Revista Brasileira de Entomologia 60 (2016) 334–340

SOCIEDADE BRASILEIRA DE ENTOMOLOGIA FUNDADA EM 1937

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution

www.rbentomologia.com

Medical and Veterinary Entomology

Intra-puparial development of the *Cochliomyia macellaria* and *Lucilia cuprina* (Diptera, Calliphoridae)

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ARTICLE INFO

Article history:
Received 25 February 2016
Accepted 23 June 2016
Available online 18 July 2016
Associate Editor: Andrzej Grzywacz

Keywords:
Insect anatomy
Insect metamorphosis
Morphology
Ontogeny
Taxonomy

ABSTRACT

The intra-puparial development of the blowflies *Cochliomyia macellaria* ($n = 310$) and *Lucilia cuprina* ($n = 470$), was studied under controlled conditions in laboratory. The 3rd instar larvae were reared until they stopped feeding, and the pre-pupae were separated according to the size in larval length and degree of pigmentation and of the cuticle. We observe a set of five continuous events or phases: (1) pupariation, (2) larva-pupa apolysis, (3) cryptocephalic pupa, (4) phanerocephalic pupa and (5) pharate adult. The total time of the intra-puparial development, larva-pupa apolysis to pharate adult, lasted for 120 h (5 days) to *C. macellaria* and 210 h (8.75 days) to *L. cuprina*.

Introduction

The majority of studies on intra-pupal development in Diptera have focused Muscoidea and Oestroidea, especially Calliphoridae and Oestridae (Cepeda-Palacios and Scholl, 2000; Pujol-Luz and Barros-Cordeiro, 2012; Richards et al., 2012; Defilippo et al., 2013; Karabey and Sert, 2014; Proença et al., 2014; Ma et al., 2015), as well as on an orthorraphous Brachycera Stratiomyoidea *Hermetia illucens* (Linnaeus, 1758) (Barros-Cordeiro et al., 2014). The information on this phase of post-embryonary development is useful in various branches of entomology, especially in the control of pests and endemic diseases, control of parasitic diseases, in forensic entomology and studies of ontogeny.

Studies about post-embryonary development of the oestroids dipterous may help with pest control of Calliphoridae, which include vector species of pathogenic microorganisms or that cause myiasis (Zumpt, 1965) such as *Cochliomyia macellaria* (Fabricius, 1775) and *Lucilia cuprina* (Wiedemann, 1830). *C. macellaria* it is one of the species that cause secondary myiasis, a lesion caused by histophagous larvae that aggravates a pre-established infection. The condition affects humans as well as other animals, making it of particular medical importance (Greenberg, 1973; Guimarães and Papavero, 1999; Marquez et al., 2007). This species is also a potential vector of various enteropathogens that cause human diseases, and is recorded as a vector of *Dermatobia hominis* (Linnaeus, 1781), a botfly responsible for cutaneous myiasis (Guimarães and Papavero, 1999). *L. cuprina* causes primary myiasis in sheep in Australia and New Zealand, being responsible for the loss of millions of dollars annually in the wool and meat industries (Sackett et al., 2006; Wall, 2012). In Brazil, the species is associated to secondary myiasis in sheep (Moreira-Lima and Moya-Borja, 1997). Records of this species causing myiasis in humans and other animals are common as reported by Foster et al. (1985) and Concha et al. (2011). *C. macellaria* and *L. cuprina* are also present in the decomposition of animal carcasses (Wolff et al., 2001; Biavati et al., 2010).

The purpose of this study is to present the description and comparison of intra-pupal developmental time in Calliphoridae of medical-sanitary and forensic importance in Brazil: the secondary screwworm *C. macellaria* and the blowfly *L. cuprina*.

Material and methods

We collected the adults of *C. macellaria* and *L. cuprina* in urban areas in the neighborhoods of Universidade de Brasília – UnB,

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http://dx.doi.org/10.1016/j.rbe.2016.06.009

Table 1
Development time of *Cochliomyia macellaria* and *Lucilia cuprina*, minimum hours (h), for each stage of development at 23 ± 1 °C.

