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The immature stages of the biting midge *Culicoides debilipalpis* Lutz (Diptera: Ceratopogonidae)

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Abstract

The fourth instar larva and pupa of *Culicoides debilipalpis* Lutz, 1913 are described, illustrated, and photomicrographed from material collected in tree-holes of *Salix* sp. in Entre Rios Province, Argentina by using binocular, phase-contrast, and scanning electron microscopy. Measurements of instars I–IV are also presented. The larva shows features typical to carnivorous-predatory larvae, as well as characters typical of larvae occurring in tree holes and clean water. Details on larval biology, habitat, and feeding behavior are given.

Key words: *Culicoides debilipalpis*, larva, pupa, bionomics

Introduction

Larvae of the worldwide genus *Culicoides* Latreille are important components in the bioenergetic cycle within aquatic and semiaquatic systems (Alencar *et al.*, 2001). The adult females are potential vectors of etiological agents that can cause diseases in humans and animals or bite in such large numbers that they thereby cause economic damages (Ronderos *et al.*, 2003b; Ronderos *et al.*, 2004).

The immature stages of Ceratopogonidae are poorly known in comparison with those of other economically important families of aquatic Diptera. Of the 267 Neotropical species of *Culicoides*, the larvae of only 23 and the pupae of 34 species are known (Borkent & Spinelli, 2007; Ronderos *et al.*, 2008b). Moreover, the knowledge of the habitats of the Neotropical species is highly limited.

The purpose of this paper is to provide not only the first detailed description of larva and pupa of *Culicoides debilipalpis* Lutz with scanning electron microscopy (SEM), binocular microscope (BCM) and phase-contrast microscopy (PCM) from specimens recently collected in Argentina, but also to give details of larval biology, habitat, and feeding behavior.

Material and methods

First to fourth instar larvae and pupae of *C. debilipalpis* were collected in large numbers by using pipettes to remove water from tree-holes of *Salix* sp. (Salicaceae) in Entre Rios Province, Argentina. The material containing the larvae and pupae was carried to the laboratory. The larvae were placed individually in Petri dishes and pupae were conditioned in vials, individually, with a drop of water. Observations were made daily until adult emergence.

In order to observe the ultrastructural characters, larvae and pupae were examined by using a scanning electron microscope (JSM6360LV) following the technique of Ronderos *et al.* (2000b, 2008a) except that we used 30% glycolid acid to clean the specimens and the exposure time of cleaning was increased to 10 minutes. For observation with BCM, specimens were slide-mounted in Canada balsam following the technique described by Borkent & Spinelli (2007). Mounted larval exuviae were placed with their ventral sides upwards to facilitate examination of the epipharyngeal combs within the head capsule and the alimentary channel; the latter is only visible when specimens are well cleared and observed with PCM. Pupal exuviae were mounted dorsally, ventrally

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and laterally to examine cuticular processes of the cephalothorax, respiratory organs, and abdominal segments. Ink illustrations were made with a camera lucida. Photomicrographs were taken with a Pentax Optio Power Shot S60 digital camera through a Leitz SM-Lux (10× or 40×).

Measurements and ratios were taken using BCM, except as noted when they were taken using SEM.

For terminology we followed Ronderos *et al.* (2008b). Studied specimens are deposited in the collection of the Museo de La Plata, Argentina (MLPA).

Abbreviations

Larva. Head capsule: AN, antennae; epy, epipharynx; DC, dorsal comb; GL, galeolacinia; hyp, hypopharynx; HY, hypostoma; LB, labrum; LC1 and LC2, lacinial sclerites; MD, mandible; MP, maxillary palpus; MX, maxilla; MS, messors PL, palatum; sb; sensilla basiconica; SC, scopae; st, sensilla trichoidea; ss, sensilla styloconica.

Chaetotaxy: j, collar pits; k, pronotal pits; n, anterolateral pits; o, parahypostomal setae; p, posterior perifrontal setae; q, postfacial setae; r, postnotal pits; s, anterior perifrontal setae; t, prefrontal setae; u, mesolateral setae; v, posterolateral setae; w, anterolateral setae; x, paranntenal setae; y, ventral setae..

Caudal Segment (CS). Chaetotaxy: d, dorsal setae; “o” outer setae; “i” inner setae; $l_{1,}$ first lateral setae; l_2, second lateral setae.

Pupa. Cephalothorax: O, operculum; ad, anterodorsal tubercle; am, anteromarginal tubercle; bs, basal sensillum; vm, ventromedian setae, vl, ventrolateral setae; d, dorsal tubercles; P, pedicel; RH, respiratory horn.

Abdomen: Fourth abdominal segment: dasm, dorsal anterosubmarginal tubercle; dpm, dorsal posteromarginal tubercle; lasm, lateral anterosubmarginal tubercle; lpm, lateral posteromarginal tubercle; vpm, ventral posteromarginal tubercle; sp, anterolateral spiracle. Caudal Segment: PP, posterolateral processes.

Abbreviations of measurements

Larva. Head capsule: HL, head length; HW, head width; HR, head ratio = HL/HW; SGW, subgenal width; SGR, subgenal ratio = HW/SGW; Epipharynx: LAW: the wide across the lateral arms of the epipharynx; DCW: wide across each of the paired dorsal comb sclerites of the epipharynx. Mandible: ML, mandible lenght. Caudal Segment: CSL, caudal segment length; CSW, caudal segment width; CSR, caudal segment ratio = CSL/CSW; OL, length of caudal setae; OD, distance between bases of caudal setae “o”; OL/OD, ratio.

Pupa. Operculum: OL, operculum length; OW, operculum width; OW/OL, ratio.

Respiratory Horn: RH, respiratory horn length; P, pedicel length; P/RH, ratio.

Caudal segment: CSL, caudal segment length; CSW, caudal segment width; CSR, caudal segment ratio.

