiracle. (C). Posterior spiracle. Online figure in color

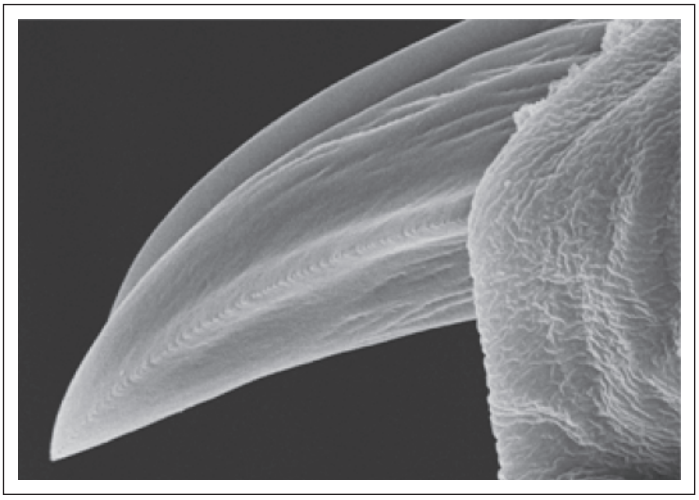

Figure 2. Scanning electron micrograph showing the sharp bladed mouth hook of the first instar of *P. ruficornis* (original picture of KL Sukontason)

Figure 3. Adult male of *P. ruficornis*. Inset displays important features of this species, yellowish orange of the third antenna (arrowhead) and palpus (arrow). Online figure in color

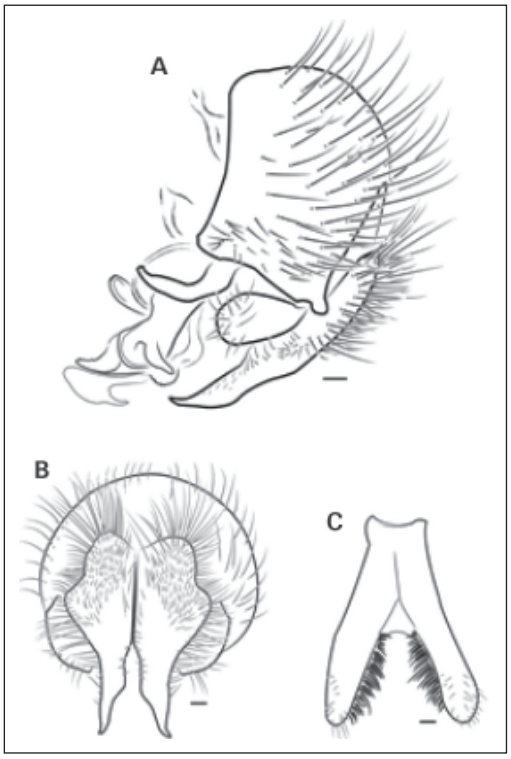

Figure 4. Male terminalia of *P. ruficornis*. (A) Lateral view. (B) Cercus, caudal view. (C) Sternite 5. Bar of all figures = 0.02 mm

Table 1. Documentation of human cases associated with larvae of *P. ruficornis*

Year	Country	Death scene	PMI_{min}	State of decay	Reference
2002	Thailand	indoor	ca. 3-4 d	bloat	(Sukontason *et al.*, 2007)
2006	Thailand	indoor, constant 25°C	unknown	mummified	(Sukontason *et al.*, 2007)
2011	Kuwait	indoor, 2nd floor (in air condition)	ca. 7.5-8.5 d	decomposed	(Al-Mesbah *et al.*, 2011)
2012	Malaysia	indoor	ND	ND	(Kumara *et al.*, 2012)

ND, no available data

Bionomics

Based on extensive study on the bionomics of sarcophagids in Thailand by Bänziger & Pape(2004), female *P. ruficornis* occasionally laid eggs on the mesh of boxes containing the breeding medium. Its laying habit is of an amphibiodotic type (larviposit on both faeces and carrion). Females typically behave in a larviparous manner by depositing 40-80 first instars (Verma & Ishikawa, 1984), but in special circumstance such as laboratory rearing, oviparous behavior also has been documented. This phenomenon was similar to that which occurred in *P. dux* in laboratory conditions (Sukontason *et al.*, 2005). Laboratory rearing conditions set at 27±4ºC and 78±4% RH demonstrated that female *P. ruficornis* laid eggs 5-7 days after emergence, with the number of eggs per batch being 19.1±11.1 (Sukhapanth *et al.*, 1988). Larval growth development (larviposition until pupariation) varied according to records. In Guam it took 7 days at 29.5ºC (Bohart & Gressitt, 1951); in Thailand 8.0±1.8 days at 27±4ºC (Sukhapanth *et al.*, 1988); and in Hawaii 7.5-10.8 days at 26ºC during experiments (Goff *et al.*, 1997). In Saudi Arabia, Amoudi *et al.* (1994) reared larvae at the constant temperatures of 13, 16, 19, 22, 25, 28, 31, 34 and 37ºC, and the larval development time took 31.5, 17.5, 10.5, 9.7, 8.5, 6.8, 6.0, 5.5 and 6.3 days, respectively. In Thailand, larvae were reared at 27±4ºC, and the pupal period was 11.7±0.14 days. Longevity of males and females ranged from 3.5 to 39 days and 2.0 to 31.8 days, respectively (Sukhapanth *et al.*, 1988).

