



---

## **Intra-Puparial Development of the Black Soldier-fly, *Hermetia illucens***

Authors: Barros-Cordeiro, Karine Brenda, Bão, Sônia Nair, and Pujol-Luz, José Roberto

Source: Journal of Insect Science, 14(83) : 1-10

Published By: Entomological Society of America

URL: <https://doi.org/10.1673/031.014.83>



## Intra-puparial development of the black soldier-fly, *Hermetia illucens*

Karine Brenda Barros-Cordeiro<sup>1,2a</sup>, Sônia Nair Bão<sup>1b</sup>, and José Roberto Pujol-Luz<sup>2c\*</sup>

<sup>1</sup>Laboratório de Microscopia Eletrônica, Departamento de Biologia Celular, Instituto de Ciências Biológicas, Universidade de Brasília, 70910-900, DF, Brazil

<sup>2</sup>Laboratório de Entomologia Forense, Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade de Brasília, 70910-900, DF, Brazil

### Abstract

The intra-puparial development of the black soldier-fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), was studied based on 125 pupae under controlled conditions in laboratory. The 6<sup>th</sup> instar larvae were reared until they stopped feeding, and the pre-pupae were separated according to the reduction in larval length and degree of pigmentation and sclerotization of the cuticle. The pupal stage lasted eight days (192 hours). The process of pupation (larva/pupa apolysis) occurred in the first six hours, extroversion of the head and thoracic appendages took place between the ninth and 21<sup>st</sup> hours, and the pharate appeared 21 hours after completing pupation. After pupariation, four morphological phases of intra-puparial development were observed and described.

**Keywords:** forensic entomology, metamorphosis, morphology, Stratiomyoidea

**Correspondence:** <sup>a</sup>[kbbc22@yahoo.com.br](mailto:kbbc22@yahoo.com.br), <sup>b</sup>[snbao@unb.br](mailto:snbao@unb.br), <sup>c</sup>[jrpujol@unb.br](mailto:jrpujol@unb.br), \*Corresponding author

**Editor:** Henry Hagedorn was editor of this paper.

**Received:** 29 August 2012 **Accepted:** 6 June 2013 **Published:** 7 July 2014

**Copyright:** This is an open access paper. We use the Creative Commons Attribution 3.0 license that permits unrestricted use, provided that the paper is properly attributed.

**ISSN:** 1536-2442 | Vol. 14, Number 83

#### Cite this paper as:

Barros-Cordeiro KB, Bão SN, Pujol-Luz JR. 2014. Intra-puparial development of the black soldier-fly, *Hermetia illucens*. *Journal of Insect Science* 14(83). Available online: <http://www.insectscience.org/14.83>

## Introduction

The Hermetiinae soldier-flies represent a relatively homogeneous group of Stratiomyidae consisting of five genera, *Chaetohermetia* (2 spp., neotropical); *Chaetosargus* (4 spp., neotropical); *Hermetia* (76 spp., cosmopolitan), *Nothohermetia* (1 sp., Australia), and *Patagiomysia* (1 sp., neotropical). The genus *Hermetia* Latreille has 76 species, 39 of them occurring in the Neotropical region, 20 with distribution including Brazil. Only four species have known larvae: *Hermetia albitarsis* (Brazil), *H. aurata* (Mexico), *H. concinna* (Mexico), and *H. illucens* (cosmopolitan) (McFadden 1967; Woodley 2001).

The black soldier fly, *H. illucens* (L.) (Diptera: Stratiomyidae), is economically important because its larvae feed on and are involved with cycling organic matter (Lardé 1990) and also inhibit and control the oviposition and development of *Musca domestica* in manure management systems (Sheppard 1983; Bradley and Sheppard 1984). There are also concerns regarding this species because of its association with cases of enteric myiasis in humans and other animals (Adler and Brancato 1995; Manrique-Saide et al. 1999; Calderón-Arguedas et al. 2005). In addition, records of the larvae and pupae of *H. illucens* occurring in human carcasses indicate that this species is also important in studies of forensic entomology, and that its development can be used to estimate post-mortem interval (Catts and Haskell 1990; Lord et al. 1994; Turchetto et al. 2001; Tomberlim et al. 2004; Pujol-Luz et al. 2008).

