doi:10.12741/ebrasilis.v11i3.808
e-ISSN 1983-0572
Publication of the project Entomologistas do Brasil
www.ebras.bio.br

Morphology and Physiology/Morfologia e Fisiologia

Ultrastructural characteristics and development time of immature stages of *Piophila casei* (Linneus) (Diptera: Piophilidae)

Karine Brenda Barros-Cordeiro[1]✉, Welinton Ribamar Lopes[2] & Sônia Nair Báo[3]

1. Laboratório de Entomologia 2-Diptera, Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade de Brasília. 2. Departamento de Ecologia, Instituto de Ciências Biológicas, Universidade Federal de Goiás. 3. Laboratório de Microscopia Eletrônica, Departamento de Biologia Celular, Instituto de Ciências Biológicas, Universidade de Brasília.

EntomoBrasilis 11 (3): 201-208 (2018)

Abstract. *Piophila casei* (Linneus), known as the cheese skipper fly, is a sarcosaprophagous dipteran, meaning it has a wide range of feeding habits: proteins, decaying animal and vegetable matter, and feces. The biological aspects of *P. casei* are relevant to several fields, including forensic entomology and human and veterinary medicine. This work presents the ultrastructure characteristics of *P. casei*, compares the structures present during its development using light microscopy and Scanning Electron Microscopy (SEM) and reports the development time of eggs, larvae and pupae under controlled laboratory conditions (23°C, 60% relative humidity and 12 h of light). Colony of adults previously maintained in the laboratory (third generation) was used in this study. The morphology of the insect and of structures such as the cephalopharyngeal skeleton, the anterior and posterior spiracles and the structures and organs of the head, thorax and abdomen of the first, second and third instar larvae were characterized. The total development time of the egg from laying until the emergence of the adult was 448 hours (18.66 days); this included 8 hours for embrionary development, 248 hours for larval development and 192 hours for pupal development.

Keywords: Cheese Skipper Fly; Forensic Entomology; Larvae; Medical-Veterinary; Morphology.

Ultraestrutura e tempo de desenvolvimento de estágios imaturos de *Piophila casei* (Linneus) (Diptera: Piophilidae)

Resumo. *Piophila casei* (Linneus), conhecida como “cheese skipper fly”, é uma mosca sarcosaprófaga, possui habito alimentar diverso. Pode alimentar-se de proteínas diversas, matéria animal e vegetal em decomposição e fezes. Os aspectos biológicos de *P. casei* são relevantes principalmente para a entomologia forense, medicina humana e veterinária. Este trabalho apresenta características da ultraestrutura de *P. casei*, comparando as estruturas dos três instares larvais do desenvolvimento, utilizando microscopias de luz e eletrônica de varredura (MEV), além de descrever o tempo de desenvolvimento de ovos, larvas e pupa sob condições controladas de laboratório (23 °C, 60% humidade relativa e 12 h de luz). A morfologia geral do inseto e estruturas como o esqueleto cefalofaríngeo, os espiráculos anterior e posterior, as estruturas e órgãos da cabeça, tórax e abdômen da larva de primeiro, segundo e terceiro instares foram caracterizadas. O tempo total de desenvolvimento de ovo até a emergência do adulto foi de 448 horas (18,66 dias), sendo 8 horas para desenvolvimento embrionário, 248 horas para desenvolvimento larval e 192 horas para desenvolvimento pupal.

Palavras-Chave: Entomologia Forense; Larva; Medico-Veterinário; Morfologia; Mosca do Queijo.

The family Piophilidae includes 14 genera and 83 species (Pape *et al.* 2011), of which 4 genera and 7 species have been recorded in Brazil (Wendt 2017). The genus *Piophila* has only two species, *Piophila megastigmata* McAlpine and *Piophila casei* (Linneus); the latter is known as the “cheese skipper fly” because it has been used in cheese production (Miokovic *et al.* 1997; Ottogalli 2001; Papavero & Pimentel 2002). The specie *P. casei* is considered a sarcosaprophagous dipteran; it feeds on a wide variety of substrates, mainly proteinaceous and fecal, and it is found in decomposing organic matter, both plant and animal (Zumpt 1965; Skevington & Dang 2002).

Due to its food and habitat behavior, *P. casei* is considered important: i) in forensic entomology, which uses its life cycle data to aid in estimating the interval since death (Martin-Vega 2011; Prado e Castro *et al.* 2012; Dekeirsschieter *et al.* 2013); ii) in medicine, because it is a vector of micropathogens and can cause gastrointestinal, nasal and urinary myiasis (Zumpt 1965; Greenberg 1971; Perez 1971; Saleh & Sibae 1993; Candida & Agatino 2004; Passos *et al.* 2004); and iii) for the economy, since it causes great damage in the dairy and meat industries (Simmons 1927; Zuska & Laštovka 1965; Haines & Rees 1989).

Morphological and developmental studies of *P. casei* may assist in the search for a method to control this species in regions in which it causes medical and economic damage (Zuska & Laštovka 1965; Greenberg 1971; Candida & Agatino 2004), in addition to assisting in forensic entomology studies. Currently, information

Edited by:
Alberto Silva-Neto
Article History:
Received: 15.viii.2018
Accepted: 05.xi.2018

✉ **Corresponding author:**
Karine Brenda Barros-Cordeiro
karine.brenda22@gmail.com
No ORCID record

Funding agencies:
Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq; Financiadora de Estudos e Projetos/FINEP; Fundação de Amparo à Pesquisa do Distrito Federal/FAPD-DF; Coordenação de Aperfeiçoamento de Pessoal/CAPES

on the morphology and developmental timing of the species is incomplete and is largely restricted to the morphology of the adults, perhaps because knowledge concerning the immature forms is scarce, especially for first and second instar larvae. The largest amount of information is available for the third larval instar and the adult fly (Hennig 1943; Brindle 1965; Cortinhas *et al.* 2016; Corriço *et al.* 2015; Zumpt 1965; Sukontason *et al.* 2001; Russo *et al.* 2006; Martín-Vega *et al.* 2012).

The present study describes the minimum total development time of *P. casei* (egg, larva and pupa) under controlled conditions of rearing and characterizes the morphological changes that occur during the larval phase from the first to the third instar.

MATERIAL AND METHODS

Adults from third generation of colony that had been maintained in the laboratory were used in this study. The animals were housed in a BOD incubator at 23.0 ± 1.0° C, 60 ± 10% RH and a 12:12 L:D cycle. We reared the colonies on a meat substrate using the methods of Barros-Cordeiro & Pujol-Luz (2010).

Obtaining and fixing samples for study. Five larvae (neolarvae) were fixed at intervals of two hours from the moment of hatching (zero hours) until the 92nd hour (n = 235) and every 12 hours thereafter until the start of pupation (n = 65). The larvae were fixed in water warmed to 80°C and later preserved in 70% ethanol. All the larvae fixed at 24 hours after hatching were digitalized and measured from the cephalic capsule to the anal papillae using ImageJ© software.

