Jurnal Sains Kesihatan Malaysia **6** (2) 2008: 35-41

Descriptions of the Larval Instars of *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae), a Species of Forensic Importance in Malaysia

(Ciri-ciri Setiap Instar Larva *Chrysomya rufifacies* (Macquart) (Diptera:Calliphoridae), Spesis Berkepentingan Forensik di Malaysia)

RAJA M. ZUHA RAJA KAMAL, MOHAMED ABDULLAH MARWI, JOHN JEFFERY, AHMAD FIRDAUS MOHD. SALLEH, WAN OMAR ABDULLAH & BAHARUDIN OMAR

ABSTRACT

The anatomical structures of the first, second and third instars of Chrysomya rufifacies (Macquart) were examined by light microscopy. Observations were documented on the three main characteristics; the cephalopharyngeal skeleton, anterior spiracle and posterior spiracle. The first instar larva bore cornuae of fairly pigmented delineation with slim hypostomal sclerite and distinct dental sclerite. First instar did not have obscured anterior spiracle but posterior spiracles were obscured with thin lining of opened peritreme. Intersegmental spines were evident. The second instar larva displayed a prominent anterodorsal process approaching closer to hypostomal sclerite while upper margin of the dorsal cornua was slightly pigmented. Each anterior spiracle consisted of nine to ten papillae, arranged in a single row. Peritreme of the posterior spiracle thick, opening at the end of peritreme was not wide and confined to two spiracular slits. The third instar larva showed a prominent arch of the ventral cornua with broad and bold appearance. It approached the dorsal cornua and became narrow at the incision median. The anterior spiracle consisted of a single row of nine to ten papillae while intersegmental spine could be identified with one to three dark pigmented tips. A dark pigmented and wide periterime was observed confining three short and thick spiracular slits while button was poorly pigmented. The most distinctive feature of this second and third instar larva was the slender, thorn-like tubercle with numerous spined tips on the middle line segment of the body. These findings provide identification features of C. rufifacies larvae instars.

Key words: Forensic entomology, Chrysomya rufifacies, Larva instar

ABSTRAK

Struktur anatomi larva instar pertama, kedua dan ketiga spesies Chrysomya rufifacies (Macquart) dikaji menggunakan mikroskop cahaya. Pemerhatian ke atas tiga ciri utama; rangka mulut, spirakel anterior dan posterior didokumentasi. Larva instar pertama memiliki struktur kornua berpigmen gelap dan digarisi pigmen terang dengan sklerit hipostomal halus serta sklerit dental yang jelas. Spirakel anterior larva instar pertama tidak kelihatan tetapi mempunyai spirakel posterior yang kabur dan peritrim terbuka dengan dikelilingi garisan halus. Duri intersegmen kelihatan. Larva instar kedua menunjukkan ciri-ciri unjuran anterodorsal yang nyata dan menghampiri sklerit hipostomal sementara bahagian sempadan atas kornua dorsal kurang berpigmen. Setiap spirakel anterior terdiri daripada sembilan hingga sepuluh papilla dan tersusun dalam satu barisan. Peritrim di bahagian posterior tebal dengan bukaan hujung lebih halus serta meliputi dua belahan spirakel. Larva instar ketiga menunjukkan lengkungan yang ketara dan tebal pada kornua ventral serta menghampiri kornua dorsal dan hampir menutupi insisi median. Spirakel anterior terdiri daripada satu barisan sembilan hingga sepuluh papilla sementara duri intersegmen memiliki satu hingga tiga hujung berpigmen gelap. Peritrim gelap dan lebar meliputi tiga belahan spirakel yang pendek dan tebal manakala butang kurang berpigmen. Ciri penting bagi larva instar kedua dan ketiga ialah tuberkel seperti tanduk yang halus dengan puncak yang berduri pada segmen tengah badan larva. Kajian ini menyenaraikan ciri-ciri identifikasi larva instar C. rufifacies.

