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Description of second and third instar larvae of *Cuterebra histrio* Coquillett, 1902 and first report of *Metacuterebra apicalis* (Guérin-Ménéville, 1835) (Diptera: Oestridae) in Veracruz, Mexico

Descripción del segundo y tercer estadios larvales de *Cuterebra histrio* Coquillett, 1902 y primer registro de *Metacuterebra apicalis* (Guérin-Ménéville, 1835) (Diptera: Oestridae) en Veracruz, México

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ABSTRACT. In this work we describe and illustrate for the first time the second and third instar of the rodent bot fly *Cuterebra histrio* Coquillett, 1902, furthermore, we discuss morphological characters that can be helpful in the identification of larvae of different species. Additionally, we report for the first time the species *Metacuterebra apicalis* (Guérin-Ménéville, 1835) for the state of Veracruz, Mexico.

Key words: myiasis; parasitism; Botfly

RESUMEN. En este trabajo se describen e ilustran por primera vez el segundo y tercer estadio larvario del tórsalo de roedor de la especie *Cuterebra histrio* Coquillett, 1902, además, se discuten caracteres morfológicos que pueden ser de utilidad taxonómica

para la identificación de las larvas de distintas especies. Sumado a esto, se reporta por primera vez la presencia de la especie *Metacuterebra apicalis* (Guérin-Ménéville, 1835) en el estado de Veracruz, México.

Palabras clave: miasis; parasitismo; Colmoyote

INTRODUCTION

Botfly larvae (Diptera: Oestridae) are obligate parasites of vertebrates, causing myiasis in their hosts (Wood, 1987; Pape, 2010). Larval development is subdermal in the subfamilies Cuterebrinae and Hypodermatinae, intestinal in Gasterophilinae, and naso-pharyngeal in Oestrinae (Pape, 2010).

The geographic distribution of the oestrids is highly-dependent on the distribution of their hosts, with some species occurring almost worldwide because they have accompanied livestock along with the human settlements. Some other species are restricted to small geographic areas due to their hosts having small distribution ranges (Sabrosky, 1986). The Subfamily Oestrinae is distributed worldwide, Hypodermatinae is present in the Nearctic, Palearctic, and Afrotropical regions, while Gasterophilinae was originally distributed in the Palearctic and Afrotropical regions, but some species have invaded other biogeographic regions and now this subfamily is considered to have a worldwide distribution (Colwell *et al*., 2006), and finally, Cuterebrinae Brauer, 1863, which is restricted to the Nearctic and Neotropical regions (Catts, 1982).

There are different opinions about the recognition of genera of the subfamily Cuterebrinae, with some authors considering only two genera *Dermatobia* Brauer, 1861 and *Cuterebra* Clark, 1815 (Pape, 2001; Pape & Thompson, 2013), while others recognizing six genera: *Andinocuterebra* Guimarães, 1984, *Cuterebra*, *Dermatobia*, *Metacuterebra* Bau, 1929, *Pseudogametes* Bischoff, 1900, and *Rogenhofera* Brauer, 1863 (Papavero & Guimarães, 2009).

Despite that Cuterebrinae is considered one of the best studied groups of Oestridae (Slansky, 2007), in Mexico, this group has not been studied sufficiently and there are only punctual and disperse reports of adults in the literature or entomological collections, and some records of larvae in a few species of mammal hosts. For those reasons, there are no systematical studies for any species up today (Manrique-Saide *et al*., 2000; Cristobal-Azkarate *et al*., 2012; Arnaud *et al*., 2016; Bravo & Hernández-Ortiz, 2016; Lara-Lagunes *et al*., 2017). In addition, the taxonomical information of larval stages and their morphological characters for several species is scarce, and most keys and descriptions are based only in adult morphology, making difficult to identify and report larvae in ecological and faunistic studies. Only a few studies have been published with larval stages descriptions (Knipling & Brody, 1940; Dalmat, 1942; Haas, 1958; Capelle, 1970; Baird, 1973; Leite & Williams, 1997; Filippis & Leite, 1998; Cristobal-Azkarate *et al*., 2012), being some of them simplified descriptions leading to further redescriptions or erroneous identifications.

