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# Description of Immature Stages and Development Time of *Paralucilia paraensis* (Mello) (Diptera: Calliphoridae) Associated with the Decomposition of a Partially Submerged Swine Carcass

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**Abstract**
We report on the bionomics and morphology of the immature stages of *Paralucilia paraensis* (Mello) (Diptera: Calliphoridae). Observations were made on a daily basis for 10 h (from 8 a.m. to 6 p.m.) on a 45-kg pig (*Sus scrofa*) whose carcass had been partially submerged in a stream of water on the 21st of November 2009, in a forested area of Manaus, Amazonas, Brazil. The collected specimens were placed in plastic vials and transferred to a growth chamber maintained at room temperature. Adults of *P. paraensis* were collected on the carcass between the 3rd and the 18th days. A total of 13 gravid females were captured; from these, 1,240 eggs were obtained and yielded 1,030 larvae that developed into 879 adults. The average time required for hatching was 13 h. On average, the larvae reached the second instar within 13 h, third instar within 18 h, and pupae within 46 h. The pupal stage lasted 96 h. The complete development time was 216 h. This is the first report on the development time and morphology of immature stages of *P. paraensis* in forested areas. Therefore, these results provided information for the implementation of future forensic studies in the state of Amazonas.

## Introduction

Forensic entomology (FE) is the study of insects and other arthropods biology for their application in medico-legal research (Byrd & Castner 2001). Dipterans are a significant portion of the fauna associated with FE due to the abundance of adults and immatures that use and/or develop on decomposing flesh (Catts & Goff 1992). Calliphoridae is among the first and most abundant arthropods to colonize decomposing carcasses (Anderson 2001, Amendt *et al* 2004). The neotropical Calliphoridae group includes approximately 27 genera and 125 species, which are grouped into four subfamilies (Mello 2003).

Initial studies on forensic species in the Amazon provided data on the oviposition behavior, morphology and/or development of several species of Calliphoridae and Sarcophagidae (Fraga 2004, Oliveira-da-Silva *et al* 2006, Ururahy-Rodrigues 2008, Souza 2009). Applied studies for the determination of the postmortem interval of humans have also been conducted (Pujol-Luz *et al* 2006).

Forensic entomology is a growing scientific field in Brazil, as well as in other countries. The existing lag in the FE in Brazil is compared to other leading countries in the field is mainly due to the lack of taxonomic and biological data on insects in Brazil. Therefore, the present study was aimed at documenting the bionomic aspects of *Paralucilia paraensis* (Mello) (Diptera: Calliphoridae) in a forested area and the morphological characterization of its immature stages. This information should contribute to the interpretation of entomological evidence for medico-legal studies.

## Material and Methods

This study was conducted at Adolpho Ducke forest reserve in Manaus, in the state of Amazonas, Brazil, at Barro

