The immatures of the Neotropical species Culicoides venezuelensis Ortiz and Mirsa (Diptera: Ceratopogonidae)

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ABSTRACT

Egg and larva of *Culicoides venezuelensis* Ortiz & Mirsa are described and the pupa Is redescribed and illustrated by using phase-contrast microscope and Scanning Electron Microscope (SEM). Larvae and pupae were collected from a muddy area in a stream margin at Sierra de la Ventana, province of Buenos Aires, Argentina, and reared in laboratory. Eggs were extracted from the abdomen of a gravid female from Encarnación, Paraguay. Larvae show characters states typical of carnivorous ceratopogonids that breed in mud. Sierra de la Ventana is the southernmost locality of the species.

INTRODUCTION

Taxonomic and biological knowledge concerning the adult stage of biting midges of the genus *Culicoides* Latreille is much more developed when compared to studies on immatures.

There are a few morphological studies of eggs and larvae of Culicoides, particularly for the Neotropical fauna. Of the almost 300 Neotropical species only the larvae of 19 have been described, ten of them (C. bermudensis Williams, C. boydi Wirth & Mullens, C. cacticola Wirth & Hubert, C. crepuscularis Malloch, C. haematopotus Malloch, C. hieroglyphicus Malloch, C. melleus (Coquillett), C. oklahomensis Khalaf, C. ousairani Khalaf and C. variipennis (Coquillett), primarily nearctic species which reach northern Mexico, Central America, Bermuda and/or Bahamas (see Murphree & Mullen, 1991 and Breidenbaugh & Mullens, 1999). The nine primarily Neotropical species from which the larvae are known are: C. albomaculus Root & Hoffman (Mexico), described by Huerta et al. (2001); C. arubae Fox & Hoffman (from Texas in USA to Colombia and Venezuela) by Murphree & Mullen (1991); C. bambusicola Lutz (Argentina, Brazil) by Ronderos & Spinelli (2000); C. barbosai Wirth & Blanton (from Florida in USA to Ecuador) by Murphree & Mullen (1991); C. furens (Poey) (from eastern USA to Ecuador and coastal Brazil) by Painter (1926), Dove et al. (1932), Wirth (1952), Forattini (1957), Linley & Kettle (1964), Jamnback (1965), Blanton & Wirth (1979), Hribar & Mullen (1991) and Murphree & Mullen (1991); *C. hayesi* Matta (Honduras, Mexico) by Matta (1967); *C. hoffmani* Fox (Caribbean) by Linley & Kettle (1964); the widely distributed *C. insignis* Lutz by Forattini et al. (1956), Forattini (1957) and Blanton & Wirth (1979), and *C. paraensis* (Goeldi) by Murphree & Mullen (1991). The only eggs described are the ones of *C. barbosai* by Linley & Davies (1971), *C. furens* by Dove et al. (1932) and Linley & Davies (1971), and *C. maruim* Lutz by Lutz (1913).

The Ncotropical biting midge *Culicoides venezuelensis* was described by Ortiz & Mirsa (1950) based on adult specimens of both sexes. Subsequently the species was mentioned again several times in the literature, Ronderos & Spinelli (1989) describing the pupa captured breeding attached to filamentous algae in castern Argentina.

The purpose of this paper is to provide the first description of egg and larva of *C. venezuelensis*, and to redescribe the pupa mainly from specimens recently collected in the province of Buenos Aires, Argentina, using a phase-contrast microscope and Scanning Electron Microscopy.

MATERIAL AND METHODS

Larvae and pupae were collected on mud from a stream margin in Sierra de la Ventana, province of Buenos Aires, Argentina, and adults were collected at the same site with aerial net; additional pupae were collected attached to filamentous algae in a small pond in the province of Entre Rios, Argentina. Pupae were placed individually in small test tubes with ca. 5 ml of water. Tubes were checked daily in the laboratory for emergence of adults. The studied eggs were extracted from the abdomen of gravid females collected with CDC light traps in Encarnacion, Paraguay.

