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Intrapuparial development of *Peckia pexata* (Wulp, 1895) (Diptera: Sarcophagidae)

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ABSTRACT

Sarcophagidae flies have great forensic importance, since different instars of their larval development are used in the determination of the postmortem interval (PMI) of corpses. However, for many species, intrapuparial developmental data are scarce. Here we analyzed the intrapuparial development of the sarcophagid *Peckia pexata* and describe the chronological and morphological changes that occur during metamorphosis. We reared approximately 330 specimens originating from the Colombian Amazon in the laboratory. Prepupae were identified by size reduction and coloration change. During the first five days of the study, four individuals were sampled every three hours, then every six hours until adult emergence. Specimens were fixed in 96% alcohol, then immersed in Carnoy solution for 24 hours and in formic acid (5%) for 48 hours for dissection and analysis of morphological changes. Four morphological phases of intrapuparial development of *P. pexata* were observed: 1) larva pupa apolysis, which lasted, in average, 9 h; 2) cryptocephalic pupa, 6 h; 3) phanerocephalic pupa, 6.5 h; and 4) pharate adult (transparent eyes, 48 h; yellow eyes, 216 h; pink eyes, 35 h; and red eyes, 52 h). The pharate adult was fully formed at 340 h and adult emergence occurred at 372.5 h or 15.5 days. We also described the formation of the prepupa, the pupariation and the final metamorphosis for the imago and compared it with the known events for *Peckia intermutans*, *Peckia lambens* (Sarcophagidae) and *Lucilia eximia* (Calliphoridae).

KEYWORDS: Amazon, Colombia, forensic entomology, immature insects, metamorphosis, postmortem interval

Desarrollo intrapupal de *Peckia pexata* (Wulp, 1895) (Diptera, Sarcophagidae)

RESUMEN

Las moscas Sarcophagidae tienen gran importancia forense, ya que diferentes etapas de su desarrollo larvario son utilizadas en la determinación del intervalo post mortem (IPM) de cadáveres. Sin embargo, para muchas especies los datos sobre el desarrollo intrapupal son escasos. Aquí analizamos el desarrollo intrapupal del sarcofagido *Peckia pexata* y describimos los cambios cronológicos y morfológicos que ocurren durante la metamorfosis. Criamos aproximadamente 330 especímenes originarios de la Amazonía colombiana en laboratorio. Prepupas se identificaron por la reducción de tamaño y cambio de color. Durante los primeros cinco días se tomaron muestras de cuatro individuos cada tres horas, y posteriormente cada seis horas hasta la emergencia de los adultos. Los especímenes se fijaron en alcohol al 96%, posteriormente se sumergieron en solución Carnoy durante 24 horas y en ácido fórmico (5%) durante 48 horas, para disección y el análisis de los cambios morfológicos. Observamos cuatro etapas del desarrollo intrapupal de *P. pexata*: 1) larva pupa apolisis, que duró, en promedio, 9 h; 2) pupa criptocefálica, 6 h; 3) pupa fanerocefálica, 6.5 h; y 4) adulto farado (ojos transparentes, 48 h; ojos amarillos, 216 h; ojos rosados, 35 h; y ojos rojos, 52 h). El adulto farado se formó completamente a las 340 h y la emergencia del adulto se produjo a las 372.5 h o 15,5 días. También describimos la formación de la prepupa, la pupariación y la metamorfosis final para el imago y lo comparamos con los eventos conocidos para *Peckia intermutans*, *Peckia lambens* (Sarcophagidae) y *Lucilia eximia* (Calliphoridae).

PALABRAS CLAVE: Amazonía, Colombia, entomología forense, insectos inmaduros, intervalo post-mortem, metamorfosis

INTRODUCTION

Forensic entomology studies insects and other arthropods associated with corpse decomposition and is used mainly as a tool to estimate the time interval between death and the discovery of the cadaver, and this period is known as postmortem interval (PMI) (Magaña 2001; Amendt et al. 2004). Frequently, when remains are found weeks, months, or even longer after death, analysis of the entomological evidence is the only method available to reliably determine the PMI (Amendt et al. 2004; Ramos-Pastrana et al. 2012).