According to Papavero and Guimarães (2009), in Mexico only three genera of the subfamily Cuterebrinae have been reported: *Cuterebra* with 14 species: *C. albipilosa* Sabrosky, 1986, *C. arizonae* Sabrosky, 1986, *C. atrox* Clark, 1848, *C. austeni* Sabrosky, 1986, *C. bajensis* Sabrosky, 1986,

C. buccata (Fabricius, 1777), *C. clarki* Sabrosky, 1986, *C. fontinella* Clark, 1827, *C. histrio* Coquillett, 1902, *C. lepusculi* Townsend, 1897, *C. postica* Sabrosky, 1986, *C. princeps* (Austen, 1895), *C. terrisona* Walker, 1849; *Dermatobia* with one species: *D. hominis* (Linneaus Jr., 1781) and *Metacuterebra* with one species: *M. apicalis* (Guérin-Ménéville, 1835). Only *Cuterebra histrio* has been reported in the state of Veracruz (Sabrosky, 1986).

The main objective of this work is to describe in detail and illustrate the second and third instar larva, and the puparium of *Cuterebra histrio*. Also, in this work we report *Metacuterebra apicalis* for the first time in the state of Veracruz.

MATERIALS AND METHODS

Larvae on which the description was based, were retrieved from rats of the species *Neotoma mexicana* Baird, 1855, captured in the locality of San Antonio Tenextepec in the municipality of Perote, state of Veracruz, Mexico. A brief description of the site can be found in Lara-Lagunes *et al*. (2017). Rats were captured using Tomahawk traps (40.64 x 12.7 x 12.7 cm) baited with a mix of oatmeal, bird seeds and vanilla essence. Rats detected with myiasis were kept in captivity to measure the time-lapse of larval development, time of host abandonment and to make it easier to retrieve the larvae.

Larvae were incubated in black dirt with a 75W lamp as a heat source at approximately 28° C and a constant relative humidity of 80%. The larvae herein described were obtained from two rats that unfortunately died in captivity. Once the larvae abandoned the dead host, we proceeded to submerge the larvae in hot water (75° C) to avoid tissue constriction before storing them in, 70° ethanol. Specimens were macerated in 10% NaOH for dissection of the cephalopharyngeal apparatus. The puparium was described based on specimens that completed the development and emerged as adults. Adults were then identified with the works of Papavero & Guimarães (1999), Sabrosky (1986), and Swenk (1905). Additionally, four unidentified specimens previously deposited at the IEXA Entomological Collection of Instituto de Ecología, A.C. in Xalapa, Veracruz, were also identified and compared with the obtained specimens.

Morphological terms for larvae are in accordance with Courtney *et al*. (2000) and Szpila *et al*. (2015). In this work we use the term "sclerotized plates" or just "plates", instead of "spines", as they are flattened, broad, and usually ending in more than one tip.

The cephalopharyngeal apparatus, posterior spiracles and cuticle surface were drawn in a Nikon stereomicroscope model (SMZ 800) with a lucid camera, and photographs were obtained with a DSLR Canon Camera, attached with a Raynox DCR-250 Macro lens.

RESULTS

***Cuterebra histrio* Coquillett, 1902**

Figures 1–4.

Cuterebra histrio Coquillett, 1902: 103. Holotype: as female, but a male (Sabrosky, 1986: 168). Type locality: Mexico, Guanajuato. *Cuterebra histrio* Coquillett: Guimarães (1967: 3, catalogue), Papavero & Guimarães (2009: 11, catalogue).

Syn. *Cuterebra americana* Fabricius: Wulp, 1888: 1 (misidentification; see Papavero & Guimarães, 2009: 4).

