

First description of immature stages and adult females of *Stilobezzia (Acanthohelea) megatheca* (Diptera: Ceratopogonidae) from Patagonia

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Abstract – The larva, pupa and adult female of *Stilobezzia (Acanthohelea) megatheca* Cazorla and Spinelli are described in this report. The immature stages were collected from the bank of a stream near Puerto Patriada, in the province of Chubut, Argentina. All described stages were photographed and illustrated with a phase-contrast microscope and scanning electron microscope. This is the first description of immature stages on the subgenus *Stilobezzia (Acanthohelea)* Kieffer from the Neotropical region. Data on the bionomics of the species and a key to Neotropical pupae of the genus *Stilobezzia* Kieffer are provided.

Key words: Biting midges / Argentina / immatures / aquatic Diptera / taxonomy

Introduction

The genus *Stilobezzia* Kieffer, 1911 is a cosmopolitan genus, occurring in all zoogeographical regions except Antarctica. Adult females are important predators on other small insects, and the immature stages are found in a wide variety of aquatic and semiaquatic habitats, including streams, lake and pond margins, puddles, swamps, rice fields, rock pools and tree holes (De Meillon and Wirth, 1991; Cazorla *et al.*, 2006).

Four subgenera are included within *Stilobezzia*, *S. (Acanthohelea)* Kieffer, 1917, *S. (Debenhamia)* Wirth and Grogan, 1988, *S. (Eukraiohelea)* Ingram and Macfie, 1921 and *S. (Stilobezzia)*. Currently, the cosmopolitan *Stilobezzia (Acanthohelea)* is represented in the Neotropical region by 40 species (Borkent, 2016; Cazorla and Spinelli, 2016), and it is one of the dominant predaceous subgenera in the family in Argentinean and Chilean Patagonia where 16 species are recognized, solely as adults. The immatures of this subgenus are poorly known. Borkent (2014)

pointed out that only a few species were known worldwide as larvae or pupae: the pupa of *Stilobezzia lutea* (Malloch, 1918) from North America, the larvae and pupae of two species from Europe [*S. gracilis* (Haliday, 1833) and *S. ochracea* (Winnertz, 1852)], the pupa of *S. orientis* De Meillon and Wirth, 1981 from South Africa and two species from Asia, the pupa of *S. soror* Johannsen, 1932 and the larva and pupa of *S. papillata* Remm, 1980.

In the last revision of the Patagonian species of the subgenus *Acanthohelea*, Cazorla and Spinelli (2015) misidentified the female of *Stilobezzia (A.) curvistyla* Cazorla and Spinelli, 2007 as *Stilobezzia (A.) megatheca* Cazorla and Spinelli, 2015. During rearing in laboratory pupae collected in the province of Chubut, Argentina, males of *S. (A.) megatheca* emerged together with females showing small-sized spermathecae, which clearly indicates to us that the female previously assigned to this species was wrongly associated with the holotype male of *S. (A.) megatheca* (Cazorla, 2015). The purpose of this contribution is to provide the first description of the larva, pupa and the adult female of *S. (A.) megatheca*, including photographs, illustrations, data on the bionomics of the species and a key to the Neotropical pupae of the genus *Stilobezzia*.

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Materials and methods

Larvae and pupae were collected on the bordering vegetation and the mud of a permanent puddle on the bank of a stream near Puerto Patriada, in Chubut Province (42°08'19"S; 71°31'57"W) (Fig. 45). The substrate was removed with the aid of a strainer and transferred to a white tray where larvae and pupae were collected with a pipette. Further substrate samples were carried to the laboratory to search for more specimens. Larvae were placed in individual containers with water, and substrate rich in microorganisms and bryophytes from their natural environment. Pupae were isolated in a vial with a drop of water, and observed daily until adult emergence. Adults were allowed to harden for 24 h before being preserved in ethanol to ensure their complete pigmentation. For detailed examination with a phase-contrast microscope, larval and pupal exuviae and adults were mounted in Canada balsam following the technique described by Borkent and Spinelli (2007). Mounted larval exuviae were oriented ventral side up to facilitate examination of the epipharyngeal combs within the head capsule. Pupal exuviae were mounted dorsoventrally. Photomicrographs were taken with a digital camera Micrometrics SE Premium, through a Nikon Eclipse E200 microscope and digital camera Leica EC3, through a Leica DM 500 microscope. 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, 2008a); measurements were taken with a (BCM) Leitz Wetzlar binocular compound microscope. The illustrations used in the key were redrawn from original descriptions or redescriptions. The temperature of the water and air was measured with an alcohol thermometer in degrees Celsius.

The determination of the character states of the pupae of *Stilobezzia* used in the key was performed through the study of material deposited in the collection of the Museo de La Plata and through the photographs or ink illustrations provided in the original publications.

For larval and pupal terms, see Cazorla *et al.* (2013) and Borkent (2014), respectively. Terms of adult structures follow those in the Manual of Central American Diptera (Brown *et al.*, 2009). Studied specimens are deposited in the collection of the Museo de La Plata, La Plata, Argentina (MLPA) and in the US National Museum of Natural History, Washington, DC, USA (USNM). We also studied specimens deposited in the Canadian National Collection of Insects, Ottawa (CNCI), collected in 1984–1985 by J. Antony Downes in northern Argentinean and Chilean Patagonia. Downes' personal collection numbers are presented after locality and other data (*e.g.*, JAD 1651/6).

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: MDL, mandible length; MDW, mandible width. Caudal Segment: CSL, caudal segment length; CSW, caudal segment width; CSR, caudal segment ratio = CSL/CSW; OL, caudal setae length; OD, distance between bases of caudal setae "o"; OL/OD, ratio.

Pupa. Dorsal apotome: DAL, dorsal apotome length; DAW, dorsal apotome width; DAW/DAL, ratio. Respiratory organ: RO, respiratory organ length; P, pedicel length; P/RO, ratio.

Results

Stilobezzia (Acanthohelea) megatheca Cazorla and Spinelli (Figs. 1–6)

Stilobezzia (Acanthohelea) megatheca Cazorla and Spinelli, 2015: 18 (male, female misidentif., Argentina); Borkent, 2016: 138 (online World catalog).

Diagnosis: Adult. The only Patagonian species of *Stilobezzia (Acanthohelea)* with scutum dark brown, with three subparallel faint yellowish vittae, the lateral vittae extending posteriorly from yellowish humeral pits. Male with posterior margin of abdominal tergite 9 with a small, rounded, hyaline projection. Female with scutum pale yellowish with 6 large setae and 5 thinner setae interleaved; fore coxa pale brown, mid and hind coxae darker. *Larva.* Hypopharynx elongate with labium well developed, triangular and hyaline. *Pupa.* Clypeal/labral sensilla as thin setae and respiratory organ with 28 pores.

Description

Adult female. Head (Fig. 1) dark brown. Antennal flagellum brown; antennal ratio 1.51–1.73 (1.63, $n = 3$). Palpus brown, segment 3 stout with subapical, rounded sensory pit; palpal ratio 2.83–3.00 (2.92, $n = 3$). Mandible with seven to nine coarse teeth.

Thorax. Scutum dark brown, with three subparallel faint yellowish vittae, lateral vittae extending from humeral pits (Fig. 2); scutellum yellowish brown with six large setae and five thinner setae; postscutellum dark brown. Pleura dark brown; legs (Fig. 3) brown, hind femur, apex of hind tibia slightly darker; fore coxa pale brown, mid and hind coxae darker; hind tibial comb with eight spines; foreleg tarsal ratio 2.00–2.08 (2.04, $n = 3$); midleg tarsal ratio 2.16–2.28 (2.21, $n = 3$), hind leg tarsal ratio 2.00–2.19 (2.13, $n = 3$). Wing (Fig. 4) length 1.87–2.10 (1.98, $n = 3$) mm, width 0.70–0.82 (0.76, $n = 3$) mm, costal ratio 0.72–0.77 (0.75, $n = 3$); membrane slightly infuscated; first radial cell rectangular, conspicuous, second radial cell 2.5–3.25 (2.94, $n = 3$) times longer than first; cubital fork at same level of base of r–m crossvein; macrotrichiae on costa, veins R₁, R₂, R₃, M₁, M₂, CuA₂,