Most of the studies that have investigated the events of metamorphosis in Diptera described only the pupariation process, which ends in the formation of the puparium. The morphological changes that occur during intra-

pupal development have been extensively studied in Muscoidea and Oestroidea (e.g., Wolfe 1954; Bennett 1962; Fraenkel and Bhaskaran 1973; Lello et al. 1985; Scholl and Weintraub 1988; Cepeda-Papacios and Scholl 2000; Colwell et al. 2006), while intrapupal development in Stratiomyoidea has never been investigated. In this paper we describe some events in *H. illucens* development, including the chronology and morphological changes observed during intrapupal development, under controlled laboratory conditions.

## Materials and Methods

Two hundred 6<sup>th</sup> instar larvae (L6) of *H. illucens* were reared and observed in a BOD incubator chamber ( $27 \pm 1.0^\circ\text{C}$ ,  $60 \pm 10\%$  RH, 12:12 L:D) until they ceased feeding, which marks the onset of the pupariation process. The pre-pupae were separated and placed in plastic containers with vermiculite. During the first 48 hours, five pupae were fixed every three hours; after this period, during the next six days, this process was repeated every 16 hours until the emergence of the adults. A total of 125 pupae were dissected in the experiment. All specimens were fixed in Carnoy's solution (48 hours), then in formic acid (5%) for another 48 hours; after that, they were transferred for permanent preservation in ethanol 70. The adults that emerged ( $n = 75$ ) were fixed at low temperature ( $-20^\circ\text{C}$ ) and maintained in ethanol (70%) with the pupae. The minimum time intervals for each event (pre-pupae, pupae) were recorded. Ten pupae were cleaned in hot lactic acid; this process permits the observation of respiratory system structures that are useful. The specimens were dissected and photographed with Leica DM2000® ([www.leica-microsystems.com](http://www.leica-microsystems.com)) and Zeiss AxioCam®MRc ([www.zeiss.com](http://www.zeiss.com)) microscopes.

The terminology and concepts used to describe the processes of pupariation and pupation and the puparium morphology were adapted from Fraenkel and Bhaskaran (1973), Costa and Vanin (1985), Cepeda-Palacios and Scholl (2000), and are defined as follows:

- (i) Pupariation: period between the time that the larvae cease feeding to complete immobilization and reduction in length of the larvae. A reduction in their mobility and a retraction of the segments gradually occur. The cuticle becomes progressively more opaque, pigmented and sclerotized.
- (ii) Pupation or intra-pupal development: all events that occur from larval-pupal apolysis until the emergence of the adult fly.
- (iii) Larva-pupa apolysis: once the pupariation process has finished, larval-pupal apolysis takes place, resulting in the formation of the adult epidermis and its separation from the last larval skin, which will form the puparium.
- (iv) Cryptocephalic pupa: a phase also known as hidden head; in this phase it is impossible to distinguish the head and the thoracic appendages externally; the imaginal discs of the appendages and the cephalic vesicle (cerebral and cephalic ganglia) are located below the thoracic and abdominal segment.

- (v) Phanerocephalic: in this phase there is the extroversion of the cephalic capsule and the thoracic appendages. This phase also marks the beginning of the apolysis process between pupa and adult.
- (vi) Pharate adult: the longest phase of intrapupal development, corresponding to the maturation of the adult.
- (vii) Imago: the final form of the insect after metamorphosis.

Results

Pupariation

The larvae of *H. illucens* remained in the vermiculite substrate between two and 15 days and buried in the substrate in scotophase, when there was a reduction in their mobility. The pupa was 1/3 the size of the puparium and, because of the reduction in the tissue in the anterior part of the puparium, there was a change in eye color from reddish to white or transparent; the abdomen also folded 45° to the ventral region, and the cuticle gradually became opaque and sclerotized (Figure 1d, e).

Larval-pupal apolysi

The process was observed in a dorsal-ventral direction and from the end of the abdomen to the head. The apolysis duration was  $4.8 \pm 1.1$  hours, being completed in a minimum time of six hours (Table 1).

Table 1. Intra-puparial development of *Hermetia illucens*.