Larval description. Second Instar. Body with 12 visible segments, oval-shaped, narrow at posterior apex. Tegument smooth and cream-colored with sclerotized spine-like plates. Pseudocephalon: with two upper plates at middle. Cephaloskeleton: in lateral view mouth hooks projecting from mouth aperture, two times longer than its basal width, with its apical third curved, and with a dorso-basal pointed projection; dental sclerite blunt, and intercalary sclerite fused with the base of mouth hooks, leaving a small fenestra between them; basal sclerite a little longer than mouth hooks, nearly straight dorsally, and with an anterior-ventral projection that could represent the intercalary sclerite fused; dorsal cornu with the sclerotized portion about as long as basal sclerite, wide at middle and ending in a very thin posterior projection, the translucent portion large with the dorsal margin convex and the ventral margin nearly straight; ventral cornu with the sclerotized portion about half the length and as broad as dorsal cornu, the membranous translucent portion stick-like, as long as dorsal cornu, but one-third to one-fourth its width. Abdominal segments I-III: dorsal area, with anterior region carrying single-pointed sclerotized plates oriented towards the posterior region of the segment, the plates extend well beyond mid-line of segment, central region almost bare, posterior region bare; on segment III the plates do not extend more than midline. Lateral area with sparse plates extending more than half the segment. Posterior area bare, except on segment III that presents some plates. Ventral area with plates restricted to the anterior region, posterior region bare. Abdominal segments IV-V: dorsal area as in segment III, the plates extend beyond mid-line of segment. Lateral area with spine-shaped plates on the whole Surface, becoming less abundant in mid-line, on anterior region the plates are directed posteriorly and vice-versa. Ventral area as previous segments. Abdominal segment VI: dorsal and lateral areas with bands of plates that go around the segment, anterior region plates oriented towards the posterior and vice-versa; mid region of segment with plates sparse, oriented posteriorly. Ventral area with plates on anterior region not extending beyond mid-line, posterior region with less plates, oriented as in dorsal area. Abdominal segment VII: dorsal area with plates restricted to posterior region, lateral area with anterior region bare, plates on the posterior region extend beyond midline, the plates near the midline are oriented towards the posterior region, while the plates on the posterior region are oriented anteriorly. Ventral area as dorsal area. Abdominal segment VIII: anterior region bare, plates only in the posterior region. Spiracle openings: well defined, with orange-brown coloration, two on each side. Plates with a single tip present in irregular bands in upper and lower region, the upper plates are directed towards lower region and vice-versa. Superior spines forward directed and inferior spines backward directed.

Third instar. Ellipsoid, robust, ten visible segments, truly 12 segments, but pseudocephalon and last abdominal segment invaginated, making them non-visible at first glance. General body coloration creamy-reddish but appears brownish due to the multiple dark brown sclerotized plates, except the pseudocephalon where the coloration is completely cream; plates present one to eight marginal tips as in Figure 1C and 1D. Pseudocephalon: with one tubercle on each side, each tubercle, at apex, is the origin point of the antenna and maxilla papillae; One band of cream-colored plates with multiple tips. Cephaloskeleton: in lateral view mouth hooks projecting from mouth aperture, about four times longer than its basal width, acuminate and evenly curved from base to tip; dental sclerite not defined; intercalary sclerite triangular in lateral view, higher than basal sclerite; basal sclerite more or less quadrate in lateral view, about one-half the length of mouth hooks, nearly straight ventrally but with a hump posterodorsally, this separated from dorsal and ventral cornuae; dorsal and ventral cornu narrowly connected; dorsal cornu with the sclerotized portion about as long as high, the translucent portion large with the dorsal margin convex and the ventral margin nearly straight; ventral cornu with the sclerotized portion similar in length but two-thirds as high as dorsal cornu, the membranous translucent portion stick-like, just shorter than dorsal cornu translucent portion. Anterior spiracle not externally visible. Thoracic segments: all three segments covered with serrated plates, with one to eight tips, plates on dorsal, lateral, and ventral view oriented towards the posterior region, but those near the junction with the next segment oriented towards the anterior region. Abdominal segments: the first six segments present plates as described for the thoracic segments in both, direction and distribution; the seventh abdominal segment presents plates with rounded border oriented towards posterior region, the plates near the junction with the segment VIII are serrated, anteriorly oriented and reddish in coloration; the eight abdominal segment presents less abundant and smaller plates with serrated border. Anal lobes with small plates. Posterior spiracles: spiracle apertures well defined, spiracle coloration dark Orange, each spiracle with three close together but well-defined dot-shaped spiracular openings, with slits intertwined; peritreme externally broad.

Puparium. Ten visible segments. Pseudocephal and eighth abdominal segments invaginated. Cuticle dark-brown, plates as described for the third instar. Anterior spiracles with pale-yellow coloration.