Species	Event	Time (h)	Mean ± S.D.	Range	*n*
Cochliomyia macellaria	Larva-pupa apolysis	6	8.45 ± 6.75	([b]00–27)	55
	Cryptocephalic pupa	6	12.6 ± 10.37	(06–42)	10
	Phanerocephalic pupa	3	28.2 ± 25	(12–78)	5[a]
	Pharate adult (ce)				
	Transparent	15	28.57 ± 7.61	(15–45)	65
	Yellowish	48	54.85 ± 15.09	(30–46)	85
	Pinkish	12	83.05 ± 4.02	(78–90)	19
	Reddish	30	107.32 ± 10.72	(90–120)	44
Lucilia cuprina	Larva-pupa apolysis	6	8.6 ± 8.4	([b]00–36)	45
	Cryptocephalic pupa	9	15.5 ± 10.4	(06–39)	25
	Phanerocephalic pupa	3	32.6 ± 11	(15–54)	38
	Pharate adult (ce)				
	Transparent	6	33.8 ± 14.7	(18–72)	24
	Yellowish	132	98.9 ± 44	(24–192)	237
	Pinkish	18	178.2 ± 16	(156–204)	30
	Reddish	36	196.4 ± 11.6	(174–210)	41

Time (h), refers to the minimum duration of each event; S.D., standard deviation; *n*, number of samples; ce, compound eyes.
[a] Of total 10 samples, were only 5 in fanerocephalic phase the other and 5 samples were apolysis process or cryptocephalic phase.
[b] Beginning of the stage.

using a modified Van Someren-Rydon trap. We set up two colonies of 150 couples, one for each species, in two cages inside a BOD incubator chamber (23.0 ± 1.0 °C, 60 ± 10% RH, 12:12 L:D). We reared the colonies on a meat substrate using the methods of Barros-Cordeiro and Pujol-Luz (2010). Once larvae migrated from the meat to the vermiculate substrate, they reduced in size, changed color, and pupated. We defined the start of pupation as the moment when the larvae changed color to brown and adopted a barrel shape. As soon as pupation started, we initiated collection of samples of 10 pupae at a time according to the following schedule: time 0; every 3 h until 48 h of development; every 6 h until adult emergence. The collected pupae were fixed in Carnoy solution for 48 h, then transferred to 5% formic acid for another 48 h, and then transferred to and stored in 70% ethanol.

The specimens were dissected and photographed with Leica M205-C® stereomicroscope. A total of 310 pupae of *C. macellaria*

Table 2
Comparison between the intervals, minimum duration, for each intra-pupal development event and total time of development of species of Calliphoridae, in hours and at different temperatures.

Event	*Cochliomyia macellaria* 23.0 ± 1.0 °C	*Lucilia cuprina* 23.0 ± 1.0 °C	*Lucilia cuprina* 30 °C
Larva-pupa apolysis	6	6	4
Cryptocephalic pupa	6	9	4
Phanerocephalic pupa	3	3	8
Pharate adult	105	192	128
Total time	120	210	144
References	This study	This study	Barritt and Birt (1971)
Total time of development (egg-adult emergence)	23.0 ± 1.0 °C	23.0 ± 1.0 °C	22.9 ± 1.1–29.5 ± 1.0 °C
	241	354	336
References	This study	This study	Greenberg & Szyska (1984)
Event	*Lucilia sericata* 25 °C	*Phormia regina* 22 °C	*Calliphora vicina* 23.0 ± 0.6 °C
Larva-pupa apolysis	8	9	48
Cryptocephalic pupa	4	14.5	84
Phanerocephalic pupa	12	18.5	67
Pharate adult	148	107	209
Total time	172	149	408
References	Karabey and Sert (2014)	Greenberg (1991)	Defilippo et al. (2013)
Total time of development (egg-adult emergence)	25 °C	22 °C	22 °C
	516	350.4	465.6
References	Marchenko (2001)	Greenberg and Kunich (2002)	Greenberg and Kunich (2002)
Event	*Chrysomya albiceps* 26.0 ± 1.0 °C	*Chrysomya putoria* 25–27 °C	*Chrysomya rufifacies* 24 °C
Larva-pupa apolysis	3	18	16
Cryptocephalic pupa	3	6	8
Phanerocephalic pupa	3	24	16
Pharate adult	81	68	96
Total time	90	116,00	136
References	Pujol-Luz and Barros-Cordeiro (2012)	Proença et al. (2014)	Ma et al. (2015)
Total time of development (egg-adult emergence)	26.0 ± 1.0 °C	22.9 ± 1.1–29.5 ± 1.0 °C	15.6–35 °C
	264	252	190 – 598
References	Kosmann et al. (2011)	Greenberg and Szyska (1984)	Byrd and Butler (1997)