Culicoides debilipalpis **Lutz**

(Figs. 1–31)

Culicoides debilipalpis Lutz, 1913: 60 (female; Brazil); Macfie, 1937: 7 (female; redescrip.; Trinidad); Floch & Abonnenc, 1942: 3 (Guyane); Barbosa, 1952: 12 (Argentina, Brazil); Forattini, 1957: 383 (in part.; redescrip.; distrib.); Wirth & Blanton, 1959: 442 (redescrip.; Panama); Franca Rodriguez, 1963: 67 (Uruguay); Messersmith, 1964: 339 (Virginia; breeding site); Smith & Varnell, 1967: 520 (Florida; tree holes); Wirth & Blanton, 1971a: 34 (redescrip.; distrib.); Wirth & Blanton, 1971b: 75 (*khalafi* as syn.; notes); Blanton & Wirth, 1979: 78 (redescrip.; distrib.); Vitale *et al.*, 1981: 149 (notes; Panama); Spinelli & Ronderos 1997: 304 (revalidation); Borkent & Spinelli, 2000: 32 (in Neotropical catalog); Ronderos *et al.*, 2003a: 22 (Yacyretá dam lake area); Spinelli *et al.*, 2005: 141 (in key; Argentina); Trindade & Gorayeb, 2005: 67 (Pará, Brazil); Borkent & Spinelli, 2007: 66 (in Neotropical catalog); Borkent & Grogan, 2009: 14 (in Nearctic catalog); Borkent, 2009: 80 (in online World catalog).

Culicoides ichesi Ronderos & Spinelli, 1995: 77 (female; Argentina, Paraguay, Uruguay); Ronderos & Spinelli, 1998: 81 (in key; Yacyretá dam lake area).

Culicoides khalafi Beck, 1957: 104 (male, female; Florida; fig.).

Culicoides lahillei: Spinelli & Wirth, 1986: 62 (misident., in part., syn. *debilipalpis*); Wirth *et al.*, 1988: 48 (in Atlas, wing photo.); Spinelli *et al.*, 1989: 735 (in list); Spinelli & Ronderos, 1991: 91 (Salto Grande dam lake area); Spinelli & Martinez, 1991: 176 (in key; Uruguay); Waller *et al.*, 1990: 352 (redescrip; Guyana); ?Lamberson *et al.*, 1992: 117 (descrip. pupa; Georgia and Missouri, USA); Borkent & Wirth, 1997: 72 (in catalog; syn. *debilipalpis*).

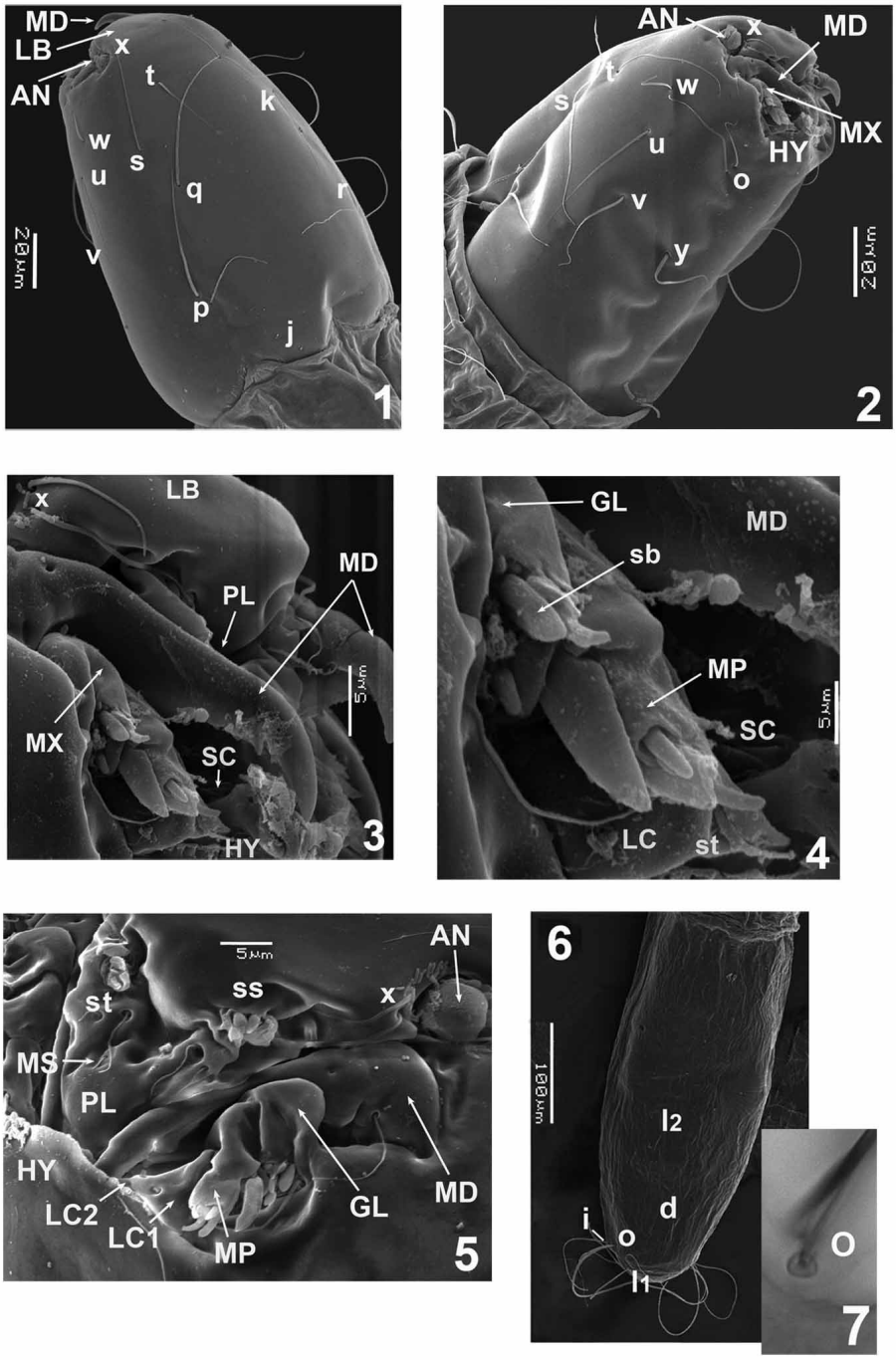

FIGURE 1–7. *Culicoides debilipalpis*, larva (SEM). Fig. 1: head capsule, chaetotaxy (dorsolateral view). Fig. 2: head capsule, chaetotaxy (ventrolateral view). Fig. 3: head capsule (anterolateral view). Fig. 4: maxilla (lateral view). Fig. 5: head capsule (anteroventral view). Fig. 6: caudal segment, chaetotaxy (dorsal view). Fig. 7: caudal segment, insertion of setae "o".