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Intrapuparial development times and pupal age can be used to estimate PMI in forensic entomology. The developmental chronology of some flies have been documented. Examples in the family Calliphoridae are *Calliphora erythrocephala* (Macquart, 1834) (Wolfe 1954), *Chrysomya albiceps* (Wiedemann, 1819) (Pujol-Luz and Barros-Cordeiro 2012), *Chrysomya rufifacies* (Macquart, 1842) (Ma et al. 2015), and *Lucilia eximia* (Wiedemann, 1819) (Ramos-Pastrana et al. 2017). In the family Muscidae, the development of *Musca domestica* Linneaus, 1758 has been described by Fraenkel and Bhaskaran (1973). In the family Sarcophagidae, known examples are *Sarcophaga bullata* (Parker, 1916) (Fraenkel and Bhaskaran 1973), *Peckia intermutans* Walker, 1861 (Sousa-Cunha 2015), *Peckia lambens* Wiedemann, 1830 (Sousa-Cunha 2015), and in the family Stratiomyidae, *Hermetia illucens* (Linnaeus, 1758) (Barros-Cordeiro et al. 2014).

The intrapuparial development of flies of medical and veterinary interest has also been described, such as *Cephenemya phobifera* (Clark, 1815) (Bennett 1962), *Cuterebra tenebrosa* Coquillet, 1898 (Baird 1972, 1975), *Dermatobia hominis* (Linnaeus Jr. 1781) (Lello et al. 1985), *Hypoderma linaetum* (Viller, 1789) and *Hypoderma bovis* (Linnaeus, 1761) (Scholl and Weintraub 1988), *Cuterebra fontinella* Clark, 1827 (Scholl 1991) and *Oestrus ovis* (Linnaeus, 1758) (Cepeda-Palacios and Scholl 2000).

Peckia pexata (Wulp, 1895) are large and robust flies that range from 11 to 13.2 mm in length (Wulp, 1895). They occur in the Nearctic and Neotropical regions (Buenaventura and Pape 2013). In Colombia, *P. pexata* has been reported from the departments of Antioquia and Sucre (Buenaventura and Pape 2013), but not in the Colombian Amazon. It is a species of medical and forensic interest, and has been reported in cadaveric succession investigations (Barros et al. 2008). Therefore, it is a species that can potentially be used to estimate the PMI in legal proceedings involving insect traces in Colombia.

In this study, we describe the stages of metamorphosis and the morphology of the different steps in the intrapuparial development of *P. pexata* form the Colombian Amazon to identify and determine the chronology of each developmental step as auxiliary tools for estimating PMI.

MATERIAL AND METHODS

This study was carried out in the Entomology Laboratory of Universidad de la Amazonía, Florencia, Caquetá, Colombia. The *P. pexata* used in the study belonged to the third generation of a colony established in the laboratory from wild specimens collected in Reserva Natural y Ecoturística La Avispa, in the rural area of the municipality of Florencia (Caquetá, Colombia) (1º37'12"N, 75º40'14"W), with an altitude between 400-600 masl, corresponding to the Amazonian foothills zone (IGAC 2019). About 430 mature larvae (L3) of *P. pexata* were reared and monitored in an incubation chamber (24 ºC, 88% relative humidity, 12:12 h light:darkness) until they stopped feeding, became pink and began leaving the carcass used for their feeding inside the incubation chamber. They were then placed into plastic containers with vermiculite from which four individuals were sampled every three hours during the first five days, then every six hours until the emergence of adults (total n = 330 individuals). The sampled specimens were fixed in ethanol (96%), followed first by a treatment with Carnoy solution for 24 hours and then with formic acid (5%) for 48 hours. This chemical treatment facilitated the removal of the pupal cuticle using the methodology proposed by Fraenkel and Bhaskaran (1973).

The pupae and adults obtained were euthanized with ethyl acetate in a killing jar. Some adults and all pupae were preserved in ethanol (96%). The remaining specimens were pinned and then used to confirm their species with the keys proposed by Buenaventura et al. (2009), Mulieri et al. (2011), and Buenaventura and Pape (2013).