Stage	Event	Time of development (hour)	Duration minimum (hours)	Sample size (n)
		Mean ± SE (Range)		
Pupa	Larva-pupa apolysis	4.8 ± 1.1 (06*-12)	6	18
	Criptocephalic pupa	15.3 ± 1.0 (6-18)	3	12
	Phanerocephalic pupa	14.3 ± 2.1 (9-21)	12	9
	Pharate			
	Yellowish eyes	40.3 ± 1.9 (21-80)	43	53
	Pinkish eyes	92.0 ± 6.6 (64-128)	32	8
	Reddish eyes	139.2 ± 7.0 (96-176)	48	10
	Brownish eyes	171.7 ± 4.7 (144-192)	48	15

\*The apolysis was complete.

**Cryptocephalic pupa (Figure 1a)**

Formation of a hardened, opaque, and pigmented puparium; this phase retained almost all the features of L6. The mandibular-maxillary complex was separated from the larva and the pupa and stayed attached to the puparium internal wall. The duration of this event was  $15.3 \pm 1.0$  hours, being completed in a minimum time of three hours, and ended with start of the extroversion of the head and thoracic appendages (Table 1).

**Phanerocephalic pupa**

Characterized by the extroversion and distinctness of the head, thorax, and abdomen of the pharate adult (Figure 1b). This process marked the pupa-adult apolysis, with a duration of  $14.3 \pm 2.1$  hours, being completed in a minimum time of 12 hours (Table 1).

**Pharate adult (Figure 1b)**

The longest phase of intra-pupal development, which can be divided into four stages according to the color of the eyes; it represents maturation of the adult (adapted from Cepeda-Palacios and Scholl 2000): (i) yellowish eyes, 21<sup>st</sup>–64<sup>th</sup> hour, with duration this period being  $40.3 \pm 1.9$  hours (Figure 2a; Table 1), followed by the definition of head, thorax, abdomen, legs and wings; (ii) pinkish eyes, 64<sup>th</sup>–96<sup>th</sup> hour, a duration of  $92.0 \pm 6.6$  hours (Figure 2b; Table 1), and the sutures of the thorax and abdomen in dorsal view were observed; (iii) reddish eyes, 96<sup>th</sup>–144<sup>th</sup> hour, a duration of  $139.2 \pm 7.0$  hours (Figure 2c; Table 1), the T-shaped dorsal thoracic suture in the puparium was observed, and the pharate showed a well-developed antennae and the beginning of the pigmentation of hair, bristles, legs, and wing veins; (iv) dark brownish eyes, 144<sup>th</sup>–192<sup>nd</sup> hours, a duration of  $171.7 \pm 4.7$  hours (Figure 2d; Table 1), the body of adult was totally formed and fully pigmented.

**Imago and emergence of the adults**

The completely formed imagoes (Figure 1c) were observed in the 144<sup>th</sup> hour, and the adults emerged from the 192<sup>nd</sup> hour (Table 1).

**Respiratory system**

In the pupal stage, the external breathing tubes (pupal-horn) appeared (Figure 3a–c). A pair of developed internal tubes was present in the first thoracic segment, and another five pairs were distributed in the abdomen segments 2 to 7. Inside the puparium, the tubes became narrowed at the distal end and were prolonged beyond the outer edge, where the ends (Figure 3a) presented two different forms: the first was horn-like (Figures 3b–d), 2<sup>nd</sup> to 5<sup>th</sup> abdominal segment, and the second had a crown-like shape (Figure 3e), 6<sup>th</sup> and 7<sup>th</sup> abdominal segments.

**Discussion**

There are a few morphological studies relating to intra-pupal development, and in some of them (Irwins-Smith 1920; May 1961; Rozkošný 1982) the subject was treated without the necessary level of detail. Most authors use the puparia to describe the last instar larvae of Stratiomyidae (Rozkošný and Kovac 1998, 2001; Pujol-Luz and Leite 2001; Xerez and Pujol-Luz 2001; Xerez et al. 2002, 2003). In Stratiomyidae and Xylomyidae, the pupa is formed within the last larval skin, which is used as a hard cocoon (puparia) impregnated with plates or calcium carbonate crystals ( $\text{CaCO}_3$ ) (Woodley 1989). The total time of post-embryonic development in soldier-flies (larva to adult) is variable, lasting for a few weeks to several months in Atlantic Rain Forest and Cerrado biomes (J.R. Pujol-Luz, personal observation). In controlled laboratory conditions, the total duration of the period between the pupal stage and adult emergence of *H. illucens* in this work lasted 192 hours, and during

this time we identified four distinct phases or stages: (i) larval-pupal apolysis, (ii) cryptocephalic pupae, (iii) phanerocephalic pupae, and (iv) pharate adult (Table 1).