Fourth instar larva (Figs. 1–14). For measurements and ratios of head capsule and caudal segment with SEM see Table 1. Larvae length 2.29 mm (SEM). Small larva**.** Head capsule (Figs. 1–2, 8, 14) yellowish, moderately elongate, apex slightly bent ventrally, HL 0.14–0.17 (0.155, n=11) mm, HW 0.09–0.14 (0.114, n=11) mm, HR 0.56–0.87 (0.738, n=11), SGW 0.096–0.10 (0.098, n=3), SGR 1.20–1.26 (1.24, n= 3). All setae simple, moderately thin, medium-sized to elongate, chaetotaxy as in Figs. 1–2. Antenna short. Labrum (Figs. 1, 3) as long as its greatest, basal width, with pair of anterolateral sensilla styloconica (Fig. 5); palatum (Figs. 3, 5) with 3 pairs of closely-spaced sensilla trichoidea (Fig. 5); messors (Fig. 5) small, thin, gently sclerotized, curved; well developed scopae (Figs. 3–4). Maxilla (Figs. 2–5) with short papillae; maxillary palpus (Fig. 3–5) short, cylindrical, with 4

subapical papillae, two long, other two short, triangular-shaped lateral lobe; galeolacinia with long seta (Figs. 4–5). Mandible (Figs. 1–5) medium length, hooked, curved, with broad base, two elongate, small pointed teeth, inner tooth smaller, with medium-sized setae on basal portion near hypocondyle, one insertion of seta on ectal margin (Fig. 3); ML 0.06 mm, MW 0.03 mm. Hypostoma (Figs. 2–3, 5, 8) with quadrangular, mesal, smooth elevation, lateral margin serrate. Labium small. Epipharynx (Figs. 8, 10–14) poorly massive, with 2 ventral combs superimposed, dorsal comb sclerites short, with 22–24 small rounded teeth on posterior edge; lateral arms membranous with lateral curtains, LAW 0.051–0.055 (0.054, n=3) mm, DCW 0.011–0.018 (0.016, n=3). Hypopharynx (Figs. 8–9, 14) elongate, thin, moderately sclerotized, arms without fringe or posterior comb. Thoracic pigmentation uniformly pale, with notorious long setae. Abdominal segments whitish. Caudal segment (Figs. 6–7) with 6 pairs of setae, four of these long, stout, pale brown, other two thin; CSL 0.305–0.309 (0.306, n=3) mm, CSW 0.129–0.156 (0.146, n=3) mm, CSR 0.38–0.51 (0.462, n=3), OL 0.150–0.204 (0.171, n= 7) mm, OD 0.022–0.026 (0.023, n=6) mm.

Instars I–III. Similar to fourth instar, but with differences in size and some proportions. For measurements and ratios with SEM see Table 1.

TABLE 1. *Culicoides debilipalpis*, larvae. Measurements with SEM and ratios of head capsule and caudal segment. All measurements are in millimeters.

		Larval instar			
		I	II	III	IV
Head Capsule	HL	0.0916	0.0973	0.117	0.134
	HW	0.0809	0.0812	0.0875	0.0915
	HR	1.13	1.19	1.33	1.46
	SGW	0.0565	0.0556	0.0490	0.0451
	SGR	1.43	1.46	1.78	2.02
Caudal Segment	CSL	---	---	0.249	0.361
	CSW	---	---	0.101	0.156
	CSR	---	---	2.46	2.31
	OL	---	---	---	0.193
	OD	---	---	0.06	0.078

Female pupa (Figs. 15–31). Exuviae pale yellowish brown. Length of cephalothorax 0.792–1.025 (0.908, n= 6) mm, its width 0.466–0.605 (0.566, n= 6) mm. Operculum (Figs. 15–16, 20, 22) slightly wider than long, distal margin nearly straight; disc surface covered by stout rounded spinules; anteromarginal tubercle (am) well-developed, 0.0329 mm (SEM) (Figs. 15–16, 20, 22), base rounded, wide, with one long, stout, pointed seta inserted in a conical-shaped area, 0.0852 mm (SEM); basal sensillum present; posterior margin slightly convex, smooth except for few small rounded spinules between anteromarginal tubercles; OL 0.122–0.186 (0.171, n=6) mm; OW 0.022–0.026 (0.023, n=7) mm; OW/OL 1.04–1.31 (1.18, n=4). Respiratory horn (Figs. 15–17, 29) medium-sized, slender, yellowish, distal half dark brown, large scale-like spines; 7–8 apical spiracles, 2–3 laterobasal ones; pedicel stout, P 0.033–0.04 (0.037, n=4) mm; RH 0.17–0.19 (0.18, n=5) mm; P/RH 0.132, 0.19–0.21 (0.20, n=4). Cephalothoracic tubercles as follows: anterodorsal tubercle (ad) prominent, with large, pointed setae (Figs. 15–16); dorsolateral tubercle (dl) (Figs. 15–17, 19–20) small, blunt, with 2 setae, one long, thin, other minute; dorsal tubercles (d): i with minute seta, ii with medium-sized, stout seta; iii with long seta; iv with very long, thin seta, v small, minute seta (Figs. 16, 21). Two ventrolateral (vl) long, thin setae (Fig. 18); 2 ventromedial (vm), medium-sized setae, one stouter (Fig. 18). Abdominal segments with smooth integument, with scarce spicules spread on anterior and posterior portions; each segment with small pigmented anterolateral spiracle (sp). First abdominal segment (Fig. 30) with setae as follows: 2 anteromesal thin setae, one long, other medium-sized; 2 posterior setae, mesal one very long, thin, other minute. Fourth segment (Figs. 23–25, 31) with all tubercles with small base: 2 dorsal anterosubmarginal tubercles (dasm), i–ii short setae, i thiner than ii; 5 dorsal posteromarginal tubercles (dpm), i–ii short seta, iii without seta, iv–v minute setae; 3 lateral posteromarginal tubercles (lpm), i, iii spur-like,

pointed setae, ii very long, thin seta; one lateral anterosubmarginal tubercle (lasm) spur-like seta; 3 ventral posteromarginal tubercles (vpm), i minute seta, ii long, thin seta, iii short, stout seta. Caudal segment (Fig. 26) approximately 1.25 times as long as greatest width, length 0.18–0.22 (0.201, n= 7) mm, width 0.10–0.19 (0.146, n= 7) mm; posteriorly directed spicules restricted to narrow anterior band, not connected to central inverted V-shaped patch of spicules; mesal spicules patch not extending to posterolateral processes, these moderately elongated, subparallel with pointed, dark tips; ventral surface of posterolateral processes with spicules present on each side of midline. Male caudal segment (Figs. 27–28) similar to female.

