

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Description of the Third-Instar of *Anastrepha leptozona* Hendel
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Descrição do Terceiro Ínstar de *Anastrepha leptozona* Hendel (Diptera: Tephritidae)

RESUMO - A morfologia da larva do terceiro ínstar de *Anastrepha leptozona* Hendel é descrita. São analisados, em microscopia de luz e de varredura, o complexo antenomaxilar, as margens da abertura oral, o bordo, o órgão oral, o esqueleto cefalofaríngeo, os espiráculos anterior e posterior e o segmento caudal. As larvas do terceiro ínstar de *A. leptozona* têm um “esclerito ventral” abaixo do esclerito faríngeo, o qual é caracterizado pela primeira vez em espécies de *Anastrepha*.

PALAVRAS-CHAVE: Morfologia, esclerito ventral, mosca-das-frutas

ABSTRACT - The morphology of the third-instar larva of *Anastrepha leptozona* Hendel is characterized using optical and scanning electron microscopy. The antenomaxillary complex, oral ridges, labium, stomal sensory organ, cephalopharyngeal skeleton, anterior and posterior spiracles and caudal segment are described and illustrated. Mature larvae of *A. leptozona* present a “ventral sclerite” below the pharyngeal sclerite which is characterized for the first time in *Anastrepha* species.

KEY WORDS: Morphology, ventral sclerite, fruit fly

Anastrepha leptozona Hendel is a species widely distributed throughout the Neotropical region including records from Mexico, Guatemala, Honduras, Costa Rica, Panamá, Guyana, Venezuela, Trinidad, Bolivia and Brazil (Hernández-Ortiz & Aluja 1993). It has been recorded breeding in several host plants species of the families Anacardiaceae (1), Icacinaceae (1), Myrtaceae (1), Quinaceae (1), Rosaceae (1) and Sapotaceae (6) (Norrbom *et al* 1999).

In Mexico, *A. leptozona* has been reported in the state of Chiapas, Morelos, Oaxaca, and Veracruz (Hernández-Ortiz 1992, 2007), usually breeding in the host *Micropholis mexicana* (Sapotaceae) and less frequently in *Crataegus* sp. (Rosaceae) (Aluja *et al* 1987)

This species belongs to the leptozona species group, along with four other species *A. barnesi* Aldrich, *A. costalimai* Autuori, *A. elongata* Fernández, and *A. steyskali* Korytkowski (Norrbom *et al* 1999). Adults of this group may be recognized by the lateral surstylus short and somewhat boot-shaped, with laterally projecting apical lobe; and the vein M strongly curved apically.

Brief description of some larval characters of *A. leptozona* was presented in studies by Carroll *et al* (2004). Also Steck

et al (1990) presented a key for the recognition of the third-instar larvae for 13 *Anastrepha* species which included *A. leptozona*. However, there is no information about the larval morphology of the other species belonging to the leptozona species group.

In this paper we made a detailed description of the morphology of the third-instar larva of *A. leptozona* for the first time. Also we discuss the relevance of some distinctive characters, such as the “ventral sclerite” which has not been previously described in any other *Anastrepha* species.

Material and Methods

The studied specimens were collected infesting fruits of “baricoco”, *Micropholis mexicana* (Gilly) on March, 2004, in the so-called “El Bucaro” neighborhood of Huehuetán, Chiapas, Mexico. The site of collection is located in the coastal region of southeastern Chiapas, at 20 m altitude above sea level (15° 01' 43" N – 91° 19' 40" W). The climate is classified as warm tropical humid (García 1973), with an average temperature of 28°C and an average annual rainfall of 2,326 mm.

The collected fruits were inspected, and the found larvae were kept alive for seven days in a maturation cage. At the end of this period the third-instar larvae were removed from the fruits and killed by dipping in hot water. In total, we obtained 350 larvae specimens, which were preserved in 70% ethanol. We selected 25 individuals which were prepared for analysis by optical and scanning electron microscopy (SEM). For optical study, the larvae were prepared following techniques described by Steck & Wharton (1988). Antennomaxillary complex, oral ridge, labium, stomal sensory organ, cephalopharyngeal skeleton, the anterior and posterior spiracles and the caudal segment were dissected and slide mounted for observations.

