



## New contributions to the knowledge of the immatures of *Stilobezzia punctulata* Lane and *Stilobezzia fiebrigi* Kieffer (Diptera: Ceratopogonidae)

CARLA G. CAZORLA<sup>1,3</sup>, FLORENTINA DIAZ<sup>1</sup>, PABLO I MARINO<sup>1</sup>

GUSTAVO R. SPINELLI<sup>1</sup> & MARÍA M. RONDEROS<sup>2</sup>

<sup>1</sup>División Entomología, Museo de La Plata, Paseo del Bosque s/n, 1900 La Plata, Argentina.

E-mail: [carlacazorla@fcnym.unlp.edu.ar](mailto:carlacazorla@fcnym.unlp.edu.ar); [mfldiaz@fcnym.unlp.edu.ar](mailto:mfldiaz@fcnym.unlp.edu.ar); [pmarino@fcnym.unlp.edu.ar](mailto:pmarino@fcnym.unlp.edu.ar); [spinelli@fcnym.unlp.edu.ar](mailto:spinelli@fcnym.unlp.edu.ar)

<sup>2</sup>División Entomología, Museo de La Plata, CCT-CEPAVE, Paseo del Bosque s/n, 1900 La Plata, Argentina.

E-mail: [ronderos@fcnym.unlp.edu.ar](mailto:ronderos@fcnym.unlp.edu.ar)

<sup>3</sup>Corresponding author

### Abstract

The fourth instar larva of *Stilobezzia punctulata* Lane is described for the first time and the one of *Stilobezzia fiebrigi* Kieffer is redescribed. They are illustrated and photomicrographed from material collected in different aquatic environments of the province of Corrientes, Argentina. Both species show features typical to carnivorous-predatory larva.

**Key words:** *Stilobezzia*, carnivorous, larvae, Corrientes, Argentina.

### Introduction

*Stilobezzia* Kieffer is a large and diverse genus of Ceratopogonidae, worldwide in distribution. The adult females are important predators on other small insects, and the immatures stages are found in a wide variety of aquatic and semiaquatic habitats, including streams, lakes and ponds margins, puddles, swamps, rice fields, rock pools, and tree holes (de Meillon & Wirth, 1991; Cazorla *et al.*, 2006).

There are at present 70 extant species of *Stilobezzia* inhabiting the Neotropical region (Borkent, 2012; Cazorla, 2013). However and despite the great importance of knowing the ontogeny, immatures of this genus have been poorly studied, the larvae of only 4 species and the pupae of 13 species have been described in this region (Borkent & Spinelli, 2007; Ronderos *et al.*, 2008a; Cazorla *et al.* 2012; Ronderos *et al.*, 2012).

During recent entomological surveys carried out in the province of Corrientes, Argentina, larvae of *Stilobezzia fiebrigi* Kieffer and *Stilobezzia punctulata* Lane were collected. The adults and pupae of both species are well known (Cazorla & Marino, 2004; Cazorla *et al.*, 2006), but the fourth instar larva of *S. punctulata* remains undescribed and the one of *S. fiebrigi* was poorly described by Lane & Forattini (1956).

The purpose of this paper is to fully redescribe the fourth instar larva of *Stilobezzia fiebrigi* and to describe the fourth instar larva of *Stilobezzia punctulata*.

### Material and methods

Larvae of *Stilobezzia fiebrigi* were collected in a temporary muddy puddle and larvae of *Stilobezzia punctulata* were collected in a lagoon associated to hydrophytes. In both cases they were carried back to the laboratory with water and sediment from their natural environments. Fourth instar larvae were separated, reared and observed daily until adult emergence.

For observation with compound microscope, larval exuviae were slide-mounted in Canada balsam following the technique described by Borkent and Spinelli (2007). They were also examined with Scanning Electron

Microscope (SEM) JSM6360LV, following the technique of Ronderos *et al.* (2000, 2008b), except that 30% glycolic acid was used to clean specimens and the exposure time of cleaning was increased to eight minutes. Measurements and ratios were taken using SEM and Binocular Compound Microscope (BCM) when possible.

Ink illustrations were made with a camera *lucida*. Photographs were taken with a digital camera Micrometrics SE Premium, through Nikon Eclipse E200 microscope and were edited in Corel DRAW X3 and Corel PHOTO-PAINT X3 graphic software.

