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Redescription of immatures of *Dasyhelea flavifrons* Guérin-Méneville (Culicomorpha: Ceratopogonidae) and new contribution to the knowledge of its larval habitats

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Abstract: The fourth instar larva and pupa of *Dasyhelea flavifrons* Guérin-Méneville are redescribed, illustrated, and photomicrographed using binocular, phase-contrast, and scanning electron microscopy. Comparisons with the American species of the grisea group were made. The immatures were collected by using a siphon bottle in tree-holes and from water collected in dead snail shells in Salta Province, Argentina, transported to the laboratory and there bred to the emergence of the adults. Details on larval habitats are given. These are the first records from Argentina and in gastrotelmata.

Key words: Ceratopogonidae, *Dasyhelea flavifrons*, gastrotelmata, immatures, tree holes.

INTRODUCTION

The biting midges *Dasyhelea* Kieffer are common and mainly diurnal dipterans occurring in all zoogeographical regions, except Antarctica. The larvae of *Dasyhelea* develop mostly in shallow aquatic and semiaquatic habitats, and in general feed on detritus and algae, though a few species feed in dead insects (Borkent and Spinelli 2007). Adult females feed on nectar from flowers and pollen. Moreover, some are important pollinators of commercial trees such as cacao (*Theobroma cacao*) and rubber (*Hevea brasiliensis*) (Borkent and Spinelli 2007, Córdoba et al. 2013).

Taxonomically, the recognition of subgenera and/or groups of species have been proposed for all regions of the world. Borkent (2016) listed 77 species for the Neotropics, of which only 12 are known also from their immatures. Subsequently, Grogan et al. (2016) described 13 new species from Guadeloupe, and Díaz et al. (2017) described

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two species from Peru and Brazil, all based on adults. Recently, Díaz et al. (2018) described two species, including their immature, from the Yungas ecoregion, Argentina.

The phytotelmata are aquatic microenvironments formed by the accumulation of water in any part of the body of plants (e.g. leaves, flowers, stems, trunks, tree holes and artificial containers). The gastrotelmata are aquatic microhabitats formed by the collection of water (usually from rainfall) in the shells of dead snails (Janetzky et al. 1995). In spite of their small size, snail shells retain water longer than any other natural container, providing a relatively permanent habitat (Lounibos 1980). The communities of organisms that live in these microenvironments are simple and based on detritus; the macroinvertebrates most frequently found are insects, immature stages of Diptera being dominant (Campos et al. 2011). The knowledge of Ceratopogonidae from Argentina that breed in these microenvironments is poor; only 7 of the 31 genera registered (Borkent and Spinelli 2007) have been associated with phytothelmata: *Atrichopogon* Kieffer, *Forcipomyia* Meigen, *Dasyhelea* Kieffer, *Culiciodes* Latreille, *Stilobezzia* Kieffer, *Bezzia* Kieffer, and *Palpomyia* Meigen (Campos et al. 2011).

During a sampling program focused on the collection and study of Diptera that was carried out in Salta province between 2011 and 2012, larvae and pupae were collected from tree holes and dead snail shells, and reared to adults. Based on the characters of adult males, they were identified as *Dasyhelea flavifrons*. This is considered a wide spread Holarctic arboreal species, whose larvae have been reported in Europe as terrestrial and inhabiting sap flows, mushrooms and tree holes (Dominiak and Szadziewski 2010). However, the original description and the different redescriptions of immatures are incomplete, except for the contribution of Keilin (1921), who described all stages of this species under the name *D. obscura* Winnertz, but also incompletely. The aim of this work is to provide the full redescription of these immature in accordance with modern standards and to present the first records from Argentina and in gastrotelmata as larval habitat.

MATERIALS AND METHODS

STUDY AREA

San Ramón de la Nueva Orán (hereafter Orán), is a city established in northwestern Argentina near the border with Bolivia (23º08' S, 64º20' W, elevation 337 m). The region has a subtropical climate, with an average summer temperature of 27.7 °C and winter temperature of 16.4 °C, and a mean annual rainfall of 1,000 mm, occurring mostly from October to April, the warmer months. The study area has been described in more detail in Mangudo et al. (2015). Briefly, Orán is located in the pedemontane floor of the Yungas subtropical montane moist forest (Brown et al. 2001), which remains mostly to the East and North of the city. Besides urbanization, other human related ecological modifications include industrial development, agriculture and forestry (Brown et al. 2001). The city is characterized by a densely built central area surrounded by suburban areas with bigger gardens and more trees; throughout the city most buildings are low.

ENTOMOLOGICAL SAMPLING

Larvae, larval exuviae and pupae were collected between February and March in 2011 and 2012, as a part of a larger study on mosquito (Diptera: Culicidae) larval habitats. Samples were collected from tree holes using a siphon bottle (Müller and Marcondes 2006, Mangudo et al. 2010) and from snail shells by overturning and examining the water content from each shell in a white plastic tray, from which larvae and pupae were collected with a pipette. Tree holes were located in the city and in yunga forest patches to the north and east of

town, while snail shells were collected from forest patches only. For details on tree holes selection see Mangudo et al. (2015, 2018) and for snail shell sampling see Mangudo et al. (2017).

Larvae were preserved in ethanol 80% and pupae were kept alive in the laboratory isolated in plastic vials (2 mm) holding water from the larval habitat and containing a piece of humid filter paper, to maintain the humidity inside the vials, until adult emergence. Emerged adults were maintained alive for 24 hours to ensure the development of their final pigmentation. Adults and their respective exuviae were stored in vials containing 80% ethanol. Larval, pupal exuviae and adults were mounted in Canada balsam following the technique described by Borkent and Spinelli (2007). The technique of Ronderos et al. (2000, 2008) was followed to prepare larvae for scanning electron microscopy (SEM). A camera lucida was used to make illustrations with pen and ink. Photomicrographs were taken with a Micrometrics SE Premium digital camera, through a Nikon Eclipse E200 microscope. For larval terms see Díaz et al. (2018) and for pupae see Borkent (2014).

