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The immatures of *Culicoides lacustris* Ronderos (Diptera: Ceratopogonidae)

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Abstract

Larva and pupa of the Patagonian species *Culicoides lacustris* Ronderos are described in detail with phase-contrast microscope at oil immersion and scanning electron microscope. Studied specimens were collected in a wetland, alongside to the Provincial Route 12, between the cities of Esquel and Gualjaina in the Chubut Province. They are compared with the more similar congener, *Culicoides venezuelensis* Ortiz & Mirsa.

Key words: Argentina, ultrastructure, immature stages, *Culicoides lacustris*

Resumen

Mediante el uso de microscopio de contraste de fase y microscopio electrónico de barrido, se describen en detalle la larva y la pupa de la especie patagónica *Culicoides lacustris* Ronderos. Los ejemplares estudiados fueron colectados en un humedal situado a la vera de la Ruta Provincial 12, entre las ciudades de Esquel y Gualjaina en la provincia de Chubut. Ellos son comparados con el congénere más similar, *Culicoides venezuelensis* Ortiz & Mirsa.

Introduction

The biting midges of the genus *Culicoides* Latreille 1809, are notorious blood-sucking pest of man and animals throughout the world. Adult females are potential vectors of etiological agents that can cause diseases or bite in such numbers that they thereby cause economic damages (Borkent & Spinelli, 2007; Ronderos *et al*., 2013). The immatures of the genus are important components in the bioenergetic cycle within aquatic and semiaquatic systems (Alencar *et al*., 2001).

Species of this genus are by far most notorious adult members of the 109 currently recognized extant genera of Ceratopogonidae. On the other hand, their immatures are relatively poorly known (Borkent & Spinelli, 2007). Borkent (2014) listed 287 species of this genus for the Neotropical region, of which adults of 43 species have recently been registered to Argentina. Of this number, the larvae of six and the pupae of seven species are known. The Neotropical biting midge *Culicoides lacustris* was described by Ronderos (1990) based on adult specimens of both sexes. The type material was collected in the subantarctic *Nothofagus* forest of the Nahuel Huapi and Lanin National Parks. The species was subsequently recorded from several places of Argentinean Patagonia by Spinelli & Ronderos (1994), Ronderos & Spinelli (2002) and Muzón *et al*. (2010).

The purpose of this paper is to provide the first description of larva and pupa of *C. lacustris* from specimens recently collected in the Chubut province in central Patagonia, as well as to give details on the larval biology.

Material and Methods

Larvae and pupae were collected in a wetland alongside to the Provincial Route 12, between the cities of Esquel

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and Gualjaina, Chubut Province 42°47'44, 2''S; 70°51' 07,7''W) (Fig. 26). The substrate was collected with the aid of an aquatic D-frame net and transferred to a white tray. Larvae and pupae were collected with the aid of a pipette and individualized to recipients and individual tubes, respectively. Samples of the substrate were carried to the laboratory in glass recipients in order to look for more specimens. Larvae were placed in individual containers with water from the same pond, covered with glass lids and were observed daily, monitoring pupal appearance, and pupae were checked daily. For specific identification, adults were compared with the type of *C. lacustris* housed in the Museo de La Plata. For detailed examination with phase-contrast microscope at oil immersion, larvae were slide mounted in Canada balsam and placed with ventral side upward to observe internal structures in the head capsule. Pupae were slide-mounted in Canada balsam, following the technique of Borkent & Spinelli (2007). Photomicrographs were taken with a digital camera Micrometrics SE Premium, through Nikon Eclipse E200 microscope. Ink illustrations were drawn with a camera lucida. Larvae were also examined using scanning electron microscopy (SEM) (JOEL 2000) following the technique of Ronderos *et al.* (2000, 2008).

Terminology follows Ronderos *et al*. (2010) for larva and Borkent (2012) for pupae, with addition of the following abbreviations of measurements: DAL (dorsal apotome length) and DAW (dorsal apotome width). Studied specimens are deposited in the collection of the Museo de La Plata, Argentina.