For larval terminology see Ronderos *et al.* (2012). Studied specimens are deposited in the collections of the División Entomología, Museo de La Plata, Argentina (MLPA).

**Abbreviations.** Antennae (AN); caudal segment (CS); collar (CO); dorsal comb (DC); fossa mandibularis (MF); galeolacinia (GL); head capsule (HC); hypostoma (HY); labrum (LB); lacinial sclerite 1 (LC1); lacinial sclerite 2 (LC2); mandible (MD); maxilla (MX); maxillary palpus (MP); medial sclerite (Ms); messors (MS); palatal bar (PB); palatum (PL); scopae (SC); sensilla campaniformia (SCa); sensilla styloconica (SS); sensilla trichoidea (ST); thoracic segments (TC); ventral comb (VC). Head capsule and caudal segment chaetotaxy are indicated by single letters: j, collar pits; o, parahypostomal setae; p, posterior perifrontal setae; q, postfrontal setae; r, postnotal pits; s, anterior perifrontal setae; t, prefrontal setae; u, mesolateral setae; v, posterolateral setae; w, anterolateral setae; x, paranntenae setae; y, ventral setae; d, dorsal setae; “o” outer setae; “i” inner setae.

## Results

### *Stilobezzia fiebrigi* Kieffer

(Figs. 1–6, 13–15)

*Stilobezzia fiebrigi* Kieffer, 1917: 309 (female; Paraguay); Lane, 1947: 199 (male; Brazil).

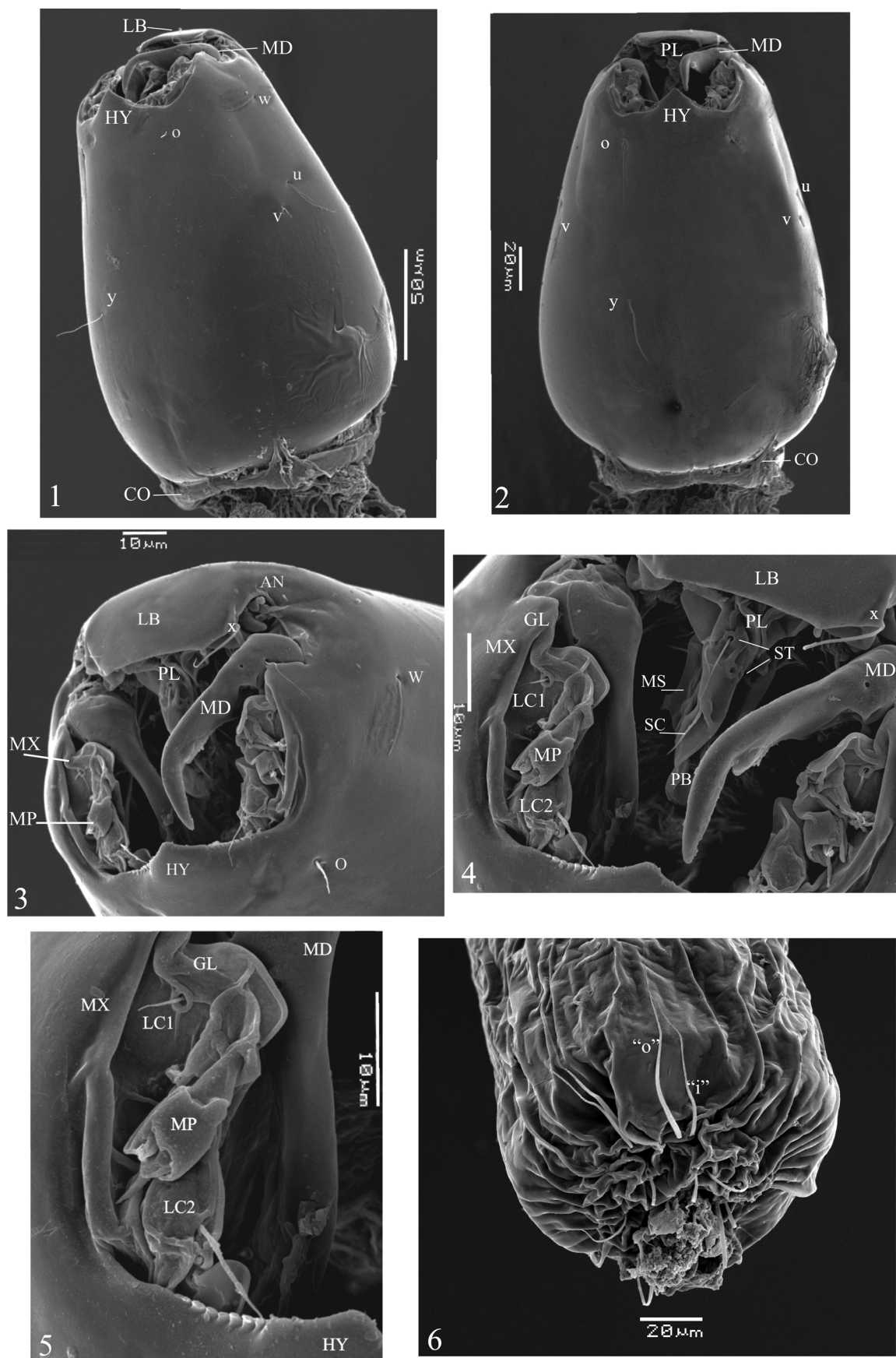
*Stilobezzia (Stilobezzia) fiebrigi*: Forattini & Rabello, 1956: 45 (larva, pupa, Brasil); Wirth 1974: 45 (catalogue of Neotropical species); Lane & Forattini, 1961: 85 (in key); Spinelli, 1983: 410 (Argentina record; Buenos Aires province); Spinelli & Wirth, 1993: 51 (in list; Argentina); Borkent & Wirth, 1997: 111 (catalogue of World species); Borkent & Spinelli, 2000: 54 (catalogue of Neotropical species); Cazorla *et al.*, 2006: 111 (adult and pupa redescription, Argentina); Borkent & Spinelli, 2007: 87 (catalogue of Neotropical species); Borkent, 2012 (catalogue of World species).