This fly species is synanthropic, that is, it lives closely associated with the human environment. In Pakistan, adult *P. ruficornis* was trapped in residential areas, where flies fed and larviposited on rabbit, fish and chicken carcasses (Shazia *et al.*, 2006). In Thailand, adults were captured from the flowers of *Bulbophyllum putidum* and the fruit of *Dimocarpus longan* (Bänziger & Pape, 2004), as well as animal waste such as putrid fish (Sucharit *et al.*, 1976). Similarly, in northeast Thailand adult *P. ruficornis* was collected from restaurants and school cafeterias, but not from fresh-food markets, garbage piles or paddy fields (Chaiwong *et al.*, 2012). In southern India, this species was collected in several villages that were hit by the tsunami on 26th December, 2004 (Srinivasan *et al.*, 2006).

Myiasis

There are limited records of myiasis caused by *P. ruficornis* and it is either reported as the sole species responsible or co-infesting with other fly larvae. In Thailand, Sucharit *et al.* (1981) reported on cases in the vagina and in India patients suffering from leprosy have been documented as being infected (Sreevatsa *et al.*, 1990). A wound co-infested with the blow fly, *Chrysomya megacephala*, and house fly, *Musca domestica*, was recorded from the scalp of a man in Brazil (Ferraz *et al.*, 2010). Adult *P. ruficornis* were caught in the intensive care unit (ICU) of a hospital in Malaysia providing further evidence of the potential for the nosocomial infestation(Nazni *et al.*, 2011).

Forensic entomology

Although blow flies (Diptera: Calliphoridae) are often used in forensic investigations, several documented cases report the presence of flesh fly larvae, suggesting that there are other fly groups of forensic importance. Of these flesh fly species, specimens of *P. ruficornis* has been recorded to associate with human death scenes, as well as from pig carcasses (*Sus scrofa*); an animal experimental model in forensic entomology in Brazil (de Souza & Linhares, 1997; Barbosa *et al*., 2009), the USA (Oahu island of Hawaii) (Davis & Goff, 2000), and Thailand (Vitta *et al*., 2007; Sukjit, 2011). Investigation on the island of Oahu, Hawaii, U.S.A. indicated that this species was an early invader and insect colonizer of the death scene (Nolte *et al*., 1992). A document reporting from Kuwait demonstrated the significance of post feeding third instar *P. ruficornis*, which was collected from the blanket which the body remain was wrapped. Based on the age of *P. ruficornis* collected and the location of the body, ~7.5–8.5 days PMI_{min} was estimated (Al-Mesbah *et al*., 2011). Based on the literature, few number of human death scenes were found to be associated with this species, mostly indoor cadavers (Table 1). Difficulty in identification of flesh fly larvae and/or incorrect identification may lead to have lower number of cases infesting with *P. ruficornis*, than the actual cases. According to the indoor death scenes associated with *P. ruficornis*, this fly species might have special features in its biology that allow them to be colonized in houses or other dwelling. This phenomenon was similarly documented in other species, *Sarcophaga caerulescens* Zetterstedt, the sarcosaprophagous flesh fly species found in indoor death scene in southern Finland (Pohjoismaki *et al*., 2010). Such phenomenon is worthy of note in forensic investigation.

In addition, study pertaining to more precise estimation and/or analysis of death related to drugs has used specimens of *P. ruficornis*. Goff *et al*. (1994) reported the larval developmental rate of this fly species when fed on phencyclidine; a common drug abused in many country and legitimate veterinary tranquilizer, from the decomposing tissue of rabbit. No significant differences in larval growth rate were observed among rabbits administered with 3.66, 7.31, and 14.62 mg of phencyclidine via ear vein infusion. Pupariation period of *P. ruficornis* were longer for animals fed on tissues containing the drug.

In conclusion, despite information on all aspects of *P. ruficornis* being relatively limited; the significance of this fly species is increasing, particularly from a forensic entomology viewpoint. Based on the literature review, the indoor environment preferred by *P. ruficornis* is worthy of note in forensic investigation. Therefore, to increase worldwide reviews of human cases involving this fly species is mandatory, and their clarification would benefit forensic investigations.

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