The thermonology used for morphology and chronological events (phases) of development followed Fraenkel and Bhaskaran (1973) and Barros-Cordeiro et al. (2014). Phase names used were larvae-pupa apolysis phase, cryptocephalic pupa phase, phanerocephalic pupa phase and pharate adult phase.

All original specimens were collected under Resolution # 01140 issued by the National Environmental Licensing Authority of the Colombian Ministry of Environment and Sustainable Development. All specimens used in this study were deposited in the Collection of the Entomology Laboratory of Universidad de la Amazonia (LEUA).

Intrapuparial development

In order to document the development of the pupa, fixed puparia were dissected, pupae extracted using a surgical scalpel and observed under a trinocular stereoscope (OLYMPUS© SZ61©, with 2x auxiliary lens). All the material and its external morphology were documented and imaged using a Leica© digital camera DFC450© coupled to a stereomicroscope Leica© M205A© and connected to a computer with Leica Application Suite (LAS©) software, with automatic mounting module (synchronization software) (http://www.syncroscopy.com/syncroscopy/). In addition, we examined the intervals of pre-pupa and pupation, and the beginning of the adult instar, to establish the chronological order to the development of metamorphosis, excluding only the larval stages. The description and morphology of all intrapuparial phases followed Fraenkel and Bhaskaran (1973) and observation began when pupation ended and the larva-pupa apolysis began.

The duration of each phase of development (start point and end point) was recorded (expressed as mean ± standard error of the mean) and were obtained using the software Estimates version 8.0 for Windows (Colwell 2006).

RESULTS

Prepupa and pupation lasted 8 ± 1.5 hours (Table 1). The larvae were in their most advanced instar of development (L3), ceased feeding, reduced their mobility and size, and their cuticles became opaque, pigmented, and hardened (Figure 1a). Intrapuparial development lasted 372.5 ± 2.6 hours (Table 1). Four phases were observed: larvae-pupa apolysis, cryptocephalic pupa, phanerocephalic pupa and pharate adult.

The larva-pupa apolysis phase took place right after the pupation process, when the formation of the adult epidermis was initiated and was subsequently separated from the last larval cuticle, which forms the pupa (Figure 1b). This phase lasted 9 ± 1.2 hours.

In the cryptocephalic pupa phase (observed from 9 ± 1.2 to 15 ± 1.3 h), the pupa had no defined shape, therefore, it was impossible to distinguish the head and thoracic appendages (Figure 1c–d). This phase lasted 6 ± 0.7 hours (Table 1).

In the phanerocephalic pupa phase (observed from 15 ± 1.3 to 21.5 ± 1.4 h), the extroversion of the head and the formation of the thoracic appendages occurred and apolysis began between the pupa and the pharate adult (Figure 1e–f). This phase lasted 6.5 ± 0.5 hours (Table 1).

The pharate adult phase was the longest intrapuparial developmental process observed, from the end of pupal-adult apolysis until the adult emerges (Table 1). This phase was divided according to the coloration of the compound eyes of the pharate adult: transparent eyes; yellow eyes; pink eyes and red eyes, which corresponds to the maturation of the adult (Figure 2).

Transparent eyes (observed from 21.5 ± 1.4 to 69.5 ± 1.7 h; duration 48 ± 1 hours; Table 1) marked the visible differentiation of the head, thorax, abdomen, thoracic appendages, buccal apparatus, and abdominal spiracles (Figure 2a,b). Yellow eyes (observed at 69.5 ± 1.7 to 285.5 ± 1.8 h; duration 216 ± 0.4 hours; Table 1) marked the appearance of thoracic sutures, the differentiation of the tergites and sternites, and the beginning of pigmentation (Figure 2c,d). Pink eyes (observed at 285.5 ± 1.8 to 320.5 ± 2.3 h; duration 35 ± 1.5 hours; Table 1) coincided with the beginning of

