Morphology of second and third instars of *Chrysomya villeneuvi* Patton (Diptera: Calliphoridae), a fly species of forensic importance

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Abstract

The morphology of the second and third instars of *Chrysomya villeneuvi* Patton, a fly species of forensic importance, was presented by use of light microscopy. Both instars were of hairy appearance, bearing elongated tubercles along the abdominal and caudal segments. The anterior spiracle had 13–15 papillae. Minute dark spots were observed to thoroughly cover the tubercle’s surface, with 4–6 strong dark tips. Regarding the third instar, the intersegmental spines between the prothorax and mesothorax were heavily pigmented. The posterior spiracle had a thick and heavily pigmented incomplete peritreme. The surface and tip of the tubercles was covered with heavily pigmented sharp spines. The integument of the body was covered with numerous distinct net-like patches. A comparison with another well-known hairy maggot, *Chrysomya rufifacies* (Macquart), was discussed.

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1. Introduction

*Chrysomya villeneuvi* Patton is a forensically important blowfly species because its larvae have been found in human corpses [1,2]. Morphologically, the larvae were of hairy appearance, bearing conspicuous tubercles along the body segments. Although its third instar is generally similar to another well-known hairy maggot in the Oriental region [*Chrysomya rufifacies* (Macquart)], the tubercles along their body are distinctive [2]. Since anatomical studies on immature stages of *C. villeneuvi* are minimal particularly in the larvae, we herein describe additional features of the second and third instars, while highlighting the most important diagnostic features to identify this species. This is the principal requirement before the species is used for subsequent forensic analysis.

2. Materials and methods

The second and third instars of *C. villeneuvi* were obtained from two unknown human male remains that were transferred for investigation to the Department of Forensic
Medicine, Chiang Mai University, Thailand, on 4 February 2004. These remains were discovered in the forested area of Muang Pan District, Lampang province, northern Thailand. During examination, numerous fly larvae (maggots) were found under their heads and bodies. Some larval specimens were collected and killed by transferring them into a beaker containing near boiling water for a few minutes. The dead larvae were cut using a sharp blade at two sites to obtain three body portions (Fig. 1). Each part was mounted by transferring them onto a clean glass slide after a few drops of Entellan (Merck: Germany) had been placed on it. A cover slip was placed over the specimens and an examination for morphological characters was made under a light microscope (Olympus , Japan) equipped with a calibrated eye piece micrometer. The images were recorded with a digital camera (Olympus Camedia, C-4040Zoom , Japan). Moreover, some larval specimens were reared to confirm the species of Chrysomya villeneuvi in the laboratory at the Department of Parasitology, Faculty of Medicine, Chiang Mai University, using pork liver as food source.

3. Results

The second instar of Chrysomya villeneuvi was muscoid-shaped, with the cephalic region being tapered anteriorly (Fig. 1). It was hairy in appearance, bearing prominent tubercles encircling the abdominal and caudal segments. While focusing on the anterior region, a dark, heavily pigmented cephalopharyngeal skeleton was located internally (Fig. 1). The fan-shaped anterior spiracle was located at the posterior margin of the prothorax (Fig. 1), appearing in a single row with 13–15 marginal papillae (Fig. 2). The intersegmental spines located between prothorax and mesothorax (Figs. 1 and 3) were fairly round at the base, and bore 1–4 heavily pigmented ends. At the posterior end of the body, a pair of posterior spiracles was located centrally at the hairy caudal segment (Fig. 4). An incomplete peritreme, with wide ends, was observed encircling two separated spiracular slits (Fig. 4).

When highlighting the elongated tubercles along the body segments, minute dark spots were observed to thoroughly cover the surface in rows, with 4–6 strong black tips existing at the apex (Fig. 5). In order to compare with the second instar of Chrysomya rufifacies (laboratory strain) clearly, the tubercle apex of this species was also shown. The inset in Fig. 5 displays the tubercle apex of Chrysomya rufifacies, which had three rows of many minute dark tips. As observed in some specimens of Chrysomya villeneuvi, the cuticle of third instar, which contained broad spines thoroughly covering the tubercle, was detected beneath the cuticle of the second instar during the molting process (Fig. 6).

Regarding the third instar of Chrysomya villeneuvi, the hairy body still existed, but some structures were found to be different. The anterior spiracle had 13–15 marginal papillae. The intersegmental spines between the prothorax and mesothorax were much more heavily pigmented, particularly at their ends. The shape of each spine was slender, with most of them entirely pigmented (Fig. 7). At the caudal segment, elongated tubercles were distinct and regularly distributed (Fig. 8). The posterior spiracle bore three wide slits, with the incomplete peritreme being thick and heavily pigmented (Fig. 9). The ends of the peritreme, which had minute projections, were close together and they encircled the poorly pigmented button (Fig. 9). The surface and tip of the tubercles of the third instar were covered with heavily pigmented sharp spines, which had longer tips (Fig. 10). The integument of the body was covered with numerous distinct net-like patches, with each patch comprising a single large denticle located centrally (Fig. 11). A comparison of the distinctive features to differentiate the second and third instars of Chrysomya villeneuvi and Chrysomya rufifacies is shown in Table 1.

4. Discussion

The morphology of Chrysomya villeneuvi, particularly at the larval stage, has been studied by a few authors [1,2]. This
study indicated that the second and third instars of this fly species were of hairy appearance, but each one held some difference in structures. This phenomenon was found in the second and third instars of *C. rufifacies* previously described [4]. In comparison between these two species on the second instar, the crowns or tips at the tubercle apex were strikingly distinctive; those of *C. villeneuvi* were stout with fewer spines (see Fig. 5), but those of *C. rufifacies* were slender.
with more spines [4]. In addition, a number of papillae at the anterior spiracle had distinctive features. The anterior spiracle of *C. villeneuvi* bore 13–15 papillae (which correlated with Greenberg and Kunich [1]), whereas 9–12 papillae were found in *C. rufifacies* [1,4–6].

Comparing the third instar of *C. villeneuvi* with that of *C. rufifacies*, the ornament of tubercles was found to have distinctive features, with the former bearing sharp-end spines that encircled the entire tubercle (see Fig. 10). However, the latter bore rounded-knobs around the lower half of
each tubercle [4]. Since the number of papillae at the anterior spiracle of the third instar was similar to that of the second instar, it was a distinctive feature.

In addition to the different morphological features previously described, the third instar of *C. villeneuvi* and *C. ruffiacies* shared some features that could not be differentiated. For example, the surface integument architecture of distinct net-like patches (see Fig. 11), which corresponded to those described in *C. ruffiacies* by means of scanning electron microscope [4], or those in another hairy maggot, *Chrysomya albiceps* (Wiedemann) [7]. The morphology of posterior spiracles, having an incomplete and a heavily pigmented peritreme (see Fig. 9), resembled *C. ruffiacies* studied by light microscopy [5,8]. We observed *C. villeneuvi* larvae in the laboratory having the aggressive feeding in both the second and third instars, which correlated with the previous report on this species [9], *C. ruffiacies* [10,11] or *C. albiceps* [7].

*C. villeneuvi* was collected geographically in many Asian countries [China (Yunnan, Hainan Is.), Vietnam, Laos, Thailand, Malaysia, Indonesia (Sumatra), Nepal, India, and Sri Lanka], which overlap in territories where *C. ruffiacies* could be found (Oriental, Australasian regions) [9]. Thus, the result presented herein enables identification of these two hairy maggot species, found within these geographical areas.

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References