The plates were made in TIFF format in Adobe Photoshop version14. The material studied is deposited in the División Entomología, Museo de La Plata (MLPA), La Plata, Argentina.

RESULTS

Dasyhelea flavifrons (Guérin-Méneville) (FIGS. 1-5)

Ceratopogon flavifrons Guérin-Méneville 1833: 165 (description, pupa, male, female, larval habitats – sap of elm, France).

Dasyhelea flavifrons: Kieffer 1919: 51 (combination); Szadziewski and Dominiak 2006: 142 (in review of European synonyms in Ceratopogonidae: syn.: *obscurus*, *versicolor*, *dufouri*, *hippocastani*, *brevitibialis*, *goetghebueri*, *lignicola*, *sensualis*, *paludicola*, *oppressa*, *septuosa*; Poland, USA, larval habitats – tree holes and sap of various tree species); Borkent and Grogan 2009: 11 (in Nearctic catalog; distribution), Borkent 2016: 65 (in online World catalog); Grogan et al. 2016: 208 (in review of Guadeloupe records, key).

Dasyhelea oppressa Thomsen 1935: 285 (New York); Waugh and Wirth 1976: 230 (in revision of eastern United States *Dasyhelea*; distribution, larval habitats – tree holes, sap of oak, elm); Graves and Graves 1985: 88 (USA: North Carolina, larval habitats – shelf fungi); Wilkening et al. 1985: 519 (Florida records); Hribar and Grogan 2005: 231 (Monroe County, Florida records); Szadziewski and Dominiak 2006: 142 (as synonym of *C. flavifrons* Guérin-Méneville); Borkent and Grogan 2009: 11 (in Nearctic catalog; distribution; as synonym of *D. flavifrons*).

REDESCRIPTION OF THE FOURTH INSTAR LARVA (FIGS. 1-3, 5) (N=3)

Total length 4.5 mm. Color in life whitish with the head capsule brown, short, wide, tapering to apex (Fig. 1c, d); chaetotaxy as in Fig. 1b, c. HL 0.29-0.30 (0.295, n=3) mm; HW 0.215-0.220 (0.216, n=3) mm; HR 0.255-0.257 (0.256, n=3); SGW 0.17-0.19 (0.18, n=3) mm; SGR 1.21 (1.16-1.26, n=3). Antenna cylindrical (Figs. 1b-d, 2a). Labrum (Fig. 1c, d) 0.80 times longer than wide; palatum (Figs. 1b, 2a, 3a) with four pairs of sensillae campaniformia (Figs. 1d, 3b, c), posterior of them three pairs of sensillae coeloconica (Figs. 1b, d, 2a, b, 3b, c): mesal one serrate, others simple; messors well developed, stout, bisegment (Figs. 2a, b, 3a-c); scopae well developed, brush-shaped (Figs. 2a, b, 3b, c). Maxilla (Figs. 1b, 3c); galeolacinia (Fig. 2a, b) with short, stout seta, 5-6 papillae and two flap-like lobe with a row of denticles on inner surface; maxillary palpus (Fig. 2a, b) short, button-like, with three-four small papillae and row of denticles near papillae; lacinial sclerite 1 stout without seta, lacinial sclerite 2 stout with medium-sized, stout seta (Fig. 2a, b). Mandible (Figs. 3a, b, 5a) strongly sclerotized, scooped, with four

