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The Immatures of The Biting Midge *Culicoides trilineatus* (Diptera: Ceratopogonidae) Potential Vector of The Bluetongue Virus (BTV)

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Abstract

The fourth instar larva and pupa of *Culicoides trilineatus* Fox, a species considered as potential vector of the bluetongue virus (BTV) in Central and South America, are described, illustrated, and photomicrographed for the first time by using binocular, phase-contrast, and scanning electron microscopy. The immatures were collected by using a siphon bottle in tree-holes in Salta Province, Argentina, transported to the laboratory and there bred to the adult's emergency. They are compared with the immatures of *C. debilipalpis* Lutz, another Neotropical species that breeds in tree holes. Details on larval biology and habitat are given.

Keywords: *Culicoides*, Ceratopogonidae, larva, pupa, phytotelmata.

Introduction

The Diptera family Ceratopogonidae is placed in the infraorder Culicomorpha (Borkent 2012). It includes six subfamilies, of which four are extant and worldwide in distribution, with 111 extant genera and 6,267 species, and fossil records include 21 genera and 284 species (Borkent 2016). The larval habitat of Ceratopogonidae are generally poorly known, but include semiaquatic habitats ranging from mud at the soil-water interface, to moist and highly organic soil substrates and intact dung pats (Purse et al. 2015). In addition, they also breed in phytotelmata, aquatic microenvironments formed by the accumulation of water in any part of plants such as leaves, flowers, stems, trunks, and tree holes (Campos et al. 2011).

The adult females of the genus *Culicoides* Latreille are haematophagous. They are known as “biting midges”, “no-see-ums” or “punkies” in English speaking countries, “polvorines”, “manta blanca”, “chaquistes” or “jejenes” (common name shared with Simuliidae) in Spanish speaking countries, and “mosquito pólvora” or “maruim” in Brazil (Spinelli and Ronderos 2005). Many species are involved in the transmission of arbovirus, protozoa and filarial nematodes that cause diseases in both humans and animals (Borkent and Spinelli 2007). One of the most important of these diseases is caused by the Bluetongue Virus (BTV), which attacks sheep, cattle, goats and wild rumiants, causing hemorrhage and ulceration in the upper gastrointestinal tract as well as laminitis, coronitis, facial and neck edema, pulmonary edema, reproductive failures and lameness (Mellor et al. 2009). Information about bluetongue virus (BTV) in Central America, the Caribbean and South America is limited. The traditional idea claims that BTV spread is limited to latitudes 35°S and 40°N or 50°N (Coetzee et al. 2012), almost all the American

continent. Latitude 35°S reaches Central Argentina and nearly all the Pampas region, where the main cattle production is carried out, whereas latitude 50°N reaches southern Canada. Nowadays, it is empirically known that BTV is widely spread between those limits, an idea supported mainly by serologic evidence (Legisa et al. 2013). Regarding the vector species suspected to be responsible for BTV transmission among ruminants, Walton and Osburn (1992), Greiner et al. (1990) and Mo et al. (1994) isolated BTV from *Culicoides insignis* Lutz, *C. filarifer* Hoffman and *C. pusillus* Lutz in Central America. Additionally, they suggested that these three species, mainly *C. insignis*, were the primary species involved in the virus transmission in the region, pointing out that other species which could act as vectors in that region are *C. pusillus*, *C. furens* Poey and *C. trilineatus* Fox. Since 1996 Argentina has been considerate serologically positive according to OIE parameters (Gorchs and Lager 2001), and the virus (serotype 4) has been isolated from cattle in Corrientes province (Gorchs et al. 2002, Lager 2004, Legisa et al. 2014, Veggiani Aybar et al. 2016). Although the southern boundary of BTV reaches the central region of the country, recent climate change around the world makes necessary a new configuration of those boundaries.

During a sampling program focused on the collection and study of Diptera carried out in Salta province between 2012 and 2013, larvae and pupae of *Culiciodes trilineatus* were collected from tree holes and reared to adults. The aim of this work is to provide for the first time the description of these immatures that belong to a species suspected to be a vector of BTV.

Materials and Methods

Study area: San Ramon de la Nueva Orán, hereafter Orán, is located in northwestern Argentina, 270 Km from the city of Salta and 44 km from the border with Bolivia (23º08' S, 64º20' W, elevation 337 m). The climate is subtropical, with an average summer temperature of 27.7°C and winter temperature of 16.4°C. The mean annual rainfall is 1.000 mm, occurring mostly during the warmer months (October to April). Orán is included in the pedemontane floor of the Yungas subtropical montane moist forest, where jungles of Palo Blanco (*Calycophyllum multiflorumy* Griseb; Rubiales: Rubiaceae) and Palo Amarillo (*Phyllostylon rhamnoides* (Poisoon) Taub; Urticales: Ulmaceae) predominate. Vines are also important in pedemontane areas (Brown et al. 2001). The area has been subjected to ecological modifications related to human activities, mainly urbanization, industrial developed, agriculture and forestry (Brown et al. 2001). The city is characterized by a densely built central area where houses with small or not front yards predominate and there are few low buildings. Suburban areas have a lower building density with bigger gardens, more trees and are closer to the border with the seminatural region.