Kata kunci: Entomologi forensik, Chrysomya rufifacies, Larva instar

INTRODUCTION

Chrysomya rufifacies (Macquart) was the second commonest species of fly larva in Malaysia from the reviewed forensic cases from 1972 to 2002 (Lee et al. 2004). This species was also reported in Australia as carrion-related Dipteran (Smith 1986), Japan (Ishijima 1967), Hawaii (Goff 2000) and Thailand (Sukontason 2001). In Malaysia, this species has a strong preference for populated area (Omar et al. 2003). It is a forensically important Dipteran with diverse habitat, based on recorded forensic specimens submitted to Hospital Kuala Lumpur and Hospital Universiti Kebangsaan Malaysia in 2001 (Hamid et al. 2003). Earlier morphological studies of this larva were comprehensive works of Ishijima (1967), and Liu and Greenberg (1989). However, their works were confined only to the third instar larvae. In a more recent study, *C. rufifacies* was compared to the characteristics of *C. villeneuvi* but the discussion focused on the second and third instars (Sukontason et al. 2005). This study provides the morphological aspects of all the three instars larvae of *C. rufifacies* especially to assist the identification process from the first to second and third instar larvae.

EXPERIMENTAL METHODS

FORENSIC SPECIMENS OF C. RUFIFACIES LARVAE

Forensic larvae of the hairy maggot, *C. rufifacies*, were obtained from Hospital Universiti Kebangsaan Malaysia in 2003.

PREPARATION OF C. RUFIFACIES LARVAE FOR OBSERVATION

Preserved larva in 70% ethanol was washed in distilled water. The posterior end of the larva was cut transversely and soaked into 10% potassium hydroxide for 24 hours. Internal organs of the larva then were removed carefully to avoid damaging the external parts. The larva was rinsed with distilled water and transferred to 10% acetic acid for ten minutes. Specimen was dehydrated by soaking into ascending series of ethanol 30%, 50%, 70%, 90% and absolute ethanol for 30 minutes each. The larva was transferred to clove oil for 30 minutes and cleared in xylene for 30 minutes. The specimen was then mounted on glass slide using Canada balsam and cover slide. The larva was dried in a 40°C incubator for three days. Morphological analysis was performed using a light microscope.

RESULTS AND DISCUSSION

MORPHOLOGY OF THE FIRST INSTAR

Cephalopharyngeal skeleton was located centrally beneath anterior and second segment of the body and layered plainly by a row of dark intersegmental spines (Fig. 1). Lateral observation of the pharyngeal section showed fairly pigmented cornuae. Dorsal cornua slender, approaching the third segment of the body producing a sharp extremity, while ventral cornua was shorter with obscure delineation. These features produce a clear wide opening of median incision. Anteriodorsal process of the cornua was distant from basal piece and pointed minimally upward. Denticle or hook part could be seen pointed ventrally, similarly described by previous study (Liu & Greenberg 1989). Hypostomal sclerite was elongated with darkened and slim characteristics, adjoining the cephalic region and pharyngeal section of the cephalopharyngeal skeleton.

Observation on the first instar larva by light microscopy technique showed absence of anterior spiracle. However, in a similarly description morphology to *C. rufifacies* larva, a prominent presence of anterior spiracle could be seen within the transition period from first to second instar larvae of *C. albiceps* (Wiedemann) (Queiroz et al. 1997). At the posterior end of the larva, a pair of posterior spiracles was observed with two spiracular opening which were bounded by opened and thin peritreme and were slightly pigmented.

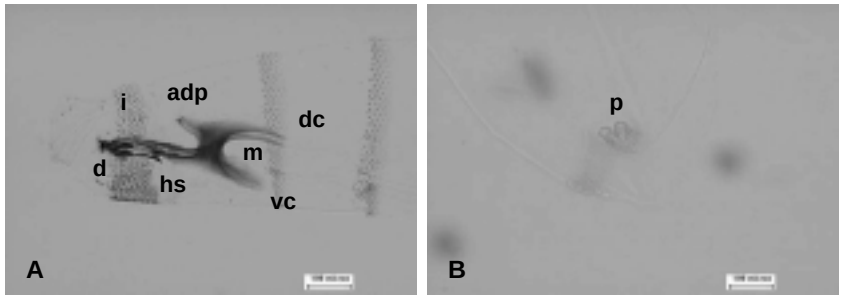

FIGURE 1. First instar of *C. rufifacies* larva. A. Cephalopharyngeal skeleton (×5), intersegmental spine (i), dorsal cornua (dc), ventral cornua (vc), median incision (m), anteriodorsal process (adp), hypostomal sclerite (hs), denticle (d). B. Posterior spiracle (×10), peritreme (p).