For scanning electron microscopy (SEM), samples were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate Buffer pH 7.4 for 2h at 4°C and postfixed in 1% OsO₄ at room temperature and darkness. Tissues dehydrated in graded acetone were critical-point dried with CO₂, mounted, and then coated with a layer of palladium-gold approximately 20 nm thick. Samples were examined using SEM JEOL JSM-5600-SV at Department of Entomology, Instituto de Ecología (Xalapa, Veracruz), Mexico, and with JEOL JSM-25-SII at the Pontificia Universidad Católica de Chile (Santiago, Chile). The terminology of larval description follows White & Elson-Harris (1992), White *et al* (1999) and Frias *et al* (2006). Voucher specimens are deposited in the Instituto de Entomología, Universidad Metropolitana de Ciencias de la Educación (Santiago, Chile), in the Instituto de Entomología A.C. Department of Entomology (Veracruz, México) and in the Moscamed (Tapachula, Chiapas) collections.

Anastrepha leptozona Hendel

Description

Third-instar larva. Body pale yellow, elongate, range of length 7.0-11.0 mm, mean length 8.6 ± 1.42 mm. There is a brown spot below the pharyngeal skeleton in ventral

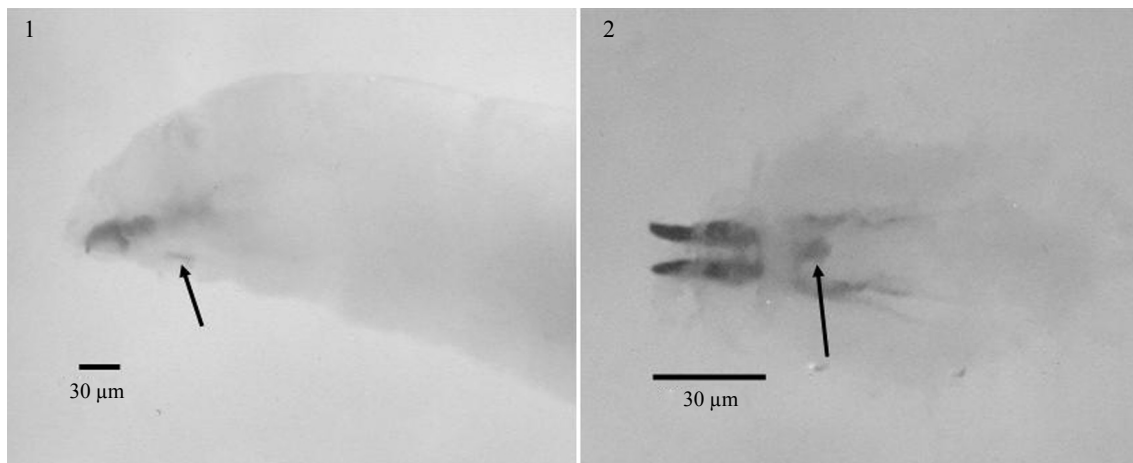
view, between the intersection of segments T1 and T2. This character, that we coined as ventral sclerite, is exclusive for *A. leptozona* and has not been previously described in *Anastrepha* (Figs 1-2).

Head dorsally smooth, without spinules; mouth opening bordered with 10-11 rows of non-serrated oral ridges, with 10-15 small accessory plates (Figs 3-4). Labium broad triangular-shaped, with two papilla sensilla and a pair of medial pits (Figs 4-5). Stomal sensory organ elongated, apically rounded, with two secondary lobes and six peg sensilla (Figs 4, 6).