Entomological sampling: Specimens were collected during field sampling carried out from January to April 2012 to 2013, as a part of a larger study on mosquitoes (Diptera: Culicidae) larval habitats. Samples were collected from tree holes using a siphon bottle, following the procedure described by Müller and Marcondes (2006) and Mangudo et al. (2010). For details on tree holes selection see Mangudo et al. (2017).

The specimens were carried to the laboratory, larvae were preserved in ethanol 80% and pupae were kept alive isolated in plastic vials (2 mm) holding water from the tree hole and containing a piece of humid filter paper, to maintain the humidity inside the vials. They were observed daily until adult emergence. After emergence adults were maintained alive for 24 hours to ensure their final pigmentation. Adults and their respective exuviae were stored in vials containing ethanol 80%. Larval, pupal exuviae and adults were mounted in Canada balsam following the technique described by Borkent and Spinelli (2007). For scanning electron microscopy (SEM), larvae and pupae were prepared following the technique of Ronderos et al. (2000, 2008). Illustrations were made with pen and ink using an attached camera lucida. Photomicrographs were taken with a Micrometrics SE Premium digital camera, through a Nikon Eclipse E200 microscope.

For larval terms see Ronderos et al. (2010) and Borkent (2014) for pupae.

The plates were made in TIFF format in Adobe Photoshop version14. The studied material is deposited in the División Entomología, Museo de La Plata (MLPA), La Plata, Argentina.

Results

Culicoides trilineatus Fox

(Figs. 1-4)

Culicoides trilineatus Fox 1946: 250 (female; Virgin Islands); Fox 1949: 30 (male; Puerto Rico); Forattini 1957: 389 (redescription; distribution); Wirth and Blanton,

1956: 189 (redescription; distribution); Cavalieri and Chiossone 1966: 149 (distribution in Argentina); Vitale et al. 1981: 146 (in key to species in the debilipalpis group); Wirth et al. 1988: 50 (in Neotropical Wing Atlas; distribution); Borkent and Spinelli 2000: 42 (in New World catalog south of the USA; distribution); Spinelli et al. 2005: 13 (in review of hematophagous Ceratopogonidae of Argentina; Paraguay record; in key; wing photograph); Borkent and Spinelli 2007: 75 (in Neotropical catalog; distribution).

Culicoides (*Oecacta*) *trilineatus*: Wirth 1974: 36 (in New World catalog south of the USA; distribution).

Fourth instar larva (Figs. 1A-D, 2A-D and 3A-C). Coloration whitish in life. Head capsule yellowish, moderately elongate, apex slightly bent ventrally, all setae simple, moderately thin, medium-sized to elongate (Figs. 1A-C and 3A); chaetotaxy as in Fig.1B-C. HL 0.205-0.210 (0.206, n=4) mm; HW 0.12-0.15 (0.13, n=4) mm; HR 1.36-1.75 (1.56, n=4); SGW 0.10-0.12 (0.11, n=4) mm; SGR 1.12-1.25 (0.19, n=4). Antennae short, cylindrical (Figs. 1C-D and 2A). Labrum (Figs. 1C-D and 3A-B) 0,64 times longer than wide, with three pairs of anterolateral sensilla styloconica (Fig. 2A-C); palatum (Figs. 1D and 2A-C) with four pairs of sensilla trichoidea closely spaced, anterior sensillum long, posterior of them three medium-sized (Fig. 2A-C); messors well developed, stout, with 4-5 angulate teeth (Fig. 2A-C); scopae well developed, with apparently 8-10 elongate, strong, pointed teeth (Fig. 2A-C). Maxilla (Fig. 1B, D and 2A) well sclerotized; galeolacinia (Fig. 2B and C) with three-four papillae and with long seta; maxillary palpus (Fig. 2C) medium-sized,

cylindrical, with two-three apical papillae; lacinial sclerite 1 without seta, lacinial sclerite 2 with long, thin seta (Fig. 2A-C).

Mandible (Figs. 1C-D and 3A-B) hooked, curved, with broad base, with distinct subapical notch and associated rounded prominence, one sensory pit and one medium-sized seta on the aboral surface, prominent point of articulation, two teeth, apical elongate, pointed tooth, the inner tooth smaller; MDL 0.040-0.055 (0.046, n=3) mm; MDW 0.012-0.015 (0.014, n=3) mm. Hypostoma (Figs. 1C-D and 2A-C) with quadrangular mesal serrate elevation, lateral margin with 5-6 strong teeth, the first smaller than the lasted tooth. Labium elongate, not extending beyond hypostoma. Epipharynx (Fig. 3A-C) massive, strongly sclerotized, two comb present, with dorsal comb moderately wide and rounded posteriorly, with 8-9 elongate, subequal teeth, the central tooth stouter than other, ventral comb with 10-12 small, lancelate, thin teeth; lateral arms stout, elongate, with lateral curtains with finely pointed teeth of moderate length (Fig. 3B-C); LAW 0.07-0.09 (0.08, n=4) mm, DCW 0.027-0.030 (0.028, n=4) mm. Hypopharynx (Fig. 3A-C) elongate, thin, moderately sclerotized, without fringe. Thoracic pigmentation diffuse. Abdominal segment whitish. Caudal segment (Fig. 2D) with four anal papillae and 6 pairs of setae: “o”, “i” long, thin setae; l_1 and l_2 medium-sized, thin setae, v, d, short, thin setae; CSL 0.46-0.50 (0.48, n=3); CSW 0.56-0.60 (0.58, n=3); CSR 2.09-3.5 (2.48, n= 3); OL 0.067-0.075 (0.070, n=3); OW 0.015-0.022 (0.019, n=3).