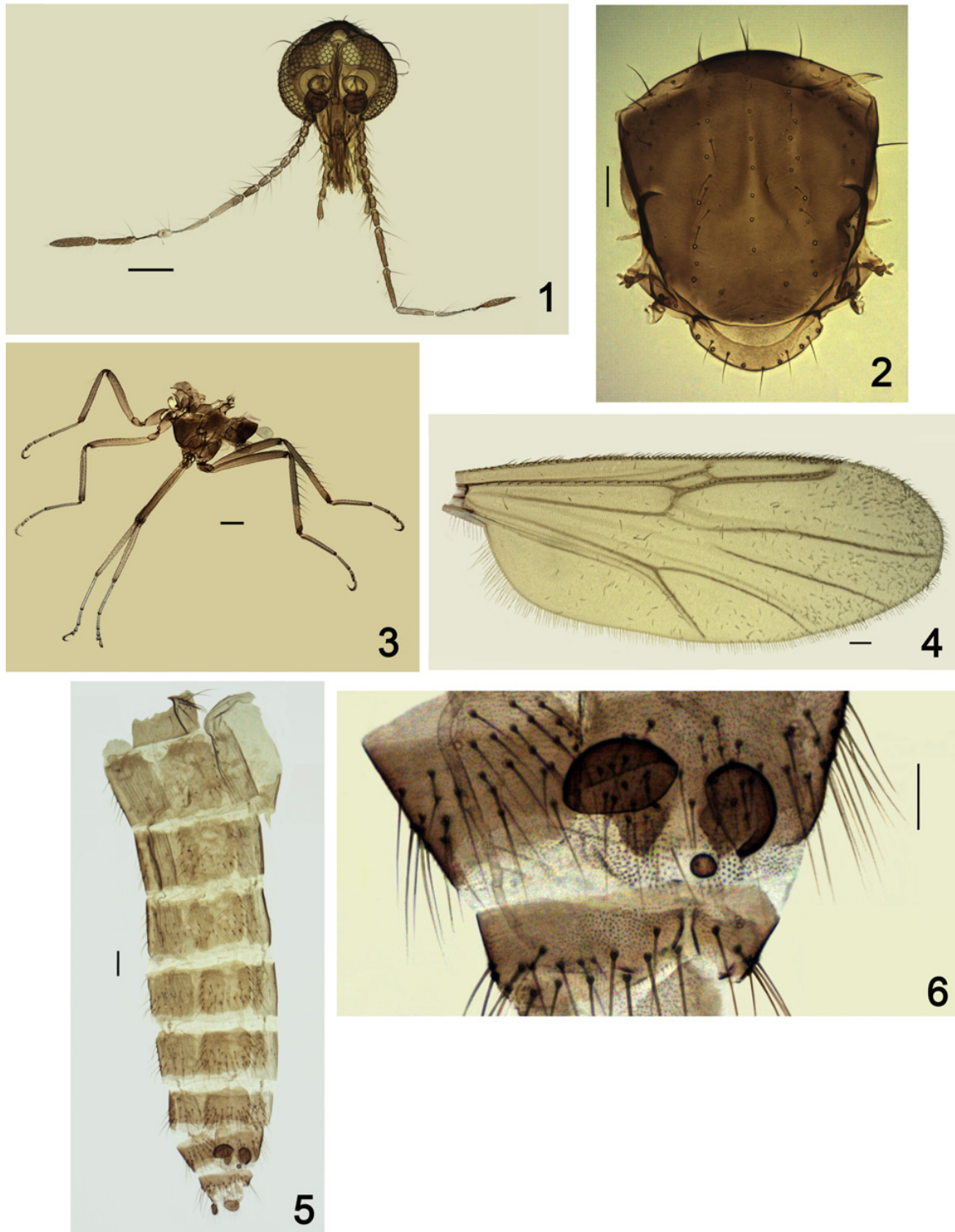


Fig. 1–6. *Stilobezzia (Acanthohelea) megatheca* Cazorla and Spinelli, adult female: 1. head, dorsal view; 2. wing; 3. scutum; 4. legs; 5. abdomen, ventral view; 6. spermathecae, ventral view. (Scale bar = 0.05 mm).

abundant on distal 1/3 of cells r_3 , m_1 , sparse on cell m_2 , cuA_1 . Halter pale.

Abdomen (Fig. 5). Uniformly dark brown. Genitalia: sternite 8 quadrangular, posteromedian excavation V-shaped; sternite 10 slender, with four pairs of setae;

cerci short, rounded; two subequal, ovoid spermathecae (Figs. 5 and 6) with thin, short necks, measuring 8.80–9.50 (9.18, $n = 3$) by 7.40–7.75 (7.55, $n = 3$) μm and 6.00–8.75 (7.67, $n = 3$) 6.00 by 4.50–6.00 (5.30, $n = 3$) μm , plus rudimentary third spermatheca present.

Fourth instar larva (Figs. 7–15). Head capsule (Figs. 7 and 8) brown, 1.82 longer than broad, conical, tapering to rounded apex, HL 0.316–0.344 ($n = 2$) mm; HW 0.200–0.248 mm ($n = 2$), HR 1.39–1.58 ($n = 2$) mm; SGW 0.22 ($n = 1$) mm; SGR 1.45 ($n = 1$) mm. Setae simple, moderately thin, medium-sized to elongate, chaetotaxy as in Figures 7 and 8. Antenna bottom-shaped, small (length 0.01 mm, $n = 1$). Labrum (Figs. 7–9) as long as wide; palatum (Figs. 8 and 9) with three pairs of anterolateral sensilla styloconica on anterior edge, inner one longer; two pairs sensilla trichoidea on mesal portion; messors (Fig. 9) small, thin, sclerotized, lateral to scopae; scopae well developed; palatal bar not observable. Mandible (Figs. 8, 10 and 12) stout, curved, strongly sclerotized, apical tooth large, two submedian teeth, stouter, equal in size, recurved to opposite side, with one sensory pit, fossa mandibularis on ectal surface; MDL 0.055–0.057 ($n = 2$) mm, MDW 0.018–0.021 ($n = 2$) mm. Maxilla (Figs. 8 and 10) sclerotized, galeolacinia with lacineal sclerite 1 (Fig. 10), lacineal sclerite 2 (Figs. 9 and 10), each with elongate conspicuous setae; maxillary palpus (Figs. 8–10) cylindrical, with three subapical papillae. Hypostoma (Figs. 8 and 9) nearly straight, smooth. Epipharynx (Fig. 13) slightly massive; ventral comb reduced; medial sclerite, stout, half-size of lateral arms, hyaline curtain with numerous thin teeth underneath; auxiliary sclerite less massive, oval-shaped; dorsal comb massive, posterior margin with five to six stout teeth; lateral arms stout, sclerotized, handle-shaped; lateral curtains present, with approximately 16–18 pointed, small teeth, with LAW 0.060–0.067 ($n = 2$) mm, DCW 0.030 ($n = 2$) mm. Hypopharynx (Fig. 14) elongate, posterior end arms thick, labium well developed, hyaline, triangular. Thorax and abdominal segments whitish. Caudal segment (Figs. 11 and 15) with one pair of long, thin setae “o”, one pair medium-sized, thin setae “i”, one pair of long, thin setae “l₁”, one pair of short, thin seta “l₂”. CSL 0.48–0.49 ($n = 2$) mm, CSW 0.244–0.280 ($n = 2$) mm, CSR 1.750–1.967 ($n = 2$), OL 0.10–0.12 ($n = 2$) mm, OD 0.048–0.750 mm.

Female pupa (Figs. 16–32). Habitus as in Figure 16. Exuviae overall pale brown. Total length 3.23–3.88 (3.26, $n = 3$) mm. Head: Dorsal apotome (Fig. 17) with ventral line of weakness, smooth area near dorsal posterior margin, slightly wider than long, distal margin nearly straight, with a few small spinules; disc surface, anterior margin covered by stout rounded spinules; antenna extending posteriorly to midleg; mouthparts (Fig. 25) with mandible well developed; palpus extending to posterolateral margin of labium; labium separated medially by labrum; apex of labrum lightly rounded; *sensilla*: dorsal apotomals (Fig. 17) D-1-H elongate, thin seta, located on well-developed tubercle, D-2-H campaniform sensillum at tubercle base; DAL 0.24–0.29 (0.26, $n = 3$) mm; DAW 0.21–0.24 (0.22, $n = 3$) mm; DAW/DAL 0.83–0.88 (0.85, $n = 3$); clypeal/labrals (Fig. 25): CL-1-H medium, thin seta, CL-2-H long, thin seta; oculars (Fig. 25): O-1-H short, thin seta, O-3-H, long, stout seta, O-2-H campaniform sensillum. Thorax: Cephalothorax rectangular,