**Description of fourth instar larva** (Figs. 1–6, 13–15). Head capsule (Figs. 1–2) pale brown, conical, small, tapering to rounded apex; ventral and ventrolateral chaetotaxy as in Figs. 1–3, dorsal chaetotaxy not in position to be photographed; HL 0.182 mm (SEM), 0.20–0.23 (0.22, n=3) mm (BCM); HW 0.137 mm (SEM), 0.18–0.23 (0.20, n=3) (BCM); HR 1.328 (SEM), 1.00–1.13 (1.08, n=3) (BCM); SGW 0.092 mm (SEM), 0.12–0.14 (0.136, n=3) mm (BCM); SGR 1.49 (SEM), 1.40–1.60 (1.50, n=3) (BCM). Labrum (Figs. 1, 3–4) wider than long; palatum (Figs. 2–4) with two pairs of sensilla trichoidea, anterior one thin, elongated, distal one absent (Fig. 4); messors well developed, sclerotized, lateral to scopae (Fig. 4); scopae thin, elongated; palatal bar not in position to be properly described (Fig. 4). Mandible (Figs. 1–5, 15) bidentate, thin, curved distally, lightly sclerotized; apical tooth long, submedian tooth short; basal portion with long, thin seta and a pore; fossa mandibularis on ectal surface, MDL 0.05–0.06 (0.056, n=3) mm. Maxilla (Figs. 3–5) sclerotized, galeolacinia (Figs. 4–5) with lacineal sclerite with short thin seta, lacineal sclerite 2 with long, thin seta; maxillary palpus (Figs. 3–5) cylindrical with 2–3 papillae. Hypostoma (Figs. 1–3) with smooth, triangular, mesal elevation flanked by serrate margins with 7 teeth. Epipharynx (Fig. 13) massive with three combs; comb 4 massive, its anterior margin irregular with 10 stout, pointed teeth; other two combs superimposed, poorly developed; medial sclerite v-shaped; dorsal comb sclerite with posterior margin with numerous, thin teeth; lateral arms stout, sclerotized, handle-shaped, without curtain or fringe; LAW 0.076–0.088 (0.082, n=2) mm, DCW 0.030–0.040 (0.035, n=2) mm. Hypopharynx (Fig. 14) elongated, thick; divergent arms connected posteriorly; U-shaped, sclerotized, internal sclerite; hypopharyngeal fringe hyaline. Thoracic pigmentation not visible. Caudal segment chaetotaxy as in Fig. 6; medium size, stout, setae “o”, seta “i”, thinner; CSL 0.200 mm (SEM); CSW 0, 098 mm (SEM).