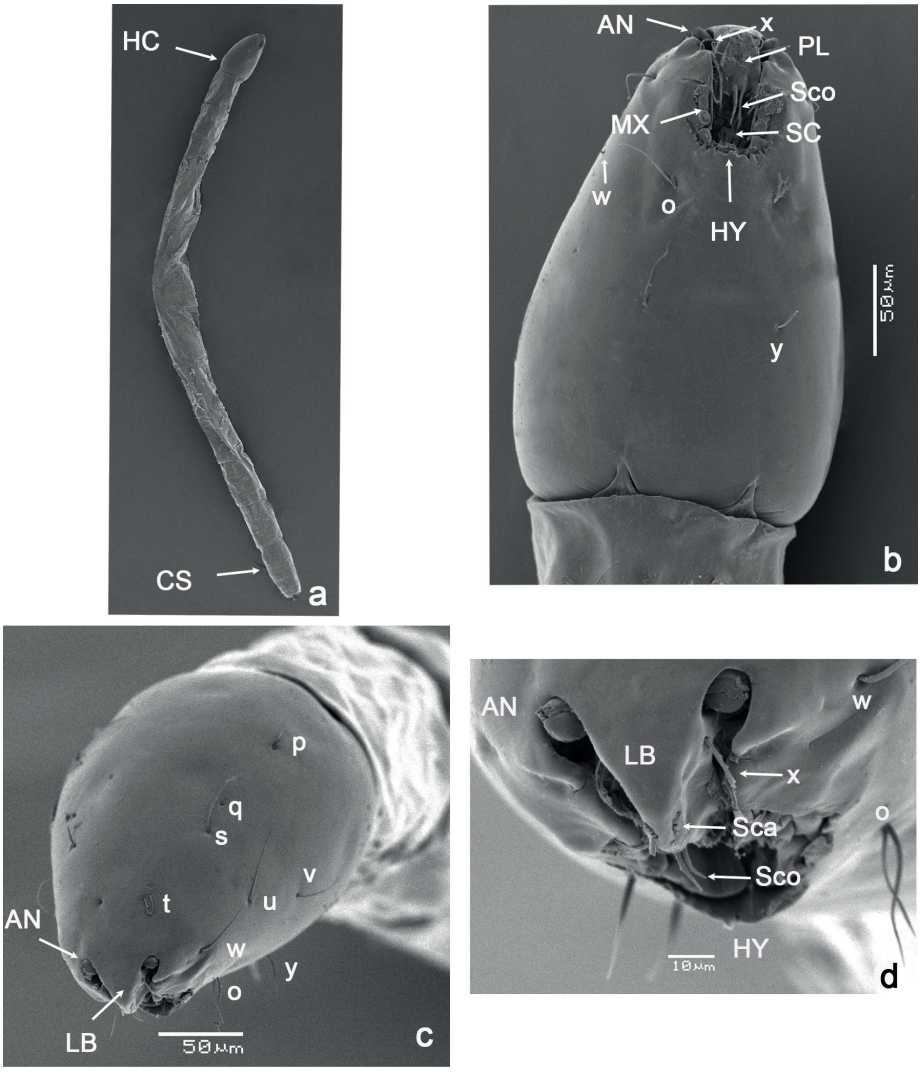

Figure 1 - *Dasyhelea flavifrons* Guérin-Méneville, larva (SEM). **a**) entire larva (lateroventral view); **b**) head capsule (chaetotaxy, ventral view); **c**) head capsule (details, laterodorsal view); **d**) details of labrum (frontal view). Antenna (AN); head capsule (HC); galeolacinia (GL); hypostoma (HY); labrum (LB); maxilla (MX); palatum (PL); scopae (SC); sensilla campaniformia (Sca); sensilla coeloconica (Sco). Head capsule chaetotaxy: o, parahypostomal setae; "q", postfrontal setae; s, anterior perifrontal setae; t, prefrontal setae; v, posterolateral setae; w, anterolateral setae; x, parantennal setae; y, ventral setae.

teeth, apical teeth elongate, medial tooth medium-sized, proximal teeth short; MDL 0.06-0.08 (0.07, n=3) mm. Hypostoma (Figs. 1b, d, 2a, b) strongly crenulate, with 3-4 stout teeth on medial portion, flanked by 5-6 angulate, strong teeth on each side. Epipharynx (Fig. 3a, d) massive, strongly sclerotized, lateral arms stout, short, with auxiliary sclerites; LAW 0.16-0.20 (0.18, n=3) mm, DCW 0.045-0.055 (0.050, n=3) mm. Hypopharynx (Fig. 3a, d) stout, sclerotized, posterior comb with fringe, labium (Fig. 3c) short, not extending beyond hypostoma. Thoracic pigmentation diffuse pale. Abdominal segment whitish, with diffuse pale brown pigmentation. Caudal segment (Fig. 2c-

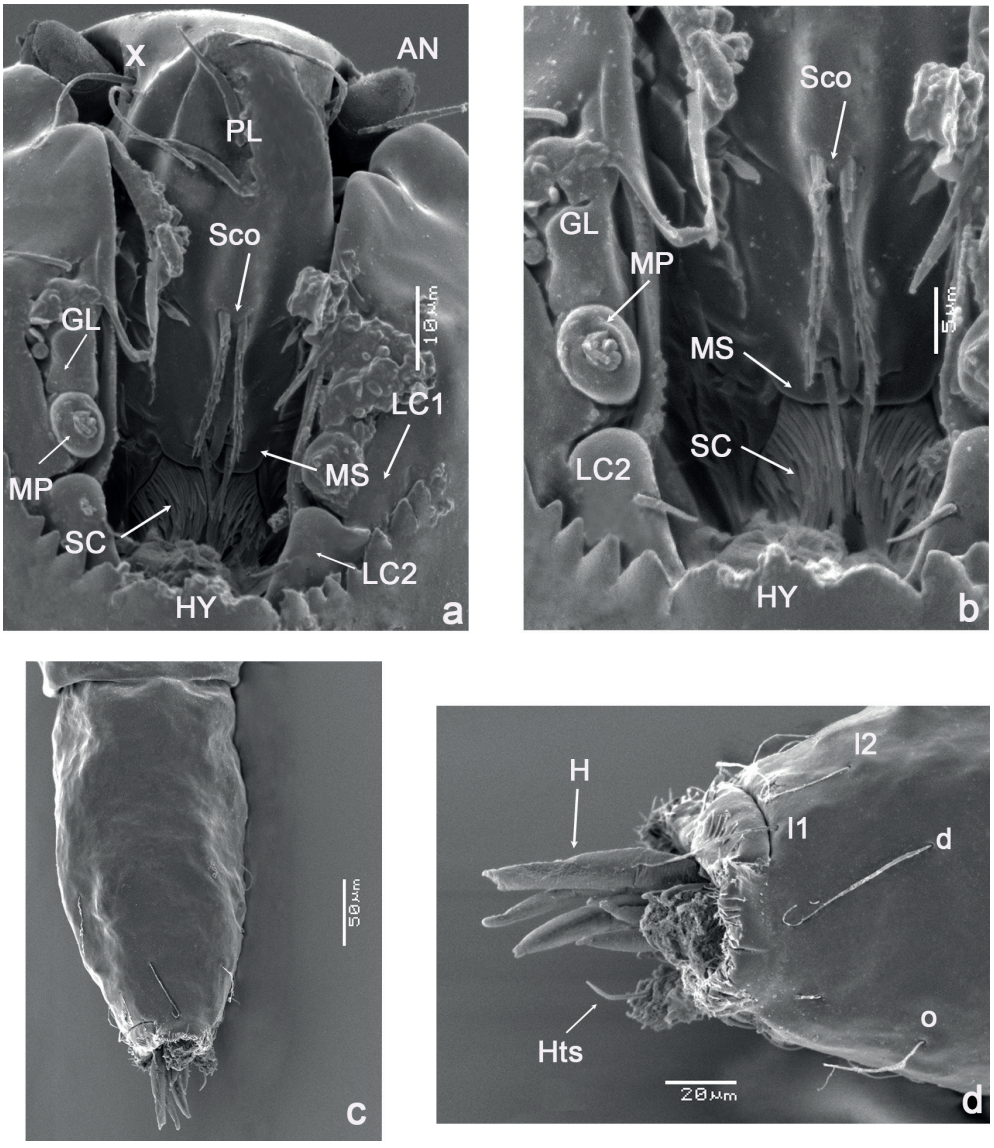

Figure 2 - *Dasyhelea flavifrons* Guérin-Méneville, larva (SEM). **a**) detail of palatum (frontal view); **b**) detail maxilla, messors and scopae (frontal view); **c**) caudal segment (lateral view); **d**) detail of caudal segment (lateral view). Antenna (AN); galeolacinia (GL); hooks (H); hooklets (Hts); hypostoma (HY); lacinial sclerite 1 (LC1); lacinial sclerite 2 (LC2); maxilla (MX); maxillary palpus (MP); messors (MS); palatum (PL); sensilla campaniformia (Sca); sensilla coeloconica (Sco), scopae (SC). Caudal segment chaetotaxy: d, dorsal setae; l_1 first lateral setae; l_2, second lateral setae; o, outer setae.