Taxonomy

Culicoides lacustris Ronderos, 1990

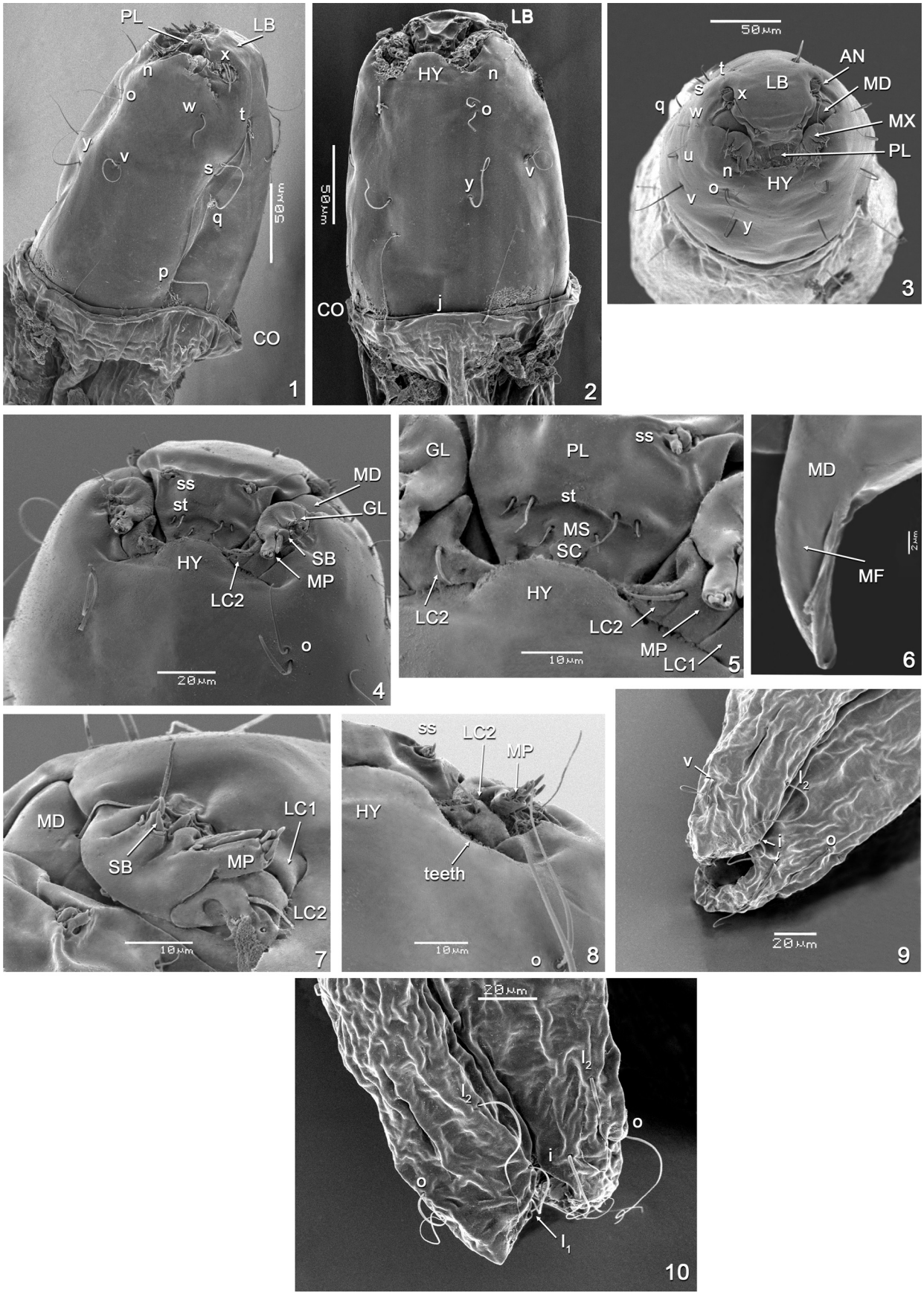
(Figs. 1–26)

Culicoides lacustris Ronderos, 1990: 116 (male, female; Argentina); Spinelli & Ronderos, 1994: 60 (Neuquén records); Borkent & Spinelli, 2000: 37 (in Neotropical catalog); Ronderos & Spinelli, 2002: 94 (Chubut and Santa Cruz records); Spinelli *et al.*, 2005: 140 (in key; Argentina); Borkent & Spinelli, 2007: 71 (in Neotropical catalog); Muzón *et al.*, 2010: 113 (localities in the Somuncura plateau); Borkent, 2014: 89 (in world catalog).

Culicoides venezuelensis: Spinelli & Wirth, 1985: 64 (misident., in part., record from Bariloche).