surface predominantly smooth with small spinules on mesonotum, between bases of respiratory organs. Length of cephalothorax 1.22–1.85 (1.52, $n = 3$) mm, width 0.87–1.10 (1.01, $n = 3$) mm. Respiratory organ (Figs. 16, 18–21) smooth, medium-sized, slightly curved with rounded apex, 28 pores closely abutting at apex in single row, five to six lateral pores; pedicel slender (Figs. 18–22), P 0.028–0.035 (0.030, $n = 3$) mm; RO length 0.235–0.287 (0.269, $n = 3$) mm, RO width 0.033–0.035 (0.034, $n = 3$) mm; P/RO 0.11–0.14 (0.12, $n = 3$); *sensilla*: two anteromedials (Figs. 18 and 20) – AM-1-T medium, thin seta, AM-2-T short, thin seta; two dorso-lateral cephalic sclerite (Figs. 18, 21 and 22) – DL-1-H long, thin seta, DL-2-H campaniform sensillum; three anterolaterals (Figs. 18, 19, 22 and 26) – AL-1-T long, thin seta, AL-2-T short, stout seta, AL-3-T campaniform sensillum; dorsals (Fig. 27) – D-1-T, D-2-T, D-4-T long, stout seta, D-3-T campaniform sensillum, D-1-T, D-2-T, D-4-T on small tubercle, closely approximated; metathoracics (Fig. 28) – M-1-T very long, thin seta, M-2-T–M-3-T campaniform sensilla, M-3-T near anterior margin of metathorax. Abdomen: Abdominal segments covered with very small spicules, segments with simple setae, with bifid, moderately elongate tubercles; segment 8 without D-3-VIII, without L-1-VIII; segment 9 (Figs. 16, 23 and 31) approximately 1.27 times as long as width, length 0.240–0.335 (0.283, $n = 3$) mm, width 0.168–0.265 (0.216, $n = 3$) mm; dorsal surface covered with pointed spicules; ventral surface densely covered on anterior area with pointed spicules on anterior; terminal process length 0.10–0.25 (0.16, $n = 3$) mm, width 0.043–0.045 (0.044, $n = 3$) mm, triangular, stout, smooth, extreme tips somewhat dark; *sensilla*: tergite 1 (Figs. 16 and 29) with two anteromesal – D-2-I medium, thin seta, D-3-I long, thin seta; four posterior sensilla – D-4-I, D-7-I campaniform sensilla, D-8-I medium, thin seta, D-9-I long, thin seta; three lateral sensilla – L-1-I, L-3-I short, thin setae, L-2-I long, thin seta; segment 4 (Figs. 16 and 30) – D-2-IV short, thin seta, D-3-IV absent, D-4-I, D-7-IV campaniform sensilla, D-5-IV, D-9-IV medium, stout setae, D-9-IV longer than D-5-IV, D-8-IV, long, stout seta; L-1-IV absent, L-2-IV long, stout seta, L-3-IV, L-4-IV shorter than L-2-IV, all on bifid tubercles with wide base and pointed apex; V-5-IV moderately long, thin seta, V-6-IV long, thin seta, V-7-IV moderately long, thin seta, all on bifid elongate tubercles; segment 9 (Figs. 16, 23 and 31) with D-5-IX campaniform sensillum.

Male pupa (Figs. 24, 26 and 32). Similar to female with usual sexual differences: Total length 3.07–3.87 ($n = 2$) mm. Dorsal apotome with DAL 0.26–0.31 ($n = 2$) mm; DAW 0.17–0.22 ($n = 2$) mm, DAW/DAL 0.65–0.71 ($n = 2$) mm. Respiratory organ, P 0.024–0.045 ($n = 2$) mm; RO length 0.23–0.30 ($n = 2$) mm, RO width 0.024–0.045 ($n = 2$) mm; P/RO 0.08–0.12 ($n = 2$) mm; *sensilla*: three anterolaterals (Fig. 26) – AL-1-T short, thin seta, AL-2-T medium, stout seta, AL-3-T long, thin seta. Cephalothorax: length 0.96–1.15 ($n = 2$) mm, width 0.88 ($n = 2$) mm. Segment 9 (Figs. 24 and 32) – length 0.24–0.33 ($n = 2$) mm, width 0.20–0.26 ($n = 2$) mm;

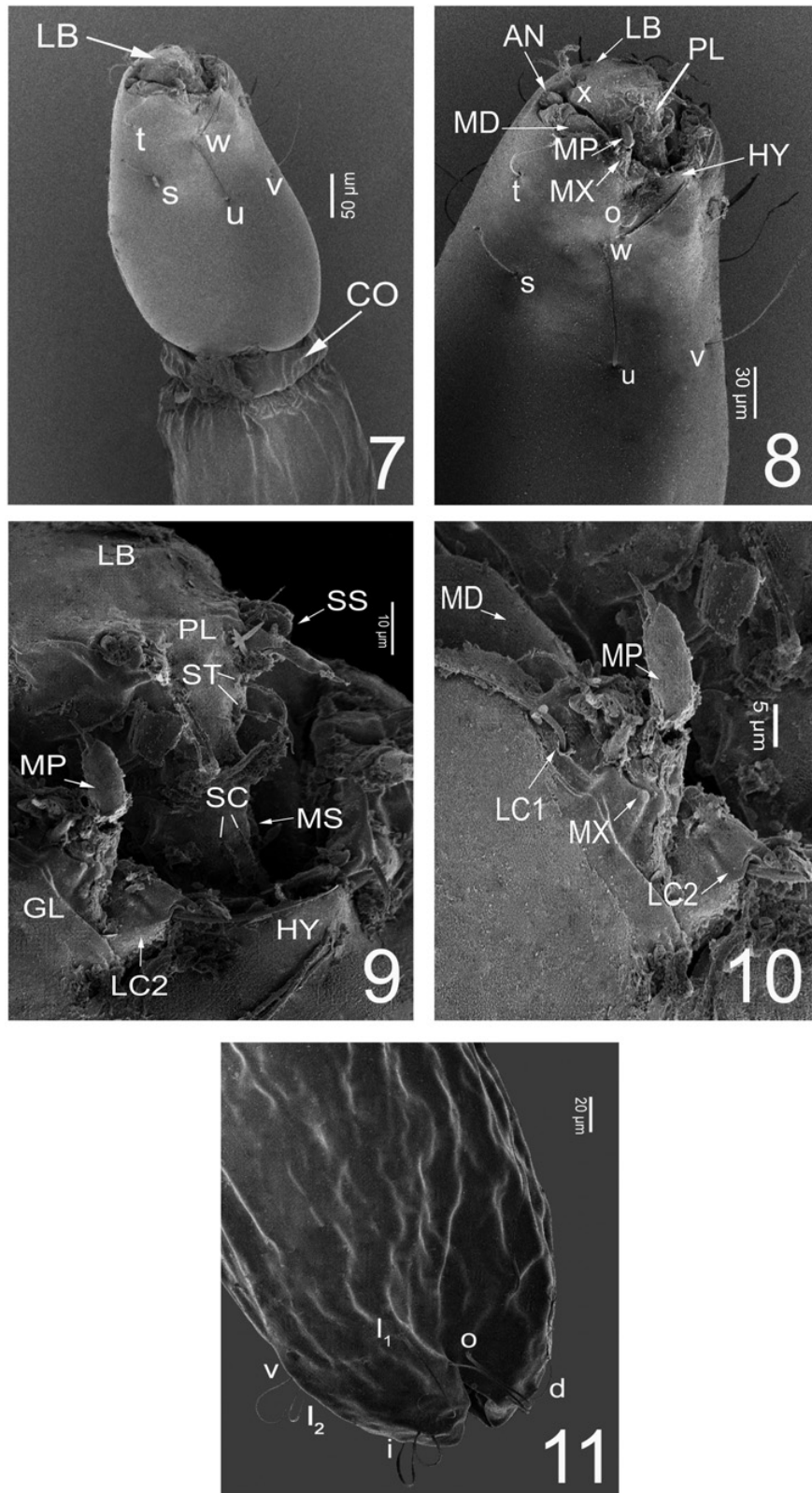


Fig. 7–11. *Stilobezzia (A.) megatheca* Cazorla and Spinelli, fourth instar larva (SEM): 7. head capsule, ventrolateral view; 8. head chaetotaxy, ventrolateral view; 9. head capsule detail, ventrofrontal view; 10. head capsule detail, ventrolateral view; 11. caudal segment, ventrolateral view. Antennae (AN); collar (CO); gaeolacinia (GL); hypostoma (HY); labrum (LB); lacinial sclerite 1 (LC1); lacinial sclerite 2 (LC2); maxilla (MX); maxillary palpus (MP); messors (MS); palatum (PL); sensilla styloconica (ss); sensilla trichoidea (st); scopae (SC). Head capsule chaetotaxy: o, parahypostomal setae; s, anteroperifrontal setae; t, prefrontal setae; u, mesolateral setae; v, posterolateral setae; w, anterolateral setae; x, parantennal setae. Caudal segment chaetotaxy: d, dorsal setae; i, inner setae, l₁, first lateral setae; l₂, second lateral setae; o, outer setae; v, ventral setae.