Preparation of samples for light microscopy. Samples of larvae from the three instars were clarified in lactic acid, dissected and mounted on slides with Entellan®. The pupae were dissected and maintained in 70% ethanol. The slides were examined and photographed under a Leica DM 2000 optical microscope.

Preparation of samples for Scanning Electron Microscopy (SEM). Larvae from the three instars were fixed in Karnovsky's fixative (2.5% glutaraldehyde, 4% paraformaldehyde, 3% saccharose and 5 mM $CaCl_2$ buffered to pH 7.2 with 0.1 M sodium cacodylate 0.1 M and pH 7.2) for 24 hours. The samples were washed in buffer and immersed in 1% osmium tetroxide in 0.1M cacodylate buffer at pH 7.2 for one hour, then washed in distilled water and dehydrated in increasing concentrations of acetone (30 - 100%) for one minute at each concentration. The material was then subjected to critical point drying with CO_2, mounted on stubs, metallized with gold and viewed under a scanning electron microscope (JEOL JSM 7001)

Terminology. The terminology used to describe the morphology of larval instars was adapted from Imms (1957), McAlpine *et al.* (1981), Brown *et al.* (2010) and Ozerov & Norrbom (2010).

RESULTS

Larvae from the three instars present 12 segments: a cephalic region (cephalic lobe), three thoracic segments (from segments II to IV) and eight abdominal segments (segments V to XII) (Figures 1A, 2A, and 3A).

First larval instar (L1). Mean length of 1.14 ± 0.40 mm (Table 2). Lateral creeping welt present from segments VII to XI. Rows of "creeping" spines with a single tip present in the ventral region of segments V to XII (Figure 1C). The cephalic lobe presents a pair of antennae arranged dorsally to the maxillary palps and anterolaterally to the body (Figure 1B). The antenna is composed of two structures, a basal ring and an apical dome (Figure 1E). One pair of maxillary palps is seen; the palps are formed by a set of basiconic and coeloconic sensilla arranged in an overlapping manner in the central region of the maxillary palp (Figure 1D). Near the set of sensilla, two accessory (antennal or mandibular) sensilla are seen. In the lower portion of the pseudocephalic region is the facial mask (=oral ridge) and the ventral organ, with two sensilla localized in the middle of a papilla (Figure 1F); oral comb present and undeveloped (Figure 1B), and the labial lobe is observed. The cephalopharyngeal skeleton is not pigmented and is slightly sclerotized in the region of the dorsal arch and in the distal portions of the dorsal and ventral cornua (Figure 4A-B). The maxilla is composed of a pair of symmetrical parts, articulated at the base and presenting at their tip a pair of teeth, each with more than one point at the apex, oriented in the ventral direction (Figure 4B). The mandible is wide such as the maxilla and is formed by a pair of symmetrical parts and a rounded apex. The hypostomal sclerite is attached to the hypopharyngeal sclerite. The pharynx and the clypeal arch are complete. Dorsal and ventral cornua are approximately the same size. There is a pair of Keilin's organs in the middle portion of each thoracic segment, and from each slit in the organ three sensilla emerge (Figure 1G). Papillae appear on the last abdominal segment, with three pairs of dorsal tubercles, three pairs of ventral tubercles and one medium pair; a basiconic sensillum is present in each papilla (Figure 1H-J). The posterior spiracle presents two slits (Figure 1K, 4E). The respiratory slits are interspersed with trichoid sensilla. The minimum duration of the instar was 46 hours (Table 1).

Pharate 1st/2nd larval instar. Mean length of 1.74 ± 0.24 mm (Table 2). A dorsal and ventral cornua developing. Maxilla and mandible appear larger and under development. The minimum duration of the transition between these two instars was two hours (Table 1).

Second larval instar (L2). Mean length of 2.77 ± 0.76 mm (Table 2). Lateral creeping welt present at segments V to XI. Rows of "creeping" spines with single tips present in the ventral region from segments VI to XII (Figure 2A). The pseudocephalic segment can be seen to have two cephalic lobes in a lateral-dorsal position (Figure 2C). There is an antenna in each lobe arranged dorsally to the maxillary palp. The antenna is composed of two structures, a basal ring and a dome; the dome is proportionally larger than the basal ring, and both are smaller than the projection of the antenna (Figure 2B). A sensillum appears in the outer part of the basal ring and between the basal ring and the antenna projection. A pair of maxillary palps can be observed; these are formed by a set of basiconic and coeloconic sensilla arranged in an overlapping manner (Figure 2D). There is also a pair of accessory sensilla. In the lower region of the pseudocephalic segment there is a facial mask (=oral ridge) with horizontal ridges; the oral comb is slightly more developed than in the previous instar; a labial lobe and the ventral organ, which is formed by two small digits over a papilla, are present (Figure 2C). Suprabuccal teeth are present (Figure 4C). The maxilla is sclerotized and pigmented with a pronounced curve and a rounded apex. The mandible is fused to the maxilla. The dental sclerite is pigmented and sclerotized. The hypostomal sclerite is wide, sclerotized and pigmented, and it has the shape of a bar linked to the pharyngeal sclerite. It appears just below the ocular depression, which is sclerotized, pigmented and pronounced, above the hypostomal sclerite. It presents a slightly pigmented and sclerotized clypeal arch. The dorsal cornu is pigmented and sclerotized. The ventral cornu presents a not very pronounced posterior tip that is sclerotized and pigmented. The median incision between the cornua is sclerotized, pigmented and pronounced (Figure 4C). A pair of fanlike prothoracic spiracles is seen on the first thoracic segment, and its tip presents ten smooth papillae that resemble a horseshoe (Figure 2F). In the ventral region of each of thoracic segments there is a pair of Keilin's organs, and three sensilla emerge from each slit in the organs (Figure 2E). In the posterior region of the last abdominal segment, there is one pair of dorsal papillae (Figure 2G), one pair of lateral papillae and one pair of internal papillae. There is a pair of posterior spiracles; on each spiracle three apertures

(slits) can be seen, and each slit contains an undeveloped trichoid sensillum. The slits are interspersed with four trichoid sensilla (Figure 2H). The minimum duration of this instar was 26 hours (Table 1).

Pharate 2nd/3rd larval instar. Mean length of 2.22 ± 1.12 mm (Table 2). The cephalopharyngeal skeleton is similar to that of the second instar, but it presents formation of the maxilla, mandible and dorsal and ventral cornua that characterizes the next instar. The minimum duration of the transition was six hours (Table 1).