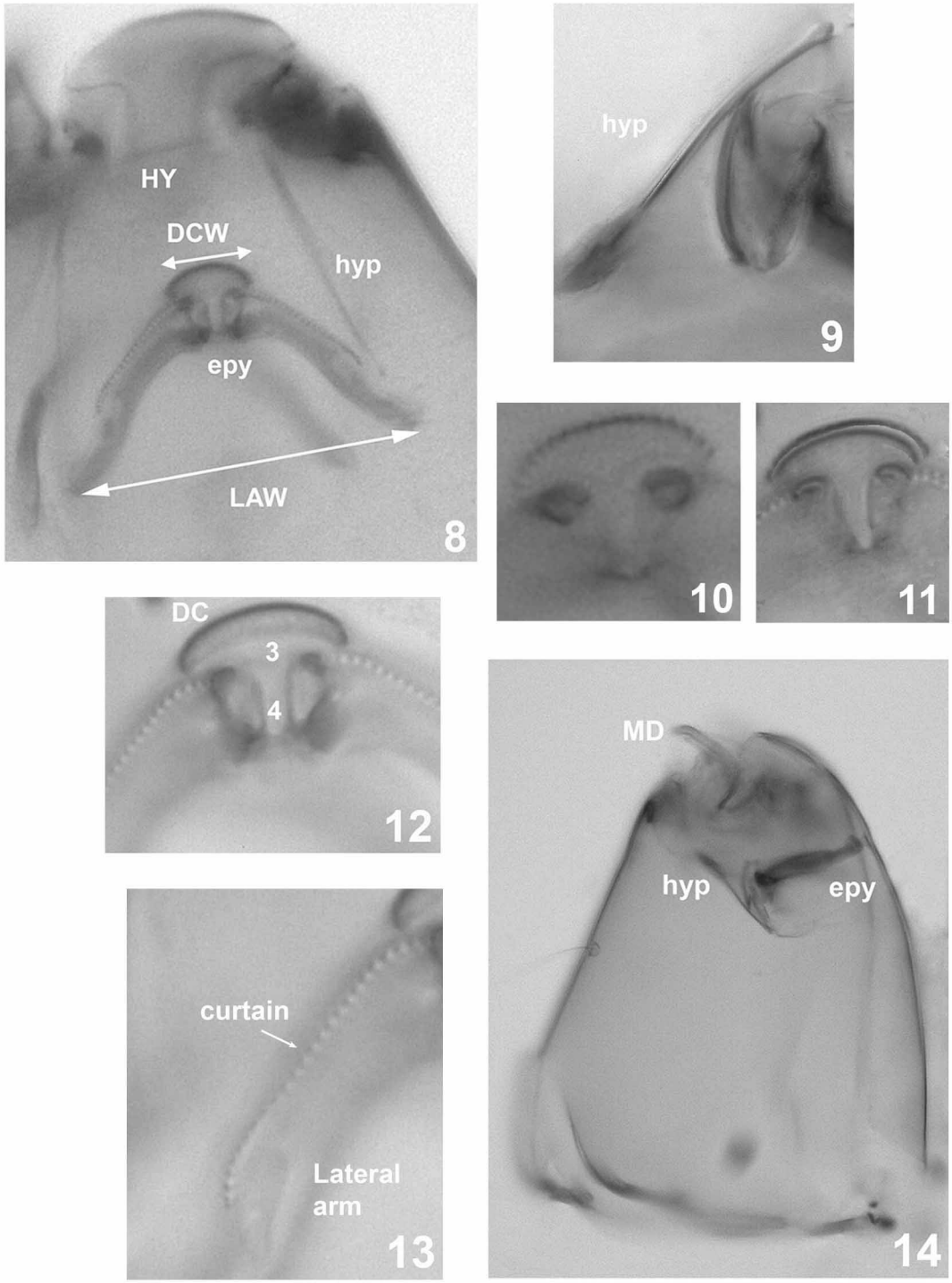

FIGURE 8–14. *Culicoides debilipalpis*, larva (digital camera). Fig. 8: head capsule, epipharynx (ventral view). Fig. 9: hypopharynx. Fig. 10: detail epipharynx comb (ventral view). Fig. 11: detail epipharynx comb (dorsal view). Fig. 12: epipharynx, detail combs 3 and 4 and curtain. Fig. 13: epipharynx, detail lateral arm and curtain. Fig. 14: head capsule (lateral view).