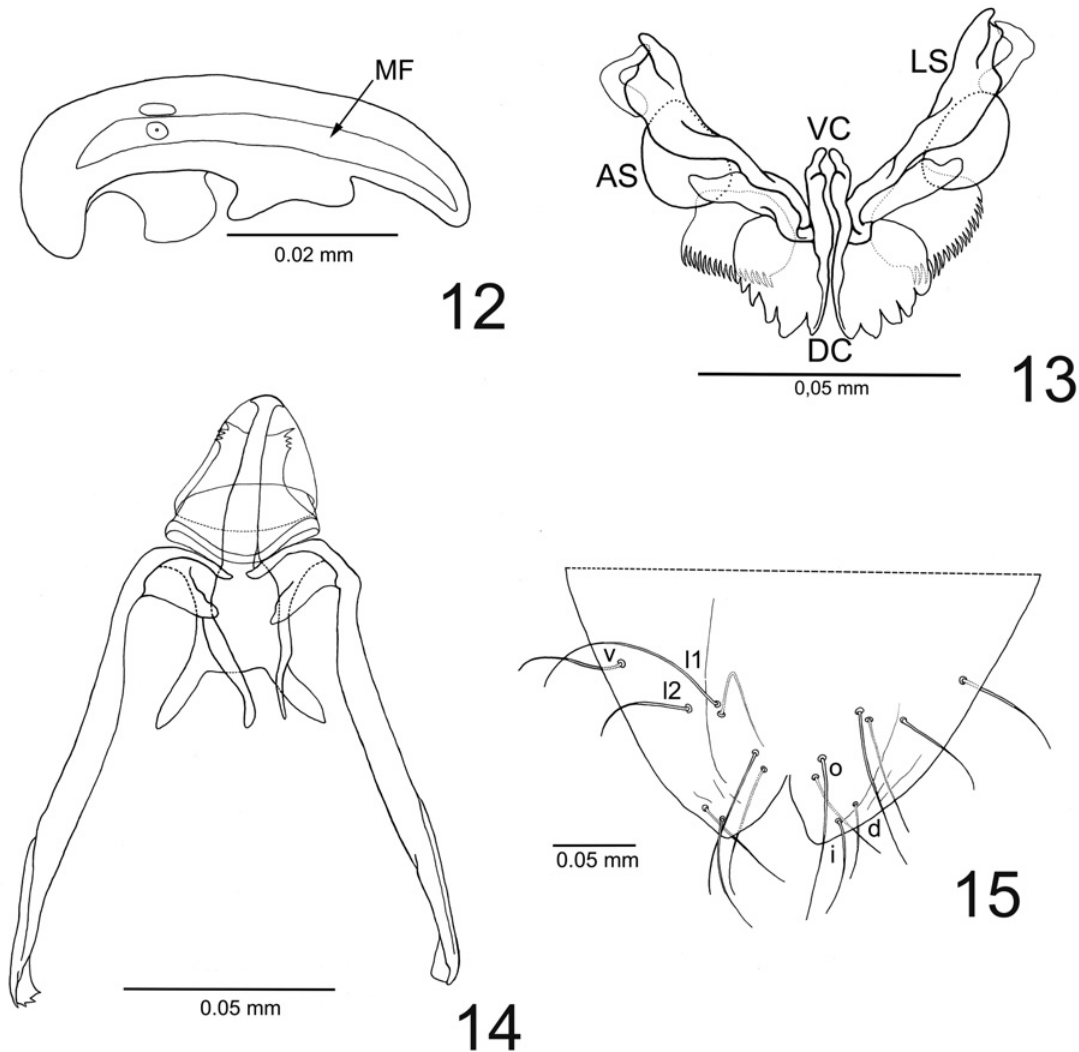


Fig. 12–15. *Stilobezzia (A.) megatheca* Cazorla and Spinelli, fourth instar larva: 12. Left mandible, lateral view; 13. epipharynx, ventral view; 14. hypopharynx, ventral view; 15. caudal segment, ventral view. Auxiliary sclerite (AS); dorsal comb (DC); Fossa mandibularis (MF); Lateral sclerite (LS); ventral comb (VC). Caudal segment chaetotaxy: d, dorsal setae; i, inner setae; o, outer setae; v, ventral setae; l1, first lateral setae; l2, second lateral setae.

terminal process length 0.09–0.18 ($n = 2$) mm, width 0.01–0.09 ($n = 2$) mm.

Material examined. *Holotype* male, Argentina, Neuquén prov., Villa La Angostura, 11–15 January 2004, G. Spinelli, light trap (MLPA). *Paratypes*: 40 males as follows: Argentina, Neuquén prov., lago Aluminé, 22 December 1996 to 3 January 1997, D. Podestá, 1 male, Malaise trap; cerro Chapelco, over lago Lacar, 27 November 1984, J. A. Downes, five males, sweep net (JAD 1653/1/6,18, 20; 1653/2/17,19) (CNCI); lago Lolog, 24 November 1984, J. A. Downes, three males, sweep net (JAD 1651/3/13,14,28) (CNCI); Río Negro prov., El Bolsón, 29 December 1999, G. Spinelli, one male, light trap; río Villegas, 57 km S Bariloche, 3–8 December 1994, L. Quate, one male, Malaise trap; lago Gutiérrez, January 2003, J. Liotta, nine males, light trap; lago Gutiérrez, 20 January 2006, Grogan and Spinelli, one male, sweep net; arroyo Los Notros, 20 km W Bariloche, 26–28 November

1994, L. Quate, four males, Malaise trap; Parque Nacional Nahuel Huapi, arroyo Ñireco (Complejo Challhuaco) 41°11' 51.9" S, 71°19'40.5" W, 962 m, 23 January to 18 February 2007, Garré and Montes de Oca, one male, Malaise trap; Chubut prov., Parque Nacional Los Alerces, margen E lago Futalaufquen, 20 January 1988, G. Spinelli, two males, sweep net; Parque Nacional Los Alerces, 9–12 December 1994, L. Quate, five males, Malaise trap; Parque Nacional Los Alerces, arroyo s/n 2 February 2005, M. Donato, 1 male, sweep net.

Chile, Osorno prov., Pucatrihue, 30 December 1992, G. Spinelli, one male, sweep net (USNM); Llanquihue prov., Yervas Buenas, 13 km N Ensenada, 1–6 December 1994, L. Quate, two males, light trap; 3 km N Ensenada, 1–2 December 1994, L. Quate, one male, Malaise trap; Ensenada, 11 December 1984, J. A. Downes, two males, sweep net (JAD1695/1/4; 1695/5/2) (CNCI). *Other specimens*: Argentina, Chubut prov., unnamed stream, 500 m

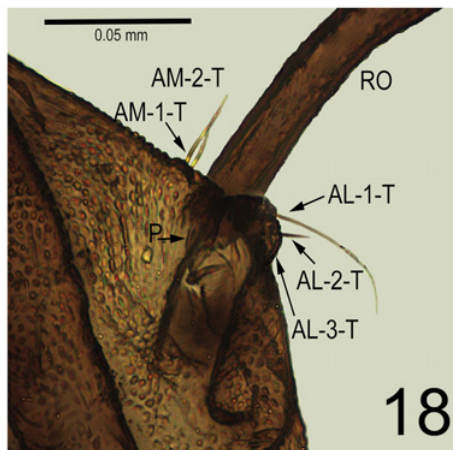
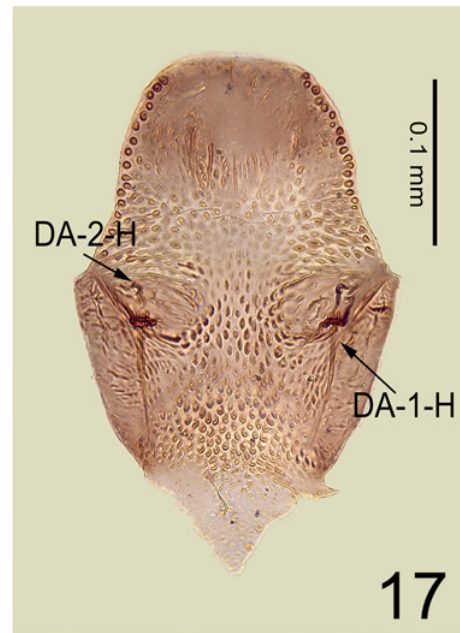
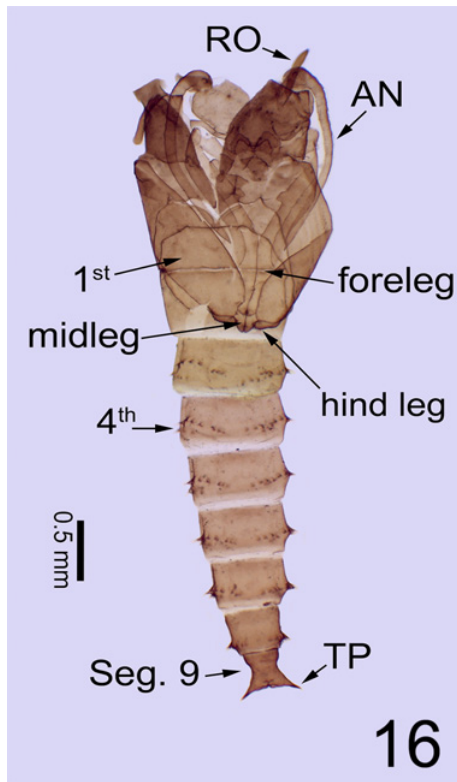