Third larval instar (L3). Mean length of 4.91 ± 1.87 mm (Table 2). Lateral creeping welt present from segments V to XI. Trailing "creeping" spines present in the ventral regions of segments IV to XII (Figure 3A, H). The pseudocephalic segment is seen in a lateral-dorsal position with two cephalic lobes (Figure 3B). In each lobe, the anterolateral region of the body presents an antenna arranged dorsally to the maxillary palp. The antenna is composed of two structures, a basal ring and a dome; the dome is proportionally larger than the basal ring, and both are much smaller than the projection of the antenna (Figure 3C). A sensillum appears in the outer part of the basal ring and between the basal ring and the antenna projection. The maxillary palps present a set of basiconic and coeloconic sensilla surrounded by smoothed folded structures similar to crests, and a pair of accessory sensilla is present on the edge of each crest (Figure 3D). In the lower region of the pseudocephalic segment there is a facial mask (=oral ridge) with ridges horizontally and vertically, an oral comb more developed than in the previous instar, a labial lobe, and the ventral organ, which is formed by two small digits over a papilla (Figure 3E). The maxilla is slightly curved, robust, strongly sclerotized and pigmented; in the anterior region it presents a rounded apex, and in the posterior region it is wider. The mandible is fused to the maxilla and has a pointed posterior apex. The dental sclerite is similar to a robust inverted L. The labial sclerite is well constituted, sclerotized and pigmented. The hypostomal and infrapharyngeal sclerites are joined to the pharyngeal sclerite. The outer edge of the dorsal cornua is less sclerotized and pigmented than the inner region, and the same occurs with the ventral cornua. The dorsal cornua present a pointed apex, and the ventral cornua have rounded tips. The median incision is strongly pronounced, with a more closed curvature than in the previous instar (Figure 4D). The first thoracic segment presents a pair of fanlike spiracles with the extremity composed of 10 to 11 smooth papillae, the form of which resembles a horseshoe (Figure 3F). The ventral region of each of thoracic segments exhibits a pair of Keilin's organs (Figure 3G). From each slit in the organ emerge three sensilla, and near the organ there is a pair of coeloconic sensilla. In the posterior region of the last abdominal segment, the papillae are more developed than in the previous instars; they contain one pair of dorsal papillae, one lateral pair and one internal pair (Figure 3J). There is a pair of posterior spiracles; on each spiracle, three apertures (slits) can be seen. Each slit contains a well-developed trichoid sensilla, and the slits are interspersed with trichoid sensilla (four) (Figures 3I, 4F). The spiracle button is visible. The minimum duration of the instar was 96 hours

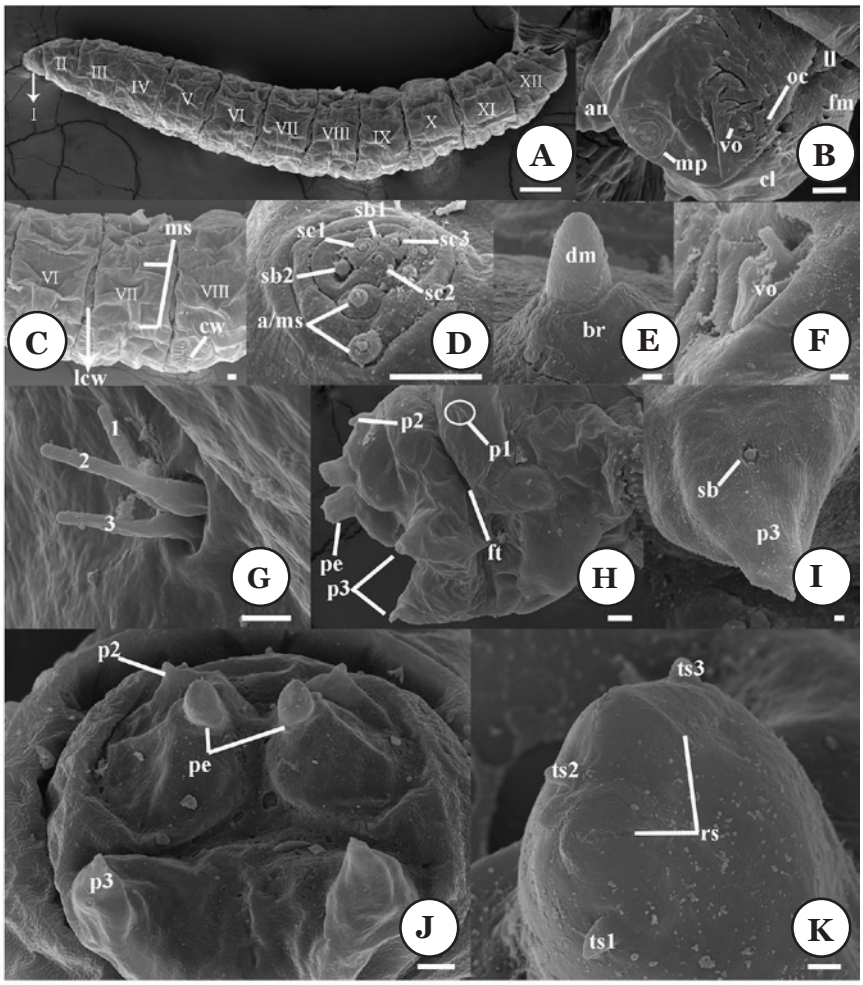

Figure 1. SEM micrographs of the first instar of *Piophila casei*. (**A**) Larval body composed of twelve segments; (**B**) cephalic region; (**C**) detail of abdominal segments; (**D**) detail of sensilla in maxillary palpus; (**E**) antenna; (**F**) detail of ventral organ; (**G**) Keilin's organ in thorax segment; (**H**) side view of abdominal segment; (**I**) abdominal papilla and basiconic sensilla; (**J**) back view of abdominal segment; (**K**) detail of posterior spiracle. **Abbreviations**: I-XII, body segments; 1-3, sensilla of Keilin's organ; an, antenna; a/ms, antennal or mandibular sensilla; br, basal ring; cl, cephalic lobe; cw, ventral creeping; dm, antennal dome; fm, face mask (oral ridge); lcw, lateral creeping welt; ll, labial lobe; mp, maxillary palpus sensilla; ms, muscle scars; oc, oral comb; p1-3, posterior papillae; pe, posterior spiracle; rs, respiratory slit; sb, basiconic sensilla; sb1-2, basiconic sensilla of maxillary palpus; sc1-3, coeloconic sensilla of maxillary palpus; ts1-3, trichoid sensillae; vo, ventral organ. **Scales:** A, 100 µm; B, C, H, and J, 10 µm; D, E, F, G, I, and K, 1 µm.

