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MORPHOLOGY OF THE FIRST AND SECOND INSTARS LARVAE OF *PECKIA* (*PECKIA*) *CHRYSOSTOMA* (WIEDEMANN, 1830) (DIPTERA, SARCOPHAGIDAE)

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ABSTRACT. The morphology of larval stages of Diptera (Insecta) is little known, especially in the case of Sarcophagidae family larvae. Interest in sarcophagid larvae studies has increased with the step forward of forensic entomology, where they are considered potential indicators of the time of death. Forensic studies show *Peckia* (*Peckia*) *chrysostoma* as one of the most important species in the Neotropical region. However, taxonomic identification of larvae is difficult due to the lack of knowledge of its morphology and useful taxonomic characters for identify them. Sealed microscope slides of first and second instar larvae were performed. The pseudocephalon, cephalopharyngeal skeleton, spinules, spiracular atrium and the posterior spiracles were studied. Morphological characteristics of larval instars I and II were described and illustrated.

Key words: Larvae, *Peckia chrysostoma*, Systematics, Forensic Entomology.

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RESUMEN. La morfología de los estadios larvales de la mayoría de los Diptera es poco conocida, especialmente en el caso de las larvas de la familia Sarcophagidae. El interés por el estudio de las larvas sarcofágidas se ha incrementado con el avance de la entomología forense, éstas se consideran potenciales indicadoras del tiempo de muerte. Estudios forenses han resaltado que *Peckia* (*Peckia*) *chrysostoma* es una de las especies con mayor importancia en la región Neotropical. No obstante, la identificación taxonómica de sus estadios larvales, puede presentar dificultades principalmente por desconocimiento de su morfología y de caracteres taxonómicos útiles para su determinación. Se realizaron montajes laminares de las larvas de primer y segundo estadio de desarrollo. Se estudió el pseudocefalon, el esqueleto cefalofaríngeo, las espínulas, el atrio espiracular y los espiráculos posteriores. Se describen e ilustran sus estadios larvales I y II.

Palabras clave: Larva, *Peckia chrysostoma*, Sistemática, Entomología Forense.

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INTRODUCTION

The biological and ecological diversity of Diptera is remarkable (Courtney *et al*. 2009). Despite its great importance, the immature larval instars of many families of this order are little known and even more complicated on those groups of which taxonomy is unstable in adult stage. This is the case of sarcophagids, of which taxonomy is the subject of few studies (Lopes 1943, Jirón & Bolaños 1986, Leite & Lopes 1989, Méndez & Pape 2002, Sukontason *et al*. 2003, Szpila & Pape 2005a, 2005b, Pérez-Moreno *et al*. 2006, Szpila & Pape 2008).

Interest in the study of sarcophagid larvae has increased with the step forward in forensic entomology, where they are considered potential indicators of the time of death (Pérez-Moreno *et al*. 2006, Buenaventura *et al*. 2009). Sarcophagid females larviposit (lay larvae instead of eggs) on the corpse and they first feed in natural orifices and injuries. Larvae development occurs on the corpse; then, they move to less humid areas to pupate. Short time after, adults emerge and complete the life cycle. The development time of the species is the reference point for dating the death (Ames & Turner 2003). Biological information of the species involved will be useful only if correct taxonomical identification is performed.

The external and internal morphology of sarcophagid larvae has not been well studied. In the case of *Peckia* (*Peckia*) *chrysostoma* (Wiedemann, 1830), the third larval instar was described by Lopes (1943), first and second instars are described in this paper.

Several studies have shown the importance of *P*. (*P*.) *chrysostoma* in forensic entomology (Ferraz 1992, D'Almeida 1988, 1989, 1993). Larvae have been found in enphysematous and colliquative stages of corpse decomposition (pers. obs.), which is coherent to the synanthropic behavior of this fly (Oliveira *et al*. 2002).

Species of *Peckia* (Diptera, Sarcophagidae) have been recorded in the Neotropical region, however, *P*. (*P*.) *chrysostoma* was recorded also in Oceania (Pape 1996). In the New World, it is distributed from USA (Florida and Texas) to Argentina (La Patagonia and Misiones) (Buenaventura & Pape 2013). In Oceania, there are some records in Cook Island and the French Polynesia (Pape 1996). This species has been found during the advanced stages of decomposition in the Cerrado area of Brazilian Federal District (Barros *et al*. 2008), Cerrado area of Minas Gerais State near to Uberlândia (Barbosa *et al*. 2009), Campinas in São Paulo State (Moretti *et al*. 2008), and in Rio de Janeiro in Brazil (D'Almeida 1988, 1989, 1993, Ferraz 1992, 1993, 1995, Oliveira *et al*. 2002, Barbosa *et al*. 2009), Santa Cruz in Costa Rica (Jirón *et al*. 1983), and Villavicencio in Colombia (pers. obs.), as well as in fresh stage in the Colombian Amazon (Pape *et al*. 2004). *P*. (*P*.) *chrysostoma* show high synanthropy in Rio de Janeiro (D'Almeida 1984, 1988, 1989, 1993), high abundance in the community of carrion flies in the Valle de Aburrá (Colombia) (Ramírez-Mora et al. 2012) and recently it has been recognized its forensic importance in South America (Carvalho & Mello-Patiu 2008).