MORPHOLOGY OF THE SECOND INSTAR

Cephalopharyngeal skeleton of the second instar larva was darker pigmented (Fig. 2). The hook part was stouter than the first instar of *C. rufifacies*. Beneath the mouth hook, a pair of dental sclerite was observed. The significant characteristics

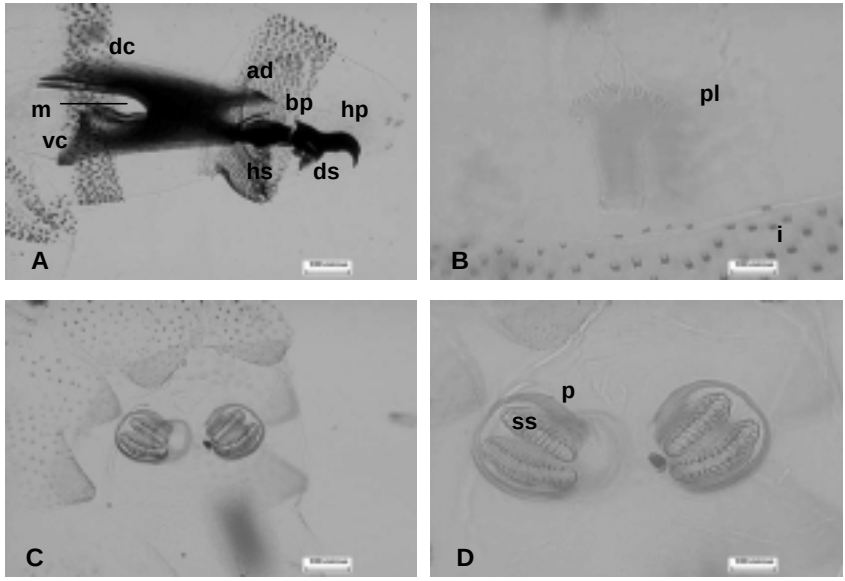

FIGURE 2. Second instar of *C. rufifacies* larva. A. Cephalopharyngeal skeleton ×5. hook part (hp), dental sclerite (ds), hypostomal sclerite (hs), dorsal cornua (dc), ventral cornua (vc), median incision (m), anteriodorsal process (adp), basal piece (bp). B. Anterior spiracle ×10. papillae (pl), intersegmental spine (i) C. Posterior spiracle ×5. D. Posterior spiracle ×10. peritreme (p), spiracular slit (ss).

of the pharyngeal region could be seen by the position of anterodorsal segment approaching closely to hypostomal sclerite. Dorsal cornua was broad, delineated by darkly pigmented inwardly and fairly pigmented outwardly. The lower region of dorsal cornua was heavily pigmented. Ventral cornua was broader, narrowing the incision median and generally shorter than dorsal cornua.

Anterior spiracle of the larvae consists of nine to ten papillae and they were arranged in a single row, a similar finding as observed by Sukontason (2005). Intersegmental spines were unicuspid, bicuspid and tricuspid with heavily pigmented tips.

A pair of posterior spiracles was observed at the centre posterior end of the second instar larva, located beneath the outer dorsal and middle tubercles and consisting of two short and wide spiracular slits. Peritreme end opened, thick and lightly pigmented.