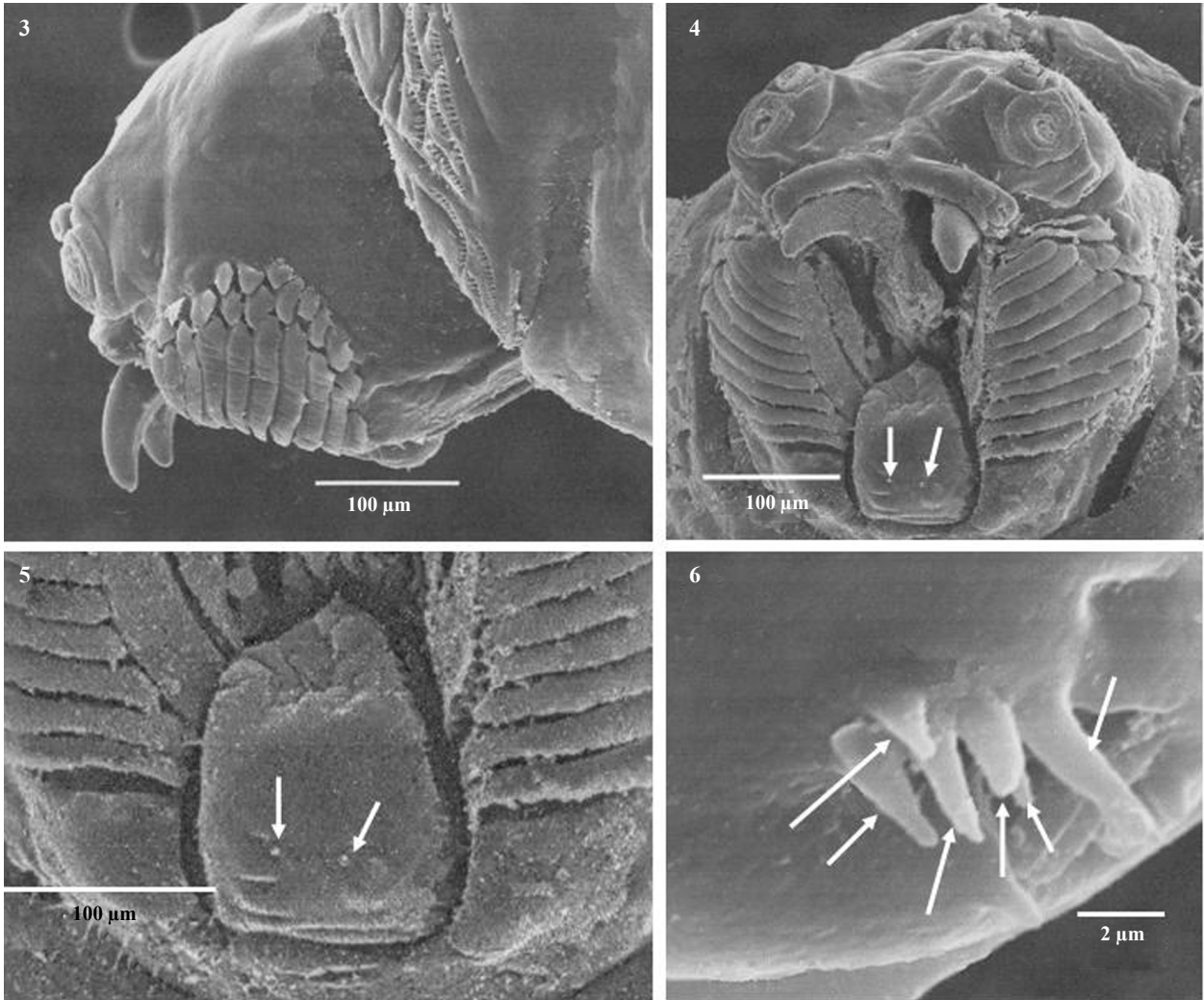
Antenna three-segmented; maxillary palpus with three papilla sensilla and two knob sensilla, dorsolateral group bearing two papilla sensilla close to maxillary palpus (Figs 7-8). Cephalopharyngeal skeleton with strongly sclerotized mandibles, apical tooth curved and black; ventral apodeme brown and broad, dorsal apodeme short; preapical teeth absent. Dental sclerite absent; labial sclerite short and brown. Hypopharyngeal sclerite anteriorly black and posteriorly yellow. Pharyngeal sclerite brown and moderately sclerotized; anterior sclerite, dorsal and ventral cornua yellow and weakly sclerotized; dorsal bridge of dorsal cornu brown, moderately sclerotized; ventral bridge of ventral cornu not evident (Figs 9-10). Below the pharyngeal sclerite, attached to the cuticle and bind with muscles to the ventral apodeme of mandible, there is a ventral sclerite that is a glove-shaped structure provided with four “fingers”-like protuberances; this character was found by Liliana López (Figs 11-13). The ventral sclerite is not directly linked to the cephalopharyngeal skeleton therefore not shown in Fig 9.

Anterior spiracles with 16-20 tubules arranged in a single row, mean 17.5 ± 1.40 , sometimes in the central area with a double row (Fig 14).