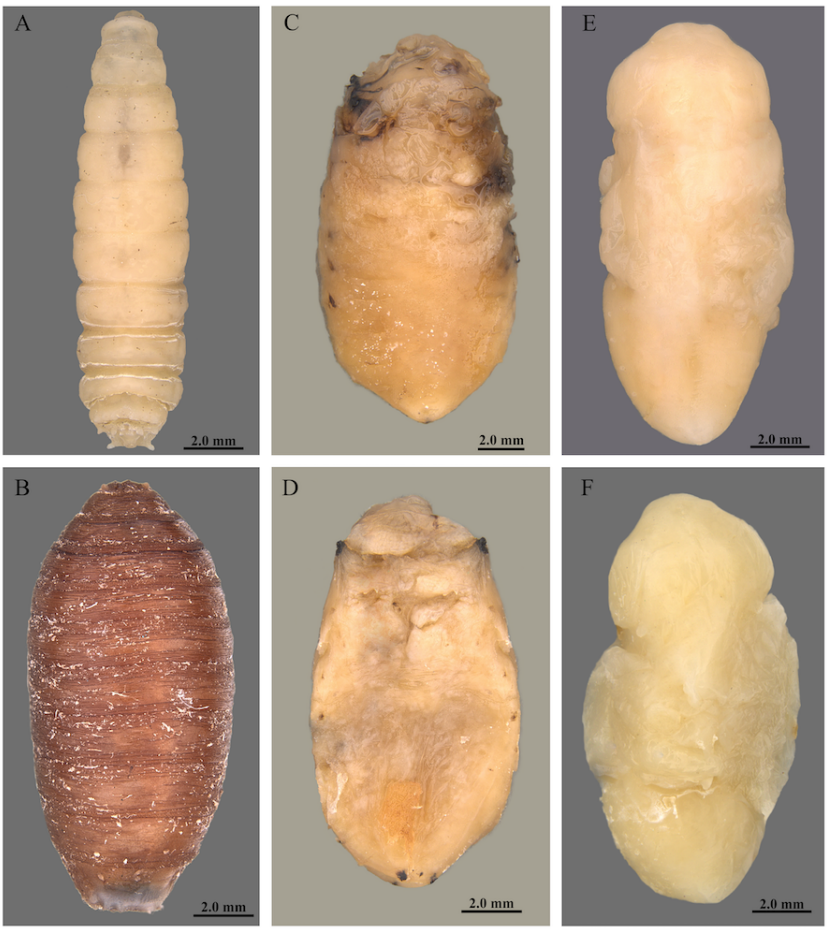

Figure 1. Morphology of the intra-puparial developmental phases of *Peckia pexata*. A – post-feeding pink larva in dorsal view; B – pupation; C – cryptocephalic pupa in ventral view; D – cryptocephalic pupa in dorsal view; E – phanerocephalic pupa in ventral view; F – phanerocephalic pupa in dorsal view.

Table 1. Onset and end time and duration (in hours) of each phase of the intrapuparial development of *Peckia pexata*. Values are the mean ± standard error. Sample size varies according to phase duration, as specimens were sampled for analysis at regular intervals of three to six hours (see Material and Methods for details).

Period of intra-puparial development	Phase	Onset and end time (in hours)		Duration (in hours)	Sample size
Pre-pupa	Pupation	8		8 ± 0	10
Pupa	Larva-pupa apolysis	0	9 ± 1.2	9 ± 1.2	12
	Cryptocephalic pupa	9	15 ± 1.3	6 ± 0.7	8
	Phanericephalic pupa	15	21.5 ± 1.4	6.5 ± 0.5	8
	Pharate adult (compound eyes)				
	Transparent eyes	21.5	69.5 ± 1.7	48 ± 1.0	56
	Yellow eyes	69.5	285.5 ± 1.8	216 ± 0.4	176
	Pink eyes	285.5	320.5 ± 2.3	35 ± 1.5	24
	Red eyes	320.5	372.5 ± 2.6	52 ± 1.1	36
Total time				372.5 ± 2.6	330