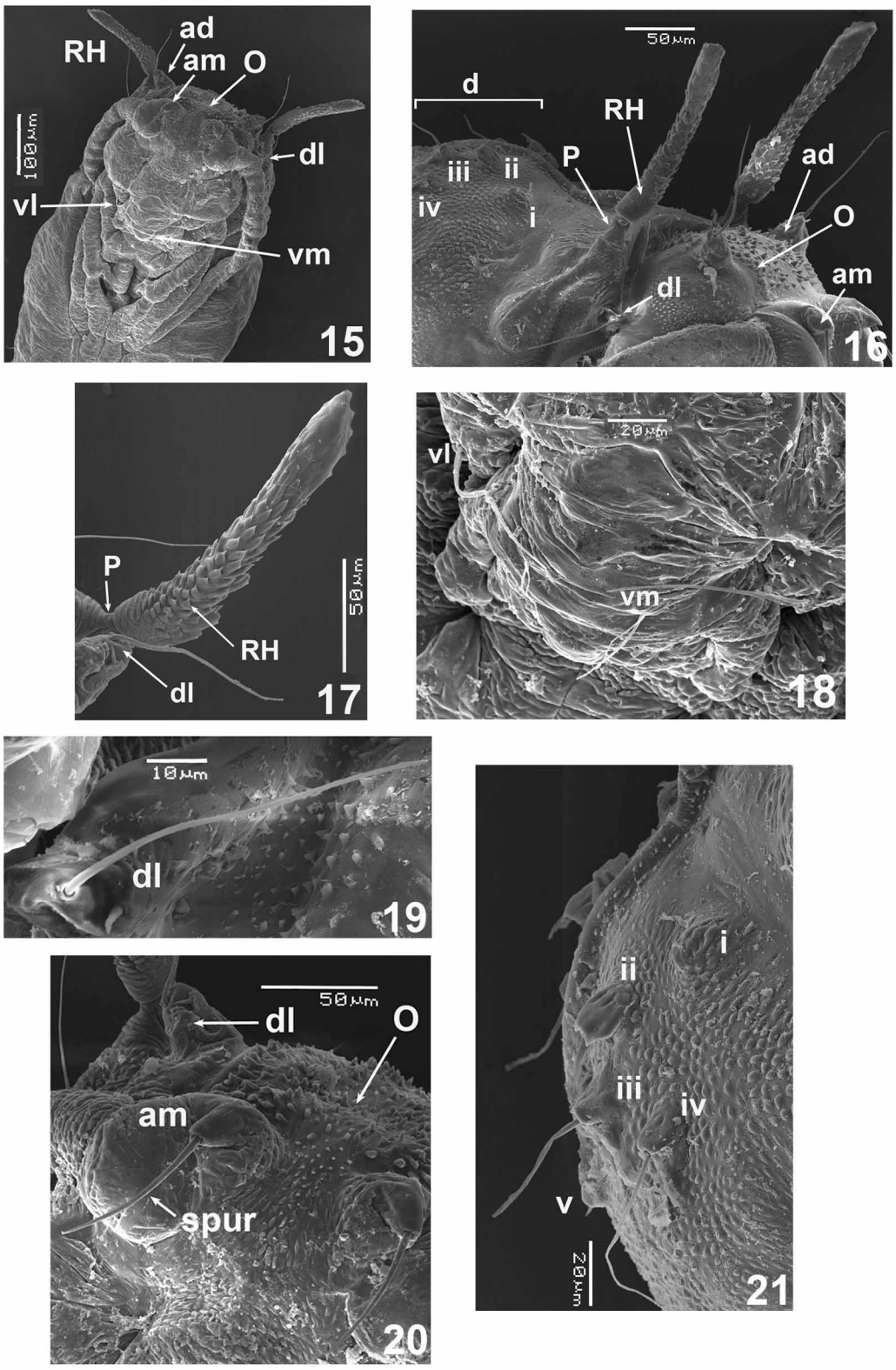

FIGURE 15–21. *Culicoides debilipalpis*, pupa (SEM). Fig. 15: cephalothorax (ventral view). Fig. 16: cephalothorax (anterodorsal view). Fig. 17: respiratory horn. Fig. 18: ventromedian and ventrolateral setae. Fig. 19: dorsolateral tubercle. Fig. 20: cephalothorax (anterior view). Fig. 21: dorsal tubercles (lateral view).

Material examined. All the specimens collected as larvae, reared in laboratory. Argentina, Entre Ríos prov., Ibicuy, from tree – holes of *Salix* sp., 7-XI-2006, R. Campos-C. Cazorla, 2 larval exuviae (instar IV), 11 larvae (instar IV), 5 female pupal exuviae, 3 pupae, 2 adult females, 1 adult male.

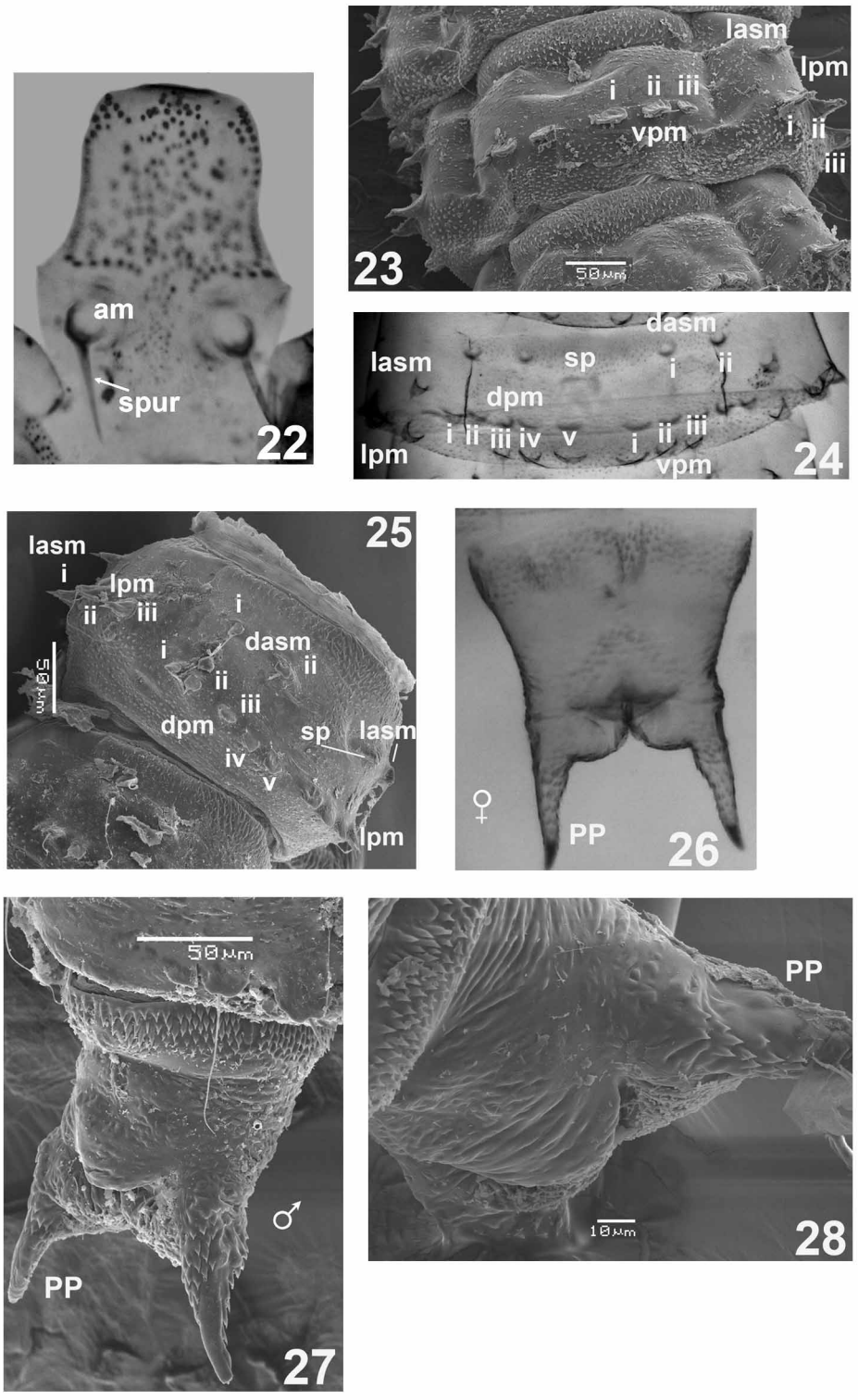

FIGURE 22–28. *Culicoides debilipalpis*, pupa. Figs. 22–26: female (digital camera). Figs. 27–28: male (SEM). Fig. 22: operculum. Fig. 23: 4th abdominal segment (lateroventral view). Fig. 24: 4th abdominal segment (dorsal and ventral views). Fig. 25: 4th abdominal segment (dorsal view). Figs. 26–27: caudal segment (ventral view). Fig. 28: caudal segment (anteroventral view).