Distribution. Known in the states of Chihuahua, Durango, Guanajuato, San Luis Potosí, Veracruz, and Zacatecas (Sabrosky, 1986).

Hosts: The only known host is the rat *Neotoma mexicana* Baird, 1855 (Rodentia, Cricetidae) (Sabrosky, 1986).

Material examined: Mexico, Veracruz, Perote, Tenextepec: Xeric shrubland: instar II, 04/IX/2018; instar III, 06/XII/2018; for both *ex*. *Neotoma mexicana*. Col. N. Lara-Lagunes.

***Metacuterebra apicalis* (Guérin-Méneville, 1835)**
Figure 5.

Cuterebra apicalis Guérin-Méneville, 1835: 101. Type locality: "America".

Metacuterebra apicalis (Guérin-Ménéville): Papavero & Guimarães (2009: 5, catalogue).
Syn. *Cuterebra analis* Macquart, 1843: 22. Type locality: Brazil.
Syn. *Cuterebra cometes* Shannon & Del Ponte, 1926: 42. Type locality: Tucumán, Argentina.
Syn. *Cuterebra pusilla* Bau, 1931: 212. Type locality: Sarampiuni, Mapiri, Bolivia.

Distribution: Southern Mexico to Argentina. Mexico: Montebello (Chiapas?) (Papavero & Guimarães, 1999; Papavero & Guimarães, 2008). This is the first record for the state of Veracruz.

Hosts: It has been reported that this species cause myasis in several rodents and marsupials (Bossi & Bergallo, 1992; Guimarães *et al*., 1983; Mello, 1978; Vieira, 1993).

Material examined: Mexico, Veracruz, Xalapa. Montane cloud forest, 1♀, III/2000, Col. S. Ibáñez-Bernal; 1♀, 19/V/2011, Col. F. Escobar-Hernández; 1♂, 25/X/2017, Col. No data; 1♀, 22/V/2018, Col. S. Jaume-Schinkel.