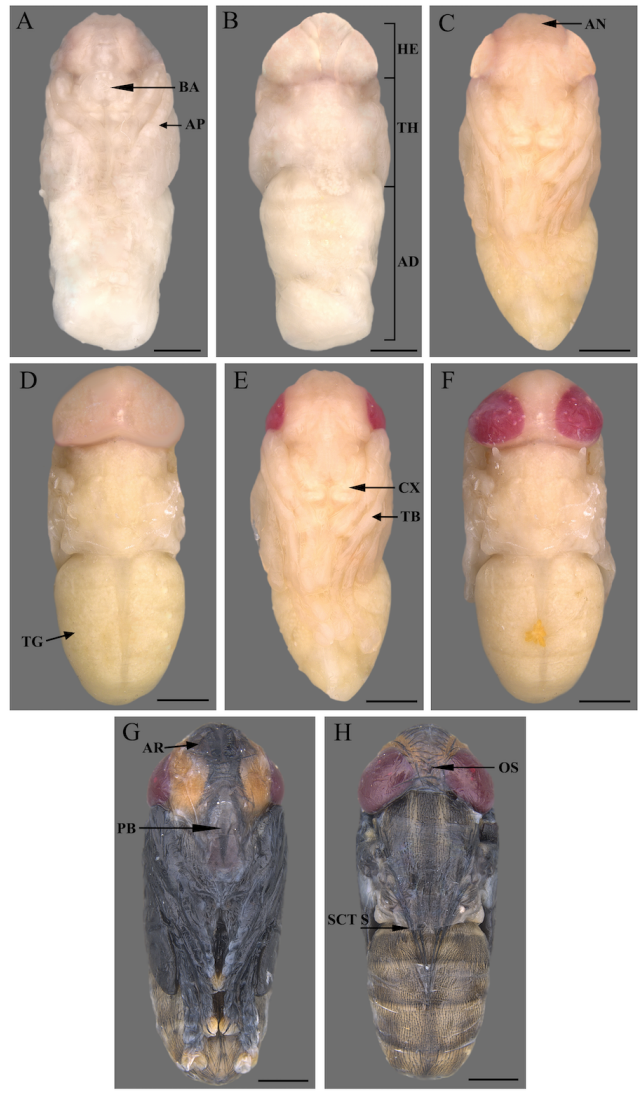

Figure 2. Morphological sequence of the pharate adult phase of *Peckia pexata*, according to the pigmentation of the eyes. A – transparent eyes in ventral view; B – transparent eyes in dorsal view; C – yellow eyes in ventral view; D – yellow eyes in dorsal view; E – pink eyes in ventral view; F – pink eyes in dorsal view; G – red eyes in ventral view; H – red eyes in dorsal view. AD = abdomen; AN = antenna; AP = appendages; AR = arista; BA = buccal apparatus; CX = coxae; HE = head; OS = ocellar setae; PB = proboscis; SCT S = scutellar setae; TB = tibiae; TG = tergites; TH = thorax.

body sclerotization, the differentiation of the postpronotum and postpronotal lobe. The differentiation of the coxae and tibiae is possible (Figure 2e,f). Red eyes (observed at 320.5 ± 2.3 to 372.5 ± 2.6 h; duration 52 ± 1.1 hours; Table 1) marked the period when the whole body was fully developed. The main feature was the beginning of the development of the ptilinum, the wings with a membranous condition, the black mouthparts, and the plumose arista. Furthermore, the setae, tergites, and sternites were strongly pigmented and well defined (Figure 2g,h).

After a period of 346.5 ± 2.4 hours, it was possible to observe the adult inside of the pupa, with the ptilinum totally formed and expanded. Emergence of the adults (n = 10) (Figure 3a) started after 372.5 ± 2.6 hours (Table 1) until mature *P. pexata* adults were obtained (Figure 3b).

DISCUSSION

Four phases of intrapuparial development were identified in *P. pexata*, which is in agreement with previous reports for others dipterous (Fraenkel and Bhaskaran 1973; Pujol-Luz and Barros-Cordeiro 2012; Barros-Cordeiro et al. 2014; Cepeda-Palacios and Scholl 2000; Ramos-Pastrana et al. 2017) (Table 2). In Diptera, there are distinct species-specific intrapuparial developmental times and life cycles (Oliveira-Da-Silva et al. 2006). In general, it is consensual that developmental rate is correlated to relative humidity and the temperature to which the larvae and pupae are exposed (Oliveira-Da-Silva et al. 2006). At low temperatures (< 16 °C) development tends to stop and dehydrate larvae and pupae (Salviano et al. 1996), while at high temperatures (20 °C to 31 °C) they have a shorter development time (Ferraz 1995).