Given the forensic importance of this species, it is outstanding to add the description of larvae to contribute to the taxonomic identification in forensic entomology's research framework.

MATERIALS AND METHODS

Specimens reviewed. Individuals were obtained from a colony under laboratory conditions. Larvae collected in Colombia were raised in a five-day decaying pork meat substrate, wrapped in aluminium foil to avoid dehydration. Larvae from Mexico were raised with substrate of blood meal, egg powder, milk, formaldehyde, cellulose fiber and water. The material studied included 48 first instar larvae and 127 second instar larvae.

Mounting and description. Larvae mounting consisted in a tissue digestion through a KOH solution (10%) during one or two days. Then, the tissues dissolved were extracted with entomological needles and forceps up to be completely removed, for which small orifices were made in the larva cuticle, without touching mandibles or spiracles. Then, each larva was immersed 15 minutes in acetic acid (5%) to neutralize the KOH and washed with distilled water during 15 minutes. Individuals were dehydrated; first, with ethanol (30%) (15 minutes); then, (70-80%) (30 minutes) and; finally (96%) (10 minutes). Larvae remained in Eugenol until the mounting.

The material preserved was mounted in microscope slides and sealed with Entellan®. Superimposed structures were avoided to observe mandibles in side view and spiracles in front view. Descriptions were done using an Olympus CH30RF100 light microscope. Measures were performed with a graticule mounted in one of the microscope eye pieces. Studied individuals were deposited in the Entomological Collection of Tecnológico de Antioquia-Institución Universitaria (CE-TdeA, by its name in Spanish).

The morphological characters examined were the pseudocephalon, cephalopharyngeal skeleton, spinules, spiracular atrium and the posterior spiracles. The nomenclature followed the proposals of Teskey (1981) and Courtney *et al*. (2000) for ventral organs.

RESULTS

First instar

General appearance. Maximum length 4.24 ± 0.01 mm, maximum width 1.27 ± 0.01 (n = 48). Cream-colored. Larvae elongated, sub-cylindrical in cross section,