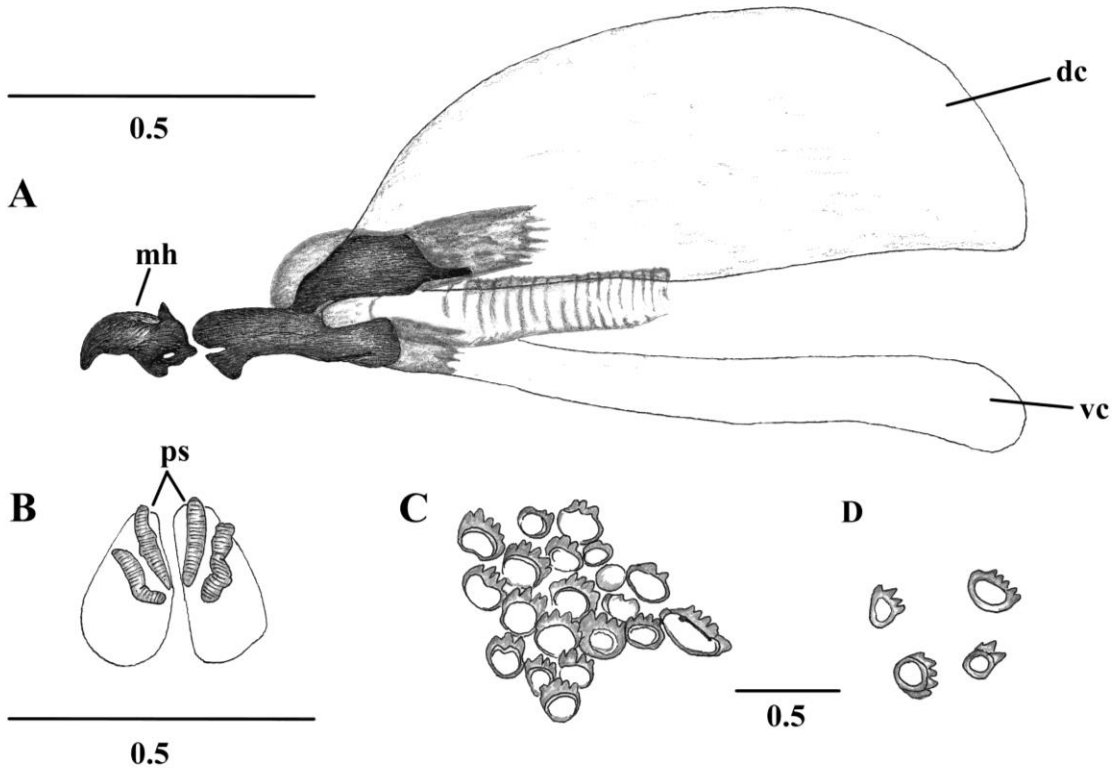

Figure 1. *Cuterebra histrio*. Second instar larva A-B, third instar C-D. A. Mouthparts lateral view. B. Posterior spiracles dorsal view. C-D. Thoracic segments plates. Abbreviations. dc: dorsal cornu, mh: Mouth hooks, ps: posterior spiracle, vc: ventral cornu.