d) with anterior ring of small spines, 3-4 pairs of short, stout, brown hooks with pointed tip, 3 pairs of elongate, slender, pale brown hooklets and setae: "o", "l_1", "l_2" and d, long, thin setae. CSL 0.40-0.42 (0.41, n=3) mm; CSW 0.23-0.28 (0.25, n=3) mm; CSR 1.49-1.77 (1.67, n= 3).

REDESCRIPTION OF THE FEMALE PUPA (FIGS. 4-5) (N=3)

Color in life brownish. Exuviae general coloration pale brown. Total length 2.52 mm. **Head**: Dorsal apotome (Fig. 4a) 2.04 X times as wide as long, apex rounded, surface covered with small rounded tubercles, anterior margin nearly straight, with 2

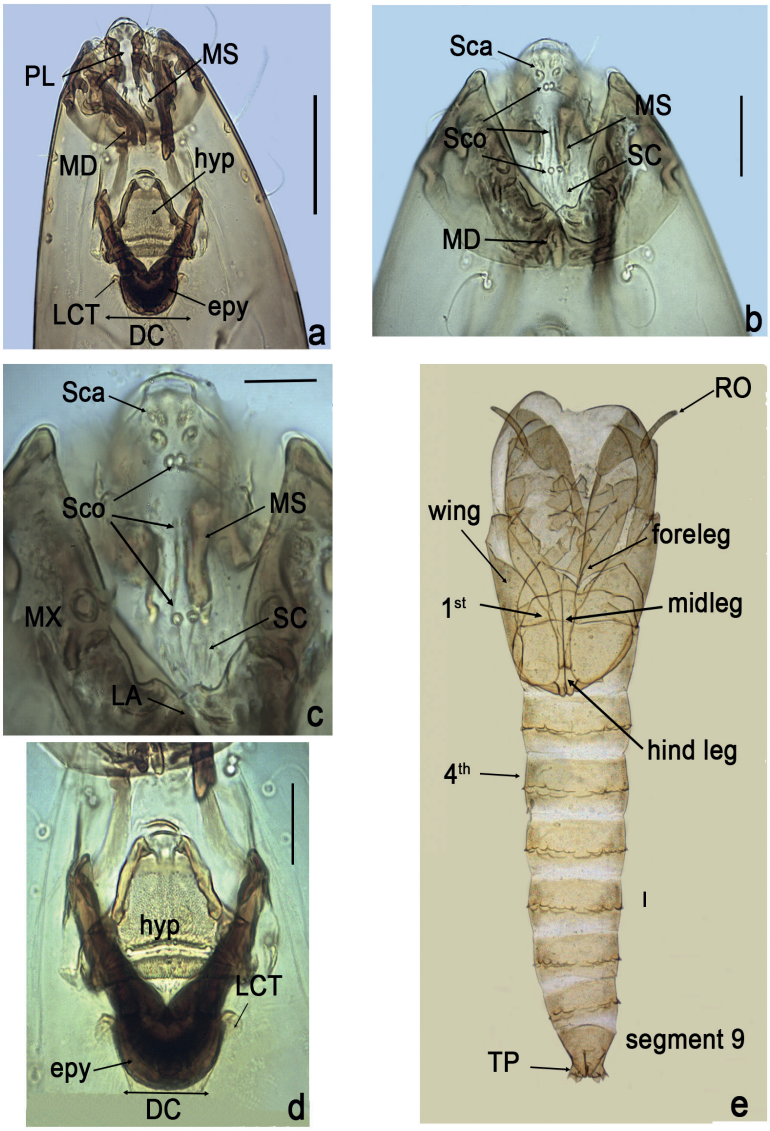

Figure 3 - *Dasyhelea flavifrons* Guérin-Méneville, **a-d**) larva, **e**) male pupa; **a**) head capsule (ventral view); **b**) head capsule (frontal view); **c**) detail of palatum; **d**) epipharynx and hypopharynx; **e**) entire pupa. Dorsal comb (DC); epypharynx (epy); hypopharynx (hyp); labium (LA); lateral curtains (LTC); mandible (MD); maxilla (MX); messors (MS); palatum (PL); respiratory organ (RO); sensilla campaniformia (Sca); sensilla coeloconica (Sco), scopae (SC); terminal processes (TP).

pairs of raised, wrinkled areas; dorsal apotome sensilla (Fig. 4a): DA-1-H medium-sized, thin seta, DA-2-H campaniform sensillum; posterior margin rounded with one stout, rounded tubercle; DAL 0.125 mm; DAW 0.255 mm; DAW/DAL 2.04. Mouthparts as in Figs. 4c, 5b. Sensilla: three dorsolateral cephalic sclerite sensilla (Figs. 4d, 5c): DL-1-H, DL-2-H short, stout setae, DL-3-H campaniform sensillum; two clypeal/labrals (Figs. 4c, 5b): CL-1-H, CL-2-H medium-sized, thin setae; one ocular (Figs. 4c, 5b): O-2-H campaniform sensillum. **Thorax**: Prothoracic extension absent; respiratory organ (Fig. 4d) brown, nearly straight, with scale-like spines with 14-16 apical and 4-5