Apart from the morphological changes associated with the change in eye color, we highlighted some modifications in the structure and pattern of the respiratory system. The respiratory system (Figure 3) of the pupa of *H. illucens* is usually described as amphipneustic, despite the presence of the vestigial breathing tubes in the 2<sup>nd</sup> to 5<sup>th</sup> abdominal segment (Rozkošný 1982; Rozkošný and Kovac 2001). However, we can provide a different interpretation based on the findings of this study. The tissular projection of the tracheal system in pharate adults forms a tube that is in contact with the internal pupal wall (Figure 3a), and that has slits opening out at the extremity of the pupal horn (Figures 3b–e), suggesting that the spiracles are indeed functional. Thus, the respiratory system should be considered hemipneustic.

## Acknowledgments

We thank Hélio Ricardo da Silva (UFRRJ), Cecília Kosmann (UnB), and João Victor Luz (Cultura Inglesa) for reading early versions of the manuscript and making helpful comments and suggestions. Érica S. Harterreiten-Souza (Embrapa-CENARGEN) and Khesller P.O. Name (UnB) kindly assisted in various stages of preparation of the manuscript. During the development of this research, we received grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Apoio à Pesquisa do Distrito Federal (FAP-DF).

## References

- Adler AI, Brancato FP. 1995. Human furuncular Myiasis caused by *Hermetia illucens* (Diptera: Stratiomyidae). *Journal Medicine Entomology* 32: 745-746.
- Bennett GF. 1962. On the biology of *Cephenemya phobifera* (Diptera: Oestridae), the pharyngeal bot of the white-tailed deer, *Odocoileus virginianus*. *Canadian Journal of Zoology* 40: 1195-1210.
- Bradley SW, Sheppard DC. 1984. House fly oviposition inhibition by larvae of *Hermetia illucens*, the black soldier fly. *Journal of Chemical Ecology* 10: 853-859.
- Calderón-Arguedas O, Barrantes JM, Solano ME. 2005. Miasis entérica por *Hermetia illucens* (Diptera: Stratiomyidae) em uma paciente geriátrica de Costa Rica. *Parasitol Latinoamerica* 60: 162-164.
- Catts EP, Heskell NH. 1990. *Entomology and death: A procedural guide*. Joyce's Print Shop.
- Cepeda- Palacios R, Scholl PJ. 2000. Intra-Puparial Development in *Oestrus ovis* (Diptera: Oestridae). *Journal Medicine Entomology* 37: 239-245.
- Colwell DD, Hall MJR, Scholl PJ. 2006. *The Oestrid Flies: Biology, Host-Parasite Relationships, Impact and Management*. CAB International.
- Costa C, Vanin SA. 1985. On the concepts of “pre-pupa”, with special reference to the Coleoptera. *Revista Brasileira de Zoologia* 2: 339-345.