Fourth instar larva (Figs.1–10). Coloration whitish in life. Head capsule (Figs. 1–3) yellowish, moderately elongate, apex slightly bent ventrally; HL 0.375 mm, HW 0.15 mm, HR 1.08. Setae simple, moderately thin, medium-sized to elongate, chaetotaxy as in Figs.1–3. Antenna short. Labrum (Figs. 1–3) shorter than greatest basal width, with 3 pairs of anterolateral sensilla styloconica (Figs. 4–5, 8); palatum (Figs. 3, 5) with 2 pairs of closely-spaced sensilla trichoidea (Fig. 4–5); messors (Fig. 5) small, thin, gently sclerotized, scopae present (no visible for detailed description). Maxilla (Fig. 3, 7) with papillae; maxillary palpus (Fig. 4–5, 7–8) medium-sized, cylindrical, with 4 subapical papillae, two medium-sized and two short, and one triangular-shaped lateral lobe; galeolacinia (Fig. 4–5, 7) with lacineal sclerite 2 bearing long, thin seta, and one pore (Fig. 4–5, 7). Mandible (Figs. 3–4, 6–7) stout, heavily sclerotized, medium-sized, hooked, curved, with broad base, apical tooth large, pointed, with long setae on basal portion near hypocondyle, one insertion of seta on ectal margin, fossa mandibularis on ectal surface (Fig. 6); ML 0.108 mm, MW 0.036 mm. Hypostoma (Figs. 2–5, 8) with rounded, mesal, smooth elevation, lateral margin serrate, with 5 truncate lateral teeth. Epipharynx LAW 0.051–0.055 (0.054, n=3) mm, DCW 0.011–0.018 (0.016, n=3). Thoracic pigmentation uniformly pale. Caudal segment (Figs. 9–10) with 6 pairs of setae, four long, stout, other two thin.