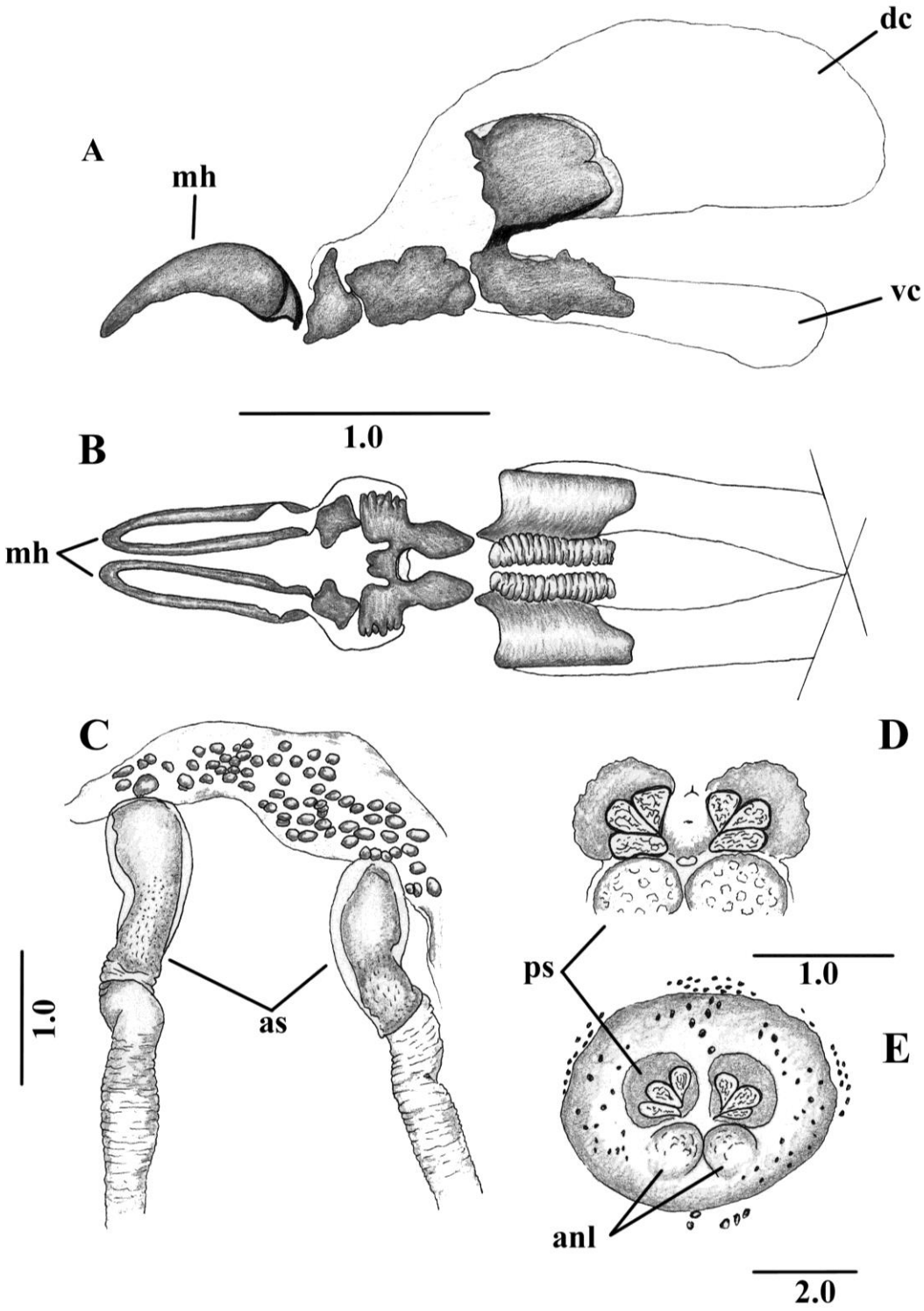

Figure 2. *Cuterebra histrio*. Third instar larva. A. Mouth parts lateral view. B. Mouth parts dorsal view. C. Anterior spiracles. D. Posterior spiracles enlarged. E. Posterior spiracles and anal lobe. Abbreviations. as: anterior spiracle, anl: anal lobe, dc: dorsal cornu, mh: Mouth hooks, ps: posterior spiracle, vc: ventral cornu.