and 470 pupae of *L. cuprina* were examined and dissected. The terminology adopted to describe the morphology of the puparium and the intra-puparial development phases follows Fraenkel and Bhaskaran (1973), Cepeda-Palacios and Scholl (2000) and Barros-Cordeiro et al. (2014).

Results

We observed five chronological events, in the intra-puparial development of *C. macellaria* and *L. cuprina*. The first phase is known as pupariation and occurs after the larva leaves the diet substrate. Gradually it begins to bury into the adjacent substrate and it mobility decreases. During this process there are a retraction and invagination of the segments of the body. The cuticle of the larva is progressively sclerotized and pigmented, usually from dark yellow to dark brown. The posterior spiracle collapses and sinks on to anal tubercle, and the larva assumes the form of a barrel. The puparium of *C. macellaria* presented a mean length of 8.87 ± 0.47 mm, which is about 40% less than the mean length of the third-instar larva, and a mean weight of 48.60 ± 5.20 mg. The minimum duration of this process was 12 h (Table 1). The puparium of *L. cuprina* presented a mean length of 7.1 ± 0.2 mm, which is about 38% less than the mean length of the third-instar larva, and a mean weight of 28.4 ± 1.7 mg. This phase lasted at least 46 h (Tables 1 and 2).

In the second event, or larval-pupal apolysis, the separation of the cuticles occurs initially in the median portion of the puparium moving to the anterior part of the body, and from the ventral to the dorsal region of the body. The extremities initially become stuck in the puparium, the anterior region by the maxilla and mandible, and the posterior part by the spiracles and intestine. The process