Eggs, larvae and pupae were examined using Scanning Electron Microscope (SEM) (JOEL 2000) by using the techniques of Ronderos et al. (2000). For observation with phase-contrast microscope with oil immersion, eggs, larvae and pupae were slide mounted in Canada balsam following the technique of Borkent (2000); larvae were placed with their ventral side upward to facilitate examination of the epipharingeal combs within the head capsule.

For terminology of eggs see Breindenbaugh & Mullens (1999), for larvae Murphree & Mullen (1991) and for pupae Nevill & Dyce (1994). Studied specimens are deposited in the collection of the Museo de La Plata, Argentina (MLPA).

RESULTS

Culicoides venezuelensis Ortiz & Mirsa

Figs. 1-21

Psycophaena pictipennis Philippi, 1865: 628 (Chile, female: fig. wing).
Culicoides venezuelensis Ortiz & Mirsa, 1950: 137 (male, female;) Venezuela; (figs.);
Wirth, 1955: 234 (= pictipennis Philippi 1865, non Steager, 1839; = ortizi Fox);
Forattini, 1957: 463 (redescrip.; distrib.; figs.); Wirth & Blanton, 1959: 372
(redescrip.; Panama; figs.); Spinelli & Wirth, 1985: 64 (in key; distrib.; wing photo); Wirth et al., 1988: 42 (in atlas; wing photo); Ronderos & Spinelli, 1989: 77

(pupa); Spinelli & Wirth, 1993: 35 (Argentina; in list.); Borkent & Wirth, 1997: 84 (in world catalog); Borkent & Spinelli, 2000: 37 (in Neotropical catalog); Spinelli & Borkent, 2004: 393 (Costa Rica record).

Culicoides ortizi Fox, 1952: 366 (female; Venezuela; figs.)

Egg (Figs. 10-11).— Eggs joined one to each other by their concave surfaces, through a thin adhesive skin. Each egg banana-shaped, yellow; chorion surface rugose, with several longitudinal rows of ansulae papillae. Average length 0.306 (0.32-0.28; n=5) mm, wide 0.036 (0.04-0.03; n=5) mm.

Larva (Figs. 1-3, 12-16).—Head capsule (Figs. 12-13) yellowish brown; elongated, tapering to rounded apex; chaetotaxy as in Figs. 12-13; HL 0.18 mm; HW 0.12 mm; HR 1.50; SGW 0.07 mm; SGR 1.71. Labrum (Fig. 13, 15) membranous; palatum (Fig. 14) with two pairs of sensilla trichoidea (one major, one minor), pair of sensilla chaetica immediately underneath; messors (Fig. 14) small, thin, well sclerotized; 5 well developed scopae (Fig. 14). Maxilla (Figs. 14) sclerotized, with one long, thick, stout seta on galeolacinia; maxillary palpus cylindrical, with 4-5 papillae of different length. Mandible (Fig. 1, 13-15) large, broad at base, strongly sclerotized; length 0.03 mm; one well defined, blunt, apical tooth; base wide with prominent, rounded point of articulation, setae, fosetae evident under SEM (Fig. 15) Hypostoma (Fig. 13-14) with rounded, mesal elevation with serrate margin. Epipharynx (Fig. 2) poorly massive, with two combs; dorsal comb sclerites with regular posterior margin with 9-10 lanceolate, subequal teeth; DCW 0.01 mm; comb 4 massive, apex not visible; each lateral arm membranous, apparently without curtain or fringe; LAW 0.06 mm. Hypopharynx (Fig. 3) lightly sclerotized; posterior end of each arm tripartite; hypopharingeal fringe with 16 fine, elongated teeth.

Thoracic pigmentation yellowish, diffuse. Caudal segment (Fig. 16) wide; CSL 0.32 mm; CSW 0.17 mm. Setae "o", "i" medium-sized, distal 1/2 hyaline; distance between bases of setae "o" subequal to the distance between bases of setae "i".