- Fraenkel G, Bhaskaran G. 1973. Pupariation and pupation in cyclorhaphous flies (Diptera): terminology and interpretation. *Annals of the Entomological Society of America* 66: 418-422.
- Irwin-Smith V. 1920. Studies in Life-Histories of Australian Diptera Brachycera. *Proceedings of the Linnean Society of North South Wales* 45: 505-530.
- Lardé G. 1990. Recycling of coffee pulp by *Hermetia illucens* (Diptera: Stratiomyidae) larvae. *Biological Waster* 33: 307-310.
- Lello ED, Gregório EA, Toledo LA. 1985. Desenvolvimento das gônadas de *Dermatobia hominis* (Diptera: Cuterebridae). *Memórias do Instituto Oswaldo Cruz* 80: 159-170.
- Lord WD, Goff ML, Adkins TR, Haskell NH. 1994. The black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) as a potential measure of human postmortem interval: observations and case histories. *Journal of Forensic Sciences* 39: 215-222.
- May BM. 1961. The occurrence in New Zealand and the life-history of the soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). *New Zealand Journal Science* 4: 55-65.
- McFadden MW. 1967. Soldier fly larvae in America north of Mexico. *Proceedings of the United States National Museum* 12:1-72.
- Manrique-Saide P, Rodríguez-Vivas RI, Rodríguez MQ, Aparicio RQ. 1999. Um caso de pseudomiasis por larvas de *Hermetia illucens* (Diptera: Stratiomyidae) em um bovino. *Revista Biomedicine* 10: 173-176.
- Pujol-Luz JR, Leite FM. 2001. Descrição do último ínstar larval e do pupário de *Ptecticus testaceus* (Fabricius) (Diptera: Stratiomyidae). *Neotropical Entomology* 30: 587-591.
- Pujol-Luz JR, Francez PAC, Ururahy-Rodrigues A, Constantino R. 2008. The Black Soldier-fly, *Hermetia illucens* (Diptera, Stratiomyidae), Used to Estimate the Postmortem Interval in Case in Amapá state, Brazil. *Journal Forensic Science* 53: 476-478.
- Rozkošný R. 1982. *A Biosystematic Study of the European Stratiomyidae (Diptera)*, volume 25. W. Junk.
- Rozkošný R, Kovac D. 1998. Descriptions of bamboo-inhabiting larvae and puparia of Oriental soldier flies *Ptecticus brunettii* and *P. flavifemoratus* (Diptera: Stratiomyidae: Sarginae) with observations on their biology. *European Journal of Entomology* 95: 65-86.
- Rozkošný R, Kovac D. 2001. New male, larva and puparium of *Odontomyia pulcherrima* Brunetti (Insecta: Diptera: Stratiomyidae) from the Oriental Region. *Raffles Bulletin of Zoology* 49: 101-108.
- Scholl PJ, Weintraub J. 1988. Gonotrophic development in *Hypoderma lineatum* and *Hipoderma bovis* (Diptera: Oestridae) with notes on reproductive capacity. *Annals Entomology of Society of America* 81: 318-324.
- Sheppard DC. 1983. House fly and lesser fly control utilizing the black soldier fly in manure management systems for caged laying hens. *Environmental Entomology* 12: 1439-1442.
- Tomberlin JK, Sheppard C, Joyce JA. 2004. Black soldier fly (Diptera: Stratiomyidae)

colonization of pig carrion in South Georgia.  
*Journal Forensic Science* 50: 152-153.

Turchetto M, Lafisca S, Costantini G. 2001.  
Postmortem interval (PMI) determined by  
study sarcophagous biocenoses: three cases  
from the province of Venice (Italy). *Forensic  
Science International* 120: 28-31.

Xerez R, Pujol-Luz JR. 2001. Description of  
the larva of *Vittiger schnusei* Kertész, 1909  
(Diptera: stratiomyidae) from Ilha de  
Marambaia, Rio de Janeiro, Brasil. *Studia  
Dipterologica* 8: 337-341.

Xerez, R, Pujol-Luz JR, Viana GG. 2002.  
Descrição da larva de *Cosmariomyia  
argyrosticta* Kertész e do pupário de  
*Dactylodeicts lopesi* Lindner (Diptera:  
Stratiomyidae). *Revista Brasileira de  
Zoologia* 19: 747-755.