**Material examined:** Argentina, Corrientes, arroyo Pay Ubre, 29°01'41.2''S, 58°10'26.6''W, 66 m, 18-III-2010, G. Spinelli, 3 larval exuviae.

**Material examined by SEM:** same data, 2 larvae (instar IV).

**Bionomics.** The larvae of *Stilobezzia fiebrigi* were collected in a temporary muddy puddle located approximately 10 meters from the bottom of a shallow stream.



**FIGURES 1–6.** *Stilobezzia fiebrigi* Lane, four instar larva. 1, head capsule ventrolateral view (chaetotaxy); 2, head capsule, ventral view (chaetotaxy); 3, head capsule, anteroventral view; 4, head capsule, frontal view; 5, head capsule, frontolateral view, detail of mouthparts; 6, caudal segment, posterior view. Scale: 0.05 mm.

Live larvae exhibited the typical fast, undulating swimming motion of other members of the subfamily Ceratopogoninae. The adults emergence occurred after a week under laboratory rear conditions.

**Taxonomic notes.** The larvae of *Stilobezzia fiebrigi* is very similar to the one of its Neotropical congener *S. coquilletti* Kieffer. They were collected in similar environments and both species show features typical to carnivorous-predatory larvae, such as a mouthparts anteriorly directed, curved and sclerotized mandible with fossa mandibularis, epipharynx gently massive and the hypostomal mesal elevation flanked by serrate margins (Thomsen, 1937; Hribar, 1993; Ronderos *et al.* 2008a). However, *S. coquilletti* can be distinguished by the absence of scopae; the palatum with three pairs of sensilla styloconica (one large, two short) and one pair of sensilla trichoidea; the galeolacinia with a bundle of four setae and the dorsal comb sclerite with an irregular posterior margin bearing 9–10 lanceolate teeth. Both species also shared the presence of medium sized setae in the caudal segment, a characteristic typical of larvae breeding in shallow water and muddy bottoms.

### ***Stilobezzia punctulata* Lane**

(Figs. 7–12, 16–18)

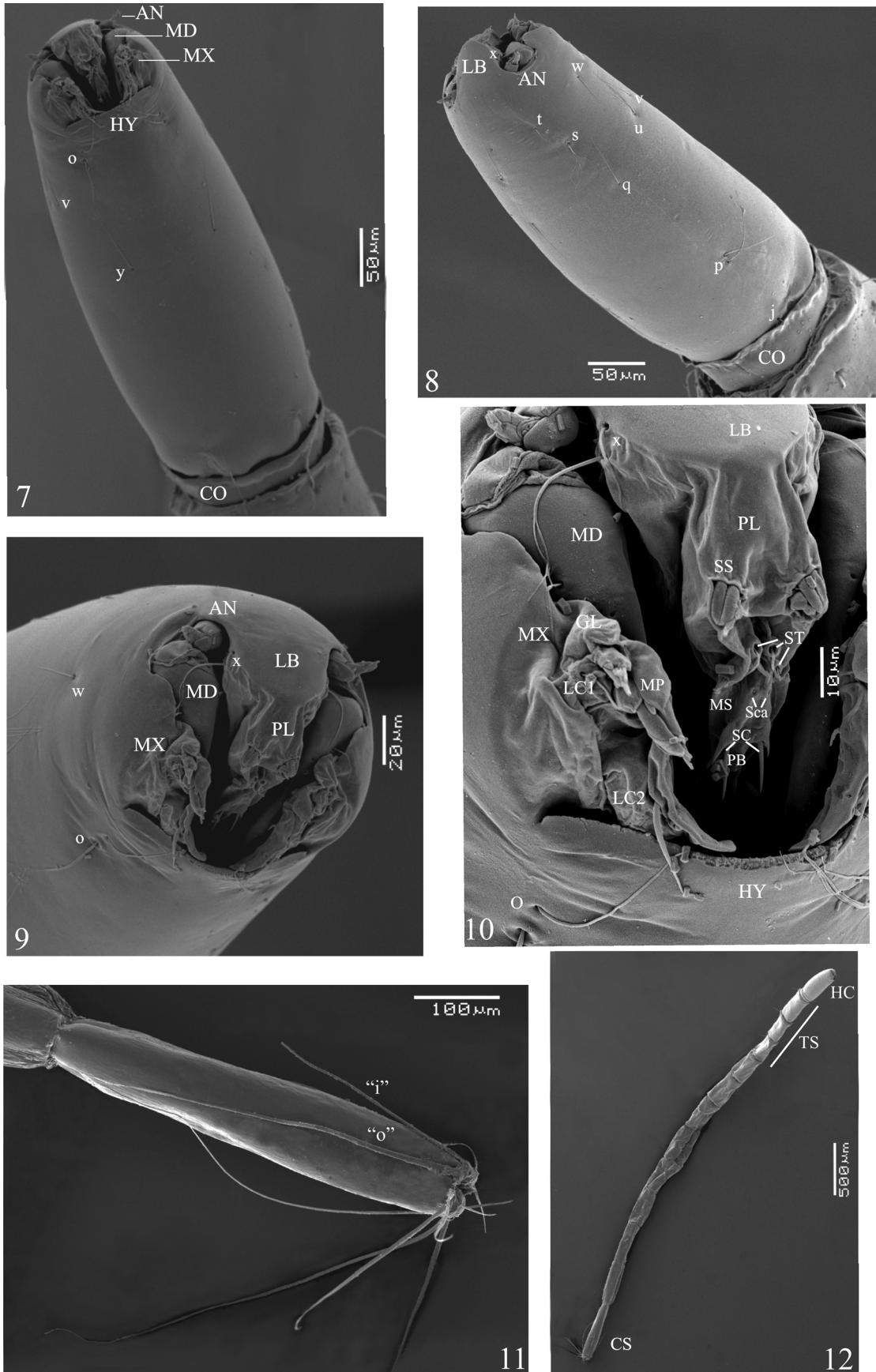
*Stilobezzia punctulata* Lane 1947: 204 (female; Brazil)