FIGURES 1–10. *Culicoides lacustris* Ronderos, larva (SEM). 1, head capsule, lateroventral view (chaetotaxy); 2, head capsule, ventral view (chaetotaxy); 3, head capsule, frontoventral view (chaetotaxy); 4, head capsule, anteroventral view; 5, detail of head capsule, ventral view; 6, detail of mandible; 7, detail of mouthparts, lateral view; 8, details of head capsule, ventral view; 9, caudal segment, ventrolateral view (chaetotaxy); 10, caudal segment, lateral view (chaetotaxy)**.** Antennae (AN); collar (CO); fossa mandibularis (MF); galeolacinia (GL); hypostoma (HY); labrum (LB); lacinial sclerite 1 (LC1); lacinial sclerite 2 (LC2); mandible (MD); maxilla (MX); maxillary palpus (MP); messors (MS); palatum (PL); sensilla basiconica (SB); scopae (SC); sensilla styloconica (ss); sensilla trichoidea (st). Head capsule chaetotaxy: j, collar pits; n, anterolateral pit; o, parahypostomal setae; p, posterior perifrontal setae; q, postfrontal setae; s, anterior perifrontal setae; t, prefrontal setae; u, mesolateral setae; v, posterolateral setae; w, anterolateral setae; x, paranntenal setae; y, ventral setae. Caudal segment chaetotaxy: o, outer setae; i, inner setae; l_1, first lateral setae; l_2, second lateral setae; v, ventral setae.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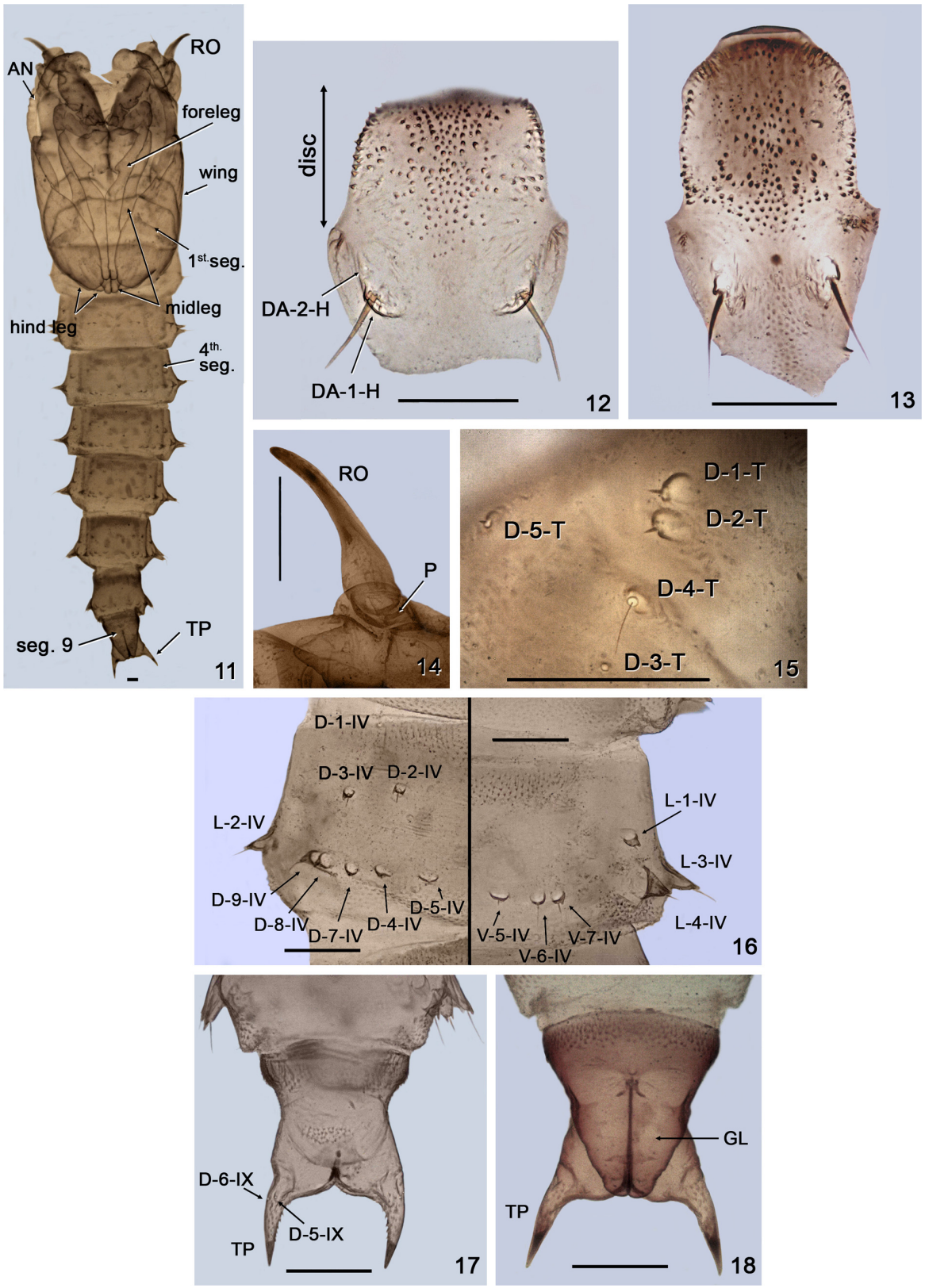


FIGURES 11–18. *Culicoides lacustris* Ronderos, pupa (SEM). 11, 13, 18, male pupa; 12, 14–17, female pupa; 11, pupa, ventral view; 12, 13, dorsal apotome; 14, respiratory organ; 15, dorsal setae (thorax); 16, fourth abdominal segment; 17–18, segment 9, ventral view. Scale bars: 0.05 mm. Antennae (AN); dorsal apotomal sensilla DA-1-H, DA-2-H; dorsal sensilla: D-1-T, D-2-T, D-3-T, D-4-T, D-5-T; dorsal of fourth abdominal segment: D-1-IV, D-2-IV, D-3-IV, D-4-IV, D-5-IV, D-7-IV, D-8-IV, D-9-IV; campaniform of Segment 9: D-5-IX, D-6-IX; lateral of fourth abdominal segment: L-1-IV, L-2-IV, L-3-IV, L-4-IV ; ventral of fourth abdominal segment: V-5-IV, V-6-IV, V-7-IV; genital lobe (GL); pedicel (P); respiratory organ (RO); terminal process (TP).