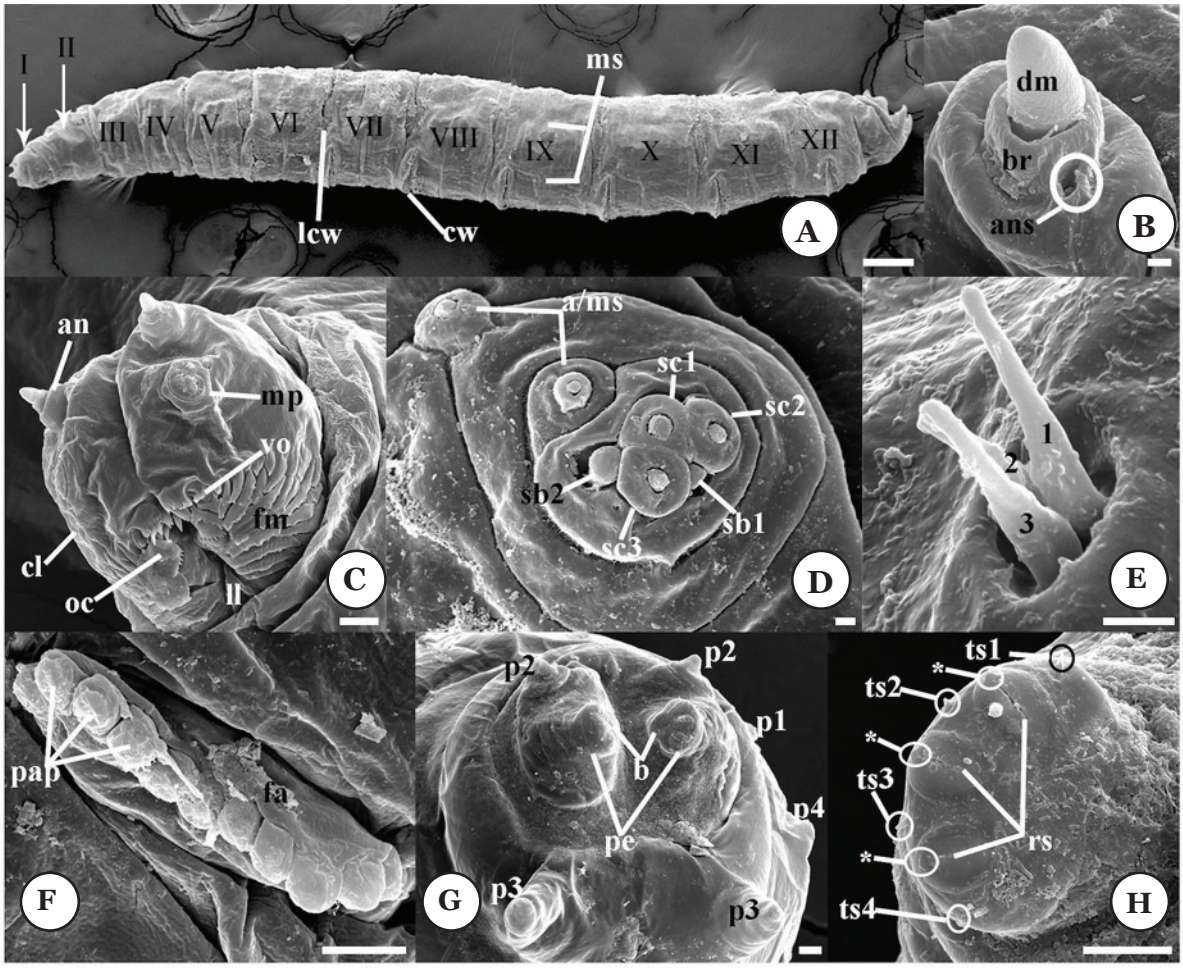

Figure 2. SEM micrographs of the second instar of *Piophila casei*. (A) Larval body composed of twelve segments; (B) antenna; (C) cephalic region; (D) detail of sensilla in maxillary palpus; (E) Keilin's organ; (F) detail of anterior spiracle; (G) back view of the larva; (H) detail of posterior spiracle. Abbreviations: I-XII, body segments; 1-3, sensilla of Keilin's organ; an, antenna; a/ms, antennal or mandibular sensilla; ans, antenna of sensilla; b, button; br, basal ring; cl, cephalic lobe; cw, ventral creeping; dm, antennal dome; fa, fan-shaped structure; fm, face mask (oral ridge); ft, folding tegument; ko, Keilin's organ; lcw, lateral creeping welt; ll, labial lobe; mp, maxillary palpus sensilla; ms, muscle scars; oc, oral comb; p1-4, posterior papillae; pap, papillae; pe, posterior spiracle; rs, respiratory slit; sb1-2, basiconic sensilla of maxillary palpus; sc1-3, coeloconic sensilla of maxillary palpus; ts1-4, trichoid sensilla; vo, ventral organ; * undeveloped trichoid sensilla. Scales: A, 100 µm; B, D and E, 1 µm; C, F, G and H, 10 µm.

(Table 1). The pre-pupal stage averaged 5.69 mm and lasted approximately 72 hours (Table 1).

DISCUSSION

The identification of Piophilidae species is often restricted to the morphology of the adults, perhaps because knowledge of the immature forms is scarce, especially with respect to the first two larval instars. Prado e Castro *et al.* (2012) believe that *P. casei* and *P. megastigmata* have been confused in many studies due to their morphological similarity.

Although they are very similar, it is possible to distinguish *P. casei* and *P. megastigmata*: i) the maxillary palps of first instar larvae (L1) of *P. casei* present two accessory coeloconic sensillum, whereas *P. megastigmata* presents one coeloconic sensillum and one basiconic sensillum; ii) in *P. casei* it was not possible to observe the presence of the clypeal arch in L1, but the clypeal arch was observed in L1 of *P. megastigmata* (Paños *et al.* 2013). The presence of this structure in *P. megastigmata* is probably due to the observation made in an L1/L2 pharate. Observing and analysing the cephalopharyngeal skeleton of these species in the first instar is very difficult because they have no pigmentation. In the present study, analysis of *P. casei* specimens was only possible after clarifying the samples and using light saturation and contrast to acquire the images (Figure 4A-B).

The larvae of first instar *Piophila* are described as being metapneustic with a pair of posterior spiracles; in the samples analyzed here, this information was confirmed. However, a rudimentary spiracle anterior has been observed in other species of flies, including *Fannia canicularis* (Linnaeus), *Chrysomya megacephala* (Fabricius), *Chrysomya bezziana* (Villeneuve) and *Lucilia cuprina* (Wiedemann) (Kitching 1976; Sukontason *et al.* 2005; Grzywacz *et al.* 2012). Kitching (1976) suggested that this structure might play a role in the breathing process in L1.