Material examined by SEM: same data, 6 larvae (1 instar I, 1 instar II, 1 instar III, 3 instar IV), 6 pupae.

Distribution. Widespread from USA (Maryland, Kentucky, Nebraska south to Lousiana and Florida), Guatemala and Belize to Argentina (Jujuy, Misiones, Corrientes, Entre Ríos, Buenos Aires provinces).

Bionomics. The freshly collected larva is basically salmon colored. When kept in the laboratory, it losses some

of this pigmentation, probably as a result of the adapted diet. The larva shows a good adaptation for swimming and typical active movement. They are positively phototrophic and their activity increases when they are observed under light. Observations during the conduct of this work indicate that this species feeds on microorganisms carried in the standing water and organic matter of their natural environment.

The life cycle of *Culicoides debilipalpis* from larva to adult lasted approximately 31 days in the laboratory. The pupa movement is slow, undulating the last seven abdominal segments, which might be used to keep floating.

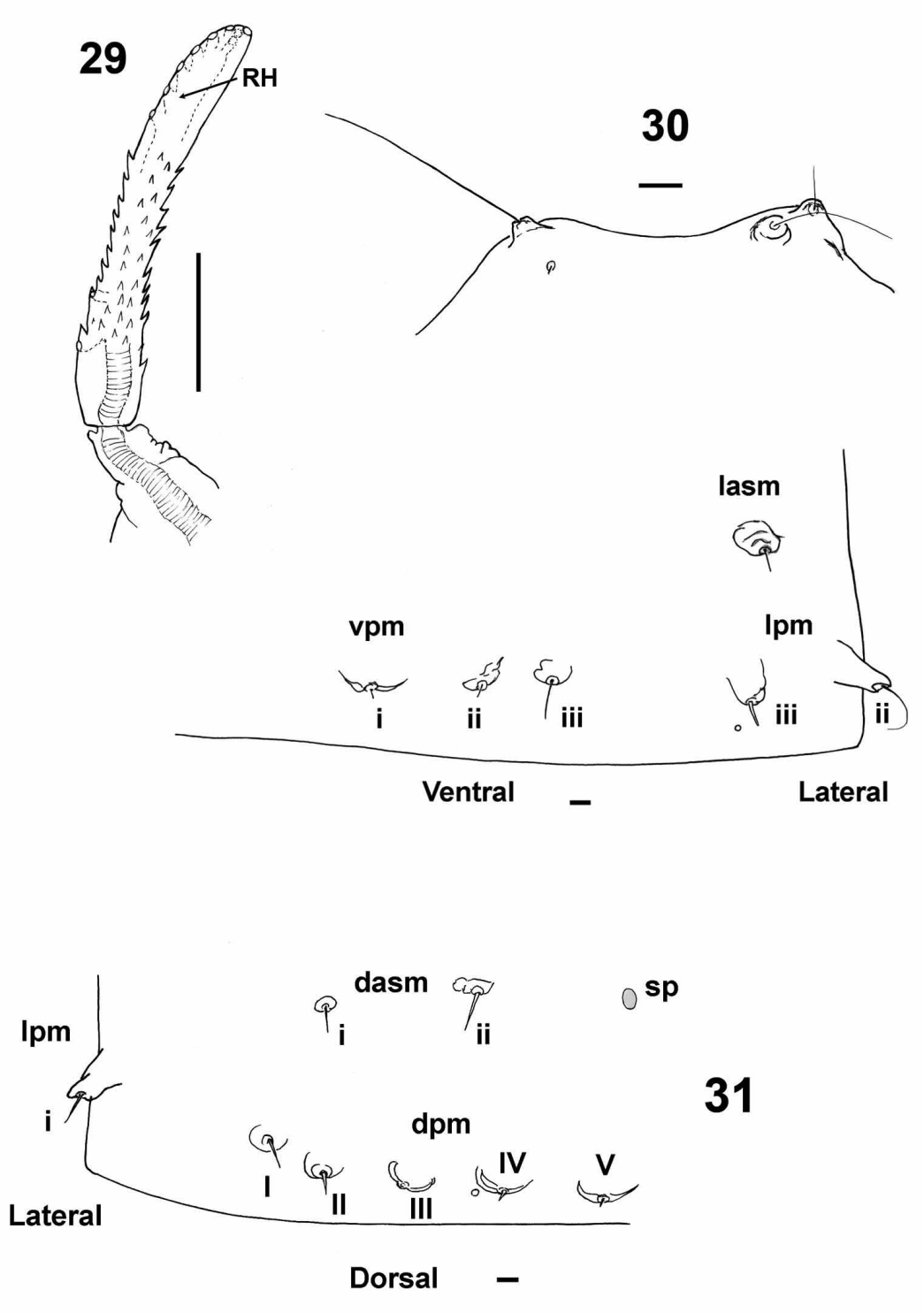

FIGURE 29–31. *Culicoides debilipalpis*, pupa. Fig. 29: respiratory horn. Fig. 30: first abdominal segment, detail tubercles. Fig. 31: fourth abdominal segment, detail tubercles.

Discussion

The accurate identification of the larval instars and pupa requires studies of their morphology and biology, being a valuable tool to predict the adult emerging time and consequently to recommend appropriate control measures. Besides, details on the biology are essential to identify key factors limiting pest populations.

As it was stated by previous authors (Murphree & Mullen, 1991; Mullen & Hribar, 1988; Ronderos *et al.*, 2008b; Spinelli *et al.*, 2005, among others) associations are evident between certain morphological structures of larvae and their feeding preference. As in other groups, mouthparts and other structures of ceratopogonid larvae often reflect their feeding habitats. The larvae of *C. debilipalpis* are interpreted to be predatory-carnivorous. They are prognathous, exhibit an elongate and narrow head, mandible hooked with a single strong tooth and a fossa mandibularis, epipharynx gently massive, hypostoma finely toothed and cylindrical maxillary palpi (Thomsen, 1937; Hribar, 1993; Díaz *et al.*, 2005).