Female pupa (Figs. 3D-E, I and 4A, C-E). Habitus as in Fig. 3D. Exuviae general coloration pale brown. Total length 2.32-2.60 (2.42, n= 3) mm. **Head**: Dorsal apotome (Fig. 3E) 1.45X broader than long, apex apparently truncated, surface

covered with small rounded spinules, distal margin truncate, smooth with 2 pairs of raised, wrinkles areas; dorsal apotome sensilla (Fig. 3E): DA-1-H elongate, thin seta, insert in well-developed tubercle, DA-2-H basal campaniform sensillum; disc surface covered by stout, rounded spinules; DAL 0.13-0.16 (0.14, n=3) mm; DAW 0.20-0.21 (0.205, n=3) mm; DAW/DAL 1.28-1.61 (1.45, n=3). Mouthparts as in Fig. 4A. Sensilla: two dorsolateral cephalic sclerite sensilla: DL-1-H long, thin seta, DL-2-H stout, short setae; without clypeal/labrals; three ocular (Fig. 4A): O-1-H, O-3-H long, thin setae; O-2-H campaniform sensillum. **Thorax**: Prothoracic extension absent; respiratory organ (Fig. 3D) pale brown, nearly straight, with scale-like spines, with 7–8 apical and 2 lateral pores; RO length 0.155-0.160 (0.158, n=3) mm, RO width 0.03 (n=3) mm; pedicel short, stout; length 0.025-0.03 (0.028, n=3); P/RO: 0.15-0.19. Sensilla: one anteromedial sensillum: AM-1-T medium-sized, thin seta, three anterolateral sensilla: AL-1-T, AL-2-T long, thin setae, AL-3-T short, stout seta; dorsals (Fig. 4C): D-1-T, D-2-T, D-4-T; D-5-T medium-sized, thin setae, D-3-T campaniform sensillum, supraalar (SA-2-T) campaniform sensillum. Metathoracics (Fig. 4D): M-2-T long thin seta, M-3-T campaniform sensillum. Cephalothorax surface with small rounded tubercles, length 0.85-0.95 (0.90, n=3) mm, width 0.60-0.67 (0.64, n=3) mm. **Abdomen**: abdominal segments covered with small spinules on anterior margin, posterior margin smooth. Sensilla: tergite 1 (Fig. 4D) with setae as follows: D-2-I, D-3-I long, thin setae; D-4-I, D-7-I campaniform sensilla; D-8-I medium-sized, thin seta; D-9-I long, thin seta; L-1-I long, thin seta, L-2-I, L-3-I short, thin setae. Second abdominal segment similar to the first one; segment 4 with sensillar pattern (Fig. 4E) as follows: D-2-IV, D-3-IV long, thin setae; D-2-IV longer than D-3-IV; D-4-IV, D-7-IV campaniform sensilla,

D-5-IV minute seta, D-8-IV, D-9-IV long, thin setae, D-8-IV stouter than D-9-IV, all located on flattened tubercles; L-1-IV, L-2-IV, L-4-IV short, stout setae, L-3-IV long, thin seta, all located on triangular tubercles; V-5-IV short, stout seta, V-6-IV, V-7-IV long, thin setae, V-7-IV longer than V-6-IV. Segment 9 (Figs. 3D and I) 1.62 X longer than wide, ventral surface with many spicules; length 0.23-0.25 (0.24, n=3) mm, width 0.14 (n=3) mm. Terminal process (Fig. 3D and I) triangular, elongated, subparallel, pointed, ventral surface of processes spiculate, with D-5-IX, D-6-IX campaniform sensilla, length 0.08-0.10 (0.09, n=3) mm.

Male pupa (Figs. 3F-H, J and 4B). Similar to female with usual sexual differences. Total length 2.30-2.40 (2.35, n=3) mm. Exuvium pale brown. Dorsal apotome (Fig. 3F) with DAL 0.17 mm; DAW 0.17 mm, DAW/DAL 1.00. Cepahalothoracic sensilla as in Figs. 3G-H and 4B. Respiratory organ (Figs. 3G-H and 4B), RO length 0.24 (0.15, n=3) mm, RO width 0.04 (n=3); pedicel length 0.025-0.30 (0.028, n=3). Cephalotorax: length 0.92-0.95 (0.93, n=3) mm, width 0.60-0.65 (0.62, n=2) mm. Segment 9 (Fig. 3J) length 0-26 (0.25-0.27, n=3) mm, width 0.15-18 (0.16, n=3) mm; genital lobe (Fig. 3J) reaching the posterior margin of segment; terminal process length 0.09-011 (0.10, n=3) mm.