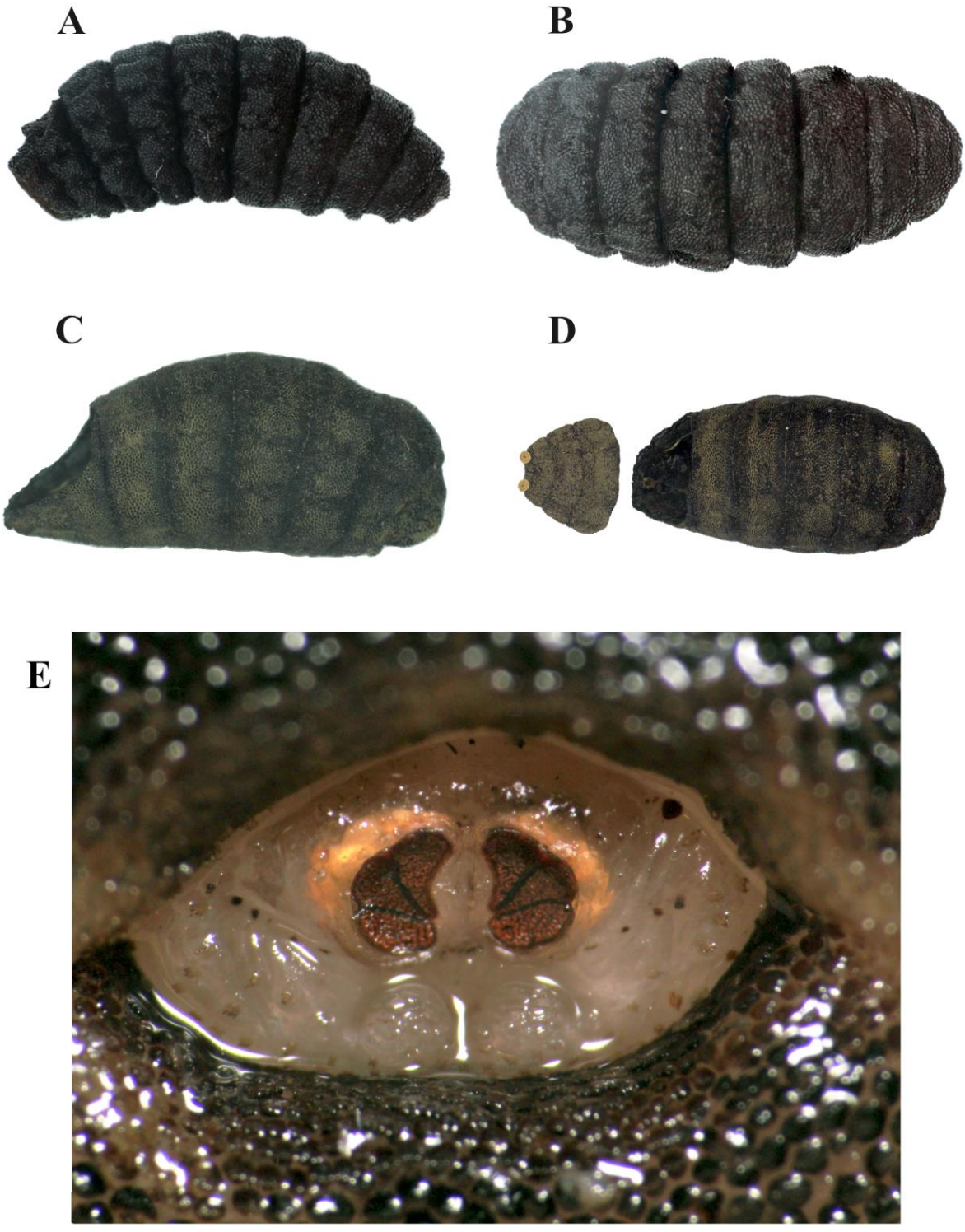

Figure 3. *Cuterebra histrio*. A-B, E. Third Instar Larva. C-D. Puparium. A. Third instar larva lateral view. B. Third instar larva dorsal view. C. Puparium lateral view. D. Puparium, dorsal view. E. Third instar larvae, posterior spiracles.