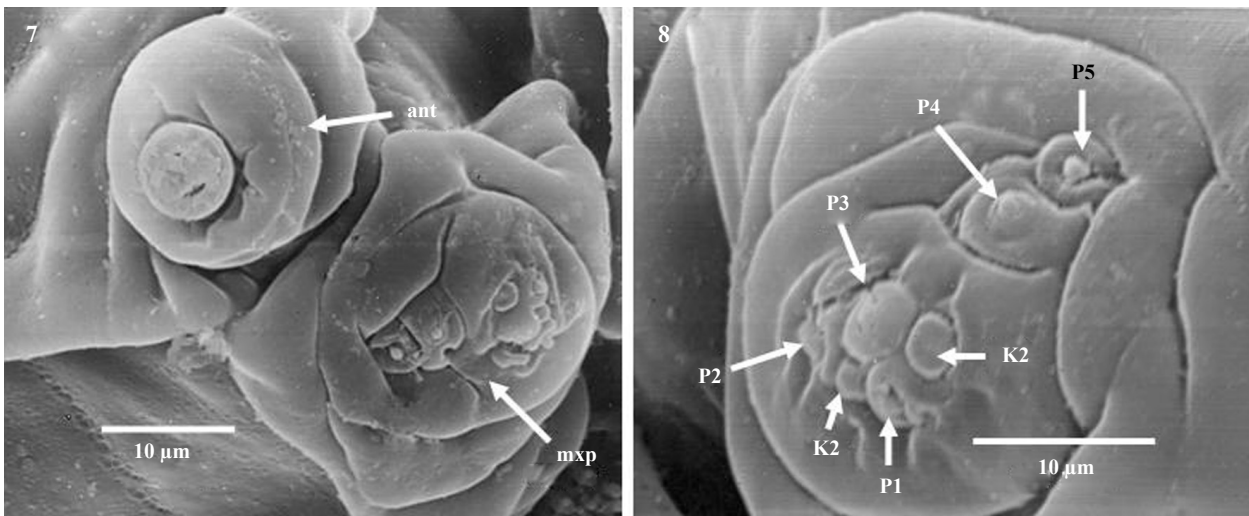
Caudal segment with two large lateral tubercles in intermediate area, intermediate sensilla I1 and I2 evident, sensilla I3 present. Lateral area with two tubercles and one lateral sensillum. Ventral area with two small tubercles over broad, triangular-shaped fold of cuticle (Fig15). Dorsal area with D1 and D2 sensilla, laterally two extraordinary



Figs 1-2 Third-instar larva of *A. leptozona*: Cephalopharyngeal skeleton in lateral view (1). ventral view (2). Arrows showing the “ventral sclerite”.



Figs 3-6 Third-instar larva of *A. leptozona*. Head in lateral view (3). Head in frontal view (4). Labium (5), arrows showing the papilla sensilla. Stomal sensory organ (6), arrows showing the peg sensilla.



Figs 7-8 Antennomaxillary complex of larva of *A. leptozona*. Antenna and maxillary palpus (7). Maxillary palpus (8).

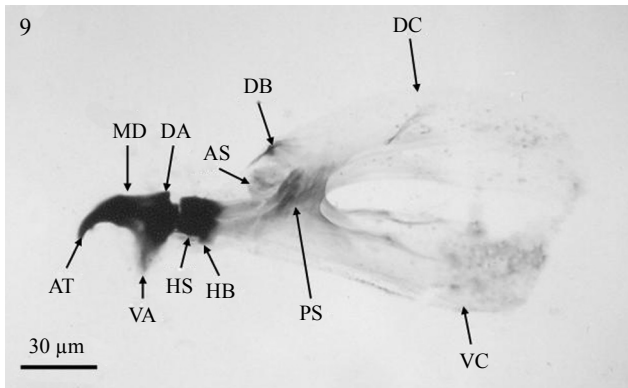


Fig 9 Cephalopharyngeal skeleton of larva of *A. leptozona*, lateral view. AS = anterior sclerite. AT = apical tooth. DA = dorsal apodeme. DB = dorsal bridge. DC = dorsal cornu. HB = hypopharyngeal bridge. HS = hypopharyngeal sclerite. MD = mandible. PS = pharyngeal sclerite. VA = ventral apodeme. VC = ventral cornu.