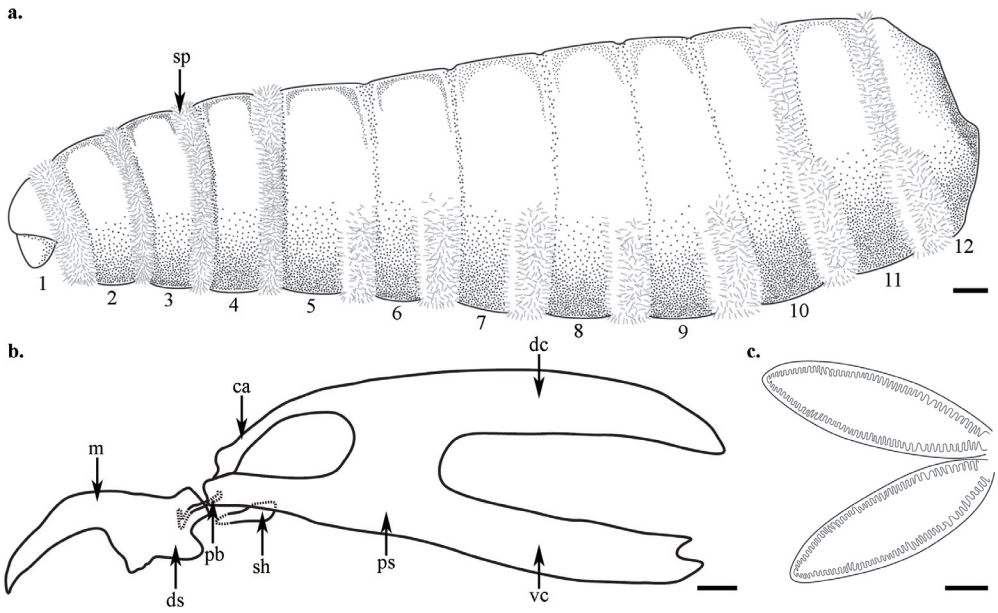

Figure 1. Morphology of the first instar larvae of *Peckia* (*Peckia*) *chrysostoma*. **a.** general pattern of spinules in left lateral view, **b.** cephalopharyngeal skeleton in left lateral view, **c.** posterior spiracle; ca: clypeal arch, dc: dorsal cornu, ds: dental sclerite, m: mouthhook, pb: parastomal bar, ps: pharyngeal sclerite, sh: subhypostomal sclerite, sp: pointed-apex spinules vc: ventral cornu. Scale bars 500µm.

slightly flattened dorsoventrally; their anterior region is blunt and the posterior truncated (Fig. 1a). First instar larvae may be recognized by the lack of obvious anterior spiracles and the simple posterior spiracles. The latter ones are represented by two simple apertures in the body wall, visible only with light microscope.

Pseudocephalon. It has a small pair of dorsal antennae and a pair of maxillary palpus in ventral position, each palpus shows two sensillae.

Thorax and abdomen. The prothorax is surrounded by a band of one and two sharp pointed spinules, grouped in a varying number and usually more densely arranged in the ventral part. Segments 2-4 with a posterior complete ring of sharp pointed spinules, 5-9 with an incomplete ring of sharp pointed spinules dorsally, 10-11 with a complete ring of sharp pointed spinules (Fig. 1a). Abdominal segments present sharp pointed spinules directed both anteriorly and posteriorly. Regions between bands do not show any spinules. Although all spinules are sharp pointed, the ones located in segments 2-4 and around the spiracular atrium are slightly more slender.

Posterior spiracles are located in a conical depression. The outer ring to spiracular atrium is covered with sharp pointed spinules. There are six dorsal tubercles; the outer pair is longer than the others. The ventral tubercles are not clearly developed, except the outer ones which are slightly longer than the inner ones. Posterior spiracles do not show ecdysial scar, peritreme is incomplete with a pair of oval spiracular openings (Fig. 1c).

Cephalopharyngeal skeleton. Uniformly pigmented cephalopharyngeal skeleton. Mandibles and maxillae are fused in a mouthhook structure with hook-like apex (Fig. 1b). Dental sclerite completely incorporated to the base of mouthhook structure. Half moon-like median subhypostomal sclerite only visible in dorsal view. Subhypostomal sclerite elongated. Pharyngeal sclerite heavily pigmented with a pointed anterior parastomal bar. The ventral edge of the pharyngeal sclerite can be slightly concave with ends curved ventrally. The dorsal cornu is more pigmented towards the ventral region and pointed posteriorly elongated. The clypeal arch is elongated and it reaches the parastomal bar.

Second instar

Differs from the first instar by the following:

General Appearance. Maximum length 11.71 ± 0.01 mm, maximum width 2.95 ± 0.01 (n = 127). Thoracic and abdominal segments show posterior rings of sharp pointed spinules (Fig. 2a).

Pseudocephalon. Similar to first instar larvae.

Thorax and abdomen. Segments 2-6 with a complete ring of spinules; 7-9 with an incomplete ring of spinules dorsally, 11-12 without spinules laterally (Fig. 2a). Posterior spiracles are located on a semi-sclerotized plate in a conical depression only visible in posterior view. The outer ring of spiracular atrium is covered with sharp pointed spinules. Each posterior spiracle consists of two elongated slots oriented vertically with openings arranged radially (Fig. 2c). Spiracular plates do not show ecdysial scar and have an incomplete peritreme.

Cephalopharyngeal skeleton. Dental sclerite separated from the mandibles (Fig. 2b). Subhypostomal sclerite pigmented, shorter than in the first instar larvae. Heavily pigmented infrahypostomal sclerite located in the middle of the pharyngeal sclerite anterior branches. Pharyngeal sclerite heavily pigmented with a pointed parastomal bar dorsally near the apex. The ventral edge of pharyngeal sclerite more concave than in first instar larvae, with ends curved ventrally. The dorsal cornu longer and