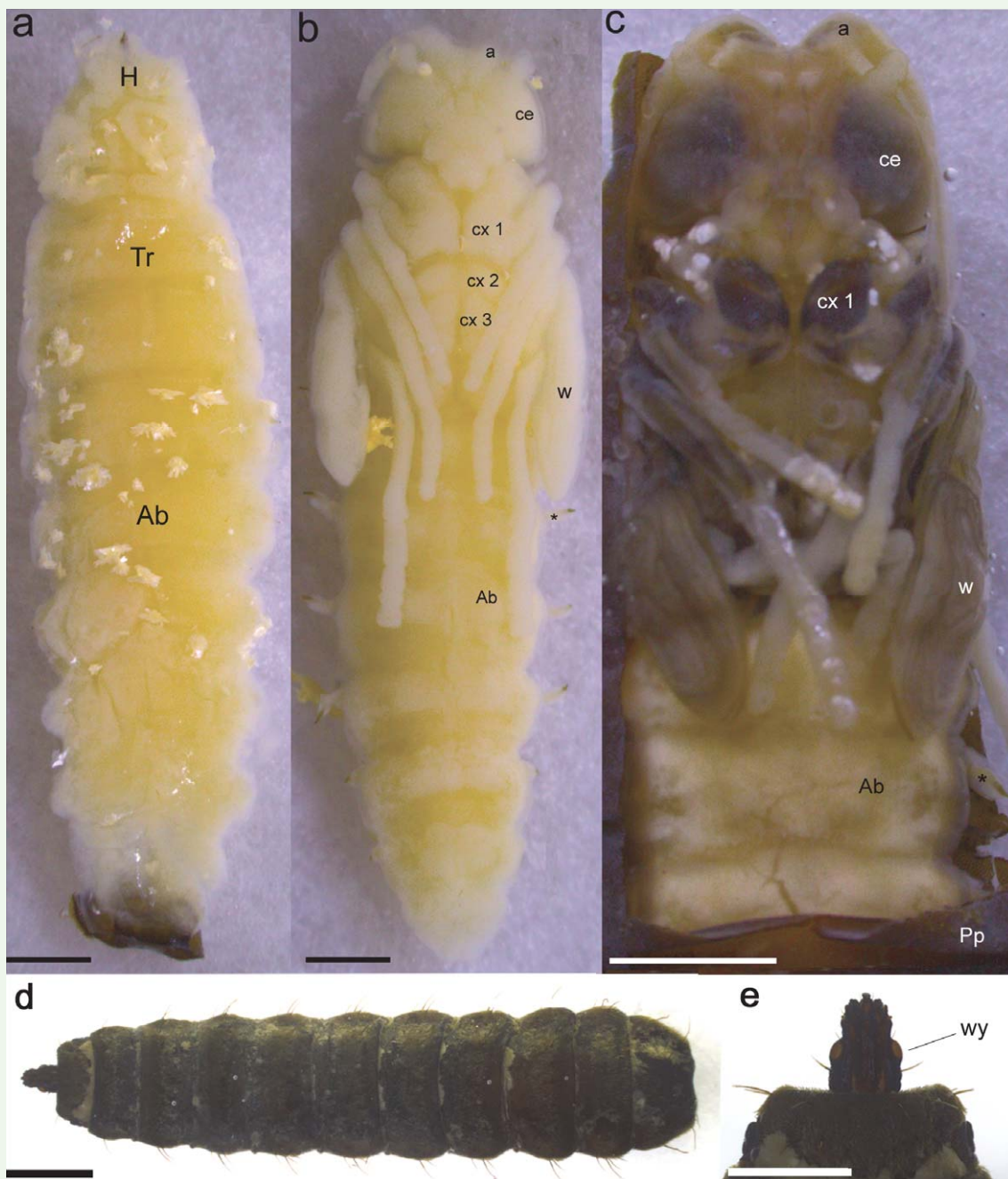
Xerez, R, Pujol-Luz JR, Viana GG. 2003.  
Descrição da larva de *Popanomyia femoralis*  
Kertész, 1909 e do pupário de *Engicerus  
major* Lindner, 1964 (Diptera, Stratiomyidae).  
*Revista Brasileira de Entomologia* 47: 403-  
408.

Wolfe LS. 1954. Studies of the development  
of the imaginal cuticle of *Calliphora  
erythrocephala*. *Quarterly Journal of  
Microscopical Science* 95: 67-78.