In this study, *P. pexata* took 372.5 hours at 24 °C to develop from larva-pupa apolysis to emergence of the adults. These results contrast with those reported by Sousa-Cunha (2015) for *Peckia intermutans* and *Peckia lambens* and by Ramos-Pastrana et al. (2017) for *Lucilia eximia* (Table 2). Intrapuparial developmental can vary as a function of factors such as diet offered to adults and larvae, collection intervals, relative humidity and to a large extent, to the developmental temperature of the pupae (Ferraz 1995; Salviano et al. 1996; Oliveira-Da-Silva et al. 2006).

The larva-pupa apolysis phase (LPA) of *P. pexata* lasted 9 hours, corresponding to 2.4% of the total time of intrapuparial development. Other *Pexia* species were reported to have a somewhat shorter duration of LPA, both at lower and higher temperatures (Table 2). In *P. lambens*, LPA duration represented similar proportions of total development time at higher temparatures, but this proportion was lower for *P. lambens* and *P. intermutans* at lower temperatures (Sousa-Cunha 2015). In *Sarcophaga bullata,* which also belongs to Sarcophagidae, LPA duration at 24 °C was much longer, at 20 hours (Fraenkel and Bhaskaran 1973), similar to that recorded for the calliphorid *Lucilia eximia* (Ramos-Pastrana et al. 2017).

The cryptocephalic pupa (CCP) was the shortest phase (6 h) in *P. pexata*, corresponding to 1.7% of the total intrapuparial development time. The congeneric *P. intermutans* also had a CCP of 6 hours, while *P. lambens* had a CCP of 3 hours, independently of temperature (Sousa-Cunha 2015; Table 2). In *L. eximia* (Calliphoridae) and *Hermetia*

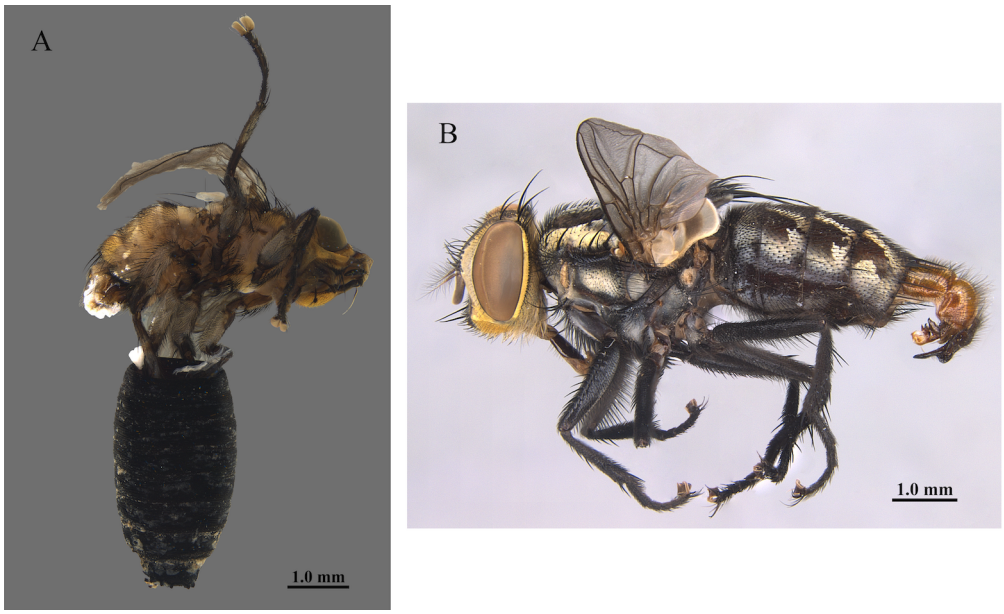

Figure 3. Emerging adult (A) and mature adult (B) of *Peckia pexata*.

Table 2. Developmental chronology (in hours) of onset of each phase of the intrapuparial development of 14 species of Diptera. T = temperature in incubation chamber; LPA = larva pupa apolysis; CCP = cryptocephalic pupa; PCP = phanerocephalic pupa; PHA = pharate adult; EAD = emergence of adult. The values separated with (-) correspond to minimum and maximum values.