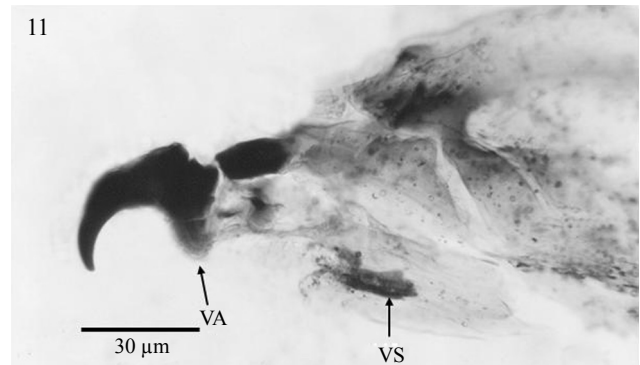


Fig 11 Cephalopharyngeal skeleton of larva of *A. leptozona* in lateral view. Ventral apodeme (VA); ventral sclerite (VS).

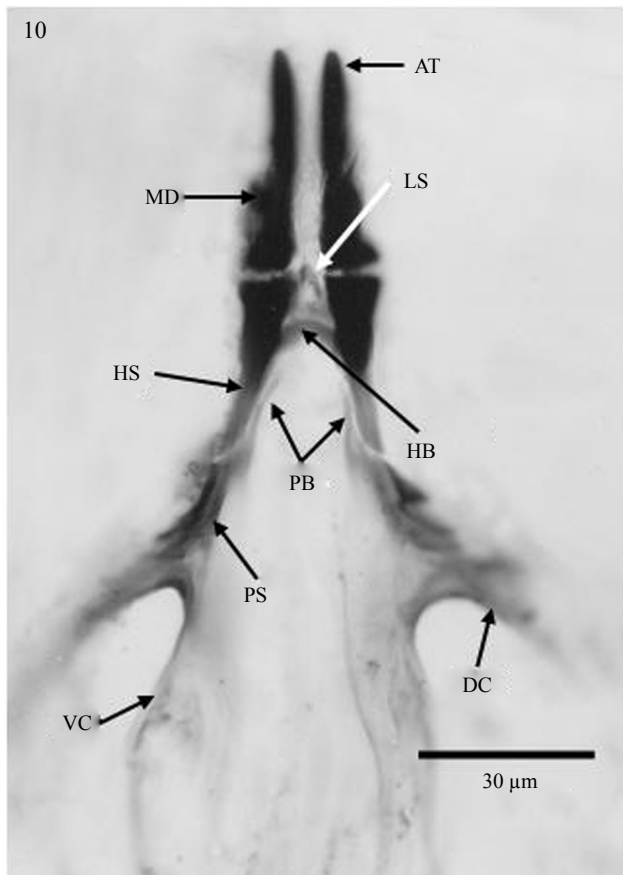
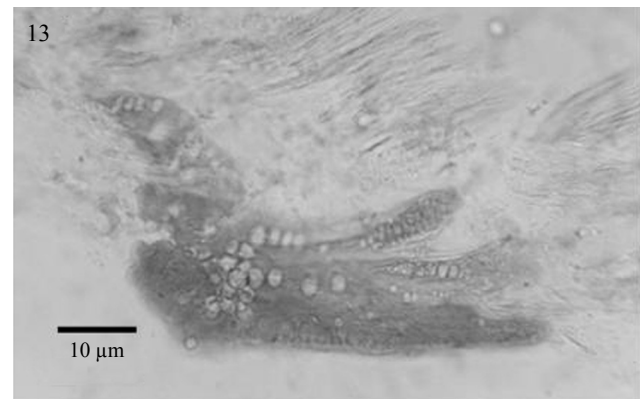
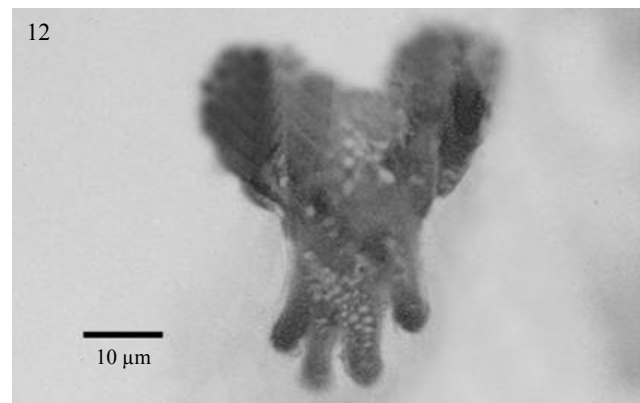