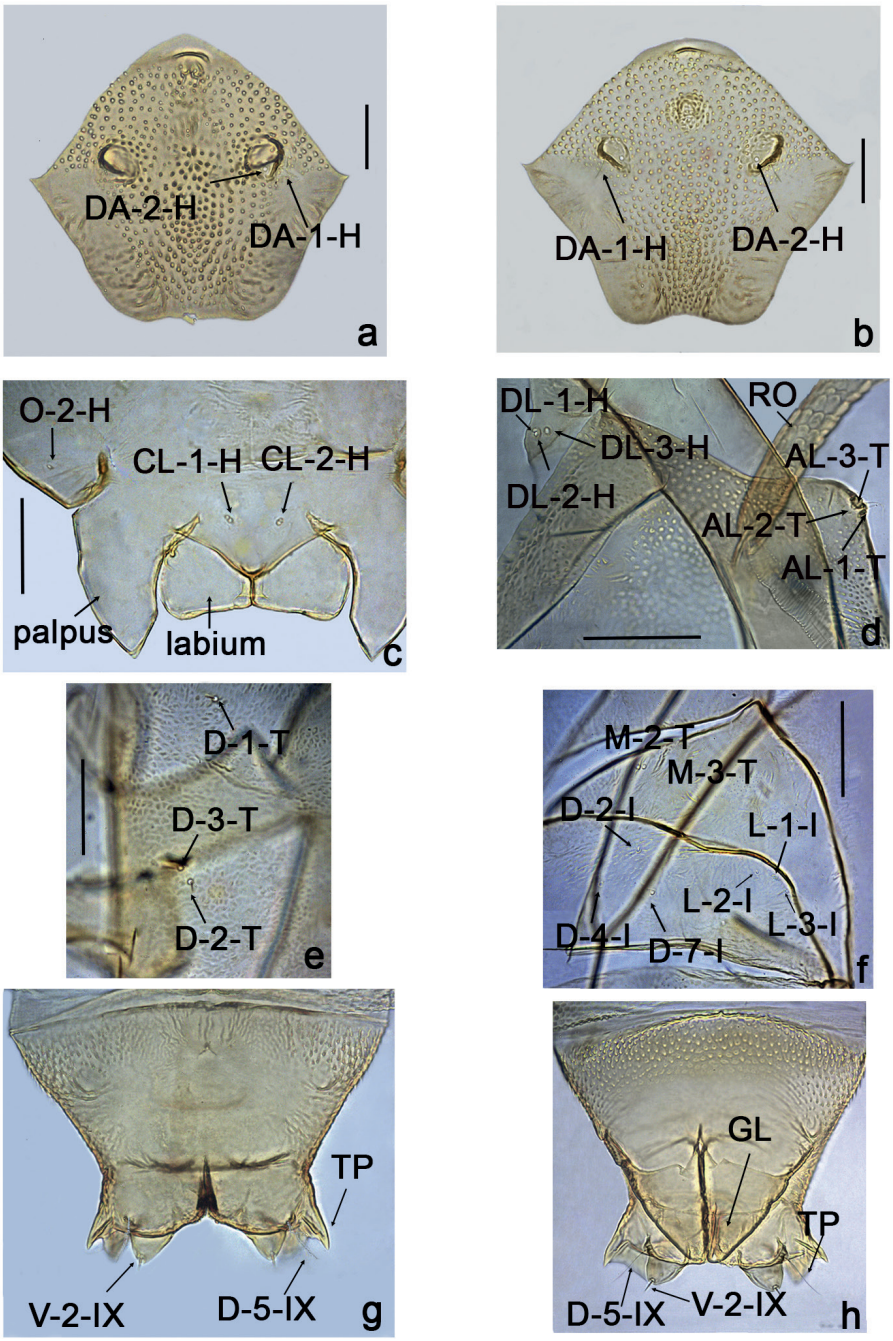

Figure 4 - *Dasyhelea flavifrons* Guérin-Méneville, **a, c-g**) female pupa; **b, h**) male pupa. **a-b**) dorsal apotome; **c**) mouthparts and clypeal/ labral and ocular sensillum (ventral view); **d**) cephalothoracis sensilla; **e**) dorsal sensilla; **f**) metathorax and tergite 1; **g-h**) segment 9. Anterolateral sensilla (AL-1-T, AL-2-T, AL-3-T); clypeal/labral sensilla (CL-1-H, CL-2-H); dorsal apotome sensilla (DA-1-H, DA-2-H); dorsolateral cephalic sclerite sensilla (DL-1-H, DL-2-H, DL-3-H); dorsal setae (D-1-T, D-2-T, D-3-T); dorsal sensilla of segment 9 (D-5-IX); genital lobe (GL); methatoracic sensilla (M-2-T, M-3-T); ocular sensillum (0-2-H);); respiratory organ (RO); tergite 1 sensilla (D-2-I, D-4-I, D-7-I, L-1-I, L-2-I, L-3-I); terminal processes (TP); ventral sensilla of segment 9 (V-2-IX).