Pupa. (Figs. 4-9; 17-21).— Length of female 2.91 mm; length of male 2.70 mm. Exuvia pale yellowish brown. Cephalothorax with prominent anterodorsal tubercle (ad) (Figs. 5,19) with base wide, subdivided; 2 stout, medium-sized, slightly unequal, pointed setae; dorsolateral tubercle (dl) (Fig. 6) with base wide, apex blunt; one subbasal, short, stout seta; one apical longer, thin seta. Operculum (Fig. 4) slightly shorter than greatest, subbasal breadth, apex blunt; surface covered by rounded, pointed, anteriorly directed tubercles, the latter more abundant laterally; well developed anteromarginal tubercle (am) with one stout, pointed seta, basal pore present; OL 0.13 (0.12-0.14; n=2) mm; OW 0.15 (0.15-0.16; n=2) mm; OW/OL 1.20 (1.07- 1.33; n=2). Respiratory horn (Fig. 18-19) long, thin, straight, annulated on basal 1/2, proximal, distal dark brown, central portion pale brown; 5-6 apical, 3-4 lateral spiracles; pedicel stout; P 0.18 (0.11-0.14; n=4) mm, RH = 0.25 (0.24-0.26; n=4), P/H 0.47 (0.44-0.56; n=4); 5 dorsal tubercles (d) (Figs. 7, 17), i,ii with stout seta (seta ii longer), iii with short seta, i-iii base raised, iv with elongated, thin seta, base rounded, v pore; 2 thin, posteriorly directed ventromedian setae (vm) (Fig. 8), anterior short, posterior one elongated; 2 ventrolateral setae (vl) (Fig. 8) on rounded tubercle, posterior one elongated. Abdominal segments integument with anteriorly directed spicules only on anterior margin; fourth segment (Fig. 9) with 5 dorsal posteromarginal tubercles (dpm),

i with minute seta, ii without seta, i-ii base flattened, iii without seta, iv with short, stout seta, iii-iv base rounded, v with thin, slightly longer seta, base rounded; 2 dorsal anterosubmarginal tubercles (dasm), i with short, stout seta, ii with thinner, longer seta; 3 ventral posteromarginal tubercles (vpm), i with medium-sized seta, ii with thin, long seta, i-ii base raised, iii with minute seta, base flattened; lateral anterosubmarginal tubercle (lasm) with medium-sized seta, base raised with biffid, pointed tip; 3 lateral posteromarginal tubercles (lpm), i, iii with short seta, ii with long seta, i, iii base raised with biffid, pointed tip. Female caudal segment (Fig. 21) approximately as a long as greatest breadth; length 0.18 (0.17-0.20; n=5) mm, wide 0.14 (0.12-0.16; n=5) mm; anterior band of spicules narrowed dorsally; dorsomedian patch of 35-40 posteriorly directed spicules; posterolateral processes of moderate length, with inner spicules, tips pointed. Male caudal segment (Fig. 20) broader than in female; length 0.19mm, wide 0.17mm; ventral process elongated.

Distribution.— Widely distributed, from Costa Rica to 39°S in Argentina.

Specimens examined.— Argentina, Buenos Aires prov., Sierra de La Ventana, Arroyo Belisario, 11-III-2004, M. M. Ronderos-F. Diaz, 1 larvae, 2 pupae, reared in laboratory; Entre Rios prov., Santa Ana, 23-IX-1984, E. Balseiro-G. Spinelli, 2 females, 1 male (collected as pupae and reared in laboratory); same data except 9-IX-1984, 1 female, 1 male. Paraguay, Itapua, Encarnacion, 1/2-XI-2000, G. R. Spinelli, 1 female, CDC light trap (eggs extracted from abdomen).

For SEM: Argentina, Buenos Aires, Sierra de La Ventana, Arroyo Belisario, 11-III-2004, M. M. Ronderos-F. Diaz, 10 eggs, 2 larvae, 2 pupae.