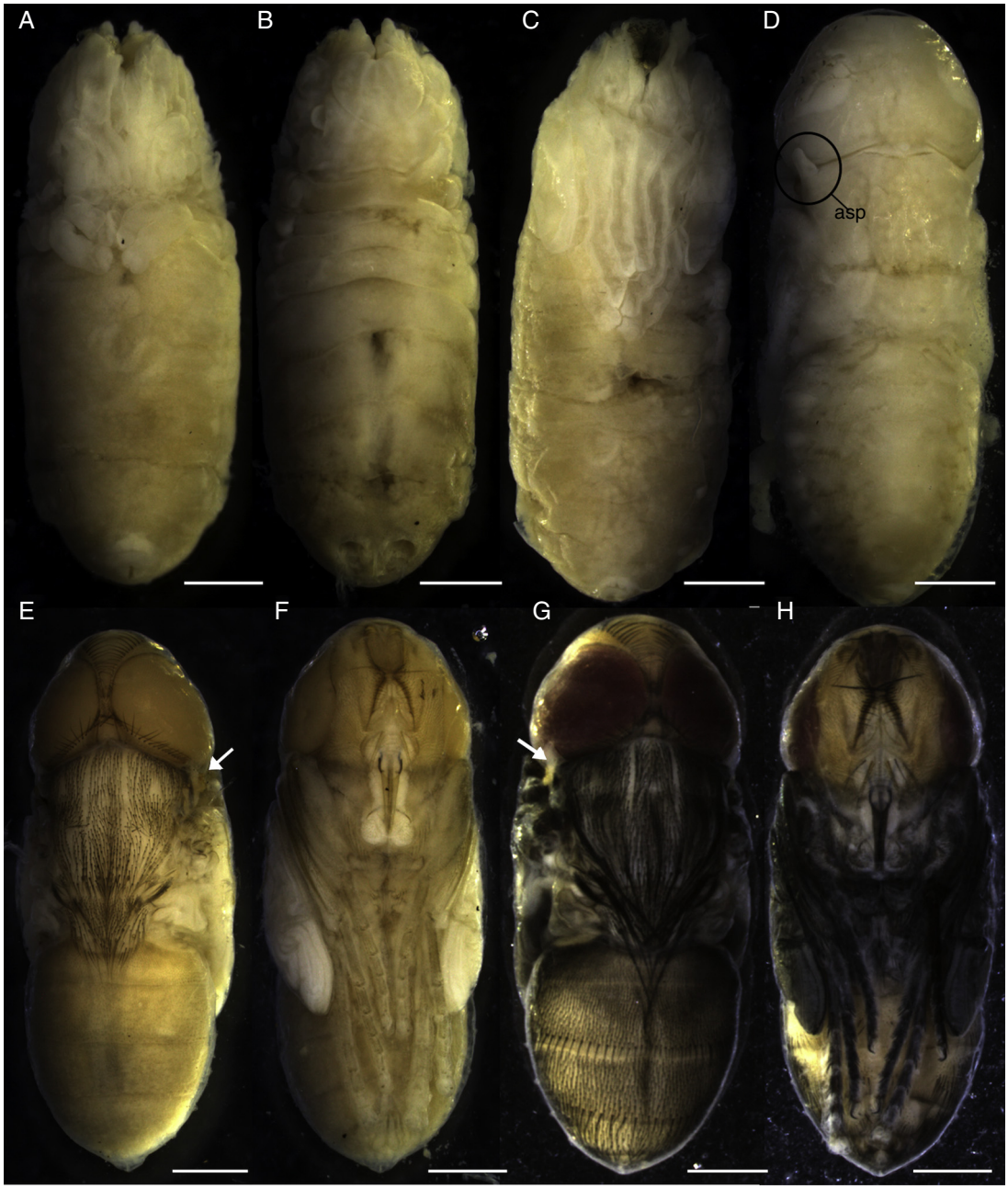

Fig. 1. Morphological sequence of the intra-puparial development of *Cochliomyia macellaria*. (A) Ventral view and (B) dorsal view, cryptocephalic pupa; (C) ventral view, phanerocephalic pupa; (D, E) dorsal and (F) ventral view, pharate adult; (G) Pharate adult in dorsal view, sequence of bristle pigmentation and body sutures; (H) Imago with formation of the ptilineal sac in ventral view. Arrow, anterior spiracle (asp). Scale: 1 mm.

of larval-pupal apolysis occurs likewise in both species. The minimum time to complete the process of apolysis was six hours in both species (Tables 1 and 2).

The third event is the cryptocephalic pupa. It starts after completion of apolysis, when the puparium becomes more pigmented and sclerotized. The mandible and maxilla are detached from the rest of the cephalopharyngeal skeleton and remain stuck to the puparium. The pupa at this moment has an undefined form and is wrapped in a fine membrane (Figs. 1–3). This event lasted six hours in *C. macellaria* and nine hours in *L. cuprina* (Tables 1 and 2).

The fourth event is the phanerocephalic pupa. It contains to the process of evagination of the cephalic capsule and the thoracic appendices. We can distinguish the head, the thorax and the abdomen of the imago. The pair of prothoracic spiracles is formed by a lateral projection, similar to a cylindrical tube, which connects to the puparium (Figs. 1–3). This process lasted an average of three hours for both species (Tables 1 and 2).

The fifth event is the pharate adult, when to the maturation of the adult insect happens in four steps defined by changes in the color of the pigmentation of the compound eyes (Tables 1 and 2). Within these steps, other structures of the body gradually became pigmented and sclerotized in both species.