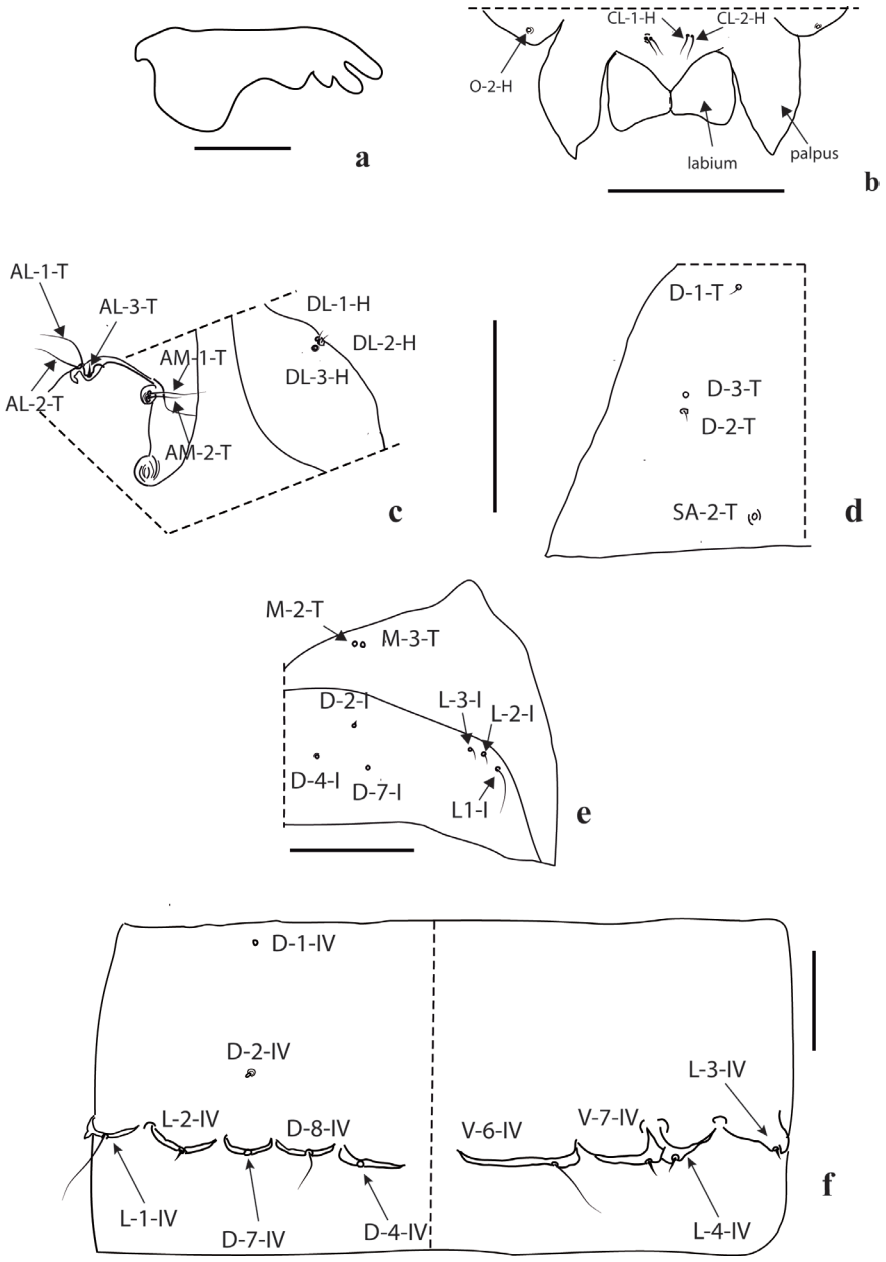

Figure 5 - *Dasyhelea flavifrons* Guérin-Méneville, **a**) larva, **b-f**) female pupa. **a**) mandible; **b**) mouthparts and clypeal/labrals and ocular sensillum (ventral view); **c**) cephalothoracic sensilla; **d**) dorsal sensilla; **e**) metathorax and tergite 1; **f**) segment 4. Anterolateral sensilla (AL-1-T, AL-2-T, AL-3-T); Anteromedial sensilla (AM-1-T, AM-2-T); clypeal/labral sensilla (CL-1-H, CL-2-H); dorsolateral cephalic sclerite sensilla (DL-1-H, DL-2-H, DL-3-H); dorsal setae (D-1-T, D-2-T, D-3-T); methatoracic sensilla (M-2-T, M-3-T); ocular sensillum (0-2-H); segment 4 sensilla (D-1-IV, D-2-IV, D-4-IV, D-7-IV, D-8-IV, L-1-IV, L-2-IV, L-3-IV, L-4-IV, V-6-IV, V7-IV); supraalar sensillum (SA-2-T); tergite 1 sensilla (D-2-I, D-4-I, D-7-I, L-1-I, L-2-I, L-3-I).

lateral pores; RO length 0.19-0.22 (0.20, n=2) mm, RO width 0.03 (n=2) mm; without pedicel. Sensilla: two anteromedial sensilla (Fig. 5c): AM-1-T long, thin seta, AM-2-T medium-sized, thin seta; three anterolateral sensilla (Figs. 4d, 5c): AL-1-T, AL-2-T long, thin setae, AL-1-T longer than AL-2-T, AL-3-T short, stout seta; dorsals (Figs. 4e, 5d): D-1-T, D-2-T short, thin setae, D-3-T campaniform sensillum, supraalar (SA-2-T) campaniform sensillum (Fig. 5d). Metathoracics (Figs. 4f, 5e): M-2-T, M-3-T campaniform sensilla. Cephalothorax surface with small rounded tubercles, length 1.01 (n=2) mm, width 0.67-0.82 (0.74, n=2) mm. **Abdomen**: abdominal segments covered with small spinules. Sensilla: tergite 1 (Figs. 4f, 5e) with setae as follows: D-2-I peg; D-4-I, D-7-I campaniform sensilla; L-1-I long thin seta, L-2-I, L-3-I short, thin setae. Second abdominal segment similar to the first one; segment 4 with sensillar pattern (Fig. 5f) as follows: D-2-IV peg; D-4-IV, D-7-IV campaniform sensilla, D-8-IV medium-sized, thin seta, all located on flattened tubercles; L-1-IV long, thin seta, L-2-IV, L-3-IV-L-4-IV short, stout setae, all located on triangular tubercles; V-6-IV long, thin seta, V-7-IV short, stout seta, both on flattened tubercles. Segment 9 (Fig. 4g) 1.52 X longer than wide, ventral surface with many spinules; length 0.19-0.20 (0.195, n=2) mm, width 0.24 (n=2) mm. Terminal process (Fig. 4g) triangular, divergent, tip pointed, with D-5-IX, D-6-IX campaniform sensilla, V-1-IX long, thin seta, V-2-IX short, stout seta; length 0.06 (n=2) mm.

DESCRIPTION OF THE MALE PUPA (N=3)

Similar to female with usual sexual differences. Habitus as in Fig. 3e. Total length 2.50 mm. Exuviae brown. Dorsal apotome (Fig. 4b) with DAL 0.13 mm; DAW 0.30 mm, DAW/DAL 2.30. Respiratory organ (Fig. 3e), RO length 0.24 (0.21-0.27, n=2) mm, RO width 0.03 (n=2); without pedicel. Cephalotorax: length 1.05 mm, width 0.70 mm. Segment 9 (Figs. 3e, 4h) length 0-26 (0.25-0.27, n=2) mm, width 0.24 (0.23-0.25, n=2) mm; genital lobe reaching (Fig. 4h) the posterior margin of segment; terminal process length 0.06 (n=2) mm.