Material examined. ARGENTINA: Salta: 3 males (with pupal exuviae), 3 females (with pupal exuviae), San Ramón de la Nueva Orán; 23°8'55.09"S, 64°19.24'.82"W, 13-III-2013, C. Mangudo, MLPA; same data except 23°8'29.16"S, 64°18'31.03"W, 26-II-2012, 4 larval exuviae.

Material examined by SEM. ARGENTINA: Salta: 2 larvae, 1 pupa, San Ramón de la Nueva Orán; 23°8'29.16"S, 64°18'31.03"W, 26-II-2012, C. Mangudo, MLPA.

Type material: Holotype female (mounted in balsam on a slide), Virgins Islands, St. Thomas, 11-IX-1937, biting in afternoon.

Distribution. Guatemala to Panama, Puerto Rico, Virgin Islands, Dominica, Barbados, Paraguay, Argentina (Salta, Formosa, Chaco, Misiones).

Discussion

Culiciodes trilineatus was originally described by Fox (1946) from St. Thomas, Virgin Islands, based on a couple of females biting humans in the afternoon. Posteriorly Fox (1949) described the male from a specimen reared after out of tree hole debris at Luquillo, Puerto Rico. Subsequently, the adult female was redescribed by Wirth and Blanton (1956). This species was assigned by Wirth (1974) to the subgenus Oecacta and Vitale et al. (1981) within to the debilipalpis group. However, Borkent and Spinelli (2000, 2007) in the Neotropical catalog did not assign it to any specific group or subgenus, including it in the Miscellaneous Unplaced species section. The reared female and male adults were identified as *C. trilineatus*, by the comparison with the above mentioned descriptions.

The larva and pupa of *C. trilineatus* are very similar to *C. debilipalpis*, a species that also breeds in tree holes, and whose immatures were fully described by Ronderos et al. (2010).

The larva of *C*. *trilineatus* resembles *C*. *debilipalpis* by virtue, the cylindrical maxillary palpus, galeolacinia with a long seta, hooked mandible, hypostoma with quadrangular mesal elevation and lateral margin with teeth; elongate and thin, hypopharynx without fringe and caudal segment with six pairs of setae. However, *C*. *debilipalpis* differs by the smaller head capsule (0.14-0.17 mm), the labrum has a pair of the sensilla styloconica, the labrum is as long as its greatest width and with three pairs of the sensilla trichoidea, the labium is distinctly smaller, the epypharynx has two ventral combs and one dorsal comb, the latter with 22-24 small teeth and caudal segment without anal papillae. The pupa of *C*. *debilipalpis* is readily distinguished from *C*. *trilineatus* by the yellowish exuviae, the yellowish respiratory organ except its distal half dark brown, and by the presence of two clypeal/labrals and the minute D-8-I. Ronderos et al. (2010), incorrectly mentioned the presence of one dorsal cephalic sclerite sensillum, two oculars and two anterolaterals for *C*. *debilipalpis*, but a detailed revision of the pupa of this species during the present study revealed the presence of the two dorsal cephalic sclerite sensilla, three oculars and three anterolateral sensilla.

Perspective. Because the change in global climate driven by global warming could contribute to the creation of more adequate conditions for the propagation and reproduction of the *Culicoides* spp. suspected to act as vectors of pathogens, the proper knowledge of the larval habitats and the accurate identification of their immatures would be extremely important in order to develop programs for the early detection of hatcheries and in this way avoid outbreaks that can spread the disease.

Biology. Larvae and pupae of *Culicoides trilineatus* were collected in tree holes between January and April 2012 to 2013. The tree holes were pans formed as branch intersections (maintaining an unbroken bark lining) in a *Delonix regia* (Bojer) Raf. (Fabales: Fabaceae) and a *Broussonetia papyrifera* (L.) Vent. (Rosales: Moraceae). The specimens described herein were collected from urban tree holes, but the species was also detected in holes from trees in yunga forest patches to the east of the city.

The *C. trilineatus* immatures were found either as single species when the volume of water was very low (>25 cc) or coexisting with *Aedes aegypti* L., *Aedes terrens* (Walker), and/or *Haemagogus spegazzini* Brèthes (water volume ranging from 300 to 600 cc). *Aedes aegypti* is the main global vector of dengue, urban yellow fever, zika, and chikungunya virus (Gubler, 2004, Ayres 2016, Marcondes et al. 2017), and *Haemagogus* species are involved in sylvan yellow fever and potentially other arbovirus transmission in South America (Karabatsos 1985). These findings highlight the importance of phytotelmata as larval habitat for dipterous of medical relevance in urban settings.