In the second larval instar (L2): i) the crests of the cephalic region of *P. casei* are oriented horizontally towards the oral cavity, whereas in *P. megastigmata* some crests have a vertical orientation; ii) the connection between the maxilla and the mandible (dorsal region) of the cephalopharyngeal skeleton of *P. casei* is almost a straight line, whereas in *P. megastigmata* the connection between the maxilla and the mandible has a depression, forming a lying-down "S"; iii) the angle between the maxilla and the dental sclerite of *P. casei* is close to 90˚, but in *P. megastigmata* this angle is greater than 90˚; iv) the anterior spiracle of *P. casei* presents 10 papillae, whereas that of *P. megastigmata* presents 11 papillae (Paños *et al.* 2013).

In the third instar (L3): i) the ocular depression of the cephalopharyngeal skeleton in *P. casei* is not very pronounced, and the maxilla, mandible and dental sclerite are more robust than those in the anterior instar, whereas in *P. megastigmata* the ocular depression is very pronounced and the maxilla, mandible and dental sclerite are very similar to those of the previous instar; ii) the anterior respiratory spiracle of *P. casei* has 9 to 11 papillae and that of *P. megastigmata* has 12 papillae; iii) in each slit of the posterior respiratory spiracle of *P. casei*, it is possible to observe a well-developed sensillum, whereas there are no

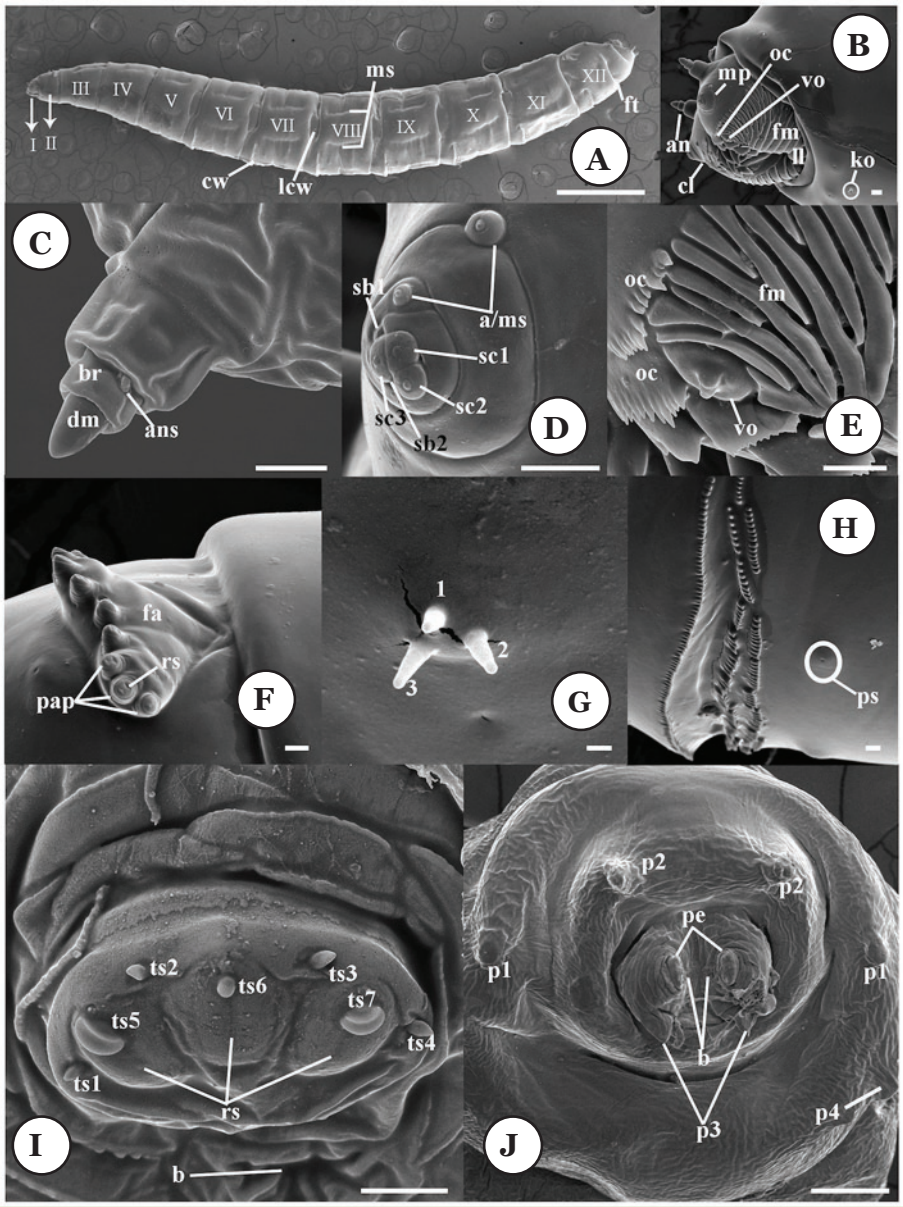

Figure 3. SEM micrographs of the third instar of *Piophila casei*. (A) Larval body composed of twelve segments; (B) cephalic region; (C) antenna; (D) detail of sensilla in maxillary palpus; (E) detail of ventral organ, oral comb and face mask; (F) detail of anterior spiracle; (G) Keilin's organ; (H) spines of the thorax; (I) detail of posterior spiracle; (J) back view of the larva. Abbreviations: I-XII, body segments; 1-3, sensilla of Keilin's organ; an, antenna; a/ms, antennal or mandibular sensilla; ans, antenna of sensilla; b, button; br, basal ring; cl, cephalic lobe; cw, ventral creeping; dm, antennal dome; fa, fan-shaped structure; fm, face mask (=oral ridge); ft, folding tegument; ko, Keilin's organ; lcw, lateral creeping welt; ll, labial lobe; mp, maxillary palpus sensilla; ms, muscle scars; oc, oral comb; p1-4, posterior papillae; pap, papillae; pe, posterior spiracle; ps, placodea sensillum; rs, respiratory slit; sb1-2, basiconic sensilla of maxillary palpus; sc1-3, coeloconic sensilla of maxillary palpus; ts1-7, trichoid sensilla; vo, ventral organ. Scales: A, 1 mm; B, C, D, E, F, H and I, 10 µm; G, 1 µm; J, 100 µm.

sensilla in the respiratory slits of *P. megastigmata* (Paños *et al.* 2013); iv) in addition to these characteristics, Keilin's organs were only observed in the thoracic segments of *P. casei,* while in *P. megastigmata* this organ was observed in all larval segments according to Paños *et al.* (2013).

The difficulty of identifying the immature forms of various species of *Piophila* also extends to the larval instar. This is probably because it has very small or transparent structures, especially in the case of the first two larval instars of *P. casei*.