DISTRIBUTION

Europe (Azores Island; Estonia, Germany, Great Britain, Belgium, Poland, Czech Republic, France, Austria, Switzerland, Spain, Croatia, Greece, Bulgaria, Ukraine, Russia, Yugoslavia); Asia (Mongolia, North China, Japan); North Africa (Algeria); America (Canada, USA; Guadeloupe; Argentina New Records).

MATERIAL EXAMINED

Argentina, Salta, San Ramón de la Nueva Orán; 23°9'15.9"S, 64°19'54.13"W, 22-II-2011, C. Mangudo, 2 males (with pupal exuviae); same data except, 23°9'4.28"S, 64°13'46.20"W, 28-II-2012, 3 larval exuviae; same data except 23°7'7.36"S, 64°18'31.03"W, 29-II-2012,1 male (with pupal exuvium), 1 female (with pupal exuvium); same data except 23°8'29.16"S, 64°18'31.03"W, 28-III-2012, male (with pupal exuvium), 1 female (with pupal exuvium); same data except 23°8'55.09"S, 64°1924.82"W, 28-III-2012, 1 female (with pupal exuvium). New Argentina records.

MATERIAL EXAMINED BY SEM

Same data except 23°9'4.28"S, 64°13'46.20"W, 28-II-2012, 2 larvae, 1 pupa.

COMPARISON

Dasyhelea flavifrons belongs to the *Dasyhelea grisea* species group as defined by Waugh and Wirth (1976), and this group is equivalent to the subgenus *Dasyhelea s. str.* (Dominiak 2012) and has a wide distribution. Guérin-Méneville in 1833 described for the first time the immatures collected from elm flux. Subsequently, various authors

described adults and/or immatures under different names and cited from different larval habitats such as sap of elm, horse chestnut, sap of oak, hornbeam, debris of chestnut tree, decaying roots of *Angelica* sp., sap of poplar, humus surrounding roots of meadowsweet, and shelf fungi (reviewed in Dominiak 2012, Szadziewski and Dominiak 2006).

Dasyhelea flavifrons is compared with all the American species of the grisea group, developing in diverse habitats including artificial containers (*D. azteca* Huerta & Grogan; *D. chani* Wirth & Linley; *D. correntina* Ronderos & Díaz; *D. eloyi* Díaz & Ronderos; *D. flavicauda* Macfie, *D. grisea* (Coquillett); *D. necrophila* Spinelli & Rodriguez; *D. paulistana* Forattini & Rabello; *D. pollinosa* Wirth; *D. pseudoincisurta* Waugh & Wirth; *D. pseudopollinosa* Díaz & Ronderos; *D. traverae* Thomsen and *D. yunga* Díaz).

Thomsen (1937) described the larva and pupa of *D. grisea* and *D. traverae*, both collected from blanket algae, but unfortunately the descriptions are poor and incomplete. Subsequently, Waugh and Wirth (1976) redescribed these species and described the pupa of *D. pollinosa* collected from pools of salt water pumped into a creek from oil wells and *D. pseudoincisurata* reared from rock pools along stream margins. The larva of *D. grisea* differs from *D. flavifrons* by the mandible with 3 teeth, the hypostoma with the medial portion smooth and flanked by 6 teeth and the caudal segment having 5 pairs of hooks. The pupa can be distinguished by the dark brown color, smaller total length (2 mm) and the respiratory organ is slightly curved and bearing 10 apical and 5 lateral pores. Likewise, the larva of *D. traverae* can be distinguished by its longer total length (5-6 mm), the mandible with one tooth and the hooks of caudal segment bifurcate. The pupa of *D. traverae* differs by the small total length (3 mm), the respiratory organ wedge-shaped bearing 40 apical pores and the apicolateral process of segment 9 very long and straight. About the pupa of *D. pollinosa* the authors only mentioned that is very similar to *D. traverae*. With regards to the pupa of *D. pseudoincisurata,* the description is very brief and differs by the respiratory organ bearing 20 apical and 5 lateral pores. Hribar (1998) only mentioned the brown thoracic pigmentation of the larva and did not describe it.

Forattini and Rabello (1957) described the pupa of *D. paulistana* and Díaz et al. (2014) completely redescribed it. The pupa was collected from matts of floating fern leaves (Salviniaceae and Azollaceae) and is readily distinguished by its smaller total length (2.3 mm); the dorsal apotome and cephalothorax smooth, two dorsolateral cephalic sclerite sensilla; the respiratory organ smooth bearing 21-22 apical pores and the segment 9 with terminal process straight and 2 x longer than length of total body.

Spinelli and Ronderos (1987) described the pupa of *D. flavicauda* collected from a lagoon and differs by the small total length (2 mm); the dorsal apotome smooth and the respiratory organ bearing 6-7 apical and 3-4 lateral pores.

Wirth and Linley (1990) described *D. chani* collected from leaves of the water lettuce, *Pistia stratiotes* L. The description of the larva is brief, but it differs by the shorter length (0.32 mm) and wider (0.20 mm) head capsule. The pupa can be distinguished by its longer total length (2.9 mm); the dorsal apotome smooth, the respiratory organ wedge-shaped and bearing 16 apical pores, and the apicolateral process of segment 9 elongate and parallel.

Ronderos et al. (2004) collected from mud in flooded soil the pupa of *D. correntina,* which distinguishes from *D. flavifrons* by its dark brown color; the dorsal apotome smooth; the respiratory organ curved and bearing 10-12 apical and 6-7 lateral pores; and the male pupa has the genital lobe not reaching the posterior margin of segment 9.