*Stilobezzia (Stilobezzia) punctulata*: Lane & Forattini, 1958: 220 (male; Brazil, Panama); Wirth 1974: 45 (catalogue of Neotropical species); Borkent & Wirth, 1997: 112 (catalogue of World species); Borkent & Spinelli, 2000: 54 (catalogue of Neotropical species); Cazorla & Marino, 2004: 75 (pupa, Mexico, Colombia, Peru, Bolivia [misident]); Borkent & Spinelli, 2007: 87 (catalogue of Neotropical species); Borkent, 2012 (catalogue of World species); Torreias *et al.*, 2013: 10 (distribution).

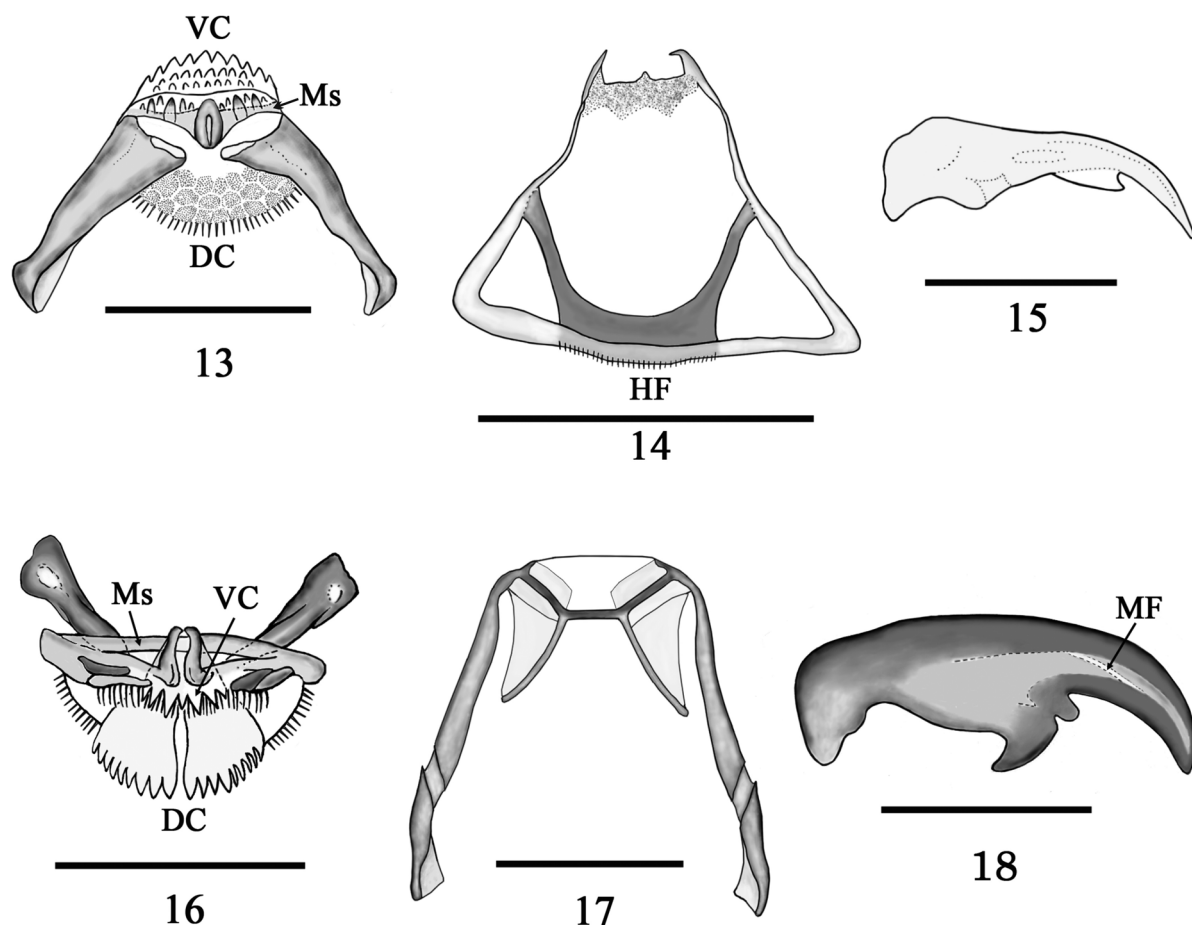
**Description of fourth instar larva** (Figs. 7–12, 16–18). Head capsule yellowish brown, elongated, cylindrical; chaetotaxy as in Figs. 7–9; HL 0.431 mm (SEM), 0.476 mm (BCM); HW 0.169 mm (SEM), 0.372 mm (BCM); HR 2.55 (SEM), 1.28 (BCM); SGW 0.140 mm (SEM), 0.312 mm (BCM); SGR 1.21 (SEM), 1.19 (BCM). Antennae well developed, stout (Figs. 7–9). Labrum (Figs. 8–10) as long as wide; palatum (Figs. 9–10) with three pairs of sensilla styloconica on its anterior edge, the inner one longer; two pairs of sensilla trichoidea on mesal portion, anterior one stouter; one pair of sensilla campaniformia immediately underneath; well developed, long, pointed scopes (Fig. 10); well developed messors (Fig. 10), lateral to scopes; palatal bar (Fig. 10) present, tongue-shaped. Mandible (Figs. 7, 9–10, 18) stout, curved, strongly sclerotized; apical tooth large, two submedian teeth, anterior one small, posterior one stouter, recurved back; basal portion with long, thin seta and a pore; fossa mandibularis on ectal surface, MDL 0.10 mm. Maxilla (Figs. 7, 9–10) sclerotized, galeolacinia (Fig. 10) with lacineal sclerite 1 with long thin setae, lacineal sclerite 2 with long, stout seta; maxillary palpus (Fig. 10) cylindrical with 3–4 apical papillae. Hypostoma (Figs. 7, 10) nearly straight, smooth. Epipharynx (Fig. 16) massive; ventral comb massive, its posterior margin irregular with 7 pointed teeth, mesal one smaller; other comb with numerous thin teeth superimposed; nearly straight, sclerotized medial sclerite; lateral arms stout, sclerotized with elongated lateral curtains; LAW 0.066 mm, DCW 0.025 mm; dorsal comb with 9 stout teeth. Hypopharynx (Fig. 17) elongate, posterior end arms thick, without hypopharyngeal fringe. Thoracic pigmentation pale brown. Caudal segment (Fig. 12), chaetotaxy as in Fig. 11; four pairs of stout, long setae and two pairs of long, thinner ones; long, stout setae "o" 0.500 mm (SEM), 0.687 mm (BCM); CSL 0.572 mm (SEM) 0.70 mm (BCM); CSW 0.140 mm (SEM) 0.200 mm (BCM).