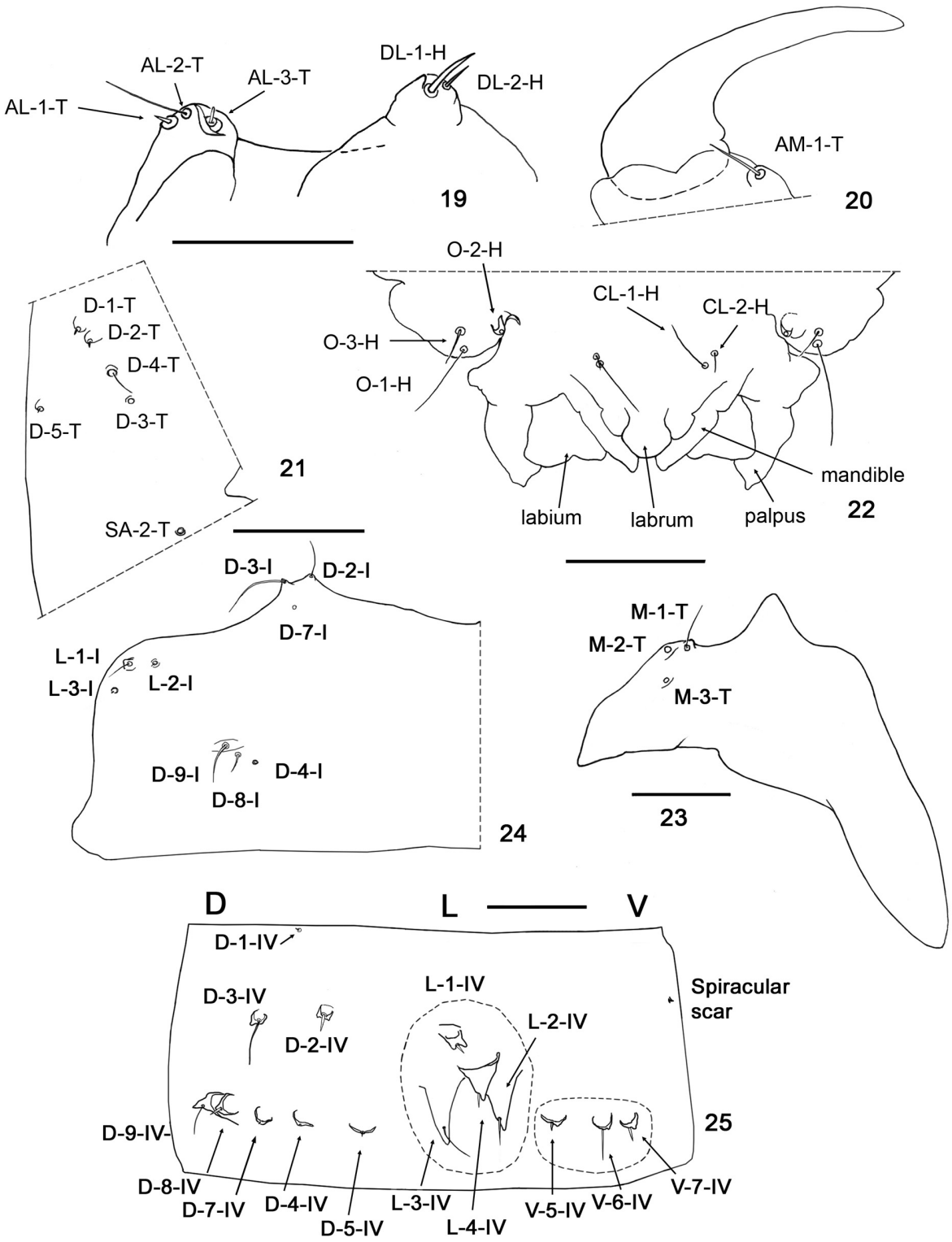
Female Pupa (Figs. 12, 14–17, 19–25). Exuviae general coloration pale brown. Flagellum appressed against lateral margin of face. Total length 2.90–3.18 (3.02, n=4) mm. Length of cephalothorax 1.23 (n= 4) mm, width 0.72–0.78 (0.75, n= 4) mm. Dorsal apotome (Fig. 12) without ventral line of weakness, dorsomedial tubercle and central dome, slightly wider than long, distal margin nearly straight, smooth except for few small spinules; disc surface and anterior margin covered by stout rounded spinules; raised areas bearing 2 dorsal apotome sensilla (Fig. 12), as follows: DA-1-H moderately elongate, thin seta, located on well developed tubercle, DA-2-H campaniform sensillum at tubercle base; DAL 0.144–0.168 (0.16, n=3) mm; DAW 0.156–0.18 (0.16, n=3) mm; DAW/DAL 0.93–1.25 (1.04, n=3). Cephalothorax rectangular, surface covered with small spinules; antenna extending posteriorly to various points along anterior margin of wing; mouthparts (Fig. 22) with mandible and lacinia well developed; palpus extending to posterolateral margin of labium; labium separated medially by labrum; apex of labrum rounded. Cephalothoracic sensilla (Fig. 19–20) as follows: one anteriomedial AM-1-T medium-sized, thin seta (Fig. 20); 2 dorsolateral cephalic sclerite sensilla (Fig. 19): DL-1-H medium-sized, stout seta, DL-2-H short, stout seta on prominent, rounded tubercle; 3 anterolaterals (Fig. 19): AL-1-T, AL-3-T short, stout setae, AL-2-T long, thin seta, all sensilla on slightly rounded tubercle; clypeal labrals (Fig. 22): CL-1-H medium-sized, thin seta, CL-2-H short, thin seta; oculars (Fig. 22): O-1-H long, thin seta, O-3-H, medium-sized, thin seta, O-2-H campaniform sensillum. Respiratory organ (Figs. 14, 20) smooth, medium-sized, slightly curved with pointed apex, wider at base, 28–30 closely abutting pores laterally and apically; pedicel stout, P 0.024 mm, 