Discussion.— As it was stated by previous authors (Murphree & Mullen, 1991; Hribar, 1993; Huerta et al., 2001; Ronderos & Spinelli, 2000; Ronderos et al., 2003, 2004a, 2004b; Spinelli et al., 2005) associations are evident between certain morphological structures of larvae and their feeding preference. In this sense, the morphology of the larva of *C. venezuelensis* indicates that it feeds on microorganisms carried in the standing water and organic matter of their natural environment, showing structures typical of carnivorous species (Thomsen, 1937; Mullen & Hribar, 1988), such as head elongated and narrow mouthparts directed anteriorly, sclerotized labium, mandible broad with pointed hooks, weak pharynx and finely serrate hypostoma. The sensilla of pallatum and messors are probably used during feeding (Becker, 1958), and Hribar (1993) stated their intimate relationship with the feeding habits; the distribution of the pallatum sensilla and messors shape in *C. venezuelensis* resembles the illustrated by Huerta et al. (2001) for *C. albomaculus* Root & Hoffman, a species which also shows morphological characters of carnivorous larvae.

With regard to the relation between larval morphology and habitat, Kettle & Elson (1976) and Kettle (1984) suggested that the long caudal setae of *Culicoides* may function to amplify the body oscillation and to increase the larval speed in water, enabling it to capture prey and/or to avoid predators. They also suggested that the setae may have tactile function, providing information on the approach of predators. The relatively medium sized "o" and "i" setae of the caudal segment of the larvae of *C. venezuelensis* could represent a good adaptation to the environment where it was captured (muddy area and filamentous algae), very different if compared to the larger ones of *C. bambusicola* and *C. paraenesis*, which are active swimmers in clean water.

The association between certain morphological structures of the pupa and

their habitats was also stated, e.g., Fox (1942) suggested that the spiculose respiratory horn and long posterolateral processes of caudal segment are typical of pupae collected in tree holes, and Borkent & Craig (2001) stated associations between the breeding places with respiratory horn, length of corporal setae and posterolateral processes of the pupa of *Stilobezzia rabelloi* Lane and other ceratopogonids. The respiratory horn and posterolateral processes of the pupa of *Culicoides venezuelensis* are very similar to the ones of *C. insignis* Lutz, particularly their shape, length and spiculate vestiture. These two species, as well as *Parabezzia balseiroi* Spinelli & Grogan, *Bezzia brevicornis* (Kieffer) and *Pachyhelea pachymera* (Williston) were collected together on filamentous algae in a small pond in eastern Argentina. Despite their belonging to different genera, they show similar morphological characters, at least the respiratory horn and posterolateral processes.

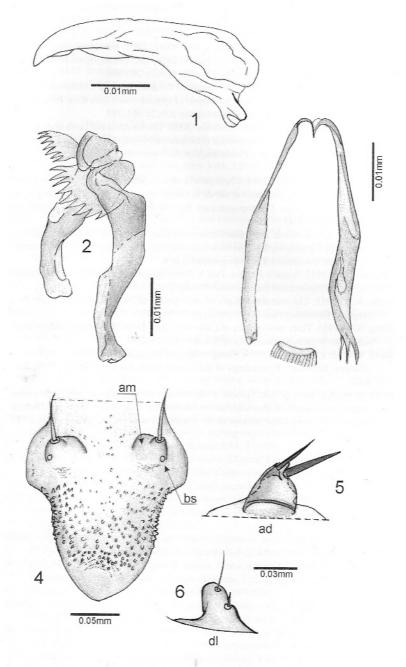
Sierra de la Ventana, in the province of Buenos Aires at 39°S, represents the southernmost locality of *C. venezuelensis*.