Identification of the larval instar of *P. casei* can be performed by observing the following structures: in L1, the cephalopharyngeal skeleton is not pigmented; from L1 to L3, the antenna, the maxillary palp and the oral comb are progressively more developed. The posterior spiracle of L2 presents sensilla in the discrete or absent respiratory slits. In L3, the posterior spiracle presents sensilla, evident and developed, in each respiratory slit.

The present morphological analyses of larvae in the first and second instars are inconsistent with the observations reported by Simmons (1927). That study described the cephalopharyngeal skeleton of L1 as pigmented and stated that insufficient information about L2 was obtained to allow a comparison. However, the present observations coincide with the schematic drawing of the cephalopharyngeal skeleton of L2 presented by Oliveira-Costa & Queiroz (2008). The morphology of L3 observed and described by us is similar to the morphology observed and described in other works (Simmons 1927; Zumpt 1965; Sukontason *et al.* 2001; Martín-Vega *et al.* 2012).

The characteristics and arrangement of the spicules, intersegmental rings and creeping welts and the presence of an anterior spiracle with four to 12 digits and a terminal segment with three to four pairs of papillae surrounding the posterior spiracles can be used to distinguish the larvae of *Piophila* from the larvae of other Piophilidae, according to the larval identification keys of Hennig (1943), Brindle (1965), Smith (1986) and Martin-Vega *et al.* (2012). Differences in the complexity of the papillae may help in identifying the larval stage. As already reported by other authors the arrangement is a potential tool for taxonomic use (Hennig 1943, Brindle 1965, Grzywacz *et al.* 2015).

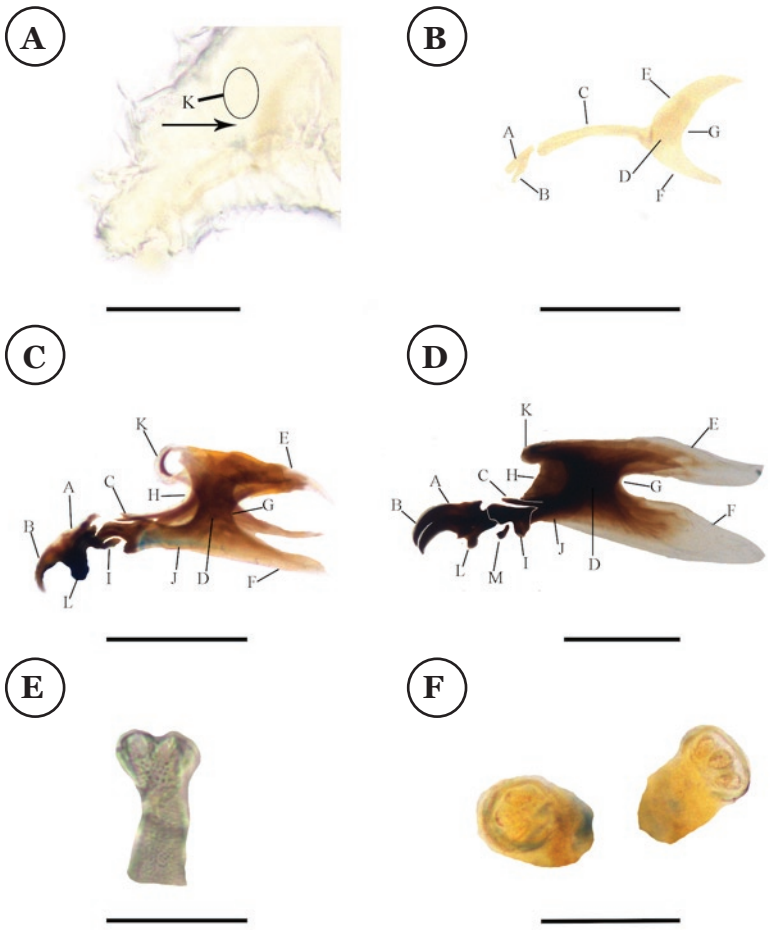

Figure 4. Cephalopharyngeal skeleton (lateral view) and posterior spiracle of *Piophila casei*. (A) Cephalopharyngeal skeleton of the first instar; (B) detail of the cephalopharyngeal skeleton of the first instar; (C) cephalopharyngeal skeleton of the second instar; (D) cephalopharyngeal skeleton of the third instar; (E) posterior spiracle of the first instar; (F) posterior spiracle of the third instar. Abbreviations: A, mandible; B, maxilla; C, hypostomal sclerite; D, pharyngeal sclerite; E, dorsal cornua; F, ventral cornua; G, median incision; H, ocular depression; I, hypopharyngeal sclerite; J, infrapharyngeal sclerite; K, clypeal arch; L, dental sclerite; M, labial sclerite. Scales: A, B, C, D and F, 50 µm; E, 20 µm.

Table 1. Minimum development time (in hours) of *Piophila casei* at each stage of development at 23 ± 1.0˚C.

Phase	Stage	Hours	Average		S.D.	Range	n
Eggs	Embryo	8				(08-15)	765
Larvae	First instar	46	22.21	±	13.39	(*00-46)	110
	Pharate 1º/ 2º instar	2	46.67	±	0.94	(46-48)	6
	Second instar	26	65.33	±	11.18	(48-104)	87
	Pharate 2º/ 3º instar	6	80.86	±	9.61	(74-104)	7
	Third instar	96	114.83	±	29.39	(80-176)	44
	Pre-pupae	72	210.88	±	22.52	(176-248)	33
Pupae	Pupae	192				(248-440)	250

S.D., standard deviation; *n*, number of samples; *, phase start.

Table 2. Mean length (in mm) of *Piophila casei* for each larval instar.

Phase		Average		S.D.	Range	n
Larvae	First instar	1.14	±	0.40	(0.44 - 2.06)	110
	Pharate 1º/ 2º instar	1.74	±	0.24	(1.45 - 2.00)	6
	Second instar	2.77	±	0.76	(1.23 - 4.15)	87
	Pharate 2º/ 3º instar	2.22	±	1.12	(1.21 - 2.81)	7
	Third instar	4.91	±	1.87	(1.48 - 7.97)	44
	Pre-pupae	5.69	±	0.93	(3.34 - 7.03)	33

S.D., standard deviation; n, number of samples.

Like *P. casei*, larvae of *Prochyliza nigrimana* (Meigen) are necrophagous and have been associated with human cadavers (Martin-Vega *et al.* 2011; Prado e Castro *et al.* 2012; Dekeirsschieter *et al.* 2013). Larvae of third instar *P. nigrimana* presents in the last abdominal segment a ventral pair of papillae that are slightly directed ventrally, and the cephalopharyngeal skeleton dorsal edge of the mouth hook is slightly convex in its basal part, whereas in *P. casei* the ventral pair of papillae is slightly directed posteriorly and the cephalopharyngeal skeleton dorsal edge of the mouth hook is slightly concave in its basal part (Martin-Vega *et al.* 2012).