**Bionomics.** The larvae of *Stilobezzia punctulata* were collected from the bottom of a temporary lagoon, associated to hydrophytes. They showed slow, undulating swimming motion, and lasted six days to reach the pupal stages under laboratory rear conditions; the only pupa that reached the adult stage took 10 days to emerged.

**Taxonomic discussion.** The larva of *Stilobezzia punctulata* shows some of the previously listed features of carnivorous-predatory larvae for *S. fiebrigi*. However, the mandible of *S. punctulata* is larger and more sclerotized than the one of *S. fiebrigi*; the head is elongated and cylindrical; the hypostoma is nearly straight and smooth, lacking the mesal elevation flanked by serrate margins; and the lateral arms of the hypopharynx are not connected posteriorly. *Stilobezzia punctulata* also presents some characters that have been observed in larvae of other genera that breed in clean water of tree holes, such as the presence of lateral curtains in the lateral arms of the epipharynx. Finally, with regard to the very long setae of the caudal segment of this species, Mullen & Hribar (1988) suggested that they may function to amplify the body oscillation and to increase the larval speed in water, enabling to capture prey and/or to avoid predators.



**FIGURES 7–12.** *Stilobezzia punctulata* Lane, four instar larva. 7, head capsule ventral view (chaetotaxy); 8, head capsule, dorsal view (chaetotaxy); 9, head capsule, anteroventral view ; 10, head capsule, anteroventral view, detail of mouthparts; 11, caudal segment, lateral view; 12, entire larva. Scale: 0.05 mm.



**FIGURES 13–18.** 13–15, *Stilobezzia fiebrigi*. 13, epipharynx, dorsal view; 14, hypopharynx, ventral view; 15, mandible, lateral view; 16–18, *Stilobezzia punctulata*. 16, epipharynx, dorsal view; 17, hypopharynx, ventral view; 18, mandible, lateral view. Scale: 0.05 mm.

**Material examined:** Argentina, Corrientes, Estación Biológica Corrientes (EBCo), 27°32'51.8" S, 58°40'44.8" W, 52 m, 26-XI-2010, P. Marino, 1 male with larval and pupal exuviae.

**Material examined by SEM:** same data, 2 larvae (instar IV).

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### References

- Borkent, A. (2012) World species of biting midges (Diptera: Ceratopogonidae). 234 pp. Available from: <http://www.inhs.illinois.edu/files/8413/4219/9566/CeratopogonidaeCatalog.pdf> (accessed 29 November 2013)
- Borkent, A. & Spinelli, G.R. (2000) Catalog 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, pp. 1–198.
- Borkent, A. & Wirth, W.W. (1997) World species of biting midges (Diptera: Ceratopogonidae). *Bulletin of the American Museum of Natural History*, 233, 1–257.