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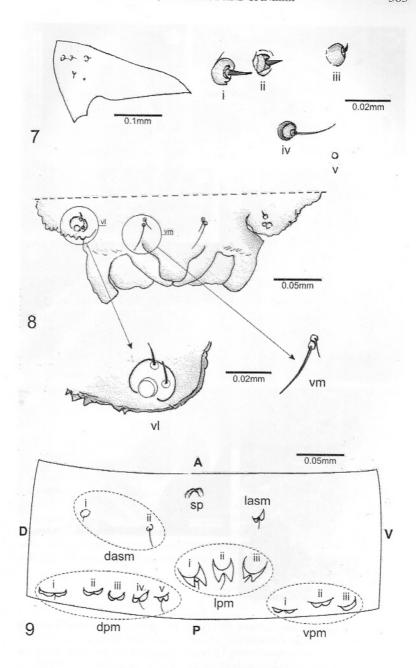
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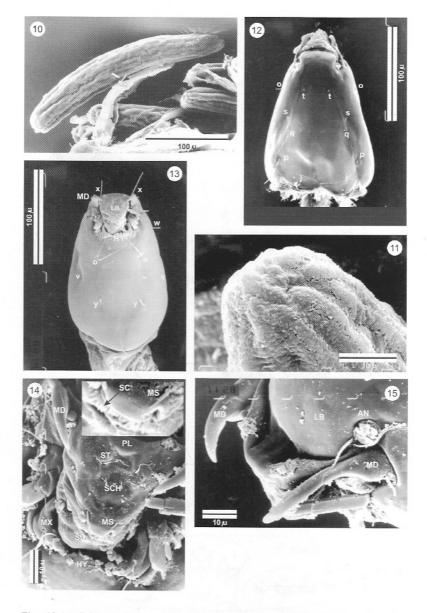
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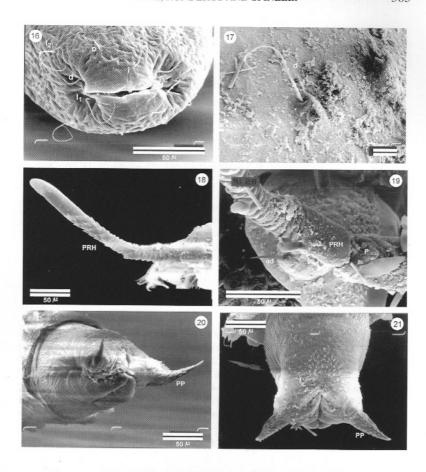
Figs. 1-6. *Culicoides venezuelensis*, 1-3 larva. 1, mandible; 2, epipharynx; 3, hypopharynx; 4-6 pupa. 4, operculum; 5, anterodorsal tubercle (ad); 6, dorsolateral tubercle (dl); anteromarginal tubercle (am); basal sensillum (bs).



Figs. 7-9. Culicoides venezuelensis, pupa. 7, dorsal tubercles (d); 8, ventrolateral setae (vl), ventromedian setae (vm); 9, 4^{th} abdominal segment, abdominal tubercles: lateral anterosubmarginal tubercle (lasm), dorsal posteromarginal tubercle (dpm), dorsal anterosubmarginal tubercle (dasm), ventral posteromarginal tubercle (lpm), anterolateral spiracle (sp).



Figs. 10-15. Culicoides venezuelensis. 10-11 eggs. 10, egg lateroventral view of entering egg, lateroventral surface with adhesive; 11. anterior end showing detail of chorion; 12-15 larva. 12, head capsule (dorsal view); 13, head capsule (ventral view); 14, head capsule (anteroventral view); 15, head capsula (frontolateral view). Head capsule chaetotaxy: j, collar pits; p, posterior perifrontal setae; q, postfacial setae; s, anterior perifrontal setae; t, prefrontal setae; o, parahypostomal setae; v, posterolateral setae; y, ventral setae; w, anterolateral setae; x, paranntenal setae; antennae (AN); labrum (LB); hypostoma (HY); mandible (MD); palatum (PL); sensilla trichoidea (ST); sensilla chaetica (SCH); messors (MS); scopae (SC); maxilla (MX).



Figs. 16-21. Culicoides venezuelensis, 16. larva. caudal segment (frontal view); 17-21. pupa. 17, dorsal tubercle (d) insertion; 18, respiratory horn; 19, respiratory horn (basal view); 20, male caudal segment; 21, female caudal segment (ventral view). Larva caudal segment chaetotaxy: "o" outer setae, "i" inner setae, 1, first lateral setae, 1, second lateral setae, d dorsal setae; respiratory horn (PRH); pedicel (P); anterodorsal tubercle (ad); dorsal tubercle (d); posterolateral processes (PP).