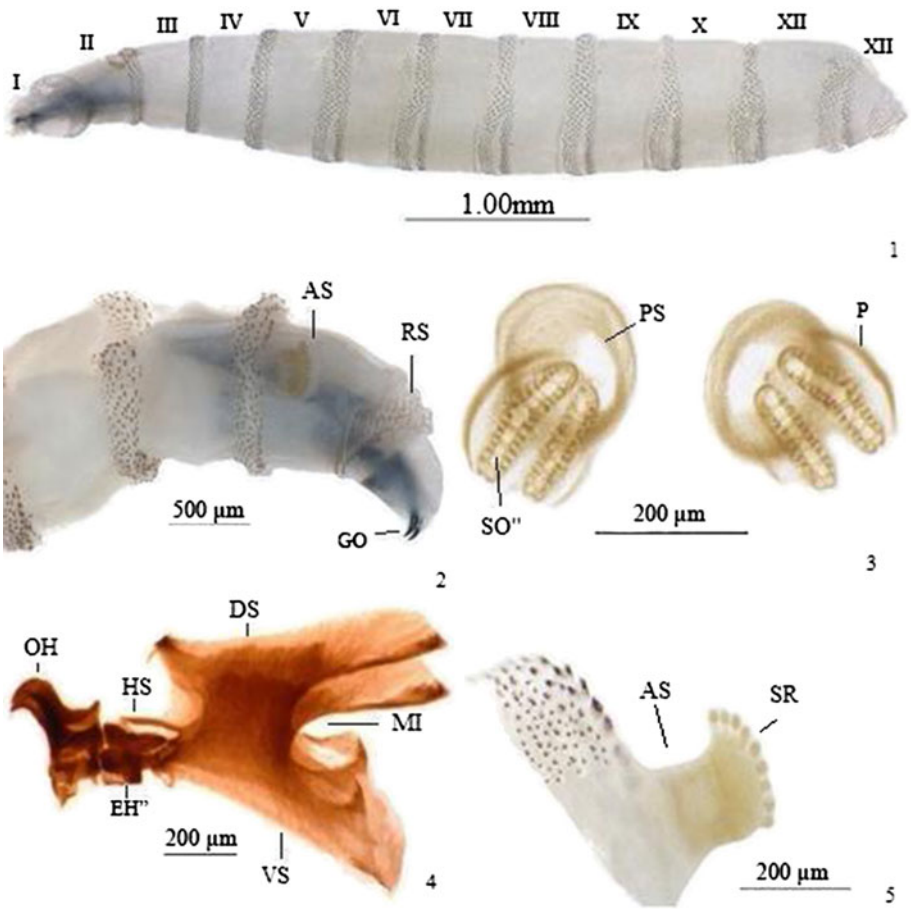
Branco water stream (02°55′49.4″S, 59°58′28.1″W), classified as a second order water stream (Delgado 1996) with clear water, 5.2 km long, pH ranging from 3.9 to 4.1, electrical conductivity between 7.5 and 8.5 µScm, temperature 24.1°C and 25.3°C, dissolved oxygen 6.5 and 8 mg1 $s^{-1}$ (Mendonça *et al* 2005). The study site was composed of a closed-canopy, primary forest with vegetation typical of a flooded lowland forest. The trees ranged from 5 to 20 m in height, and there were many species of tall palm trees on the banks of the stream (Ribeiro da Silva *et al* 1999). According to Köppen, the region's weather is classified as Afi (tropical and humid) with constant rain in all seasons. The average rainfall level is between 1,500 and 2,500 mm (Alencar *et al* 1979, Ribeiro & Adis 1984)

Samplings were made during the rainy season in November 2009 and they complement the information obtained for studies of aquatic insects associated with a partially submerged carcass of a 45-kg swine *Sus scrofa*. The animal was taken alive to the study site and was killed with a shot of a 38 mm caliber pistol in the frontal head. The carcass was placed in an iron cage (1.00×0.63×0.74 cm) and was covered with an aluminum screen (3×3 cm mesh) to protect the carcass from large vertebrate scavengers.

A collection pilot was developed to identify the species of *Paralucilia* occurring in the area. *Paralucilia paraensis* and *Paralucilia fulvinota* were recorded and stock colonies of both species were established from gravid females collected from the carcass at the initial stage flotation using bottles with screw caps (100 ml) at 7 a.m., 1 p.m., and 6 p.m. and transferred to wide-mouth plastic vials (33 cm in diameter and 15 cm high).

After oviposition, eggs were counted and transferred to Petri dishes containing ground meat as a food source for the larvae. At the third instar, larvae were transferred to wide-mouth plastic vials (33 cm in diameter and 15 cm high) containing 250 g of ground meat and fine sawdust, and kept at room temperature until adult emergence. Adults were killed by freezing and identified according to Mello (2003).

For analysis and the determination of the three developmental larval stages, ten *P. paraensis* larvae were separated and fixed in KAAD solution (Cruz *et al* 2009). The characterization of the instars was performed according to the number of spiracle openings and to the presence or absence of prothoracic spiracles (Knipling 1937). Adult specimens were dry-preserved with entomological pins, labeled, and kept in entomological boxes. All specimens




Figs 1–5 Second instar of *Paralucilia paraensis*. *1* Larva, *2* Detail of the head, *3* Posterior spiracle, *4* Cephalopharyngeal skeleton, *5* Detail of the anterior spiracle. *RS* spine bands, *SO*" spiracle opening, *DS* dorsal spine, *VS* ventral spine, *AS* anterior spiracle, *HS* hypostomal sclerite, *EH*" hypopharyngeal sclerite, *PS* posterior spiracle, *OH* oral hooks, *MI* medial incision, *P* peritreme, *SR* spiracle ramifications.

collected were housed as part of the "Coleção de Invertebrados do Instituto Nacional de Pesquisa da Amazônia (INPA)".