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470 Figure legends
471
472 Figure 1. Scanning electron micrographs of larva *Culicoides trilineatus* Fox
473 (Diptera: Ceratopogonidae) collected in Argentina: entire larva (**A**); head capsule,
474 lateroventral view (**B**); head capsule ventral view (**C**); head capsule laterofrontal
475 view (**D**). AN, antennae; CS, caudal segment; GL, galeolacinia; HC, head capsule;
476 HY, hypostoma; LB, labrum; MD, mandible; MX, maxilla; PL, palatum; ss, sensilla

477 styloconica; st, sensilla trichoideaa. Head capsule chaetotaxy are indicated by
478 single letters: o, parahypostomal setae; t, prefrontal setae; u, mesolateral setae; v,
479 posterolateral seta; w, anterolateral setae; x, paranntenal setae; y, ventral setae.
480
481 Figure 2. Scanning electron micrographs of larva *Culicoides trilineatus* Fox
482 (Diptera: Ceratopogonidae) collected in Argentina: palatum, messors and scoope
483 (**A**); detail palatum (**B**); detail maxilla (**C**); caudal segment dorsal view (**D**).
484 AN, antennae; AP, anal papillae; GL, galeolacinia; HY, hypostoma; LC2, lacinial
485 sclerite 2; LCT, lateral curtains; MD, mandible; MX, maxilla; MP, maxillary palpus;
486 MS, messors; PL, palatum; SC, scopae; ss, sensilla styloconica; st, sensilla
487 trichoidea. Caudal segment chaetotaxy: “d”, dorsal setae; “i”, inner seta; “l_1”, first
488 lateral seta; “l_2”, second lateral seta; “o”, outer seta; “p”, posterior perifrontal seta;
489 “q”, postfrontal setae; “v”, ventral setae.
490
491 Figure 3. Larva and pupa of *Culicoides trilineatus* Fox (Diptera: Ceratopogonidae)
492 collected in Argentina: Larva (**A**-**C**); female pupa (**D**-**E**, **I**); male pupa (**F-H**, **J**): head
493 capsule (**A**); head capsule, anteroventral view (**B**); caudal segment (**C**); entire pupa
494 (**D**); dorsal apotome (**E**-**F**); mouthparts and ocular sensilla (**G**); anteromedial
495 sensilla (**H**); respiratory organ (**I**); segment 9 (**J**-**K**).
496 AL-1-T, AL-2-T, AL-3-T, anterolateral sensilla; AM-1-T, AM-2-T, AM-3-T,
497 anteromedial sensilla; DA-1-H, D-2-H, dorsal apotome sensilla; DC, dorsal comb;
498 ep, epipharynx; GL, genital lobe, hyp, hypopharynx, HY, hypostoma: LTC lateral
499 curtains; MD, mandible; LB, labrum; O-1-H, O-2-H, O-3-H, ocular sensilla; P
500 pedicel; p, pore, RO, respiratory organ; TP, terminal process; VC, ventral comb.

Figure 4. Draw of pupa *Culicoides trilineatus* Fox (Diptera: Ceratopogonidae) collected in Argentina: Female pupa (**A**, **C**-**E**); male pupa (**B**): Mouthparts and ocular sensilla (**A**); Cephalothoracis sensilla (**B**);) dorsal sensilla (**C**); metathorax and tergite 1 (**D**); Segment 4 (**E**). Scale bars: 0.05 mm.

AL-1-T, AL-2-T, AL-3-T, nterolateral sensilla; AM-1-T, anteromedial sensilla; D-1-T, D-2-T, D-3-T, D-4-T, D-5-T, dorsal sensilla; DL-1-H, DL-2-H, dorsolateral cephalic sclerite sensilla; M-2-T, M-3-T, metathoracic sensilla; O-1-H, O-2-H, O-3-H, ocular sensilla; P, pedicel; RO, respiratory organ; D-1-IV, D-2-IV, D-3-IV, D-4-IV, D-5-IV, D-7-IV, D-8-IV, D-9-IV, L-1-IV, L-2-IV, L-3-IV, L-4-IV,V-1-IV, V-5-IV,V-6-IV, V7-IV, segment 4 sensilla, D-1-I, D-2-I, D-4-I, D-7-I, D-8-I, D-9-I, L-1-I, L-2-I, L-3-I, tergite 1 sensilla.