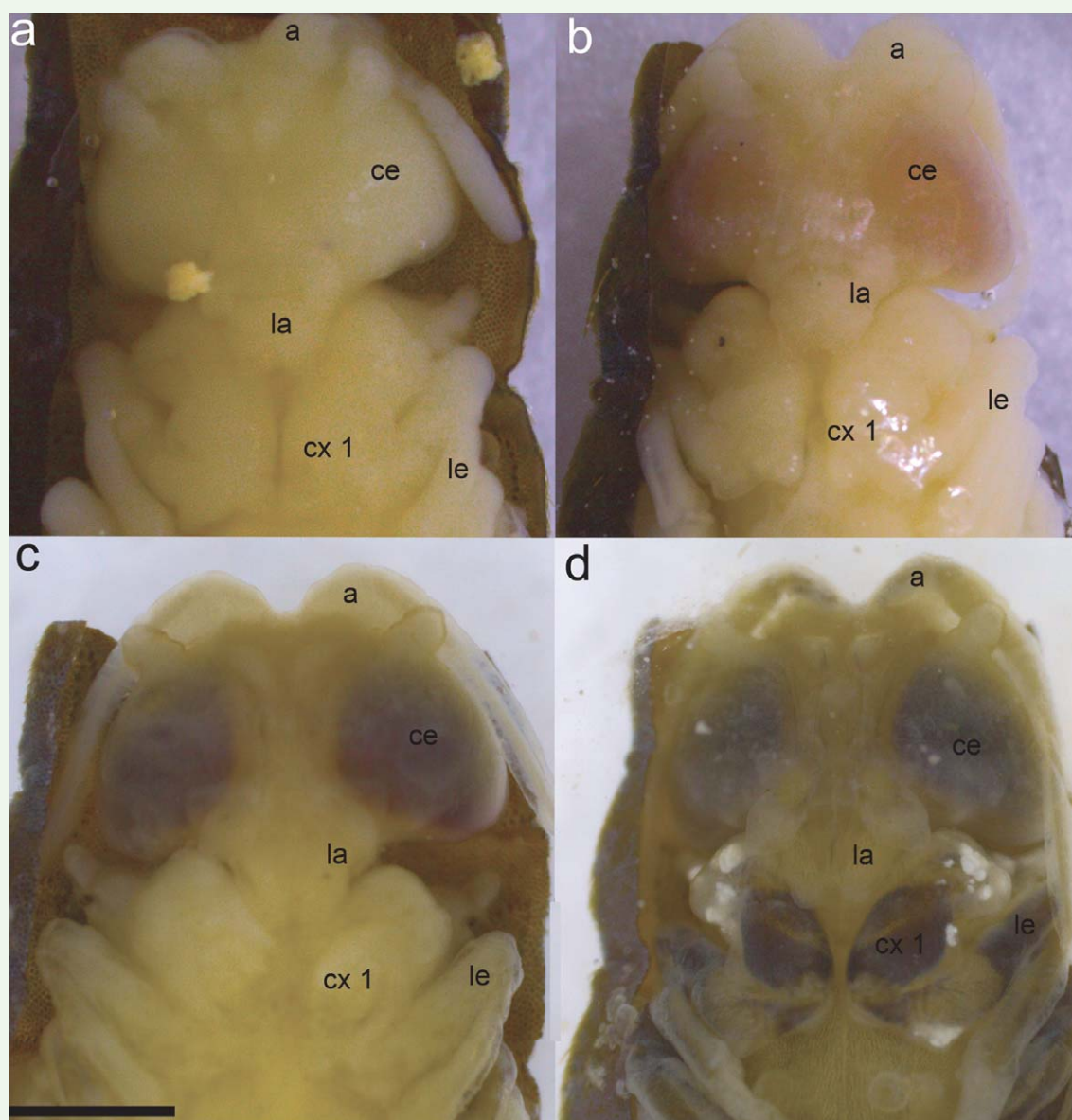
Woodley NE. 1989. Phylogeny and  
classification of the “orthorrhaphous”  
Brachycera. In: McAlpine JF, Wood DM,  
Editors. pp. 1371-1395. *Manual of Nearctic  
Diptera*. Monograph 32, Volume 3. Research  
Branch, Agriculture Canada.

Woodley NE. 2001. A world catalogue of the  
Stratiornyidae (Insecta: Diptera). *Myia* 11: 1-  
473.