MORPHOLOGY OF THE THIRD INSTAR

Cephalopharyngeal skeleton of third instar larva showed heavily pigmented appearance and located at one-third of the ventral part of the body (Fig. 3). The arch of the ventral cornu was broad and bold in appearance, approaching the dorsal cornua and narrowing the incision median. The lower region of dorsal cornua was heavily pigmented. Body of cephalopharyngeal skeleton anterior to cornua was wide and stout. Anterodorsal process was closely reaching the basal piece. The oral hook consists of dental sclerite and its hypostomal sclerite was short and stout, similar to description by Ishijima (1967). The strong oral hook of the third instar larva supports its cannibalistic and predatory feeding behavior (Sukontason et al. 2001).

Located dorsally to the middle section of the pharyngeal sclerite was the anterior spiracle, consisting a single row of nine to ten papillae. Intersegmental spine could be identified with unicuspid, bicuspid and tricuspid dark pigmented tips.

At the posterior end, a dark pigmented and wide periterime was observed confining three rows of short and wide spiracular slits. Buttons were poorly pigmented.

The most distinctive feature of this second and third instar larva was the thorn-like tubercle with numerous spined tips on the middle line segment of the body. The step of the tubercle was devoid of spine (Figure 3C). A complete description of spinations and tuberculations has been enumerated extensively (Ishijima 1967). Morphology of larvae with hairy appearance other than *C. rufifacies*, the *C. villeneuvi* Patton, has been studied and revealed striking difference of tubercles structure. Tips at the tubercle of *C. rufifacies* were slender with more spines than those were found in *C. villeneuvi* (Sukontason et al. 2005).

This study has provided important information to forensic scientist examining *C. rufifacies* maggot for PMI estimation. More often than not, the stages of maggot received for identification should not only be limited to third instar larva but should also be extended to the first and second instars larvae.

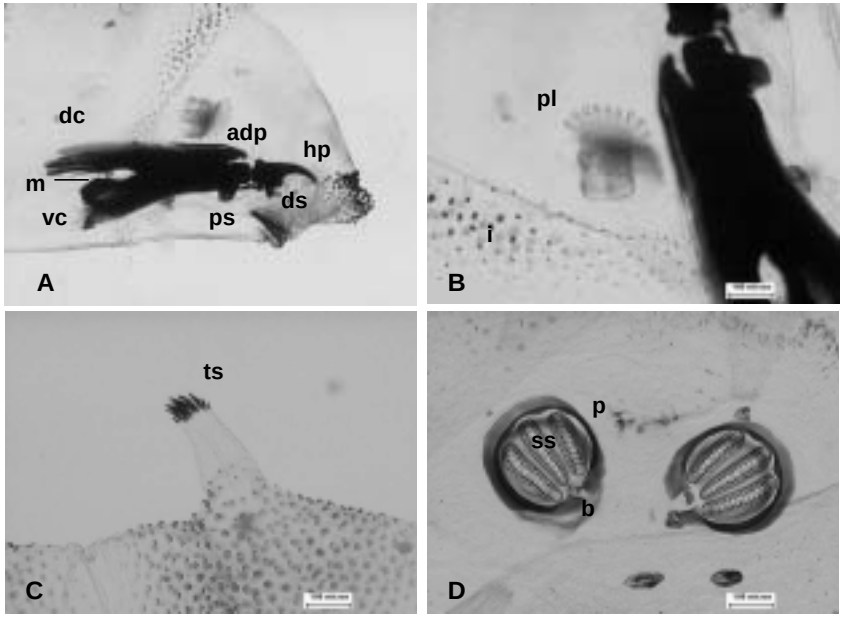

FIGURE 3. Third instar of *C. rufifacies* larva. A. Cephalopharyngeal skeleton ×2.5. hook part (hp), dental sclerite (ds), pharyngeal sclerite (ps), dorsal cornua (dc), ventral cornua (vc), median incision (m), anteriodorsal process (adp). B. Anterior spiracle ×5. papillae (pl), intersegmental spine (i). C. Tubercle ×5, tubercle spine (ts) D. Posterior spiracle ×5. peritreme (p), spiracular slit (ss), button (b).