The morphological analysis of the cephalopharyngeal skeleton, prothoracic, and caudal spiracles were done after the clarification of these structures in warm 10% KOH for 15 min. KOH was neutralized with acetic acid for 10 min followed by washing in distilled water. The larval structures were subsequently mounted on microscope slides with glycerin.

The larvae were photographed in lateral and ventral views using a stereoscopic microscope (Leica M165) coupled to a mounting program to determine the position of the spines from the cephalic region to the 12th body segment. The caudal spiracle was photographed to visualize the peritreme, spiracle openings, prothoracic spiracle (when present), and exposed fingerlike extensions. The cephalopharyngeal skeleton and the prothoracic and caudal spiracles and spines were photographed in lateral views to visualize structural details. The pupae were photographed in dorsal and ventral views under a stereomicroscope.

Measurements of the larvae and puparium were taken under a stereomicroscope (Leica Application Suite). The terminology used for the morphological descriptions of immature specimens was made according to Shewell (1981).

## Results

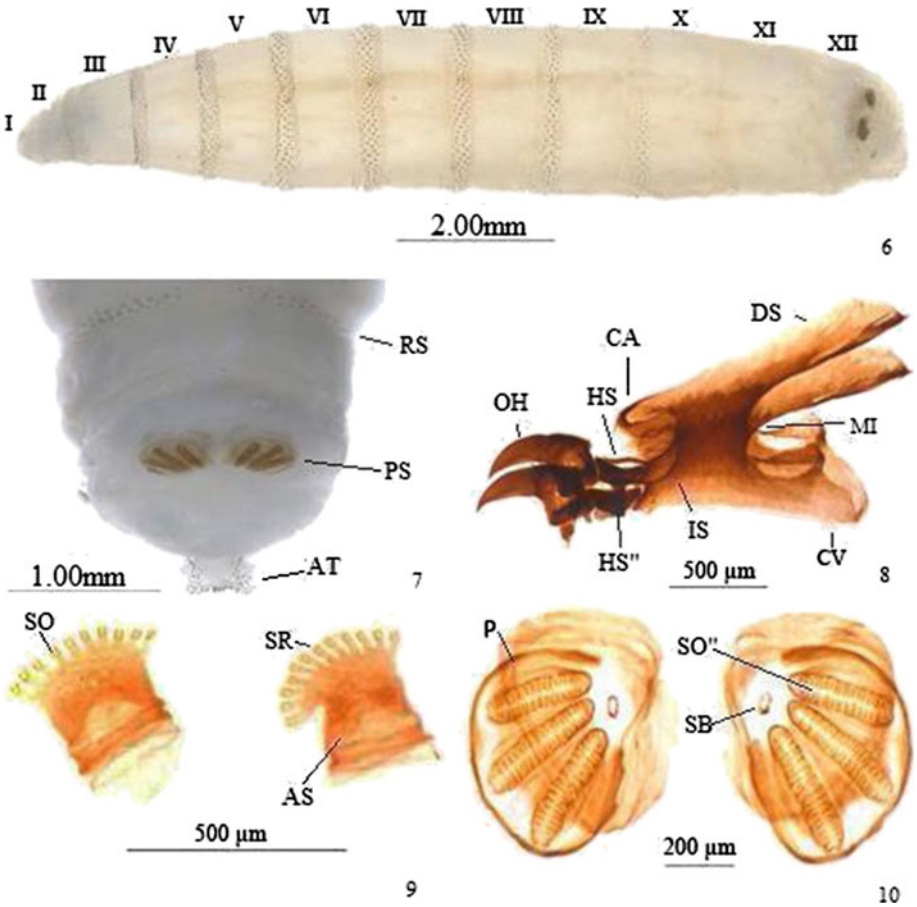
A total of 13 gravid *P. paraensis* females were collected. From these, approximately 1,240 eggs were obtained, which yielded 1,030 larvae. The presence of *P. paraensis* was identified on the third day of sampling, as opposed to Ururahy-Rodrigues (2008) who collected this species since the first day of the PMI and observed peaks of abundance within 3 days of the PMI.