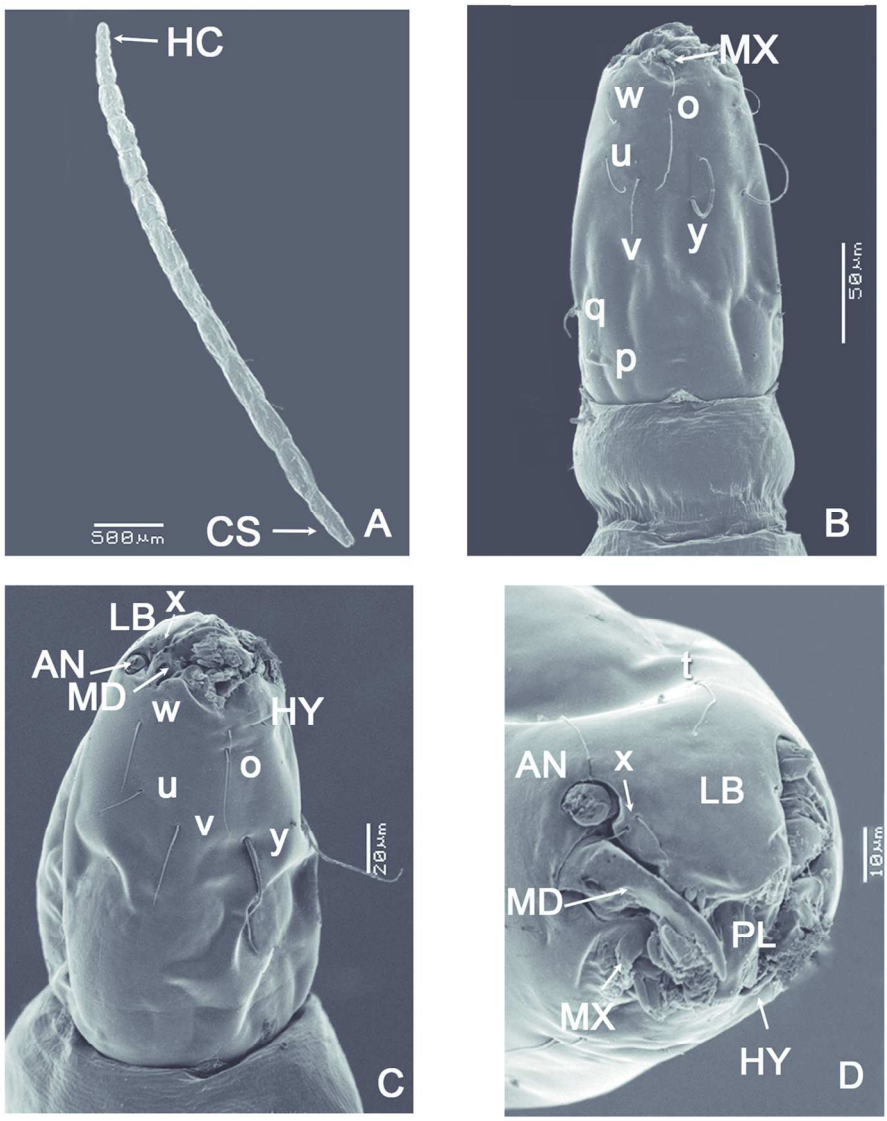

Figure 1. Scanning electron micrographs of larva *Culicoides trilineatus* Fox (Diptera: Ceratopogonidae) collected in Argentina: entire larva (A); head capsule, lateroventral view (B); head capsule ventral view (C); head capsule laterofrontal view (D). AN, antennae; CS, caudal segment; GL, galeolacinia; HC, head capsule; HY, hypostoma; LB, labrum; MD, mandible; MX, maxilla; PL, palatum; ss, sensilla styloconica; st, sensilla trichoideaa. Head capsule chaetotaxy are indicated by single letters: o, parahypostomal setae; t, prefrontal setae; u, mesolateral setae; v, posterolateral seta; w, anterolateral setae; x, paranntenal setae; y, ventral setae.

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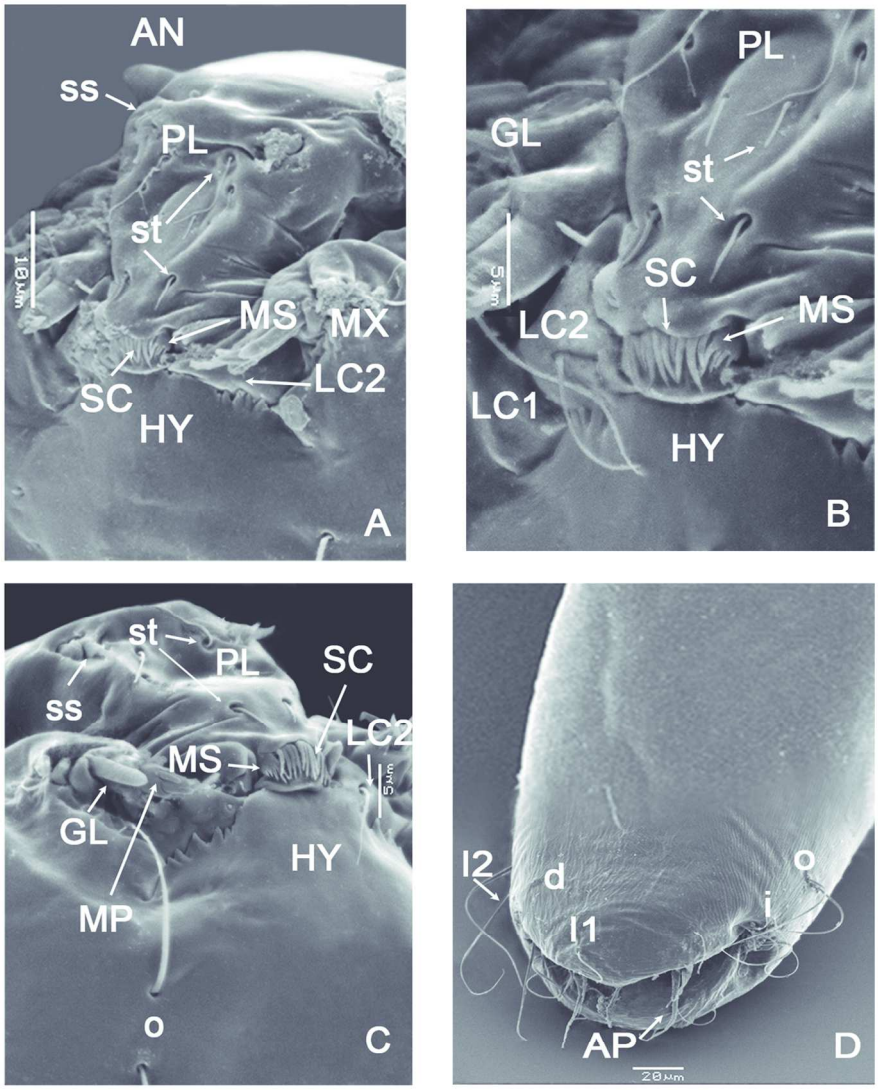