The length of the "o" and "i" setae of the caudal segment represent a good adaptation to the environment where it the larva was captured (Glukhova, 1977). Murphree and Mullen (1991) pointed out that the elongate caudal setae, the presence of epipharyngeal curtains and a diffuse yellowish brown thoracic pigmentation are typical of tree-hole breeding species. *Culicoides debilipalpis* shows the first two mentioned characters, lacking a thoracic pigmentation pattern. The presence in the pupa of scale-like spines are also typical species breeding in tree-holes (Fox, 1942; Ronderos & Spinelli, 2000a; Huerta *et al.*, 2001).

Spinelli & Wirth (1986) erroneously synonymyzed *C. debilipalpis* under *C. lahillei* (Iches). This nomenclatorial action was accepted by subsequent authors, and therefore *C. lahillei* was recorded several times from northern South America, Central America and even North America, when its actual distribution is northern Argentina, southeastern Brazil, Paraguay and Peru. This appears to be the case of the citation of *C. lahillei* from Georgia and Missouri (USA) by Lamberson *et al.* (1992) when describing its pupa. In fact, the respiratory horn, operculum, abdominal tubercles, spiculate vestiture and shape of posterolateral processes of the pupa herein described are identical to these features as described by Lamberson *et al.* (1992).

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References

Alencar Y. B., Ludwig T. A. V., Soares C. C. & Hamada N. (2001) Stomach content analyses of *Simulium perflavum* Roubaud 1906 (Diptera: Simuliidae) larvae from streams in Central Amazonia, Brazil. *Memórias do Instituto Oswaldo Cruz*, 96: 561–576.

Barbosa F. A. S. (1952) Novos subsidios para o conhecimento dos Culicoides neotropicos (Diptera: Heleidae). *Thesis, Universidade do Recife, Recife*: *Imprensa Industrial*, 21 pp.

Beck E. C. (1957) Two new species of *Culicoides* from Florida (Diptera: Heleidae). *Florida Entomologist*, 40: 103–105.

Blanton F. S. & Wirth W. W. (1979) The sand flies (*Culicoides*) of Florida (Diptera: Ceratopogonidae). *Arthropods of Florida and Neighboring Land Areas*, 10: 1–204.

Borkent A. (2009) World Species of Biting Midges (Diptera: Ceratopogonidae)-http://www.inhs.uiuc.edu/research/FLYTREE/CeratopogonidaeCatalog.pdf, 236 pp.

Borkent A. & Grogan W. L. Jr. (2009) Catalog of the New World Biting Midges North of Mexico (Diptera: Ceratopogonidae). *Zootaxa*, 2273: 1–48.

Borkent A. & Spinelli G. R. (2000) Catalogue of the new world biting midges south of the United States of America (Diptera: Ceratopogonidae). *Contributions on Entomological International*, 4 (1): 1–107.

Borkent A. & Spinelli G. R. (2007) Neotropical Ceratopogonidae (Diptera: Insecta). In: Adis J., Arias J. R., Rueda-Delgado G. & Wantzen K. M. (Eds.): *Aquatic Biodiversity in Latin America (ABLA)*. Vol. 4. Pensoft, Sofia-Moscow, 198 pp.

Borkent A. & Wirth W. W. (1997) World Species of Biting Midges (Diptera: Ceratopogonidae). *Bulletin of the American Museum of Natural History*, 233: 257 pp.

Díaz F., Ronderos M. M. & Spinelli G. R. (2005) The immatures of the Neotropical species *Culicoides venezuelensis* Ortiz and Mirsa (Diptera: Ceratopogonidae). *Transactions of the American Entomological Society*, 131 (3–4): 375–385.

Floch H. & Abonnenc E. (1942) Cératopogonides hématophages de la Guyane Francaise. *Publication de l'Institut Pasteur de la Guyane et du Territory de L'Inini*, 37: 1–10.

Forattini O. P. (1957) *Culicoides* da Regiao Neotropical (Diptera, Ceratopogonidae). *Arquivos da Faculdade de Higiene e Saúde Pública da Universidade de São Paulo*, 11: 159–526.

Fox I. (1942) La trompa respiratoria y el segmento anal en las pupas de algunas especies de *Culicoides* (Diptera: Ceratopogonidae). *The Puerto Rico Journal of Public Health and Tropical Medicine*, 17: 426–434.

Franca Rodriguez M. E. (1963) Primeros estudios sobre los *Culicoides* (Diptera: Ceratopogonidae. *Revista Uruguaya de Patología Clínica*, 1:65–70.

Glukhova V. M. (1977) Midges of the family Ceratopogonidae (synonym Heleidae) p. 431–457. In: Kutikova L. A. & Starobogatov Y. L. (Eds). *Determination of the Freshwater Invertebrates of the Europen Regions of the USSR* (*Plankton and Benthos*) (*In Russian*). Leningrad, 127 pp.

Huerta H., Ronderos M. M. & Spinelli G. R. (2001) Description of larva and pupa and redescription of the adult of *Culicoides albomaculus* Root and Hoffman (Diptera: Ceratopogonidae). *Transactions of the American Entomological Society*, 127 (4): 545–561.

Hribar L. J. (1993) Mouthpart morphology and feeding behavior of biting midges larvae (Diptera: Ceratopogonidae), p. 43–58. In: Schaefer C. W & Leschen R. A. B (Eds.). *Functional Morphology of Insect Feeding*. Thomas Say Publications in Entomology: *Proceedings Entomological Society of America*. Lanham, MD.

Lamberson C., Pappas C. D. & Pappas L. G. (1992) Pupal taxonomy of the tree-hole *Culicoides* (Diptera: Ceratopogonidae) in Eastern North America. *Annals of the Entomological Society of America*, 85 (2): 111–120.

Lutz A. (1913) Contribuçao para o conhecimento das Ceratopogoninas hematófagas de Brasil. Parte Sistematica. Segunda Memoria. *Memórias do Instituto Oswaldo Cruz*, 5:45–73.

Macfie J. W. S (1937) Ceratopogonidae from Trinidad. *Annals and Magazine of Natural History*, Ser. 10, 20: 1–18.

Messersmith D. H. (1964) The breeding site of *Culicoides debilipalpis* Lutz (Diptera: Ceratopogonidae) in Virginia. *Mosquito News*, 24: 339.

Mullen G. R. & Hribar L. J. (1988) Biology and feeding behavior of Ceratopogonidae larvae (Diptera: Ceratopogonidae) in North America. *Bulletin of the Society of Vector Ecology*, 13: 60–81.