In *C. macellaria* (Figs. 1 and 2) we observed in the sequential steps: (i) transparent eyes, head, thorax and abdomen defined, legs and wings not membranous. This event lasted at least 15 h; (ii) yellowish eyes, sutures of the thorax and abdomen defined, visible terminalia, start of pigmentation of hairs and bristles. In this step, we observed filiform maxillary palps, and the start of the formation of the three longitudinal vitae in the dorsal region of the thorax. This period lasted at least 48 h; (iii) pinkish eyes, greater pigmentation of hairs, bristles, veins of wings and legs; proboscis and antennae clear. This step lasted at least 12 h; (iv) reddish eyes, body completely formed, antennae, palps and ocelli well defined; wings membranous and veins blackened; longitudinal strips in the dorsal region of the thorax strongly marked; external genitalia visible; sclerites defined and delimited; ptilineal sac is formed. This period lasted 30 h.

In *L. cuprina* (Figs. 3 and 4) we observed in the sequential steps: (i) transparent eyes, head, thorax, abdomen and legs defined, wings not membranous. This period lasted at least six hours; (ii) yellowish eyes, sutures of the thorax and abdomen defined, the terminalia are visible, start of pigmentation of setae and bristles. This period lasted at least 132 h; (iii) pinkish eyes, strong pigmentation of setae, bristles, veins of wings and legs; proboscis and antennae