Fig 10 Cephalopharyngeal skeleton of larva of *A. leptozona*, dorsal view. AT = apical tooth. DC = dorsal cornu. HB = hypopharyngeal bridge. HS = hypopharyngeal sclerite. LS = labial sclerite. MD = mandible. PS = pharyngeal sclerite. VC = ventral cornu.



Figs 12-13 Ventral sclerite of larva of *A. leptozona*. Ventral view (12). Lateral view (13).

15, 18). Posterior spiracles with long spiracular slits, nearly two times as long as wide, dorsal and ventral spiracular bundles with short spatulate hairs (Fig 17).

Remarks

Based on the morphology of the third-instar larva of *A. leptozona* some traits could be used for the recognition of this species as follows: the presence of the ventral sclerite below the pharyngeal sclerite observed under optical microscopy appears glove-shaped and it is absent in all other larvae of *Anastrepha* previously described. The

D3 and D4 sensilla. Anal lobes unilobate with anal opening surrounded by wrinkled surface with minute spinules (Figs

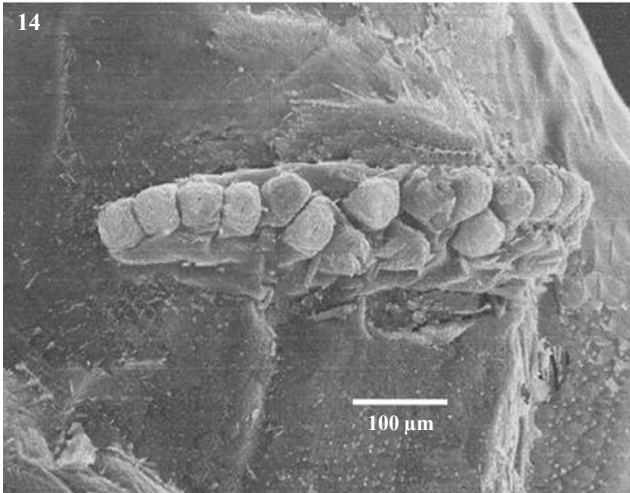
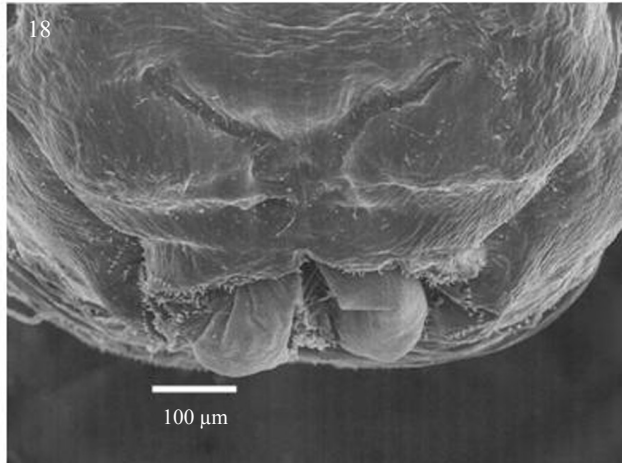
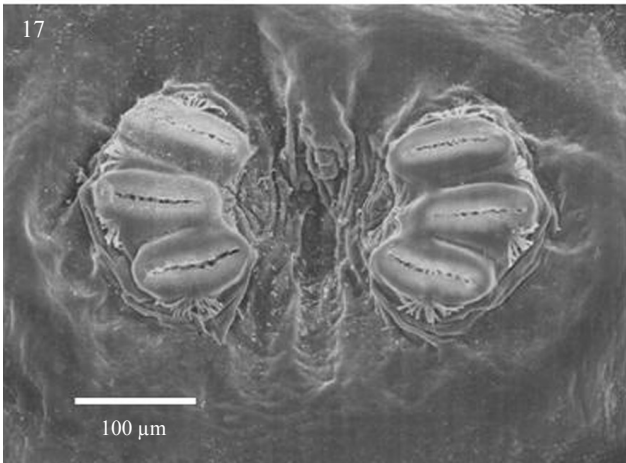
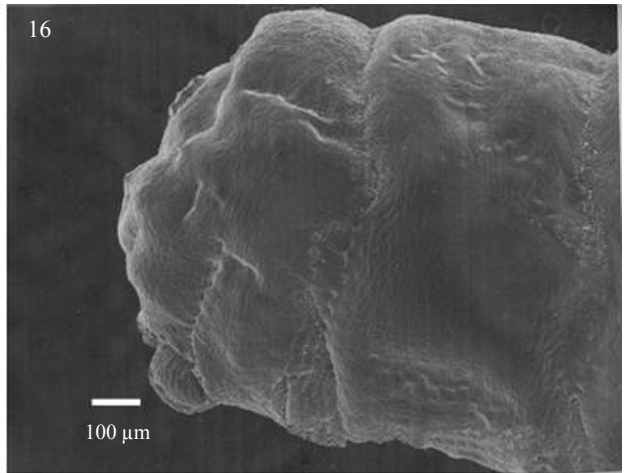
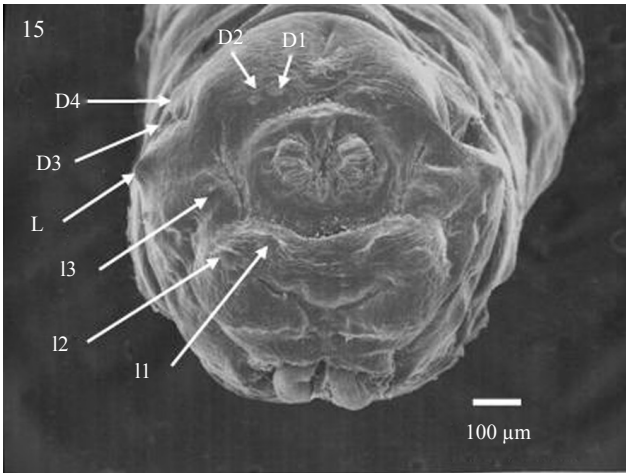