Murphree C. S. & Mullen G. R. (1991) Comparative larval morphology of the genus *Culicoides* Latreille (Diptera: Ceratopogonidae) in North America with a key to species. *Bulletin of the Society of Vector Ecologists*, 16 (2): 269–399.

Ronderos M. M. & Spinelli G. R. (1995) Redescripción de *Culicoides lahillei* y descripción de *Culicoides ichesi*, n. sp. de la Argentina, Paraguay y Uruguay (Diptera: Ceratopogonidae). *Neotrópica*, 41: 77–81.

Ronderos M. M. & Spinelli G. R. (1998) Las especies de *Culicoides* en el área de influencia de la represa de Yacyretá (Diptera: Ceratopogonidae). *Revista de la Sociedad Entomológica Argentina*, 57: 79–83.

Ronderos M. M. & Spinelli G. R. (2000a) The larva and pupa of *Culicoides bambusicola* Lutz observed with SEM, and additional notes on the adult (Diptera: Ceratopogonidae). *Transactions of the American Entomological Society*, 126 (1): 133–134.

Ronderos M. M., Spinelli G. R. & Sarmiento P. (2000b) Preparation and mounting of biting midges of the genus *Culicoides* Latreille (Diptera: Ceratopogonidae) to be observed with Scanning Electron Microscope. *Transactions of the American Entomological Society*, 126 (1): 125–132.

Ronderos M. M., Greco N. M. & Spinelli G. R. (2003a) Diversity of biting midges of the genus *Culicoides* Latreille (Diptera: Ceratopogonidae) in the area of the Yacyreta dam lake between Argentina and Paraguay. *Memórias do Instituto Oswaldo Cruz*, 98 (1): 19–24.

Ronderos M. M., Spinelli G. R., Lager I. & Díaz F. (2003b) La importancia sanitaria de los jejenes del género *Culicoides* (Díptera: Ceratopogonidae) en la Argentina. *Entomología y Vectores*, 10 (4): 601–612.

Ronderos M. M., Díaz F., David F. (2004) Clave gráfica de adultos de los géneros hematófagos de Ceratopogonidae (Díptera: Ceratopogonidae) presentes en la región Neotropical. *Entomología y Vectores*, 11 (3):505–519.

Ronderos M. M., Díaz F. & Sarmiento P. (2008a) A new method using acid to clean and a technique for preparation of eggs of biting midges (Diptera: Ceratopogonidae) for Scanning Electron Microscope. *Transactions of the American Entomological Society*, 134 (3–4): 471–476.

Ronderos, M. M., G. R. Spinelli & Borkent A. (2008b) A description of the larva and pupa of *Culicoides charruus* Spinelli & Martinez (Diptera: Ceratopogonidae) from leaf axils of *Eryngium panddanifolium* (Apiaceae). *Russian Entomological Journal*, 17(1): 115–122.

Smith W. W., Vannell J. H. Jr. (1967) Hydrogenion concentration (pH) as related to the occurrence and abundance of tree-holes dwelling *Culicoides* spp. (Diptera: Ceratopogonidae) in northern Florida. *Mosquito News*, 27: 519–521.

Spinelli G. R. & Martinez M. (1991) The genus *Culicoides* in Uruguay (Diptera: Ceratopogonidae). *Insecta Mundi*, 5: 175–179.

Spinelli G. R. & Ronderos M. M. (1991) Los polvorines del género *Culicoides* en áreas de influencia de la represa de Salto Grande (Diptera: Ceratopogonidae). *Neotrópica*, 37: 83–94.

Spinelli G. R. & Ronderos M. M. (1997) Description of the male and revalidation of *Culicoides debilipalpis* (Diptera: Ceratopogonidae). *Transactions of the American Entomological Society*, 123 (4): 303–309.

Spinelli G. R. & Wirth W. W. (1986) Clave para la identificación de las especies del género *Culicoides* Latreille presentes al sur de la cuenca amazónica. Nuevas citas y notas sinonímicas (Diptera: Ceratopogonidae). *Revista de la Sociedad Entomológica Argentina*, 44: 49–73.

Spinelli G. R., Ronderos M. M. & Balseiro E. G. (1989) Análisis de la actividad diaria de dípteros nematóceros en Punta Lara (Pdo. Ensenada, Prov. de Buenos Aires). II. Ceratopogonidae, con la descripción de una especie nueva del género *Atrichopogon*. *Limnobios*, 2: 733–737.

Spinelli G. R., Ronderos M. M., Díaz F. & Marino P. I. (2005) The bloodsucking midges of Argentina (Díptera: Ceratopogonidae). *Memórias de Instituto Oswaldo Cruz*, 100 (2): 137–150.

Thomsen L. C. (1937) Aquatic Diptera. Part V, Ceratopogonidae. *Memoirs of the Cornell University Agriculture Experimental Station*, 210: 57–80

Trindade, R. L. & Gorayeb, I. S. (2005) Maruims (Ceratopogonidae: Diptera) do Estuario do Río Pará e do Litoral do Estado do Pará, Brasil. *Entomología y Vectores*, 12 (1): 61–74.

Vitale G. W., Wirth W. W. & Aitken T. H. (1981) New species and records of *Culicoides* reared from arboreal habitats in Panama, with a synopsis of *debilipalpis* group (Diptera: Ceratopogonidae). *Proceedings of the Entomological Society of Washington*, 83: 140–159.

Waller J., Lancelot R., Lefevre P. C. & Kremer M. (1990) Note faunistique sur les *Culicoides* de Guyane (Diptera: Ceratopogonidae). *Revue d'élevage et de Medecine Vétérinaire des pays tropicaux*, 43: 349–364.

Wirth W. W. & Blanton F. S. (1959) Biting midges of the genus *Culicoides* from Panama (Diptera: Heleidae). *Proceedings of the United States National Museum*, 109: 237–482.

Wirth W. W. & Blanton F. S. (1971a) New Neotropical sandflies of the *Culicoides debilipalpis* group (Diptera: Ceratopogonidae). *Proceedings of the Entomological Society of Washington*, 73: 34–43.

Wirth W. W. & Blanton F. S. (1971b) New species and synonymy of Florida *Culicoides* (Diptera: Ceratopogonidae). *Florida Entomologist*, 54: 73–78.

Wirth W. W., Dyce A. L. & Spinelli G. R. (1988) An atlas of wing photographs, with a summary of the numerical characters of the Neotropical species of *Culicoides* (Diptera: Ceratopogonidae). *Contributions of the American Entomological Institute*, 25: 1–72.