Figure 2. Scanning electron micrographs of larva *Culicoides trilineatus* Fox (Diptera: Ceratopogonidae) collected in Argentina: palatum, messors and scoope (A); detail palatum (B); detail maxilla (C); caudal segment dorsal view (D).
AN, antennae; AP, anal papillae; GL, galeolacinia; HY, hypostoma; LC2, lacinial sclerite 2; LCT, lateral curtains; MD, mandible; MX, maxilla; MP, maxillary palpus; MS, messors; PL, palatum; SC, scopae; ss, sensilla styloconica; st, sensilla trichoidea. Caudal segment chaetotaxy: "d", dorsal setae; "i", inner seta; "l1", first lateral seta; "l2", second lateral seta; "o", outer seta; "p", posterior perifrontal seta; "q", postfrontal setae; "v", ventral setae.

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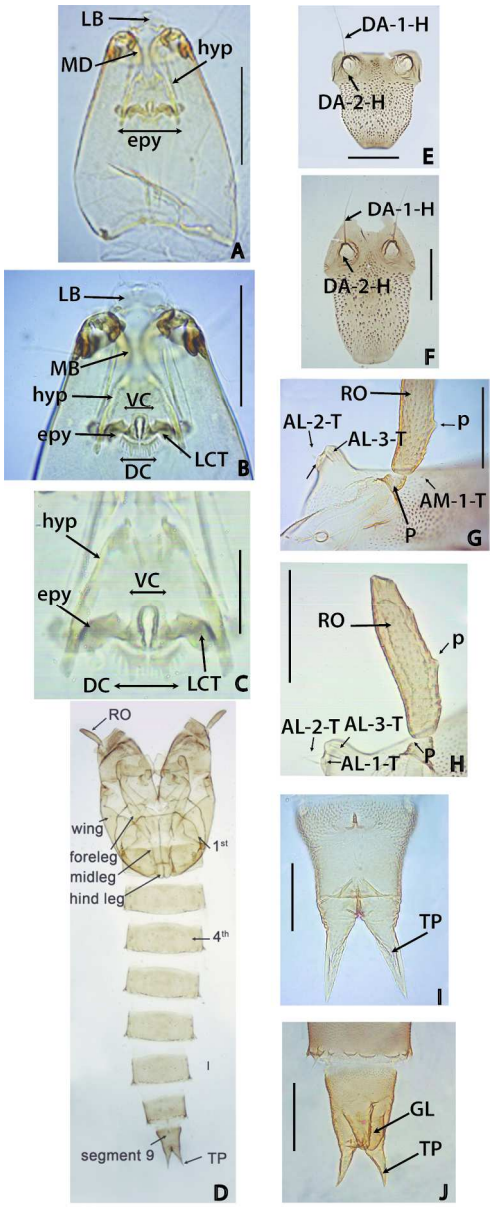

Figure 3. Larva and pupa of *Culicoides trilineatus* Fox (Diptera: Ceratopogonidae) collected in Argentina: Larva (A-C); female pupa (D-E, I); male pupa (F-H, J): head capsule (A); head capsule, anteroventral view (B); caudal segment (C); entire pupa (D); dorsal apotome (E-F); mouthparts and ocular sensilla (G); anteromedial sensilla (H); respiratory organ (I); segment 9 (J-K).┣ ┳ AL-1-T, AL-2-T, AL-3-T, anterolateral sensilla; AM-1-T, AM-2-T, AM-3-T, anteromedial sensilla; DA-1-H, D-2-H, dorsal apotome sensilla; DC, dorsal comb; ep, epipharynx; GL, genital lobe, hyp, hypopharynx, HY, hypostoma: LTC lateral curtains; MD, mandible; LB, labrum; O-1-H, O-2-H, O-3-H, ocular sensilla; P pedicel; p, pore, RO, respiratory organ; TP, terminal process; VC, ventral comb.