Fig 14 Anterior spiracle of larva of *A. leptozona*.

anterior spiracles comprise 17-19 tubules in a single row laterally and double row centrally in all the individuals studied, similar to *A. grandis* (Macquart) (Steck & Wharton 1988) and *A. bistrigata* Bezzi (Steck & Malavasi

1988), in most other *Anastrepha* species these tubules are arranged in a single row, e.g. *A. fraterculus* (Wiedmann), *A. interrupta* Stone, *A. limae* Stone, *A. ludens* (Loew), *A. obliqua* (Macquart), *A. serpentina* (Wiedmann), *A. striata* Schiner and *A. suspensa* (Berg 1979, Steck & Wharton 1988, Carroll & Wharton 1989, White & Elson-Harris 1992, Frías *et al* 2006). In the posterior spiracles the bundle of hairs of the spiracular slits are shorter than width of spiracular slits, in other species such as *A. fraterculus*, *A. interrupta*, *A. grandis*, *A. limae*, *A. ludens*, *A. obliqua*, *A. serpentina*, *A. striata* *A. suspensa* these hairs are as long as or longer than the width of spiracular slits (Berg 1979, Steck & Wharton 1988, Steck & Malavasi 1988, Carroll & Wharton 1989, White & Elson-Harris 1992, Frías *et al* 2006). The labium has two papilla sensilla and a small pair medial pits which have been previously described only in mature larva of *A. ludens* (Carroll & Wharton 1989). The intermediate area of caudal segment presented two big papilla sensilla, which were never described before (Berg 1979, Steck & Malavasi 1988, Steck & Wharton 1988, Carroll & Wharton 1989, Steck *et al* 1990, White & Elson-Harris 1992, White *et al* 1999, Carroll *et al* 2004, Frías *et al* 2006). These traits may be diagnostic for the identification of third-instar larva of *A. leptozona*.



Figs 15-18 Caudal segment of larva of *A. leptozona*. Posterior view (15), D1, D2, D3, D4 = dorsal sensilla; I1, I2, I3 = intermediate sensilla, L = lateral sensillum. Lateral view (16). Posterior spiracles (17). Anal lobes (18).

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