Spinelli and Rodriguez (1999) described the immature of *D. necrophila* collected from artificial containers and Ronderos et al. (2003) redescribed

with SEM the immature. The larva of *D. flavifrons* is very similar in having the mandible with four teeth, the maxillary palpus short and button-like and the caudal segment with anterior ring of small spines. The larva of *D. necrophila* differs in the labrum with two pairs of sensilla campaniformia; the epypharynx lacking the auxiliary sclerite and the caudal segment with 5-6 pairs of hooks. On the other hand, the pupa of *D. necrophila* differs by the total length 2.70 mm, the posterior margin of dorsal apotome with two tubercles; the clypeal labrals long and thin; the respiratory organ 30 apical and 4 lateral pores and tergite 1 with L-2-I, L-3-I short seta. The male pupa has the genital lobe not reaching posterior margin of segment 9.

Díaz et al. (2013) described the larva and pupa of *D. eloyi* collected from flooded soil and from water lettuce (*Pistia stratiotes* L.) and differs in the longer head capsule; the labrum with three pairs of sensillae campaniformia; the scopae with 4-6 teeth; the lateral arms of epypharynx smaller and the caudal segment longer. The pupa of *D. eloyi* is readily distinguished from *D. flavifrons* in the following characters: the posterior margin of dorsal apotome with two tubercles and DA-1-H long; the clypeal labral sensilla long and thin; the anteromedial sensillum AM-2-T short; the respiratory organ with 16-18 apical and 6-8 lateral pores, and the D-2-T represented by a peg; segment 4 with L-1-IV medium-sized and segment 9 longer than in *D. flavifrons*.

Díaz et al. (2014) described the pupa of *D. pseudopollinosa* that was collected together with *D. paulistana* and can be distinguished from *D. flavifrons* by its small total length (2.8 mm); the dorsal apotome smooth; the respiratory organ smooth bearing 18-20 apical pores and the segment 9 with terminal process straight, as long as the length of the body of segment 9. The male pupa has the genital lobe reaching the posterior margin of segment 9.

Recently, Díaz et al. (2018) described the immature of *D. azteca* and *D. yunga* that were collected from small temporary ponds and stream. The larva of *D. flavifrons* shares with the larva of *D. azteca* the following characters: the total length 4-5 mm; the palatum with four pairs of sensillae campaniformia; the galeolacinia with short, stout seta, 5-6 papillae and two flap-like lobes; the maxillary palpus short and button-like; the lateral arms of epipharynx with auxiliary sclerites and hypopharynx with posterior comb with fringe. However, *D. azteca* differs in having two pairs of sensilla coeloconica; the scopae with 14-16 strong teeth; the mandible with three teeth, and caudal segment with 10-12 hooks. The pupa differs in the smaller total length (2.22 mm); respiratory organ bearing 22-24 apical and 3-4 lateral pores; dorsal apotome with DA-1-H short seta; the clypeal-labral sensilla CL-1-H medium-sized, thin seta, and CL-2-H short, thin seta; anterolateral sensilla AL-1-T long, thin seta, AL-2-T medium-sized, thin seta and AL-3-T campaniform sensillum; the respiratory organ with 16-18 apical and 6-8 lateral pores, and D-2-IV short seta. The male pupa has genital lobe extending slightly beyond of posterior margin of segment 9.

Finally, the pupa of *D. yunga* Díaz differs clearly by its exuviae yellowish, with dorsal apotome smooth and without clypeal/labrals, respiratory organ pale brown, smooth, with 18-20 apical and 3-4 lateral pores and genital lobe of pupa male moderately wide and globose extending beyond posterior margin of segment 9.

BIONOMICS

The tree holes where the larvae and pupae of *Dasyhelea flavifrons* were collected were pans formed as branch intersections (maintaining an unbroken bark lining) and rot holes (lacking bark lining and penetrating into the wood of the tree). The specimens described herein were collected

from urban tree holes in *Delonix regia* (Bojer) Raf. (Fabales: Fabaceae), and in holes from unidentified trees in yunga forest patches to the east of the city. Other specimens were also collected in the city from *Bauhinia* sp. (Fabales: Fabaceae), *Citrus sinensis* Osbeck (Sapindales: Rutaceae), *Morus* spp. (Rosales: Moraceae), and *Thevetia nereifolia* Juss. (Gentianales: Apocynaceae). The finding of immature *D. flavifrons* in tree holes in northwest Argentina is consistent with the described larval habitats in the Holartic region, since larvae and pupae have been usually collected from tree holes and sap flows of various species, such as *Quercus, Fagus, Ulmus* and *Fraxinus* (Oboňa and Dominiak 2014; see also revisions on Dominiak and Szadziewski 2010, Dominiak 2012).

This species was found in tree holes either as single species or coexisting with other species of Ceratopogonidae, *Culicoides trilineatus* Fox, the latter species suspected to be a vector of BTV. The immatures of *D. flavifrons* were also found coexisting with *Aedes aegypti* L. (vector of dengue, urban yellow fever, zika, and chikungunya virus (Gubler 2004, Ayres 2016, Marcondes et al. 2017)), *Haemagogus spegazzini* Brèthes (potentially vector of arbovirus (Karabatsos 1985), or *Toxorhynchites guadeloupensis* Dyar and Knab (Diptera: Culicidae).

In the shell of dead *Megalobulimus lorentzianus* (Doering) snails, *D. flavifrons* were found as a single species (specimens described herein). The snail shells width and length of aperture were approximately 3 cm and 5.5 cm and held less than 0.5 ml of water. Other *Megabulimus* shells held larvae or pupae of the mosquito *Limatus durhamii* Theobald (Diptera: Culicidae) and/or immature Psychodidae. To our best knowledge, this is the first report of Ceratopogonidae collected from gastrotelmata in Argentina.

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AUTHOR CONTRIBUTIONS

RMG, FD, and CM conceived the research. CM collected the samples and raised the larvae; FD and MR identified and redescribed the specimens FD, CM, MMR and RMG contributed material. FD, CM and RMG wrote the manuscript. RMG and FD secured funding. All authors read and approved the manuscript.

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