Fig. 16–19. *Stilobezzia (A.) megatheca* Cazorla and Spinelli, female pupa: 16. Habitus, ventral view; 17. dorsal apotome, dorsal view; 18. cephalothorax chaetotaxy, dorsal view; 19. Respiratory organ and anterolateral sensilla, dorsal view. Antenna (AN); anterolateral sensilla (AL-1-T, AL-2-T, AL-3-T); anteromedial sensilla (AM-1-T, AM-2-T); dorsal apotome sensilla (D-1-H, D-2-H); dorsolateral cephalic sclerite sensilla (DL-1-H, DL-2-H); genital lobe (GL); lateral sensilla of segment 1 (L-1-I, L-2-I, L-3-I); pedicel (P); respiratory organ (RO); segment 1 (1st); segment fourth (4th); terminal process (TP).

before lago Epuyen, access road to Puerto Patriada, 42°08'19"S; 71°31'57"W, 412 m, 20 September 2012, pupa in laboratory 7 October 2012, one female (with larval exuvium); same data except, 18 October 2012, emerged in laboratory 21 October 2012, D. Anjos-Santos, one female (with pupal exuvium); same data except, 13 November 2012, emerged in laboratory 16 November 2012, Anjos-Santos, D. leg, one male (with pupal exuvium); same data except, 24 February 2015, emerged in laboratory 25 February 2015, one male (with pupal exuvium); same data except, 29 October 2015, emerged in laboratory

31 October 2015, one female (with pupal exuvium); same data except, pupa in laboratory 3 November 2015, emerged in 10 November 2015, one female (with larval and pupal exuviae).

Material examined with SEM. Argentina, Chubut prov., unnamed stream, 500 m before Epuyen Lake, access road to Puerto Patriada, 42°08'19"S; 71°31'57"W, 412 m, 13 November 2012, D. Anjos-Santos, one pupa male and one pupa female.

Distribution. Argentina (Neuquén, Chubut), Chile (Valdivia, Osorno, Chiloé).

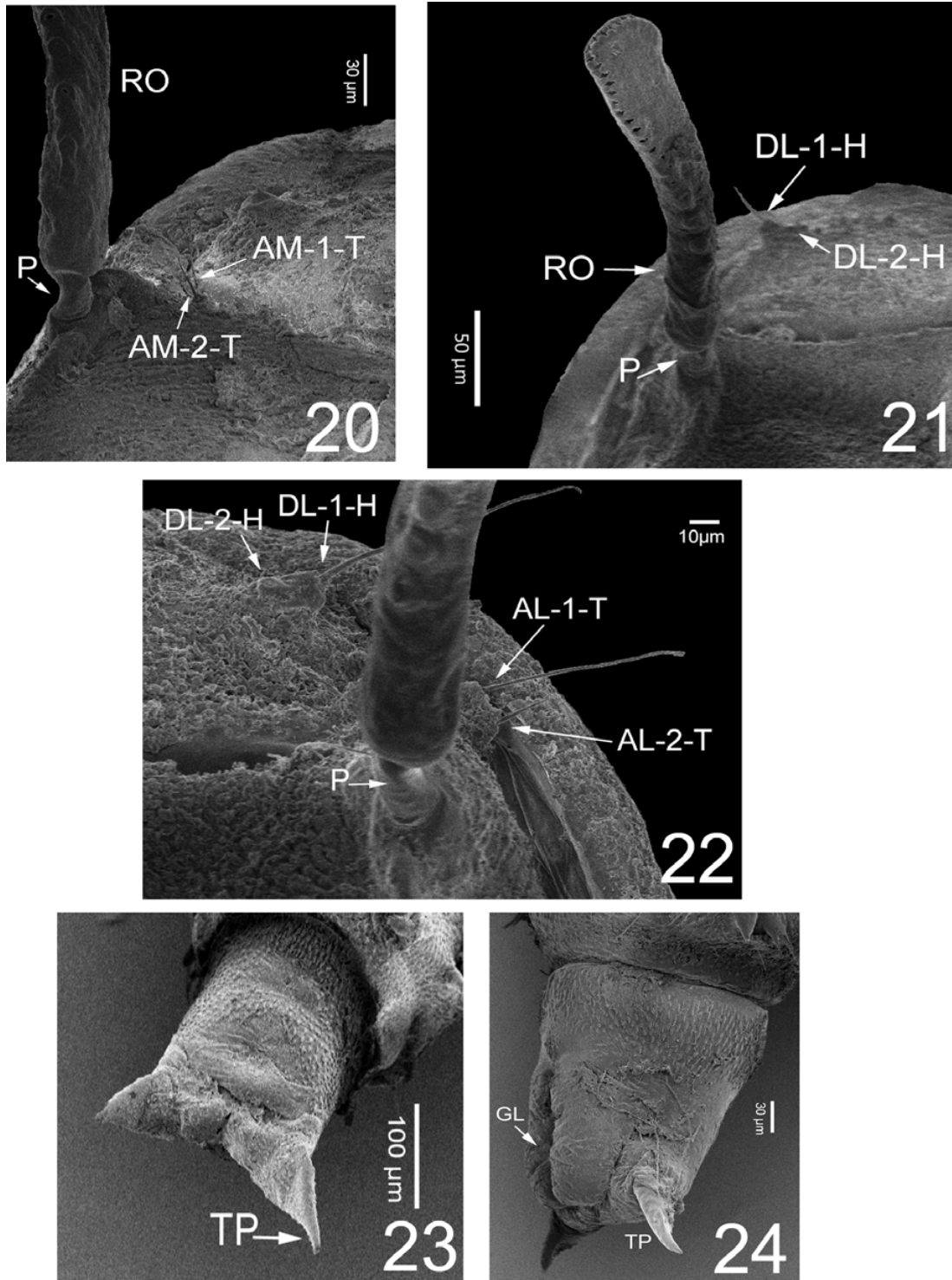


Fig. 20–24. *Stilobezzia (A.) megatheca* Cazorla and Spinelli, female pupa (20–23), male pupa (24), (SEM): 20. anteromedial sensilla and respiratory organ, dorsal view; 21. respiratory organ and dorsolateral cephalic sclerite, dorsal view; 22. dorsolateral cephalic sclerite and anterolaterals sensilla, dorsal view; 23. segment 9, female, ventral view; 24. segment 9, male, anterolateral view. Anterolateral sensilla (AL-1-T, AL-2-T, AL-3-T); anteromedial sensilla (AM-1-T, AM-2-T); dorsolateral cephalic sclerite sensilla (DL-1-H, DL-2-H); pedicel (P); respiratory organ (RO); terminal process (TP).

