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Research Note

Morphological descriptions of second and third instar larvae of *Hypopygiopsis violacea* Macquart (Diptera: Calliphoridae), a forensically important fly in Malaysia

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Abstract. *Hypopygiopsis violacea*, a species of fly of forensic importance, was recovered from a corpse and described for the first time. The morphological structures of the second and third instar larvae of four specimens were examined using light microscope. Observations were focused on three main morphological characters: cephalopharyngeal skeleton, anterior and posterior spiracles. Cephalopharyngeal skeleton of second instar larva is darkly pigmented and without accessory sclerite below the mouth hook. The anterior spiracles of second and third instar larvae have 8-9 papillae each, arranged in a single row. The posterior spiracle of second instar larva has two spiracular slits with no thickening of peritreme. This differentiates it from the third instar, whereby the latter has three slits for each posterior spiracle. Cephalopharyngeal skeleton of third instar larva is heavily pigmented. An accessory sclerite is found below the hook part of third instar larva but is absent in second instar. Peritreme of the posterior spiracle of third instar larva is thick almost complete encircling a button. The intersegmental spines of the cuticular surface are dome-shaped and unicuspid. Third instar larva of this species is large with size approximately 15 mm long. These findings provide important identification features of immature stages of *Hy. violacea* which could be useful in forensic entomology.

Hypopygiopsis violacea is a forensically important calliphorid since this species was reported as the first fly to arrive at monkey carcasses (Omar *et al*., 1994; Dhang, 2008). However, to date, the anatomical features of immature stages of these larvae have not been described. Morphologically, the larvae are large (approximately 15 mm) and non-hairy (non-tuberculated), but has numerous dome-shaped and unicuspid spines on the intersegmental body surface. Grossly it appears similar to that of *Chrysomya megacephala*, *Chrysomya pinguis* and *Hemipyrellia ligguriens* larvae except for its larger size. This paper describes for the first time the important diagnostic features of second and third instar larvae for the purpose of species identification during forensic investigations.

The second and third instar larvae of *Hy. violacea* were obtained from a corpse in Johor Bharu, Malaysia. The corpse was found lying on a ground in palm oil estate. The preserved larvae were sent to the Forensic Unit, Department of Parasitology and Medical Entomology PPUKM for Post

Mortem Interval analysis. The sub-posterior end of the preserved larvae was cut transversely and soaked in 10% potassium hydroxide overnight. Internal body contents of the larvae were then removed carefully to avoid damaging the external parts. The larvae were rinsed several times with distilled water and transferred into 10% acetic acid for seven minutes. Larvae were then dehydrated by soaking into ascending series of 80%, 85%, 90%, 95% and absolute alcohol solution for 30 minutes each. Larvae were then transferred into clove oil for 30 minutes and cleared in xylene for another 30 minutes. They were mounted onto glass slide with a few drops of Canada Balsam (BDH) and subsequently kept in an incubator at 40°C to dry for three days. An examination for morphological characters was then performed using light microscope (Leica®). The images were captured with a Microscope Image Analyser (Leica® Application EZ4D).

Cephalopharyngeal skeleton of the second instar larva is darkly pigmented (Fig. 1A). A pair of dental sclerite is observed posterior to the mouth hook. The hook part of this instar has no accessory sclerite. A pair of fan-shaped anterior spiracles are located at the posterior margin of the second segment and each has be a single row of eight papillae (Fig. 1B). The papillae are long and thin. A pair of posterior spiracles are located at the posterior end of the body. The posterior spiracle of second intar larvae has an incomplete and pale peritreme. Each spiracle encircles two short spiracular slits (Fig. 1C). Intersegmental spines are unicuspid. The lengths of the second instar larvae ranged from 4.25 mm to 4.65 mm.