Family/Species	T (ºC)	LPA	CCP	PCP	PHA	EAD	Reference
Calliphoridae							
Calliphora erythrocephala (Macquart, 1834)	24	–	–	24–25	–	80	Wolfe (1954)
Chrysomya albiceps (Wiedemann, 1819)	26	3	3–6	6–9	81	90	Pujol-Luz and Barros-Cordeiro (2012)
Lucilia eximia (Wiedemann, 1819)	26.8	23	5	92	182	302	Ramos-Pastrana et al. (2017)
Muscidae							
Musca domestica Linnaeus, 1758	30	4	6–9	16–18	28	96	Fraenkel and Bhaskaran (1973)
Oestridae							
Cuterebra tenebrosa Coquillet, 1898	24	120–144	–	–	–	1248	Baird (1975)
Dermatobia hominis (Linnaeus Jr. 1781)	24	72–120	–	168–192	–	720	Lello et al. (1985)
Oestrus ovis (Linnaeus, 1758)	24	18	48	120	31	528	Cepeda-Palacios and Scholl (2000)
Sarcophagidae							
Peckia intermutans Walker, 1861	23	6	6	30	276	318	Sousa-Cunha (2015)
Peckia lambens Wiedemann, 1830	21	3	3	3	225	234	Sousa-Cunha (2015)
Peckia lambens	26	3	3	3	117	126	Sousa-Cunha (2015)
Peckia lambens	31	3	3	3	108	114	Sousa-Cunha (2015)
Peckia pexata (Wulp, 1895)	24	9	6	6.5	351	372.5	This study
Sarcophaga bullata (Parker, 1916)	24	20	20–28	46–48	168–192	–	Fraenkel and Bhaskaran (1973)
Stratiomyidae							
Hermetia illucens (Linnaeus, 1758)	27	6	3	12	171	192	Barros-Cordeiro et al. (2014)

illucens (Stratiomydae), CCP was also the shortest phase (Barros-Cordeiro et al. 2014; Ramos-Pastrana et al. 2017).

The phanerocephalic pupa phase lasted 6.5 hours in *P. pexata*, also lasted 3 hours in *P. lambens*, but was much longer in *P. intermutans,* with 30 hours at a lower temperature of 23 °C (Sousa-Cunha 2015; Table 2). During this phase in the three species the extroversion of the head, the formation of the thoracic appendages occurred, and apolysis began between the pupa and the pharate adult.

The pharate adult (PHA) phase was by far the longest in *P. pexata* (351 h), in agreemen with almost all other species with data for all intrapuparial developmental phases (Table 2). In the congenerics *P. intermutans* and *P. lambens*, despite PHA duration also being much longer than that of other phases, in no case reached 300 hours, even at temperatures below 24 °C (Sousa-Cunha 2015; Table 2).

During the pharate adult phase, we were able to identify four morphological changes in the pigmentation of the eyes, as previously reported for *P. intermutans* and *P. lambens* (Sousa-Cunha 2015), *Chrysomya albiceps* (Pujol-Luz and Barros-Cordeiro 2012) and *L. eximia* (Ramos-Pastrana et al. 2017). The duration of each color, however, varied among the species (Table 2), even among congenerics, which may be due to differences in temperature and humidity in which the larvae of each species were reared. Abiotic factors directly influence development time of insects of forensic importance, with temperature being more influential than diet, geographic origin and photoperiod (Villet et al. 2006; Nassu et al. 2014).

The maturation time of a pupa in a case involving a human death is fundamental for the determination of the PMI, and the knowledge about the internal processes of intrapuparial development enables more accurate PMI estimations, as intrapuparial development is the longest process during dipteran metamorphosis (Ferraz 1995; Salviano et al. 1996).

CONCLUSIONS

Our results set laboratory-controled values for the onset and duration of the different phases of the intrapuparial development of *Peckia pexata* that can be used as a reference for the estimation of the PMI in forensic cases where pupae or adults of this species are found on cadavers at a crime scene.

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