The mean lengths of *P. casei* larvae at L1, L2 and L3 were 1.14 mm, 2.77 mm and 4.91 mm, respectively. However, Simmons (1927) reported the lengths of L1, L2 and L3 larvae as 1.5-1.8 mm, 4 mm and 10 mm, respectively, whereas Peterson (1960) and Martín-Vega *et al.* (2012) reported the length of L3 larvae as 8 mm and 7.6 mm, respectively. These differences are likely to be associated with the diet consumed by the immature forms and/or with other abiotic conditions that influence larval development, for example, light and temperature.

The development time of immature stages described in other works appears to be little different from the development time noted in this work considering the different temperatures and diets used in the experiments. Moreover, studies have shown that *P. casei* displays great variation in development time as a function of temperature; larvae of this species can survive for 10 days at 4°C, 30 days at 5°C, 6 months at 9-10°C and 23 days at 15°C (Sacchi *et al.* 1971; Hegazi *et al.* 1978; Busvine 1980). In this study, the minimum development times of the embrionary, larval and pupal phases of *P. casei* under controlled conditions of 23 ± 1°C, 60 ± 10% RH and a 12-h photoperiod were 8 h, 248 h (10.3 days) and 192 h (8 days), respectively.

Belcari & Antonelli (1992) observed that the development of the embryo took place occurred over 1.2 days at 25˚C, 1 day at 28˚C and 0.9 day at 33˚C; Costa *et al.* (1986) recorded 2 days at 27˚C. Using diets containing different protein sources and fermented milk the time required for larval development was between 8.4 and 14.3 days at 27˚C (Hegazi *et al.* 1978); Costa *et al.* (1986) observed a larval development time of 6 days at 27˚C in meat.

According to Simmons (1927), in the warmest period of the year, the embrionary development of *P. casei* occurs in less than one day, the larval stage lasts 7 days, and the pupal stage lasts approximately 5 to 8 days. Russo *et al.* (2006) worked with this species under controlled conditions of 25 ± 1°C, 70 ± 5% RH and an 8-h photoperiod and obtained results that differ from ours; the total development time of *P. casei* under their conditions was 22.7 days (33.6 h for the egg, 14.4 days for the larva and 6.9 days for the pupa). These differences in the observed development times are most likely related to differences in the photoperiod and in the other conditions under which the insect was raised, with special attention to the diet offered to the larval phase. Biotic and abiotic variations greatly influence the time required for insect development.

ACKNOWLEDGMENTS

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq, to Financiadora de Estudos e Projetos/FINEP, to Fundação de Amparo à Pesquisa do Distrito Federal/FAPD-DF, to Coordenação de Aperfeiçoamento de Pessoal/CAPES, to Postgraduate Program in Animal Biology for grants and to Laboratório de Entomologia-Diptera UnB.

REFERENCES

Barros-Cordeiro K.B. & J.R. Pujol-Luz, 2010. Morfologia e duração do desenvolvimento pós-embrionário de *Chrysomya megacephala* (Diptera: Calliphoridae) em condições de laboratório. Papéis Avulsos Zoologia, 50: 709-717.

Belcari, A. & R. Antonelli, 1992. Limiti termici ed optimun di sviluppo degli stadi preimmaginali di *Piophila casei* (L.), (Diptera Piophilidae), p. 107-117. In: Atti 5 Simposio sulla difesa antiparassitaria nelle industrie alimentari e la protezione degli alimenti, Piacenza, Italy.

Brindle, A., 1965. Taxonomic notes on the larvae of British Diptera No. 21-The Piophilidae. Entomology 78:158-160

Brown, B.V., A. Borkent, J.M. Cumming, D.M. Wood, N.E. Woodley & M.A. Zumbado, 2010. Manual of Central American Diptera, 2:1031-1039.

Busvine, J.R., 1980. Houseflies and blowflies. Insect and hygiene. The biology and control of insect pests of medical and domestic importance. Chapman & Hall, London, 211 p.

Candida, V.M. & R.R. Agatino, 2004. *Piophila casei* L. (Diptera: Piophilidae) monitoring in Cheese Ripening Storehouses. IOBC/WPRS Bulletin Journal, 27, 109-114.

Cortinhas, L.B., P.M. Mendonça Barbosa & M.M.C. Queiroz, 2016. Ultrastructure Of Immature Stages Of The Black Dump Fly, Ophyra Aenescens (Wiedemann, 1830) (Diptera: Muscidae: Azeliinae). Acta Tropica, 158: 125-129. DOI: https://doi.org/10.1016/j.actatropica.2016.02.022.

Carriço, C., P.M. Mendonça, J.R. Santos-Mallet & M.M.C. Queiroz, 2015. Ultrastructural studies of some character of Diptera (Muscidae) of forensically importance. Acta Tropica, 142: 96-102.

Costa, P.R.P., R. Gurvitz, G. Muller & P.B. Ribeiro, 1986. Ciclo vital de Piophila casei L. (Diptera: Piophilidae). Arquivos Brasileiros Medicina Veterinaria e Zoologia 38: 191-196.

Dekeirsschieter, J., C. Frederickx, F.J. Verheggen, P. Boxho & E. Haubruge. 2013. Forensic Entomology Investigations from Doctor Marcel Leclercq (1924–2008): A Review of Cases from 1969 to 2005. Journal of Medical Entomology, 50: 935-954. DOI: https://doi.org/10.1603/me12097.

Greenberg, B., 1971. Flies and disease, ecology, classification and biotic associations. Vol. 1. Princeton University Press, New Jersey, 856 p.

Grzywacz, A., T. Pape & K. Szpila, 2012. Larval morphology of the lesser housefly, *Fannia canicularis*. Medical and Veterinary Entomology, 26: 70-82. https://doi.org/10.1111/j.1365-2915.2011.00968.x.

Haines, C.P. & D.P. Ress, 1989. A field guide to the types of insects and mites infesting cured fish. FAO fisheries, Rome, Italy. Available on: <http://www.fao.org/docrep/003/t0146e/T0146E00.HTM>.

Hegazi, E.M., F.H. El-Gayar, I.A. Rawah & S.A. Ali, 1978. Factors affecting the bionomics of Piophila casei (L.). Zeitschrift für Angewandte Entomologie, 85: 327-335. DOI: https://doi.org/10.1111/j.1439-0418.1978.tb04044.x.

Hennig, W., 1943. Piophilidae, p. 1-52. In: Lindner, E. (ed.) Die Fliegen der Palaearktischen Region, vol 5. Schweizerbart, Stuttgart.

Imms, A.D., 1957. A general textbook of entomology. 9a Ed. London, Methuen and CO LTD.