The third instar larva, which is approximately 15mm in length, has some

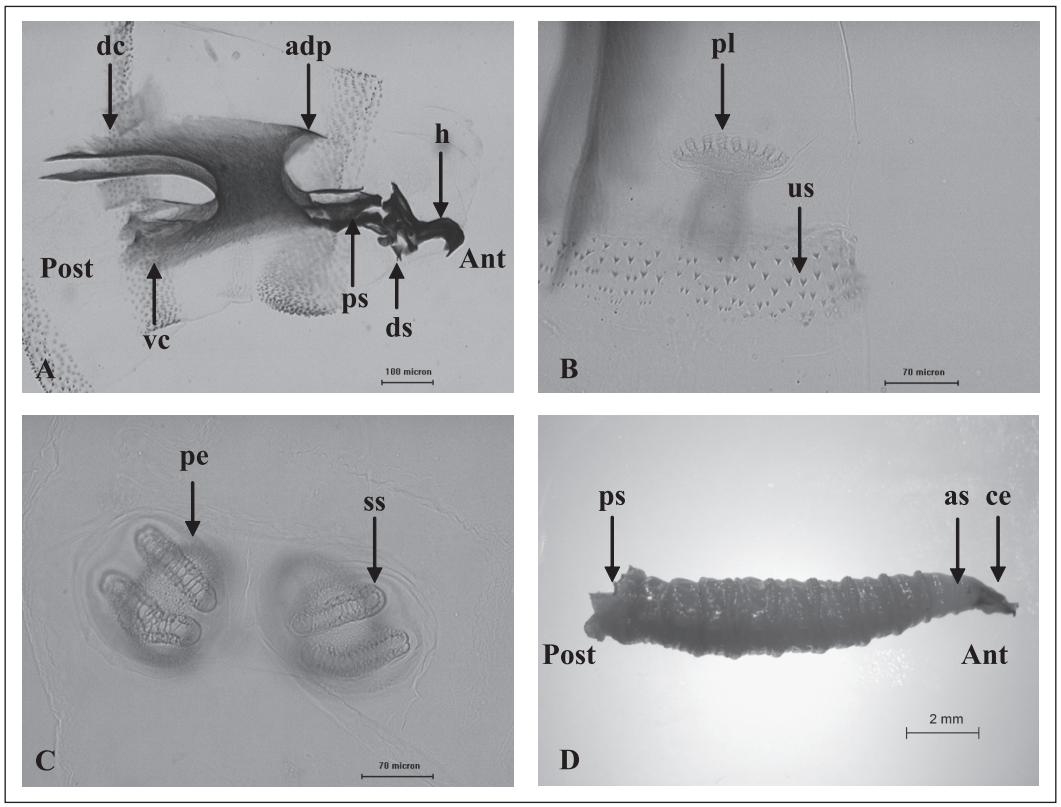

Figure 1. Second instar larva of *Hy. violacea*. A. Cephalopharyngeal skeleton x5. Hook (h), dorsal cornua (dc), dental sclerite (ds), ventral cornua (vc), pharyngeal sclerite (ps), anteriodorsal process (adp), anterior (Ant), posterior (Post). B. Anterior spiracle x10. Papillae (pl), unicuspid spine (us) C. Posterior spiracle x10. Peritreme (pe), spiracular slit (ss). D. Gross specimen x8. Cephalopharyngeal skeleton (ce), anterior spiracle (as), posterior spiracle (ps).