- Cazorla, C.G. (2013) A new species of *Stilobezzia* (Diptera: Ceratopogonidae) from northern Argentina. *Acta Entomologica Musei Nationalis Pragae*, 53 (1), 323–327.
- Cazorla, C.G., Diaz, F. & Ronderos, M.M. (2006) Redescription of pupa and adult of *Stilobezzia fiebrigi* Kieffer 1917 (Diptera: Ceratopogonidae). *Transactions of the American Entomological Society*, 132, 111–119.
- Cazorla, C.G. & Marino, P.I. (2004) The pupa of *Stilobezzia punctulata* Lane from Peruvian Amazonia. (Diptera: Ceratopogonidae). *Amazoniana*, 18, 75–80.
- Cazorla, C.G., Ronderos, M.M., Spinelli, G.R., Torreias, S.R.S. & Ferreira-Keppler, R.L.A. (2012) A new species of *Stilobezzia* Kieffer from the Neotropical Region (Diptera: Ceratopogonidae). *Revista Brasileira de Entomologia*, 56 (4), 399–404. <http://dx.doi.org/10.1590/s0085-56262012000400002>
- De Meillon, B. & Wirth, W.W. (1991) The genera and subgenera (excluding *Culicoides*) of the Afrotropical Biting Midges (Diptera: Ceratopogonidae). *Annals of the Natal Museum*, 32, 27–147.
- Forattini, O.P. & Rabello, E.X. (1956) As forma imaturas de *Culicoides guayanensis* Floch e Abonnenc, 1942 e de algumas espécies de *Stilobezzia* (Diptera, Ceratopogonidae). *Revista Brasileira de Entomologia*, 56, 43–50.
- Hribar, L.J. (1993) Mouthpart morphology and feeding behavior of biting midges larvae (Diptera: Ceratopogonidae). 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, pp. 43–58.
- Kieffer, J.J. (1917) Chironomides d' Amérique conservés au Musée National Hongrois de Budapest. *Annales Historico-Naturales Musei Nationalis Hungarici*, 15, 292–364.
- Lane, J. (1947) Espécies Brasileiras de *Stilobezzia* (Diptera: Ceratopogonidae) e *Zigoneura stonei* nov. nom. (Diptera: Mycetophilidae). *Revista Brasileira de Entomologia*, 18, 197–214.
- Lane, J. & Forattini, O.P. (1956) Neotropical *Stilobezzia* Kieffer, 1911 I. Nine new Panamanian species (Diptera: Ceratopogonidae). *Revista Brasileira de Malariologia*, 8, 207–226.
- Lane & Forattini (1958) Neotropical *Stilobezzia* II. Fourteen new species, chiefly from Panama (Diptera: Ceratopogonidae). *Revista Brasileira de Entomologia*, 8, 203–224.
- Lane, J. & Forattini, O.P. (1961) Neotropical *Stilobezzia* Kieffer, 1911. III Key for the adults of this genus and description of one new species (Diptera: Ceratopogonidae). *Revista Brasileira de Entomologia*, 10, 83–94.
- 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.
- Ronderos, M.M., Cazorla, C.G., Spinelli, G.R. & Carrasco, D.S. (2008a) Description of immature stages and adult diagnosis of *Stilobezzia coquilletti* Kieffer 1917 (Diptera: Ceratopogonidae). *Zootaxa*, 1958, 31–40.
- Ronderos, M.M., Diaz, F. & Sarmiento, P. (2008b) 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. <http://dx.doi.org/10.3157/0002-8320-134.3.471>
- Ronderos, M.M., Spinelli, G.R. & Borkent, A. (2012) A peculiar new species of *Stilobezzia* Kieffer breeding in bamboo internodes in northeastern Argentina (Diptera: Ceratopogonidae). *Aquatic Insects: International Journal of Freshwater Entomology*, 34, 1–17. <http://dx.doi.org/10.1080/01650424.2012.718079>
- Ronderos, M.M., Spinelli, G.R. & Sarmiento, P. (2000) Preparation and Mounting of Biting Midges of the Genus *Culicoides* Latreille (Diptera: Ceratopogonidae) to be observed with a Scanning Electron Microscope. *Transactions of the American Entomological Society*, 126 (1), 125–132.
- Spinelli, G.R. (1983) Notas sobre Ceratopogonidae (Diptera, Nematocera) de la República Argentina. Una nueva especie del género *Alluaudomyia* Kieffer, redescrpción de *Dasyhelea penthesileae* Macfie, y nuevas citas para el género *Stilobezzia* Kieffer. *Limnobiós*, 2, 403–411.
- Spinelli, G.R. & Wirth, W.W. (1993) Los Ceratopogonidae de la Argentina (Insecta: Diptera). In: de Castellanos, Z.A. (Ed.), *Fauna de agua dulce de la República Argentina, La Plata*, 38 (3), 1–121.
- Thomsen, L.C. (1937) Aquatic Diptera. Part V, Ceratopogonidae. *Memoirs of the Cornell University Agriculture Experimental Station*, 210, 57–80.
- Torreias, S.R.S., Ferreira-Keppler, R.L. & Ronderos, M.M. (2013) Biting midges (Ceratopogonidae: Diptera) present in aquatic macrophytes from wetlands of Marchantaria Island, Iranduba, Central Amazonia, Brazil. *Journal of Natural History*, 1–14. <http://dx.doi.org/10.1080/00222933.2013.791934>
- Wirth, W.W. (1974) Family Ceratopogonidae. In: Papavero, N. (Ed.), *A Catalog of the Diptera of the Americas south of the United States, Fasc. 14*. Museu de Zoologia, Universidade de Sao Paulo, pp. 89.