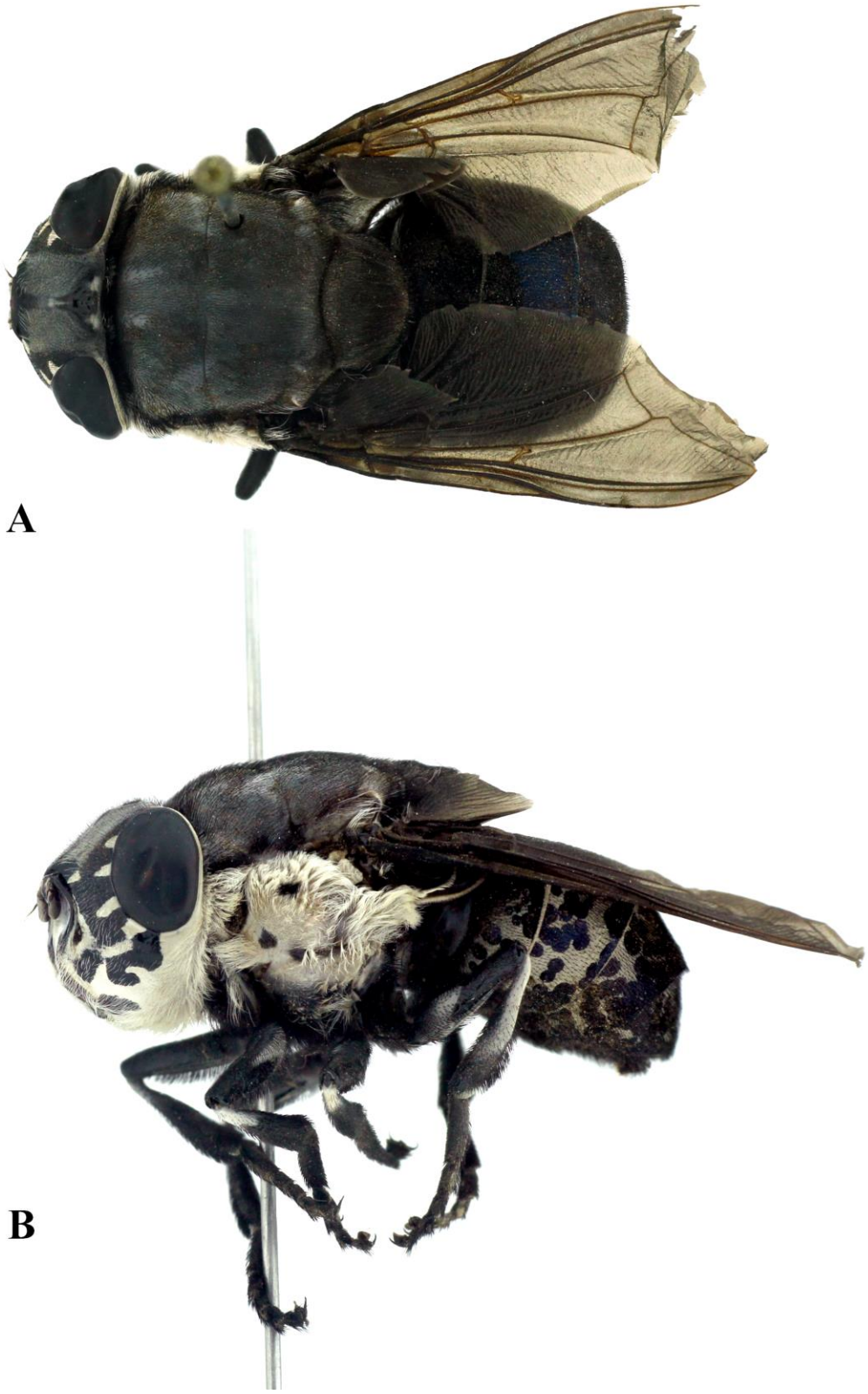

Figure 4. *Cuterebra histrio*. A-B. Adult. A. Habitus dorsal view. B. Habitus lateral view.

Figure 5. *Metacuterebra apicalis.* A-B. Adult. A. Habitus dorsal view. B. Habitus lateral view.

DISCUSSION

Despite there are approximately 70 species of cuterebrids, the knowledge of larval states of this family is very scarce (Leite & Williams, 1997), principally because the larval full development and rearing of adults is necessary to properly identify to species level. At the same time, larvae are similar between species and difficult to identify.

In this study we observed external and internal characteristics between larval stages and compared them with other available larval descriptions (Knipling & Brody, 1940; Dalmat, 1942; Haas, 1958; Capelle, 1970; Baird, 1973; Leite & Williams, 1997; Filippis & Leite, 1998; Cristobal-Azkarate *et al*., 2012) which include the shape, relative number and disposition of cuticular sclerotized pales, shape of cephaloskeleton, and the shape and position of posterior spiracles. These characters are proven to be diagnostic characters for other groups within the Oestroidea such as Calliphoridae and Sarcophagidae (Szpila, 2015).

According to Baird (1973) the shape and distribution of cuticular plates (spines) can be useful for the identification of cuterebrid larval stages, as it has been determined that the number of plates is related to the host species preferred by the botfly (Baird, 1971). Cuterebrid species that use lagomorphs as hosts only present plates with a single tip (Knipling & Brody, 1940; Haas & Dicke, 1958), on the contrary, species that prefer rodents as hosts present multiple tips that are mentioned here as sclerotized plates or squamae (Cameron, 1926; Dalmat, 1942; Capelle, 1970; Baird, 1973), with shape variating significantly between species. These cuticular ornamentations (plates) present single-tipped plates in the second stage of *C. histrio* and *C. tenebrosa*, but in *C. latifrons* they are double-tipped. In the third larval stage of *C. histrio* are present as sclerotized plates with multiple tips (from one to eight tips) (as in Figs. 1 C, D), while in *C. polita* they have only two to four tips (Capelle, 1970) in contrast with *C. latifrons* that presents four to 14 tips on each plate.

Additionally, the shape of the cephaloskeleton has variation between species, the more notable differences can be found in the shape of the mouth hooks, intercalary segments and the ventral/dorsal cornu. The use of size and shape of these structures as taxonomically important characters is useful to separate larvae from different species. However, we observed notable differences in *Cuterebra histrio* in the shape of these structures between second and third instar, which states the importance of the description of all the developmental stages (Knipling & Brody, 1940; Capelle, 1970) and the lack of knowledge of this family.

Finally, the shape of the posterior spiracles varies between larval stages and between species. In the second instar, the larva presents only two slits on each spiracle, this is similar to what has been previously reported in the majority of second instar larval descriptions (Knipling & Brody, 1940; Dalmat, 1942; Capelle, 1970), with the exception of *C. tenebrosa* (Baird, 1973) which presents three to four slits per side. On the contrary the third instar presents three clear divisions and multiple slits intertwined on each spiracle. When compared to other species, the differences are notable in shape and disposition of the spiracle (Cameron, 1926; Knipling & Brody, 1940; Dalmat, 1942; Benett, 1955; Haas & Dicke, 1958; Capelle, 1970; Baird, 1973; Lara-Lagunes *et al*.,

2016). Therefore, these characters can serve as useful taxonomical characters for the proper identification of larval stages inside this genus.

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