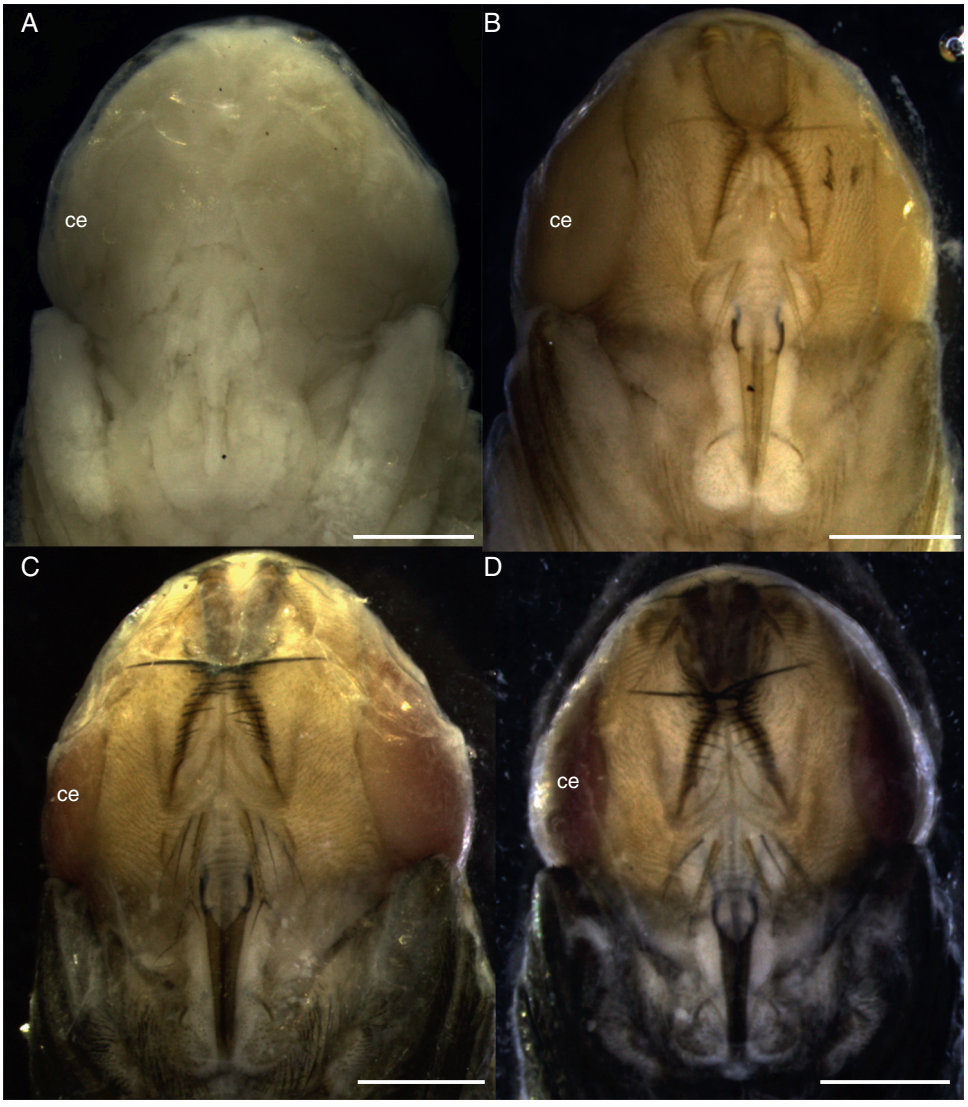

Fig. 2. The pharate adult of *Cochliomyia macellaria*, according to the color of the compound eyes. (A) Transparent eyes; (B) yellowish eyes; (C) pinkish eyes; (D) reddish eyes. ce, compound eyes. Scale: (A) 0.5 mm; (B–D) 1 mm.