ACKNOWLEDGEMENT

Authors would like to thank the Department of Parasitology dan Medical Entomology, University Kebangsaan Malaysia Medical Centre and Biomedical Science Department, Faculty of Allied Health Sciences, for providing specimens, laboratory facility and staff support.

REFERENCES

Goff, M. L. 2000. *A fly for the prosecution: How insects evidence helps solve crimes.* Cambridge, Massachusetts: Harvard University Press.

Hamid, N. A., Omar, B., Marwi, M. A., Ahmad, A. M., Mansar, A. H., Siew, S. F. & Mokhtar, N. 2003. A review of forensic specimens sent to forensic entomology laboratory Universiti Kebangsaan Malaysia for the year 2001. *Trop. Biomed.* 20(1):27-31.

Ishijima, H. 1967. Revision of the third stage larvae of synanthropic flies of Japan (Diptera: Anthomyiidae, Muscidae, Calliphoridae and Sarcophagidae). *Jpn. J. Sanit. Zool.* 18(2,3): 47-100.

Lee, H. L., Krishnasamy, M., Abdullah, A. G. & Jeffery, J. 2004. Review of forensically important entomological specimens in the period of 1972-2002. *Trop. Biomed*. 21(2): 69-75 (Supp.).

Liu, D. & Greenberg, B. 1989. Immature stages of some flies of forensic importance. *Ann. Entomol. Soc. Amer.* 82(1): 80-93.

Omar, B., Marwi, M. A., Ahmad, A., Zuha, R. M. & Jeffery, J. 2003. Synanthropic index of flies (Diptera:Muscidae and Calliphoridae) collected at several locations in Kuala Lumpur and Gombak, Malaysia. *Trop. Biomed.* 20(1):77-82.

Queiroz, M. M. C., de Mello, R. P. & Lima, M. M. 1997. Morphological aspects of the larval instars of *Chrysomya albiceps* (Diptera, Calliphoridae) reared in the laboratory. *Mem. Inst. Osw. Cruz.* 92(2): 187-196.

Smith, K.G.V. 1986. *A manual of forensic entomology.* British Museum (National History). NewYork, USA: Cornell University Press.

Sukontason, K., Sukontason, K. L., Piangjai, S., Narongchai, P., Samai, W., Boonchu, N., Sripakdee, D., Ngern-klurn, R. & Siriwattanarungsee, S. 2005. Morphology of second and third instars of *Chrysomya villeneuvi* Patton (Diptera:Calliphoridae), a fly species of forensic importance. *For. Sc. Int.* 154: 195-199.

Sukontason, K. L., Sukontason, K., Narongchai, P., Lertthamnongtham, S., Piangjai, S. & Olson, J. K. 2001. *Chrysomya rufifacies* (Macquart) as a forensically-important fly species in Thailand: A case report. *J. Vect. Ecol.* 26(2): 162-164.

Raja Muhammad Zuha Raja Kamal Bashah
Forensic Science Programme
Faculty of Allied Health Science
Universiti Kebangsaan Malaysia
50300 Jalan Raja Muda Abdul Aziz
Kuala Lumpur, Malaysia

Wan Omar Abdullah
Faculty of Medicine and
Health Sciences,
Universiti Putra Malaysia,
43400 Serdang, Selangor Darul Ehsan
Malaysia

Mohamed Abdullah Marwi
John Jeffery
Ahmad Firdaus Mohd. Salleh
Department of Parasitology and
Medical Entomology
Faculty of Medicine
Universiti Kebangsaan Malaysia
50300, Jalan Raja Muda Abdul Aziz
Kuala Lumpur, Malaysia

Baharudin Omar
Department of Biomedical Science
Faculty of Allied Health Science
Universiti Kebangsaan Malaysia
50300, Jalan Raja Muda Abdul Aziz
Kuala Lumpur, Malaysia

Corresponding author: Raja Muhammad Zuha Raja Kamal Bashah
Email address: raja_mzuha@hotmail.com
Tel: 603-26923132; Fax: 603-26923132

Received: September 2007
Accepted for publication: May 2008