Kitching, R.L., 1976. On the protoracic spiracles of the first instar larvae of calyptrate Cyclorrhapha (Diptera). Journal of Australia Entomology Society, 15: 233-235. DOI: https://doi.org/10.1111/j.1440-6055.1976.tb01698.x.

Martín-Vega, D., 2011. Skipping clues: forensic importance of the family Piophilidae (Diptera). Forensic Science Intternational, 212: 1-5. DOI: https://doi.org/10.1016/j.forsciint.2011.06.016.

Martín-Vega, D., A. Gómez-Gómez, A. Baz & L.M. Díaz-Aranda, 2011. New piophilid in town: the first Palaearctic record of *Piophila megastigmata* and its coexistence with *Piophila casei* in central Spain. Medical and Veterinary Entomology, 25:64-69. DOI: https://doi.org/10.1111/j.1365-2915.2010.00907.x.

Martín-Vega, D., A. Baz & L.M. Díaz-Aranda, 2012. The immature stages of the necrophagous fly, *Prochyliza nigrimana*: comparison with *Piophila casei* and medicolegal considerations (Diptera: Piophilidae). Parasitology Research,

111: 1127-1135. DOI: https://doi.org/10.1007/s00436-012-2943-5.
McAlpine, J.F., V.B. Peterson, G.E. Shewell, J.H. Teskey, J.R. Vockeroth & D.N. Wood, 1981. Manual of Neartic Diptera. Research Branch Agriculture Canada, Monograph, 674 p.
Miokovic, B., J. Zivkovic & L. Kozacinski, 1997. Authochthonous Croatian "rotten" cheese. Veterinarski Archives, 67: 25-32.
Oliveira-Costa, J. & M.M.C. Queiroz, 2008. Bionomia de Dípteros de interesse forense. *In*: Entomologia forense: quando os insetos são vestígios. Oliveira-Costa. 2 ed. Campinas, SP: Millenium Ed., 420 p.
Ottogalli, G., 2001. Atlante dei formaggi. Hoepli, Milano, Italy, 462 p.
Ozerov, A.L. & A.L. Norrbom, 2010. Piophilidae, p. 865-869. In: Brown, B.V., A. Borkent, J.M. Cumming, D.M. Wood, N.E. Woodley & M. Zumbado (Eds). Manual of Central American Diptera. Ottawa, Canada: National Research Council Press.
Paños, A., M.I. Arnaldos, M.D. García & N. Ubero-Pascal, 2013. Ultraestructure of preimaginal stages of *Piophila megastigmata* McAlpine, 1978 (Diptera, Piophilidae): a fly of forensic importance. Parasitology Research, 112: 3771-3788. DOI: https://doi.org/10.1007/s00436-013-3567-0.
Papavero, N. & T.J. Pimentel, 2002. Insecta-Diptera-Piphilidae (Incl. Thyreophoridae). Fauna Amazônia Brasileira, 15: 1-3.
Pape, T., V. Blagoderov & B.M Mostovski, 2001. Order Diptera Linnaeus, 1758. Zootaxa 3148: 222-229.
Passos, M.R.L., N.A. Barreto, R.Q. Varella, G.H.S. Rodrigues & D.A. Lewis, 2004. Penile myasis: a case report. Sexually Transmitted Infections, 80: 183-184.
Perez, I.C., 1971. Dipterous and Coleopterous pseudoparasites of the human intestine. Graellsia, 2: 161-176.
Peterson, A., 1960. Larvae of Insects: an introduction to nearctic species, Part II-Coleoptera, Dispetera, Neuroptera, Siphonaptera, Mecoptera, Trichoptera. Bookseller Image. Published by Edwards Brothers. Columbus, Ohio. Michingan, U.S.A., 395 p.
Prado e Castro, C., E. Cunha, A. Serrano & M.D. García, 2012. *Piophila megastigmata* (Diptera: Piophilidae): First records on human corpses. Forensic Science International, 214: 23-26. DOI: https://doi.org/10.1016/j.forsciint.2011.07.009.
Russo, A., G.E. Cocuzza, M.C. Vasta, M. Simola & G. Virone, 2006. Life fertility tables of *Piophila casei* L. (Diptera: Piophilidae) reared at five different temperatures. Environmental Entomology, 35: 194-200. DOI: https://doi.org/10.1016/j.forsciint.2011.07.009.
Sacchi, L., A. Gigolo & G. Cestari, 1971. Un metodo di allevamento di Piophila casei L. Rivista di Parassitologia, 32: 289-301.
Saleh, M.S. & M.M. Sibae, 1993. Urino-genital myiasis due to *P. casei*. Journal Egypt Society of Parasitology, 23: 737-739.
Smith, K.G.V., 1986. A manual of forensic entomology. British Museum (Natural History) and Cornell University Press, London, 205 p.
Simmons, P., 1927. The cheese skipper as a pest in cured meats. U.S. Department of Agriculture Bulletin, 1453: 1-55.
Skevington, J.H. & P.T. Dang, 2002. Exploring the diversity of flies (Diptera). Biodivesity, 3: 3-27. DOI: https://doi.org/10.1080/14888386.2002.9712613.
Sukontason, K.L., K. Sukontason, S. Piangjai, W. Choochote, R.C. Vogtsberger & J.K. Olson, 2001. Scanning electron microscopy of the third-instar *Piophila casei* (Diptera: Piophilidae), a fly species of forensic importance. Journal of Medical Entomology, 38, 756-759. DOI: https://doi.org/10.1603/0022-2585-38.5.756.
Sukontason, K.L., R,C. Vogtsberger, N. Boonchu, T. Chaiwong, D. Sripakdee, R. NgernKlun, S. Piangjai & K. Sukontason, 2005. Larval morphology of *Chrysomya nigripes* (Diptera: Calliphoridae), a fly species of forensic importance. Journal of Medical Entomology, 42: 233-240. DOI: https://doi.org/10.1603/0022-2585(2005)042[0233:lmocnd]2.0.co;2.
Wendt, L.D., 2017. Piophilidae in Catálogo Taxonômico da Fauna do Brasil. PNUD: Available on: <http://fauna.jbrj.gov.br/fauna/faunadobrasil/2399>.
Zumpt, F. 1965. Myiasis in man and animals in the old world. London, Butterworths, 267 p.
Zuska, J. & P. Lestovka, 1965. A review of the Czechoslovak species of the Piophilidae with special references to their importance to food industry (Diptera, Acaliptrata). Acta Entomology Bohemoslovaca, 62: 141-157.

****

Suggestion citation:

Barros-Cordeiro, K.B., W.R. Lopes & S.N. Báo, 2018. Ultrastructural characteristics and development time of immature stages of *Piophila casei* (Linneus) (Diptera: Piophilidae). EntomoBrasilis, 11 (3): 210-208.
Available on: doi:10.12741/ebrasilis.v11i3.808