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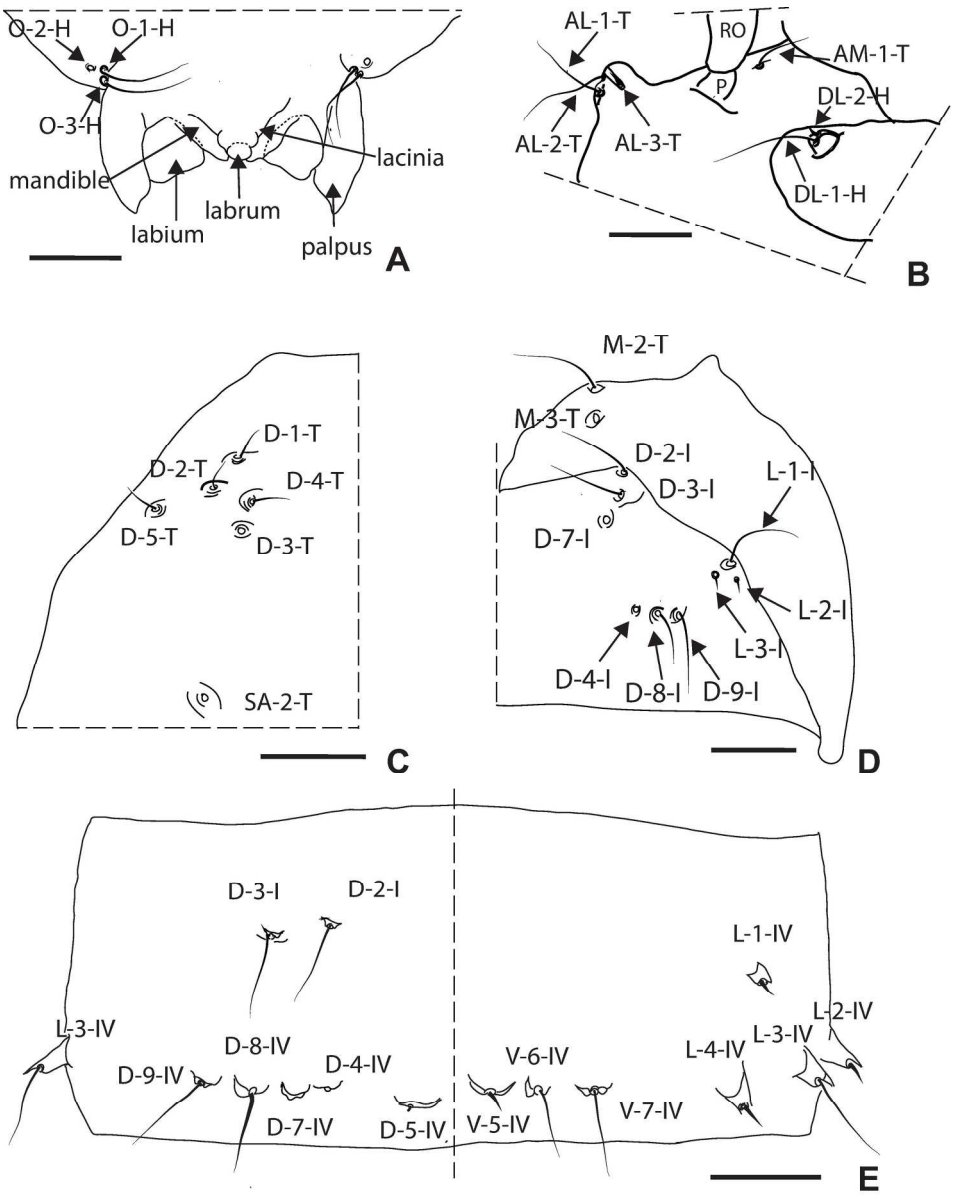

Figure 4. Draw of pupa *Culicoides trilineatus* Fox (Diptera: Ceratopogonidae) collected in Argentina: Female pupa (A, C-E); male pupa (B): Mouthparts and ocular sensilla (A); Cephalothoracis sensilla (B);) dorsal sensilla (C); metathorax and tergite 1 (D); Segment 4 (E). Scale bars: 0.05 mm.
AL-1-T, AL-2-T, AL-3-T, nterolateral sensilla; AM-1-T, anteromedial sensilla; D-1-T, D-2-T, D-3-T, D-4-T, D-5-T, dorsal sensilla; DL-1-H, DL-2-H, dorsolateral cephalic sclerite sensilla; M-2-T, M-3-T, metathoracic sensilla; O-1-H, O-2-H, O-3-H, ocular sensilla; P, pedicel; RO, respiratory organ; D-1-IV, D-2-IV, D-3-IV, D-4-IV, D-5-IV, D-7-IV, D-8-IV, D-9-IV, L-1-IV, L-2-IV, L-3-IV, L-4-IV,V-1-IV, V-5-IV,V-6-IV, V7-IV, segment 4 sensilla, D-1-I, D-2-I, D-4-I, D-7-I, D-8-I, D-9-I, L-1-I, L-2-I, L-3-I, tergite 1 sensilla.

190x241mm (300 x 300 DPI)