Bionomics. Immatures of *Stilobezzia megatheca* were collected from a first-order stream, a tributary of Epuyen Lake in northern Chubut Province (Patagonia, Argentina). The site is surrounded by riparian forest

composed mainly of pine tree plantations [*Pinus radiata* (D. Don), with patches of native vegetation *Austrocedrus chilensis* (D. Don) Pic. Ser. et Bizzarri, *Chusquea* Kunth, *Fuchsia* L., *Nothofagus dombeyi* (Mirb.) Oerst.], and is

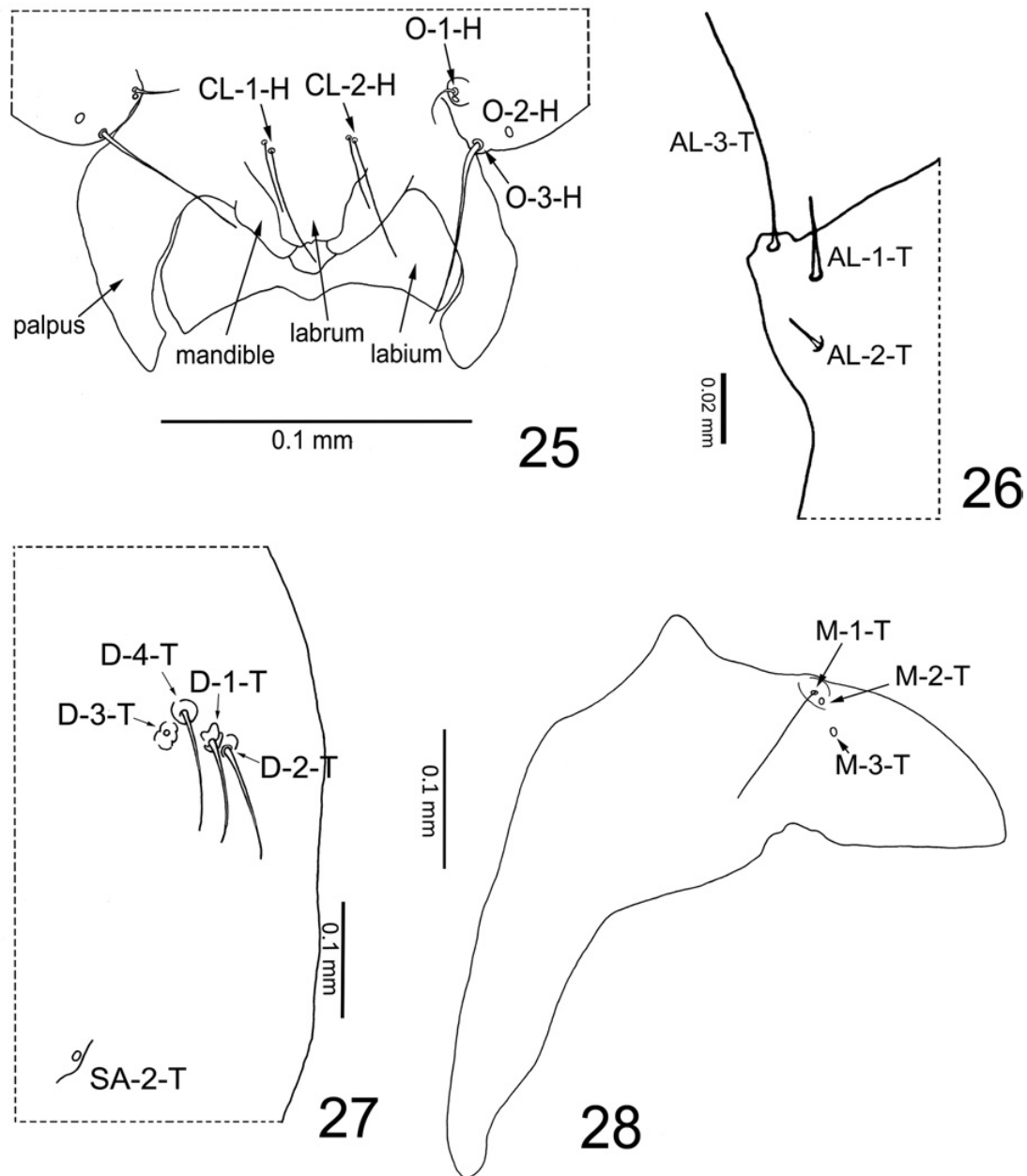


Fig. 25–28. *Stilobezzia (A.) megatecha* Cazorla and Spinelli, female pupa (25, 27, 28), male pupa (26): 25. mouthparts, ventral view; 26. anterolateral sensilla, dorsal view; 27. dorsal sensilla and supraalar sensillum, dorsal view; 28. metathoracic chaetotaxy, dorsal view. Anterolateral sensilla (AL-1-T, AL-2-T, AL-3-T); clypeal/labral sensilla (CL-1-H, CL-2-H); dorsal sensilla (D-1-T, D-2-T, D-3-T, D-4-T); ocular sensilla (O-1-H, O-2-H, O-3-H); metathoracic sensilla (M-1-T, M-2-T, M-3-T); supraalar sensillum (S-2-T).

used as a water source for cattle. The area suffered a major fire in early 2012 that consumed much of the forest. It is now recovering naturally and some burned pine have been removed. Larvae were collected in a permanent small puddle on the bank of the stream, with abundant vegetation (grasses and herbaceous), filamentous algae and decomposing organic matter in 18 October 2012 (10, 15 °C) and November (12, 30 °C). At the same site coexist larvae of *Didicrum* Enderlein, 1937 and *Psychoda* Latreille, 1796 (Psychodidae), *Parochlus* Enderlein, 1912 (Chironomidae) and Scirtidae (Coleoptera). Pupae were collected in bordering vegetation (mainly bryophytes) on

24 February 2014 (28, 12 °C), 20 September 2012 (7, 6 °C), 18 October 2012 (15, 10 °C), 29 October 2015 (16, 9 °C) and 13 November 2012 (30, 12 °C). Under laboratory conditions, the larvae took 5–17 days to reach the pupal stage, and 7 days to complete its development to the adult stage. Pupae found in the site completed their development in 1–3 days.

Larvae of *Stilobezzia (A.) megatecha* showed the typical movement reported by Mullen and Hribar (1988) for the genus *Stilobezzia*, crawling slowly over algae. Pupae observed on trays showed a semi-circular, slow abdominal movement typical of other ceratopogonid pupae.

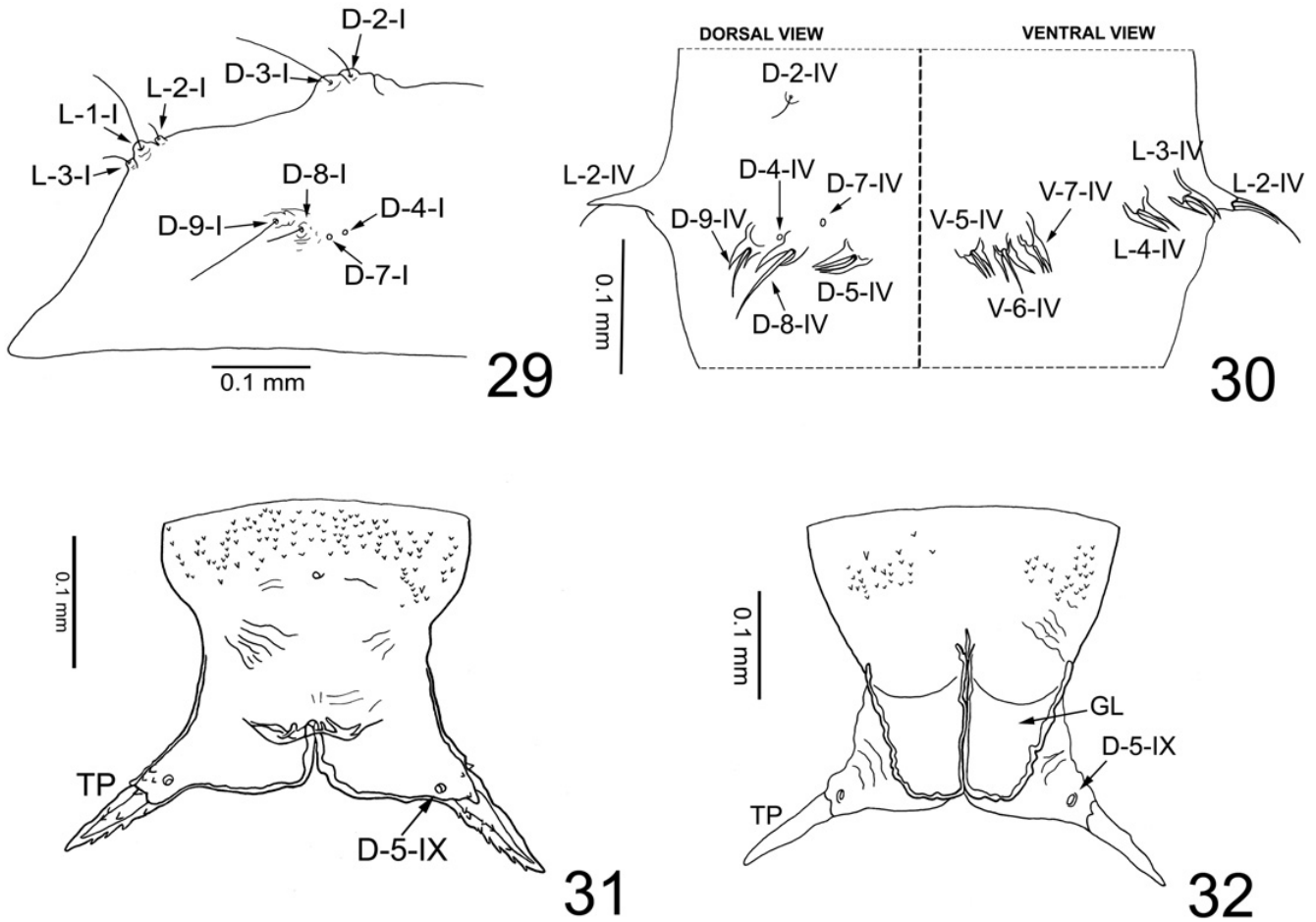


Fig. 29–32. *Stilobezzia* (*A.*) *megatheca* Cazorla and Spinelli, female pupa (25–31), male pupa (32): 29. tergite 1 chaetotaxy, dorsal view; 30. Segment 4 chaetotaxy, dorsal and ventral view; 31, 32. segment 9, ventral view. Dorsal sensilla of segment 1 (D-2-I, D-3-I, D-4-I, D-7-I, D-8-I, D-9-I); dorsal sensilla of segment 4 (D-2-IV, D-4-I, D-5-IV, D-7-IV, D-8-IV, D-9-IV); lateral sensilla of segment 4 (L-2-IV, L-3-IV, L-4-IV); pore (p); sensilla of segment 9 (D-5-IX); supraalar sensillum (S-2-T); terminal process (TP); ventral sensilla of segment 4 (V-5-IV, V-6-IV, V-7-IV).