### *Egg*

The average time required for the embryonic development was 13 h. Eggs were cylindrical in shape with rounded edges, whitish-yellow to cream colored and mainly grouped together, with a mean length of 1.87 mm.

### *First instar*

First instar larvae remaining aggregated once hatched. Larvae are vermiform, cylindrical, with a white-colored body, with an average length of 3.55 mm ($N$=10). Body was divided in head (segment I), prothorax (segment II), mesothorax (segment III), and metathorax (segment IV).

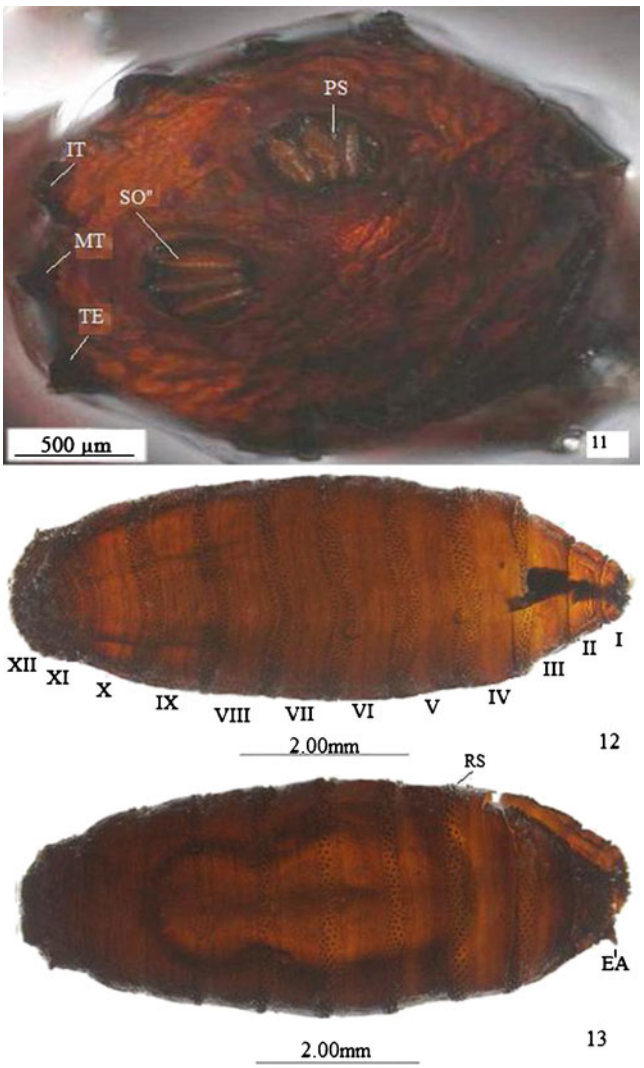



Figs 6–10 Third instar of *Paralucilia paraensis*. *6* Larva, *7* Posterior spiracle, *8* Cephalopharyngeal skeleton *9* Anterior spiracle *10* Detail of the posterior spiracle. *RS* spine bands, *CA* clypeal arch, *SO*" spiracle opening, *SB* spiracle button, *DS* dorsal spine, *CV* ventral spine, *AS* anterior spiracle, *HS* hypostomal sclerite, *HS*" hypopharyngeal sclerite, *IS* infrapharyngeal sclerite, *PS* posterior spiracle, *OH* oral hooks, *MI* median incision, *SO* spiracle orifice, *P* peritreme, *SR* spiracles ramifications, *AT* anal tubercle.

These were followed by eight abdominal segments (V to XII). There was a complete row of spines in segments I–IX, and the remaining segments had incomplete rows of spines. Double rows of spines began at segment V, ventrally positioned. Segment I–XII separated by rows of spines.