0.133–0.166 (0.155, n=3) mm; RH length 0.144–0.18 (0.156, n=4) mm, RH wide 0.06 (n=4) mm; P/H 0.133–0.166 (0.155, n=3); 5 dorsal setae (Figs. 15-21) and SA-2-T (Fig. 21): D-1-T minute seta, D-2-T short, stout seta, D-3-T campaniform sensillum, D-4-T very long, thin seta, D-5-T minute seta. Metathoracics (Fig. 23): M-1-T medium-sized, thin seta, M-2-T-M-3-T campaniform sensilla, M-3-T near anterior margin. Abdominal segments with smooth integument, scarce spicules spread on anterior and posterior portions; each segment without pigmentation pattern. First abdominal segment (Fig. 24) with setae as follows: 3 anteromesal setae, D-2-I short, stout seta, D-3-I long, thin seta, D-7-I campaniform sensillum; 3 posterior setae, D-4-I campaniform sensillum, D-8-I short, thin seta, D-9-I medium-sized, thin seta; L-1-I medium-sized, thin seta, L-2-I-L-3-I minute setae. Fourth segment (Fig. 16, 25) with sensilla as follows: D-1-IV minute seta, D-2-IV short seta, D-3-IV medium-sized seta, D-3-IV thinner than D-2-IV; D-4-I without setae, D-5-IV minute seta, D-7-IV minute seta, D-8-IV short, stout seta, D-9-IV medium-sized, thin seta; V-5-IV minute seta, V-6-IV medium-sized, thin seta, V-7-IV short seta, all on rounded small tubercles; L-1-IV minute seta, L-2-IV, L-4-IV short setae, L-4-IV shorter than L-2-IV, L-3-IV medium-sized, thin seta, all on triangular tubercles. Segment 9 (Fig. 17) approximately 1.6 times as long as greatest width, length 0.264–0.276 (0.27, n= 4) mm, width 0.17–0.18 (0.174, n= 4) mm; posteriorly directed spicules restricted to narrow anterior band, not connected to central patch of spicules; mesal spicules patch not extending to posterolateral terminal processes, terminal processes closely approximated basally, moderately elongated, subparallel with pointed, dark tips; ventral surface of processes with spicules on each side of midline, D-5-IX-D-6-IX campaniform sensilla.