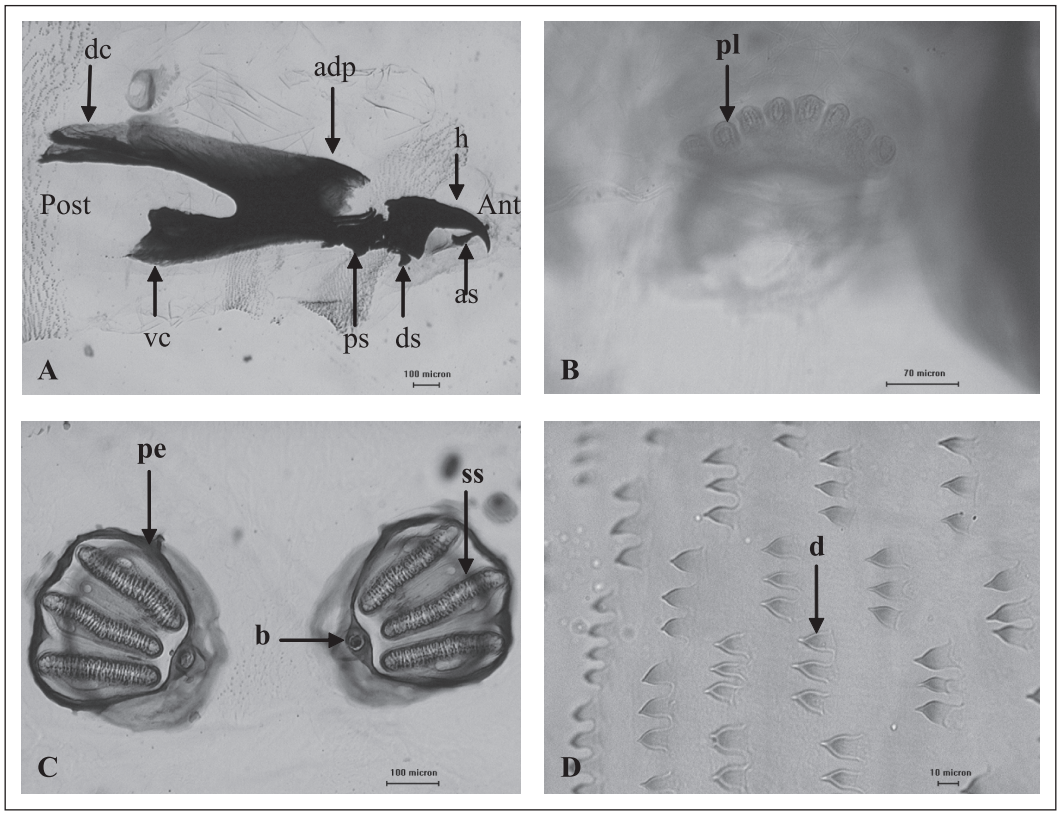

Figure 2. Third instar of *Hy. violacea*. A. Cephalopharyngeal skeleton x2.5. hook (h), accessory sclerite (as), dorsal cornua (dc), dental sclerire (ds), ventral cornua (vc), pharyngeal sclerite (ps) anteriodorsal process (adp), anterior (Ant), posterior (Post). B. Anterior spiracle x10. Papillae (pl). C. Posterior spiracle x5. Peritreme (pe), spiracular slit (ss), button (b). D. Spine x20. Dome-shape spine (d).

structures which are unique to the stage. Cephalopharyngeal skeleton is heavily pigmented (Fig. 2A). A comma-shaped accessory sclerite is found below the hook of this third instar larva. The lower region of both the dorsal and the ventral cornuae are heavily pigmented. The anterior spiracle has eight marginal papillae which are more robust than the second instar larvae (Fig. 2B). As for the posterior spiracle, it is well developed and has a highly pigmented peritreme (Fig. 2C). The peritreme encircles three rows of short cigar-shape spiracular slits. At the posterior tip of the slits is a button-like feature. The integuments of the intersegmental part of the body are covered by numerous dome-shape and unicuspid spines. The spines are highly pigmented (Fig. 2D). A comparison of the distinctive features of the second and third instar larvae of *Hy. violacea* is shown in Table 1.

Generally the third instar larva of calliphorids shared some similar features, but at a careful observation of the cephalopharyngeal sclerites, anterior spiracles, posterior spiracles and intersegmental spines as described above differentiate *Hy. violacea* larvae from other species (Table 2).

Note: The specimens examined above were confirmed as *Hy. violacea* based on reference specimens that were reared earlier from eggs to adult stage in our laboratory. The eggs were obtained from a gravid of *Hy. violacea* caught in Universiti Malaya Forest Reserve in Kuala Lumpur by the fifth author.

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Table 1. Comparison of distinctive features of the second and third instar larvae of *Hy. violacea*

Character	Second instar	Third instar
Accessory sclerite of cephalopharyngeal sclerite	Absent	Present
Intersegmental spines	Weakly pigmented, unicuspid, pointed end	Highly pigmented, unicuspid, dome-shaped, pointed end
Button of posterior spiracle	Absent	Present
Peritreme of posterior spiracle	Weakly pigmented, incomplete peritreme	Highly pigmented, complete peritreme

Table 2. Characteristic features differentiating third instar larvae of *Hy. violacea*, *Hemipyrellia ligurriens* and *Lucilia cuprina*

Characters	*Hy. violacea*	*Hemipyrellia ligurriens*	*Lucilia cuprina*
Length	15 mm	13 mm	13 mm
Anterior spiracle	8-9 papillae	6-9 papillae	5-7 papillae
Accessory sclerite	Present	Present	Absent
Intersegmental spines	Unicuspid, dome-shaped, pointed end	Unicuspid, pointed end	Unicuspid, pointed end
Posterior spiracle	Big, heavily pigmented withinter-slit projection	Small, heavily pigmented with inter-slit projection	Very small, peritremefine without inter-slit projection
Button of posterior spiracle	Present and distinct	Present and distinct	Present and projected inward of close peritreme
Posterior spiracle slit	Long and thin	Narrow and thin	Wide and thin

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