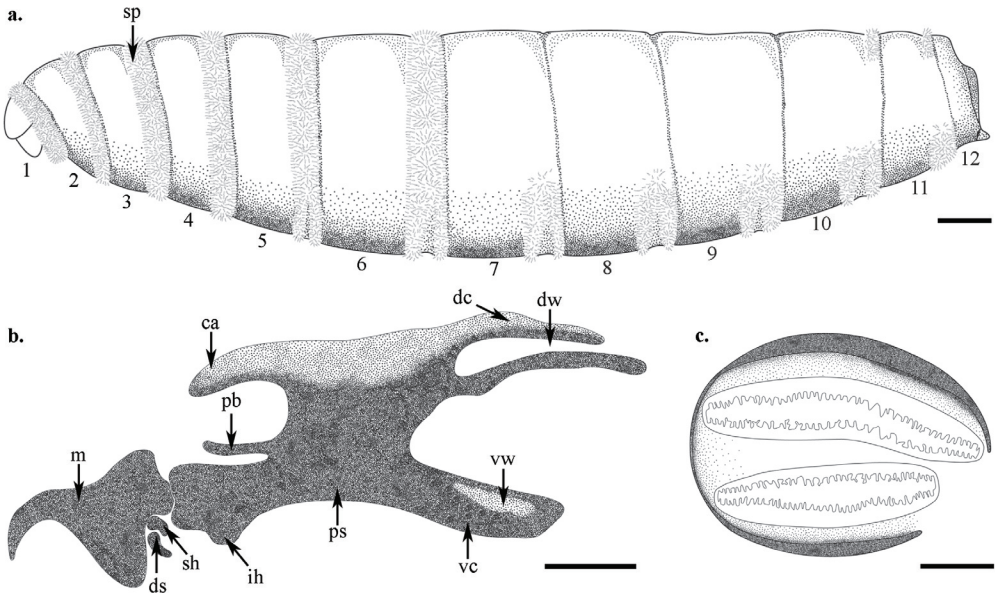

Figure 2. Morphology of the second instar larvae of *Peckia* (*Peckia*) *chrysostoma*. **a.** general pattern of spinules in left lateral view, **b.** cephalopharyngeal skeleton in left lateral view, **c.** posterior spiracle; ca: clypeal arch, dc: dorsal cornu, ds: dental sclerite, hs: hypopharyngeal sclerite, ih: infrahypostomal sclerite, m: mouthhook, pb: parastomal bar, ps: pharyngeal sclerite, sp: pointed-apex spinules arranged in rings, sh: subhypostomal sclerite, vc: ventral cornu. Scale bars 500µm in a, 100µm in b and 250µm in c.

wider than the ventral cornu. The ventral cornu is more pigmented towards the ventral region and truncated posteriorly, approximately twice longer than wide. Dorsal and ventral cornu have windows, the dorsal one is opened distally, but the ventral is closed distally. Clypeal arch elongated, but it does not reach the parastomal bar.

Material examined. MEXICO. **Chiapas**: 28 first instar larvae, Chiapa de Corzo, Km 2 de la carretera a La Angostura, COMEXA, 420 m, Bishop trap, decomposing liver, viii.2010, *M. S. Gómez Dorantes*. CE-TdeA. 107 second instar larvae, same data than before. COLOMBIA. **Meta:** 20 first instar larvae, Villavicencio, Estación Biológica Roberto Franco, sweep net, decomposing fish, 467 m, 04.v.2008, *E. Buenaventura*. CE-TdeA. 20 second instar larvae, same data.

DISCUSSION

Morphological characters mentioned in this paper correspond to the variation considered by Dahlem (1991) to define the family. In general, the morphology of the

structures studied also corresponds to that one found in other Diptera behaving like sarcosaprophagus as *P*. (*P*.) *chrysostoma.*

Partial descriptions of larvae of *P*. (*P*.) *chrysostoma* were provided by Lopes (1943) and Lopes (1982) with illustrations of the cephalopharyngeal skeleton of the third instar larvae, and by Leite & Lopes (1989) with SEM photos of externar surface of first larval instar. The detailed description of the arrange of spines (here as spinules) of Leite & Lopes (1989) is combined here, with new data and illustrations of the specific pattern of spinules in the body wall and posterior spiracles, as well as the description of internal structures as the cephalopharyngeal skeleton.

Some differences in morphology of larval instars I and II were found. Among them, the re-arrangement of spinules and the dorsal cornu which is elongate in first instar larvae and it reaches the parastomal bar. This latter character state may have a phylogenetic potential to define the genus *Peckia* (Giroux *et al*. 2010), which should be better studied due to some differences of shape and degree of development can be seen at subgeneric level.

As it has been found in other genera of Sarcophagidae (Giroux *et al*. 2010), there is a uniformity in the external morphological characters of adults, reason why the morphology of larvae and females continue been a potential source of information for designing any taxonomic tool and phylogenetic studies. The exploration of others characters through a scanning electron microscope (SEM) can be useful (Cantrell 1981).

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