Key to Neotropical pupae of the genus *Stilobezzia* Kieffer [*S. (S.) glauca* Macfie, 1939, *S. (S.) wygodzinskyi* Lane and *S. (S.) dubitans* Lane *et al.*, 1955 not included]

1. Abdominal segment 9 with terminal processes long, directed posteriorly (Fig. 33) 2
 - Abdominal segment 9 with terminal processes short, directed posteriorly or laterally (Figs. 34 and 35)..... 4
2. Respiratory organ strongly curved on basal third, symmetrical apex and tapering towards the tip, with 20–23 pores (including lateral pores) (Fig. 36) 3
 - Respiratory organ straight on basal third, asymmetrical apex (outer side curved and inner side straight), with 12–16 pores opening at tubercles on curved lateral margin.....
..... *S. (S.) enigma* Ronderos *et al.*, 2012
3. Abdominal segment 4 with D-5-IV, D-8-IV, D-9-IV sensilla bifurcating with plumose apex (Fig. 37);

respiratory organ with basal row of five dorsal pores and medially-facing row of 15 pores on distal half (Fig. 36) *S. (S.) rabelloi* Lane, 1947

- Abdominal segment 4 with D-5-IV, D-8-IV, D-9-IV sensilla bifurcating without plumose apex; respiratory organ with basal row of five dorsal pores and 18 apical pores on distal portion..... *S. (S.) punctulata* Lane, 1947
4. Dorsal apotome with strong and elongate anteromarginal tubercle (Fig. 38)
..... *S. (S.) coquilletti* Kieffer, 1917
 - Dorsal apotome without strong and elongate anteromarginal tubercle..... 5
 5. Respiratory organ with pores separated on basal, mesal and apical portion 6
 - Respiratory organ with pores distributed continuously from mesal to apical portion (Fig. 40)..... 10

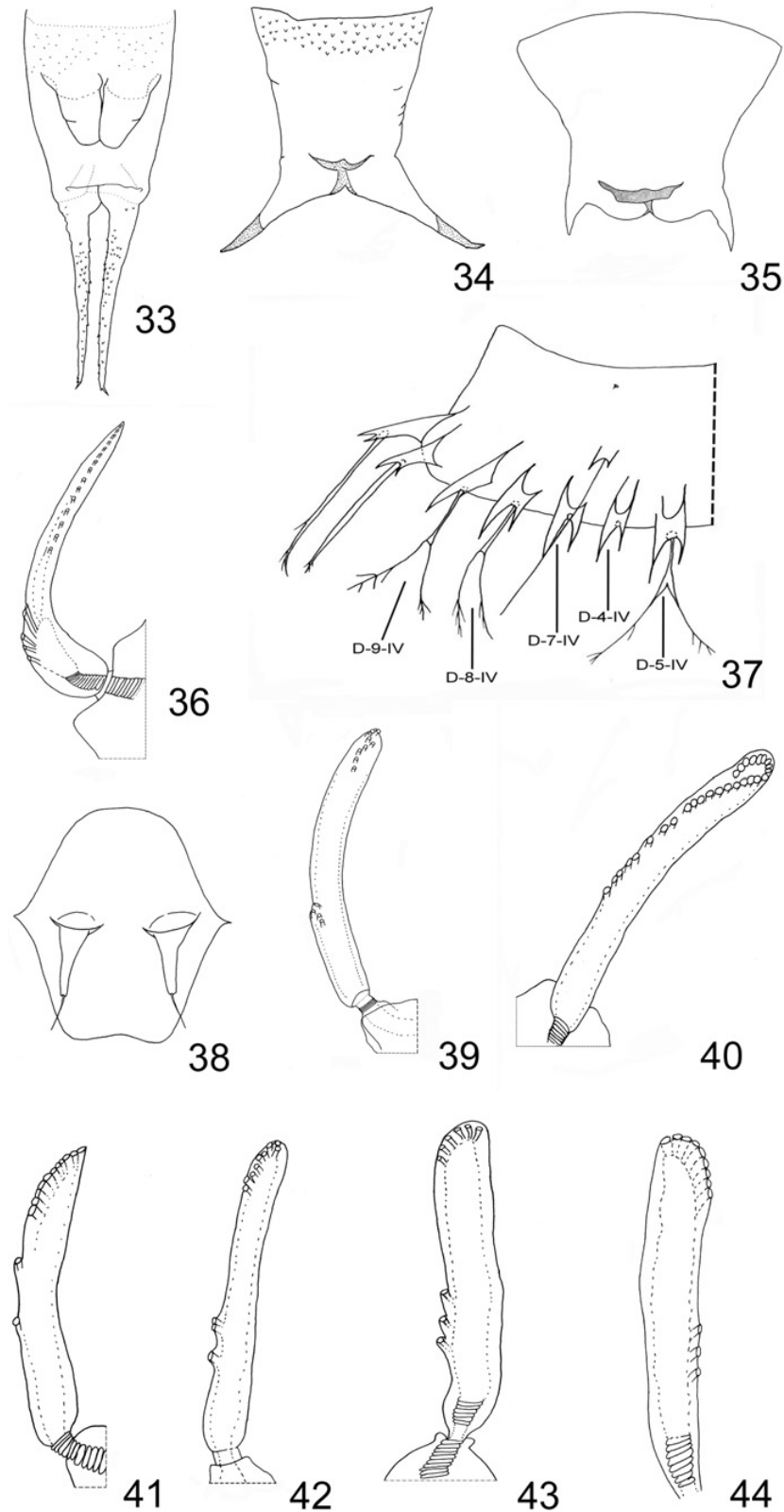


Fig. 33–44. Neotropical pupae of the genus *Stilobezzia* Kieffer. 33. *S. (Stilobezzia) enigma*, segment 9, ventral view (from [Ronderos *et al.*, 2012](#)); 34. *S. (S.) merceri*, segment 9, ventral view (from [Cazorla *et al.*, 2005](#)); 35. *S. (S.) pseudopunctulata*, segment 9, ventral view (from [Cazorla *et al.*, 2012](#)); 36. *S. (S.) rabelloi*, respiratory organ, dorsal view ([Lane, 1947](#)); 37. *S. (S.) rabelloi*, segment 4, dorsal view; 38. *S. (S.) coquilletti*, dorsal apotome, dorsal view (from [Kieffer, 1917](#)); 39. *S. (S.) fiebrigi*, respiratory organ, dorsal view (from [Ronderos *et al.* 2008b](#)); 40. *S. (Acanthohelea) megatheca*, respiratory organ, dorsal view; 41. *S. (S.) panamensis*, respiratory organ, dorsal view (from [Lane and Forattini, 1958](#)); 42. *S. (S.) merceri*, respiratory organ, dorsal view (from [Cazorla *et al.*, 2005](#)); 43. *S. (S.) chaconi*, respiratory organ, dorsal view (from [Lane and Forattini, 1961](#)); 44. *S. (S.) antennalis*, respiratory organ, dorsal view (from [Wirth and Grogan, 1981](#)).