*Second instar*

Vermiform, cylindrical, and white-colored larvae. Mean length of 4.80 mm ($N$=10). Twelve visible body segments with rows of spines at the edge of each segment. Segments I–IX had complete rows of spines that were dark colored (as in the first stage). Segments V–XII had double rows of spines in the ventral surface of the segments. Segments X–XII had incomplete rows of spines at the dorsal portion (Figs 1, 2). Posterior spiracle with two slits in parallel to the incomplete peritreme (Fig 3). Cephalopharyngeal skeleton dark brown, with a pair of separated and symmetrical mouth hooks. The hypostomal sclerite was connected to the infrapharyngeal sclerite, barlike, and was the same length as the hypopharyngeal sclerite (Fig 4). The prothoracic spiracle had 9–12 fingerlike extensions (Fig 5). Second instars were also observed aggregated for 18 h before molting into the third instar.




Figs 11–13 Pupa of *Paralucilia paraensis*. *11* Detail of posterior spiracle, *12* Ventral view, *13* Dorsal view. *RS* spine bands, *PS* posterior spiracle, *SO"* spiracle opening, *IT* internal dorsal tubercle, *MT* median dorsal tubercle, *TE* external dorsal tubercle, *EA* anterior spiracle.

*Third instar*

Vermiform, cylindrical, with a white- or cream-colored body with 12 distinguishable segments. Average length of 11.35 mm ($N$=10). There were rows of spines at the edges of the segments. A double row of spines was located ventrally on segment V (as in other stages). Segments I–IX had complete rows of spines, and the other segments had incomplete rows (Fig 6). The last segment had a row of spines around the anal tubercles (Fig 7). Dark brown cephalopharyngeal skeleton fully formed, visible. The tip of a bar-shaped hypostomal sclerite reached the hypopharyngeal sclerite (Fig 8). Anterior spiracle with 9–12 fingerlike extensions, and each one with an opening (Fig 9). The posterior spiracle had an incomplete peritreme, three slits and a button (Fig 10). Third instars took approximately 46 h to disperse in search of a pupation site. At the prepupal stage (2 days after reaching the third instar), the larvae exhibited reduced movement and the cuticle shrunk and hardened resembling a barrel (puparium) which the adults would develop inside.

*Puparium*

Reddish brown, barrel-shaped and strongly sclerotized. Average length of the puparium was 7.35 mm ($N$=10). Twelve visible segments, with segments I to X separated by rows of spines. Anterior portion narrower than posterior. Ventral side with an inconspicuous double row of spines, which became increasingly conspicuous at segment V (Fig 11). The posterior spiracle was sclerotized and barely visible, and it had three dark brown and barely visible spiracle slits. Dark brown tubercles without dorsal spines (Fig 12). Prothoracic spiracle barely visible (Fig 13). The pupal stage lasted approximately 96 h.

Adults emerged through an opening in the mid-anterior portion of the puparium, and they had soft bodies with curled wings, which expanded slowly over a period of approximately 50 min. On average, the total time for development was 216 h.

## Discussion

Larvae of *P. paraensis* share several morphological characteristics with those from *P. fulvinota*, whose immature stages have been described by Greenberg & Szyska

(1984). Larvae at different instars will all have the same number of fingerlike extensions in the anterior spiracle and the pigmentation of the posterior spiracle, as well as pupae with the same shape and color. However, third instars of *P. paraensis* have full rows of spines (Fig 6) in segments I–IX, while in *P. fulvinota* these rows are in segments II—IX.

The cephalopharyngeal skeleton of *P. paraensis* (Fig 8) is the most diverse morphological trait from *P. fulvinota*. While it has a bar-shaped hypostomal sclerite that nearly touches the hypopharygeal sclerite in *P. paraensis*, the bar-shaped hypostomal sclerite of *P. fulvinota* is located slightly above, and do not reach the hypopharygeal sclerite. Both species also differ in the shape of the clypeal arch. It is round in *P. paraensis* whereas in *P. fulvinota* it is pointed. The dorsal spine in *P. paraensis* is round and posteriorly projected. In *P. fulvinota* it is pointed.

Immatures of *P. paraensis* raised in the forest area did follow the same development pattern and period observed in urban areas as described by Souza (2009).

The presence and the development of *P. paraensis* in swine carcasses demonstrate the forensic potential of this species in aiding in the determination of postmortem intervals. The description of the anatomical structures of *P. paraensis* provided here will allow for the development of identification keys for the immature stages of *Paralucilia* and other Calliphoridae. Bionomic and taxonomic knowledge is essential to forensic entomology and could contribute to the use of entomological evidence in medico-legal investigations in the state of Amazonas.

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