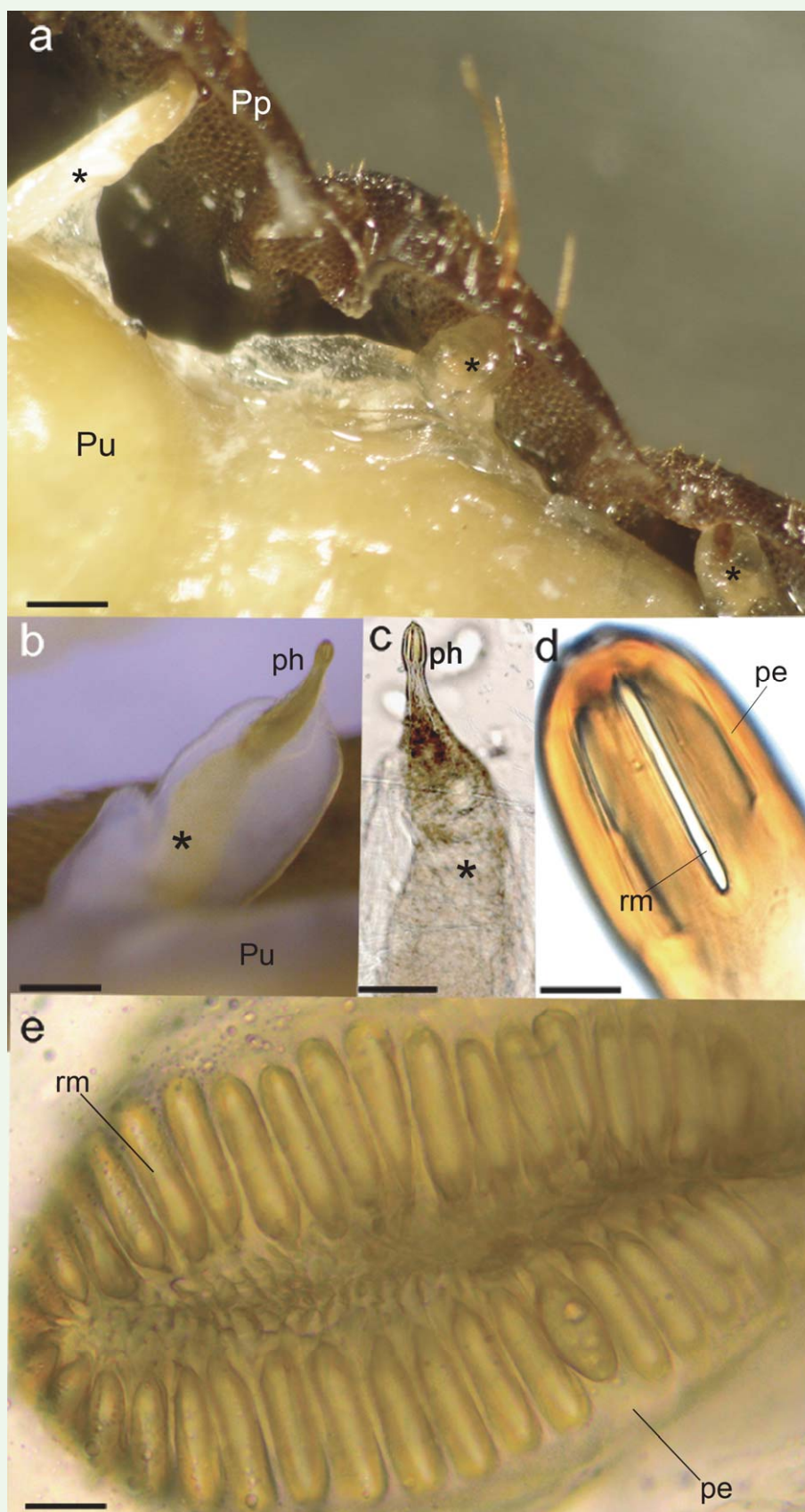


**Figure 1.** Sequence of the intra-pupal development of *Hermetia illucens* (ventral view). (a) Cryptocephalic pupa; (b) pharate adult; (c) imago; (d) pupae; (e) detail of the head (dorsal view). Abbreviations: Ab, abdomen; a, antenna; ce, compound eyes; cx, coxa; H, head; Pp, puparium; Tr, thorax; w, wing; wy, white-eyes; \* respiratory tubes. Scale bars: 1.6 mm (a,b), 2.5 mm (c) 1.2 mm (d), and 0.7 mm (e). High quality figures are available online.



**Figure 2.** The pharate of *Hermetia illucens*, according to the color of the compound eyes. (a) Yellowish eyes; (b) Pinkish eyes; (c) Reddish eyes; (d) Brownish eyes. Abbreviations: a, antenna; ce, compound eyes; cx, coxa; la, labrum; le, legs. Scale bar: 1.5 mm. High quality figures are available online.





**Figure 3.** Morphology of the respiratory system of *Hermetia illucens*. (a) Lateral projections of respiratory tubes; (b, c) pupal-horn; (d) tip of the pupal-horn; (e) crown-like respiratory opening. Abbreviations: pe, peritreme; ph, pupal respiratory horn; Pp, puparium; Pu, pupa; rm, rima; \* respiratory tubes. Scale bars: 0.3 mm (a), 2.5 mm (b), 3.4 mm (c), 0.6 mm (d), and 1.6 mm (e). High quality figures are available online.