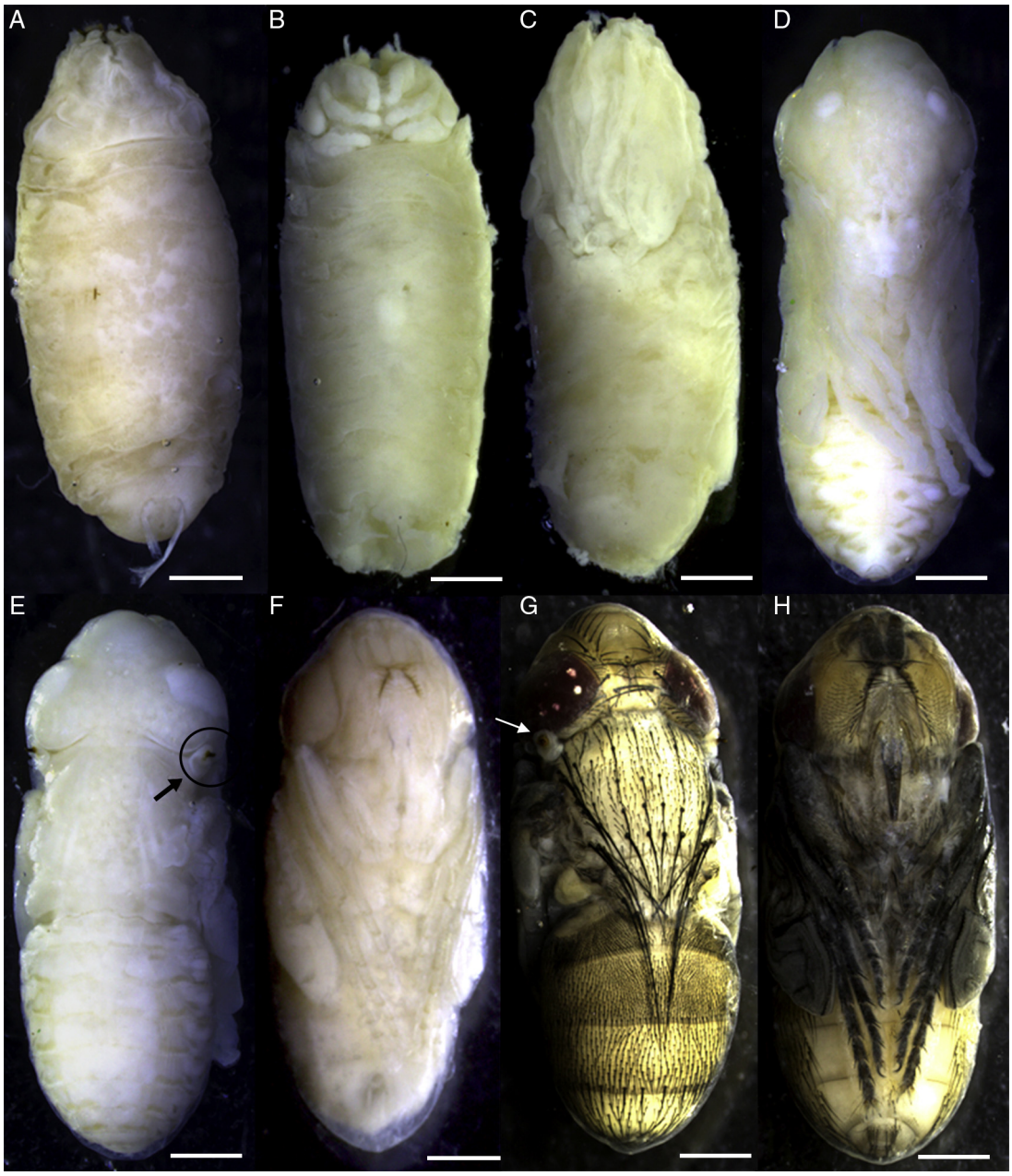

Fig. 3. Morphological sequence of the intra-puparial development of *Lucilia cuprina*. (A) ventral view of the cryptocephalic pupa; (B, C) ventral view of the phanerocephalic pupa; (D) ventral view of the pharate adult and (E) dorsal view, anterior spiracle of the pupa (arrow); (F) ventral view of the pharate adult, sequence of pigmentation of the hairs, bristles and body structures; (G) formed adult and formation of the ptilineal sac in the dorsal view, anterior spiracle of the pupa (arrow) and (H) ventral view. Scale: 1 mm.

translucent; the three acrostical bristles visible and highly pigmented. This period lasted at least 18 h; (iv) reddish eyes, body completely formed, antennae, palps and ocelli well defined; wings and veins blackened; external genitalia visible; bristles of the 5th sternite strongly pigmented; sclerites defined and delimited; ptilineal sac is formed. This period lasted 36 h.

Discussion

Intra-puparial development in *C. macellaria* and *L. cuprina* (Table 1) are similar to those observed for other Cyclorrhapha viz. Fraenkel and Bhaskaran (1973): *Musca domestica* Linnaeus, 1758; and *Sarcophaga bullata* (Parker, 1916); Cepeda-Palacios and Scholl (2000): *Oestrus ovis* Linnaeus, 1758. Most of the similarities are restricted to the sequence of the chronological events (larva-pupa apolysis; cryptocephalic pupa; phanerocephalic pupa; pharate adult) that occurs intra-puparially as already described for other species of Cyclorrhapha (Table 2) (Fraenkel and Bhaskaran, 1973; Pujol-Luz and Barros-Cordeiro, 2012).

Extrinsic factors, e.g. temperature and humidity, affect the developmental timing, as already suggested elsewhere (e.g. Denlinger and Ždárek, 1994; Cepeda-Palacios and Scholl, 2000). *C. macellaria* and *L. cuprina* may spend about 50% of the total time of its development (egg to adult) in the pupal stage, as other blowflies do (Table 2). The pharate adult phase (Table 1) represents more than 60% of the total time intra-puparial. However, it is among the pharate adult pinkish eyes and pharate adult reddish eyes that some unique morphological changes take place. In *L. cuprina* the hardly pigmentation of the thoracic setae and the appearance of a dense pubescence of 5th abdominal tergite abdominal and, in *C.*