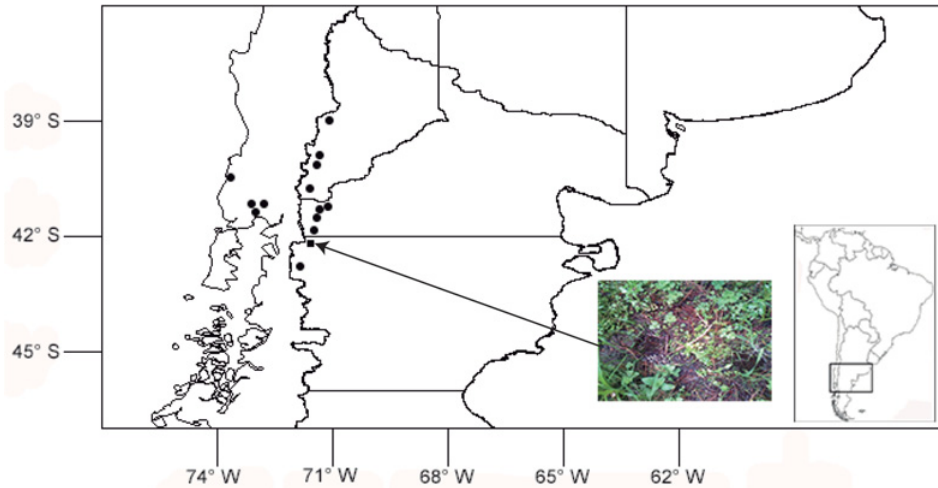


Fig. 45. Distribution map of *Stilobezzia megatheca*. Cazorla and Spinelli. (■) collection site of the immature stages.

6. Respiratory organ with two or three posterolateral pores situated below or above mesal line..... 7
 - Respiratory organ with three to five posterolateral pores situated below mesal line (Fig. 39).....*S. (S.) fiebrigi* Kieffer, 1917
7. Respiratory organ with two posterolateral pores situated below and/or above mesal line (Figs. 41 and 42)..... 8
 - Respiratory organ with 3 posterolateral pores situated below mesal line (Fig. 43)..... 9
8. Respiratory organ with one posterolateral pore situated below mesal line, the other above it (Fig. 41); abdominal segment 9 with terminal processes directed posteriorly *S. (S.) panamensis* Lane and Forattini, 1958
 - Respiratory organ with posterolateral pores situated below mesal line (Fig. 42); abdominal segment 9 with terminal processes directed laterally (Fig. 34) *S. (S.) merceri* Cazorla and Spinelli in Cazorla *et al.*, 2005
9. Respiratory organ with six to seven apical pores (Fig. 43) *S. (S.) chaconi* Macfie, 1938
 - Respiratory organ with eight to ten apical pores (Fig. 44) *S. (S.) antennalis* Coquillett, 1901
10. Dorsal apotome with D-1-H short, thick seta, located on very small tubercle; respiratory organ with 11–12 pores opening on apical margin; abdominal segment 9 with posterior processes very short, slender, directed posteriorly (Fig. 35).....*S. (S.) pseudopunctulata* Cazorla and Ronderos, in Cazorla *et al.*, 2012
 - Dorsal apotome with D-1-H elongate, thin seta, located on well-developed tubercle; respiratory organ with 28 pores distributed from mesal portion to apex (Fig. 40); abdominal segment 9

with posterior processes triangular, stout, directed laterally (Figs. 31 and 32) *S. (A.) megatheca* Cazorla and Spinelli, 2015

Taxonomic discussion

The adult female of *S. (A.) megatheca* resembles that of *S. (A.) estepae* Cazorla and Spinelli, 2015 by its general coloration, but the latter species presents eight large and eight thinner scutellar setae and the wing has the r-m crossvein interrupted at mid portion.

This is the first description of the immature stages of a Neotropical species of the subgenus *S. (Acanthohelea)*. Knowledge of the larvae and pupae of this subgenus is still very incomplete, and there are not enough ultrastructural features described to allow us to compare species adequately.

The pupae of *S. (S.) antennalis*, *S. (S.) chaconi* and *S. (S.) panamensis* are briefly described and illustrated. However, the main differences between them and *S. (A.) megatheca* are provided in the above key to Neotropical pupae. On the other hand, the pupal descriptions of *S. (S.) glauca*, *S. (S.) wygodzinskyi* and *S. (S.) dubitans* are more brief and incomplete, and it is almost impossible to determine the most relevant structures even at the generic level. Therefore, they have not been included in our key.

We have compared the larva and pupa of *S. (A.) megatheca* with the immatures of Neotropical species of the subgenus *Stilobezzia*: *S. (S.) coquilletti*, *S. (S.) enigma*, *S. (S.) fiebrigi*, *S. (S.) merceri*, *S. (S.) pseudopunctulata*, *S. (S.) punctulata* and *S. (S.) rabelloi*.

The larva of *S. (A.) megatheca* shares with the compared species some features typical of predatory larvae, such as curved and sclerotized mandible with fossa

mandibularis, epipharynx less massive and a cylindrical maxillary palpus (Hribar, 1993; Ronderos *et al.*, 2008b, 2012; Cazorla *et al.*, 2013). This species shares with *S. (S.) coquilletti* and *S. (S.) fiebrigi* the presence of medium-sized setae on the caudal segment, a character state typical of larvae breeding in shallow water and muddy bottoms (Cazorla *et al.*, 2013). However, *S. (S.) coquilletti* can be distinguished by the absence of scopae, mesal elevation of hypostoma flanked by serrate margins, galeolacinia with a bundle of four setae and the epipharynx dorsal comb sclerite with posterior margin bearing nine to ten lanceolate teeth. *Stilobezzia (S.) fiebrigi* differs by having the labrum wider than long, palatum with three pairs of sensilla trichoidea, hypostoma with smooth, triangular, mesal elevation flanked by serrate margins, epipharynx with irregular anterior margin with ten stout, pointed teeth and the hypopharynx connected posteriorly.

The larva of *S. (S.) enigma* differs from that of *S. (A.) megateca* by possessing the following features: palatum with a pair of small parallel furrows underneath sensilla styloconica; scopae with six lanceolate teeth; hypostoma with mesal groove flanked by serrate margins; epipharynx with dorsal comb bearing six to eight lanceolate teeth; hypopharynx with small, broad labium.

Finally, the larva of *S. (S.) punctulata* differs from that of *S. (A.) megateca* by having the following features: head elongated and cylindrical; antennae stout and well developed; scopae well developed and long; epipharynx with seven pointed teeth in posterior margin of ventral comb; dorsal comb with nine stout teeth; caudal segment with very long setae.

The pupa of *S. (A.) megateca* shares with *S. (S.) coquilletti* and *S. (S.) fiebrigi* some features, especially with regards to their general shape. All of them have respiratory organs with a rounded apex, most sensilla of segment 4 on pointed tubercle, and segment nine longer than wide, terminal process triangular and with a smooth, wide base directed laterally with extreme tips somewhat dark. *Stilobezzia (S.) coquilletti* is distinguished by the dorsal apotome, with a strong and elongate anteromarginal tubercle, only one anterolateral sensillum (AL-1-T), CL-1-T and CL-2-T campaniform sensilla, O-3-H absent, respiratory organ with 16–19 pores and *S. (S.) fiebrigi* by CL-1-T and CL-2-T campaniform sensilla, O-1-H stout, O-3-H absent, D-2-IV minute seta, L-1-V short, thin seta, respiratory organ with 11–15 pores.

In the case of the pupae of *S. (S.) enigma*, *S. (S.) punctulata* and *S. (S.) rabelloi*, they can be easily distinguished from *S. (A.) megateca* by the very long distal processes of segment 9. *Stilobezzia (S.) enigma* also presents D-7-I campaniform sensillum on the anterior margin of the segment near D-2-I and D-3-I, and most sensilla of abdominal segment 4 on tubercles. Among the differences, the following stand out: respiratory organ short with 12–16 pores, L-4-I long, thin seta, D-2-I and D-3-I absent, L-1-V long, thin seta.

Stilobezzia (S.) merceri differs from *S. (A.) megateca* by the dorsal apotome wider than long, with D-1-H

spur-like seta, AM-2-T campaniform sensillum, CL-1-T and CL-2-T campaniform sensilla, respiratory organ with 7–9 apical and two postolateral pores, all sensilla of fourth abdominal segment on prominent tubercle, V-2-IV and L-3-IV present.

Stilobezzia (S.) pseudopunctulata differs from *S. (A.) megateca* by the dorsal apotome wider than long, AM-1-T minute seta, AM-2-T long, thin seta with associate pore, CL-1-T and CL-2-T campaniform sensilla, the respiratory organ with 11–12 apical pores, D-2-T minute seta, D-5-I absent, L-1-I minute seta, L-2-I short seta, L-3-I long, thin seta, D-3-IV, D-4-IV and D-5-IV minute setae, D-7-IV long, thin seta, V-5-IV and V-6-IV campaniform sensilla, tubercles of segment 4 rounded and distal processes of segment 9 slightly wider than long with short tips posteriorly directed. Both species share a medium-sized respiratory organ and tips of segment 9 triangular, short and pointed, typical of ceratopogonids that live in shallow and clean waters (Cazorla *et al.*, 2012).

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