Male pupa (Fig. 11, 13, 18). Similar to female with usual sexual differences. Total length 3.03–3.25 (3.13, n=4) mm. Exuviae pale brown. Dorsal apotome (Fig. 13) with DAL 0.144–0.168 (0.156, n=2) mm; DAW 0.144–0.168 (0.15, n=2) mm, DAW/DAL 0.86–1.08 (0.97, n=2). Respiratory organ (Fig. 11): RO length 0.144–0.204 (0.17, n=5) mm, RO width 0.048–0.06 (0.050, n=5) mm; pedicel length 0.024 (n=4) mm, P/RO 0.060–0.076 (0.069, n=4). Cephalothorax (Fig. 11) length 1.23–1.26 (1.248, n=5) mm, width 0.72–0.81 (0.76, n=5) mm. Segment 9 (Fig. 18) length 0.288–0.312 (0.3, n=5) mm, width 0.18–0.24 (0.197, n=5) mm; terminal processes (Fig. 18) length 0.084–0.096 (0.09, n=5) mm, width 0.024–0.03 (0.0252, n=5) mm, ventral genital lob moderately long, slightly globose, extending just beyond posterior margin, surface wrinkled.

Distribution. Argentina, in *Nothofagus* forests and steppes of Neuquén, Río Negro, Chubut and Santa Cruz provinces.

Bionomics. Immatures of *Culicoides lacustris* were found in a 3.14 m^2 pond located in a semi-arid steppe area of the Chubut province, which is connected to a "mallin", a typical Patagonian wetland fed by groundwater, with intermittent flooding regime and devoid of fishes (Epele & Archangelsky, 2012). The climate in this region is generally dry, cold and windy. This pond presents clayey sediment, bordered by shrubs and grass steppe on one side and by the road on the other (Fig. 26). This site is used as pasture and water supply for sheep and domestic horses.

A total of 14 pupal exuviae, two pupae and three larvae were collected in the site, at 2:00–2:30 pm, with a temperature of 20°C. The water temperature was 17°C and the pH 7.5. Only one larva took 12 days to reach the



FIGURES 19–25. *Culicoides lacustris*, Ronderos, female pupa. 19, cephalothoracic sensilla; 20, anteromedial sensillum; 21, dorsal setae; 22, monthparts, clypeal labrals and oculars (details, ventral view); 23, metathoracics chaetotaxy; 24, first abdominal segment chaetotaxy; 25, fourth abdominal segment chaetotaxy. Scale bars: 0.05 mm. Anterolaterals: AL-1-T, AL-2-T, AL-3-T; anteromedial: AM-1-T; campaniform sensilla of terminal processes: D-5-IX, D-6-IX; clypeal/labrals: CL-1-H, CL-2-H; dorsolateral cephalic sclerite sensilla: DL-1-H, DL-2-H; dorsal sensilla of first abdominal segment: D-2-I, D-3-I, D-4-I, D-7-I, D-8-I, D-9-I; dorsal of fourth abdominal segment: D-1-IV, D-2-IV, D-3-IV, D-4-IV, D-5-IV, D-7-IV, D-8-IV, D-9-IV; lateral sensilla of first abdominal segment: L-1-I, L-2-I, L-3-I; lateral of fourth abdominal segment: L-1-IV, L-2-IV, L-3-IV, L-4-IV; metathoracic sensilla: M-1-T, M-2-T, M-3-T; oculars: O-1-H, O-2-H, O-3-H; supraalar : SA-2-T; ventral of fourth abdominal segment:V-5-IV, V-6-IV, V-7-IV.

pupal stage, but did not reach the adult stage. Two larvae died after 10 days. Larvae were kept at a temperature ranging between 18 and 21°C.

The medium-sized "o" and "i" setae of the pupal caudal segment could represent a good adaptation to the environment where it was captured.

Discussion. The larva of *C. lacustris* is very similar to the Neotropical congener *C. venezuelensis* Ortiz and Mirsa by virtue of the labrum, the hooked mandible, the maxillary palpus cylindrical and the caudal segment with 6 pairs of setae (4 long, 2 thinner ones). However, *C. venezuelensis* differs by its yellowish brown coloration *in vivo*, the palatum has 3 pairs of sensilla trichoidea (one major, one minor), a pair of sensilla chaetica immediately underneath, 5 well developed scopae and the mandible apical tooth is more elongated and pointed.

The pupae of *C. venezuelensis* is clearly disting from that of *C. lacustris* by the respiratory organ long, thin and straight with rounded apex, annulated on basal ½, dark brown except central portion paler, and bearing 5–6 apical and 3–4 lateral pores. Moreover, the exuvia of *C. venezuelensis* is yellowish brown, and the apex of the dorsal apotome is blunt and its surface is covered by rounded, pointed, strong and anteriorly directed tubercles.

Specimens examined. Argentina, Chubut, Provincial Route 12, "El Tropezón", 42°47'44, 2"S; 70°51' 07,7"W, 781 m, 13.xii.2012, Anjos-Santos, D. *leg*, 2 ♂, 2 ♀, with pupal exuviae, 5 pupal exuviae ♂, 5 pupal exuviae ♀, 1 larva. All the specimens were collected as larvae and pupae, and were reared in laboratory.

Specimens examined by SEM: same data, 2 larvae.

FIGURE 26. Collecting site.

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