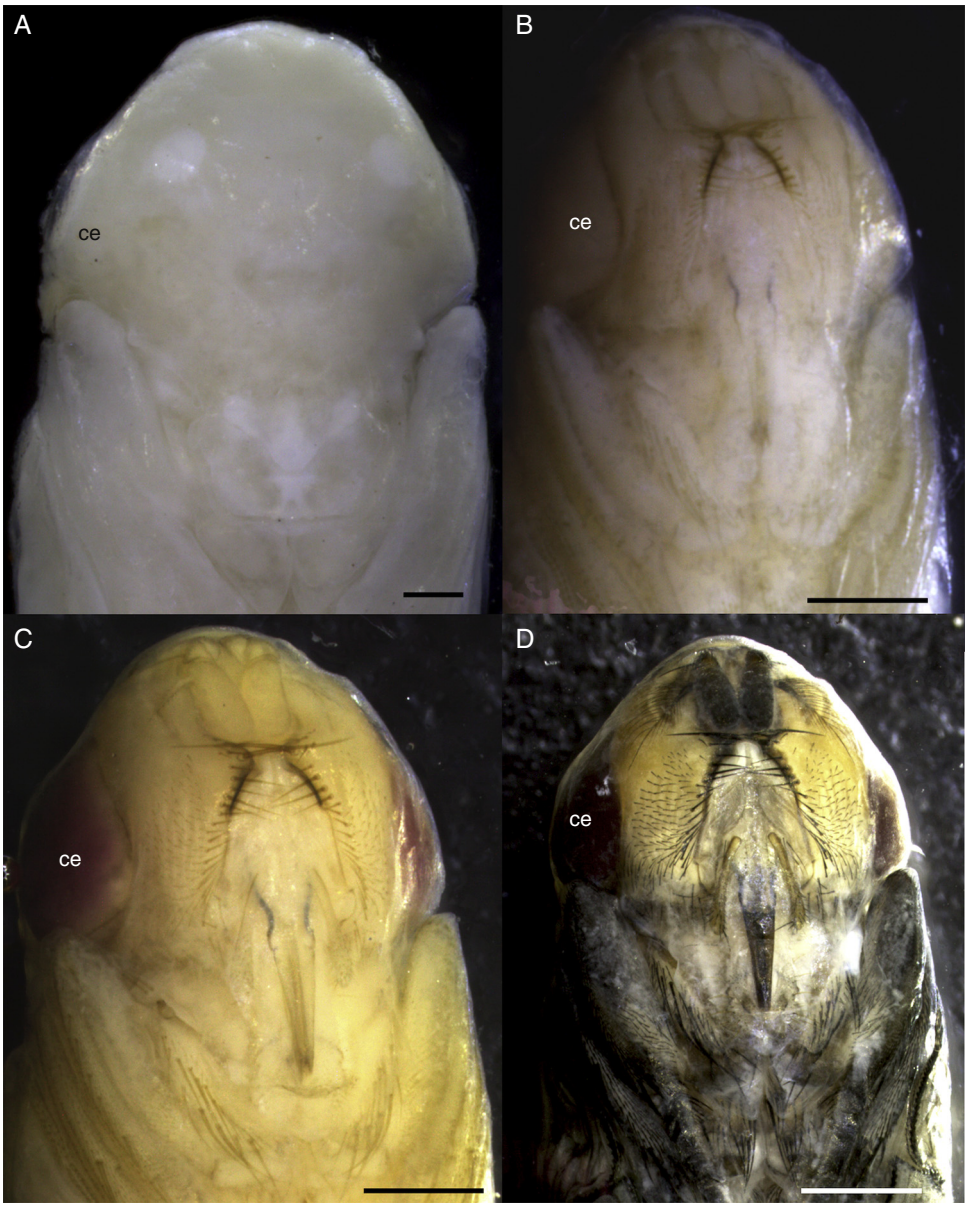

Fig. 4. The pharate adult of *Lucilia cuprina*, according to the color of the compound eyes. (A) Transparent eyes; (B) yellowish eyes; (C) pinkish eyes; (D) reddish eyes. ce, compound eyes. Scale: (A) 0.5 mm; (B–D) 1 mm.

macellaria appear the distinctive thoracic pigmentation, with a conspicuous vitae and a dense white pollinosity in abdominal ventral surface.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

This research was developed with grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, Fundação de Apoio à Pesquisa do Distrito Federal – FAP-DF, Financiadora de Estudos e Projetos – FINEP. We are also grateful to Érica S. Harterreiten-Sousa, Ana Carolina Franco Pereira, Caroline Demo and André Gardelino Savino, for help in different parts of this research. Professor R.B. Cavalcanti reading the manuscript and making helpful comments and suggestions.

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