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The pupa of *Stilobezzia (Eukraiohelea) elegantula* (Johannsen) (Diptera: Ceratopogonidae)

Florentina DÍAZ[1*], Mahia M. AYALA[1], Alan LEGUIZAMÓN[1], Ruth L. FERREIRA-KEPPLER[2], Gustavo R. SPINELLI[3]

[1] Centro de Estudios Parasitológicos y de Vectores (CEPAVE), CONICET—UNLP. La Plata, Buenos Aires, Argentina

[2] Instituto Nacional de Pesquisas da Amazônia, Coordenação de Pesquisas de Entomologia, Manaus, Amazonas, Brazil

[3] ILPLA—CONICET, UNLP-División Entomología, Museo de La Plata, Buenos Aires, Argentina

* Corresponding author: florentina.diaz@gmail.com

ABSTRACT

The pupa of *Stilobezzia* (*Eukraiohelea*) *elegantula* is described and illustrated, being the first description of a pupa for a species belonging to this subgenus. The studied material was collected large banks of floating aquatic macrophytes in the Marchantaria Island, in the municipality of Iranduba, Amazonas, Brazil, located at 15 km from Manaus, and surrounded by the white waters of the Solimões river. The collected pupae were isolated and observed in the laboratory until the adult emergence and then identified as *S.* (*E.*) *elegantula*. The described pupa is compared with those of Neotropical species *Stilobezzia* (*Stilobezzia*) *punctulata* and *S.* (*S.*) *rabelloi*.

KEYWORDS: Immatures, Predaceous midges, taxonomy

A pupa de *Stilobezzia* (*Eukraiohelea*) *elegantula* (Johannsen) (Diptera: Ceratopogonidae)

RESUMEN

Se describe e ilustra la pupa de *Stilobezzia* (*Eukraiohelea*) *elegantula* siendo la primera descripción de una pupa de una especie perteneciente al subgénero. El material estudiado fue colectado en la Isla Marchantaria sobre grandes bancos de macrófitas acuáticos flotantes, municipio de Iranduba, Amazonas, Brasil, localizada a 15 km de Manaus, y rodeada por las aguas del río Solimões. Las pupas colectadas fueron aisladas y observadas en el laboratorio hasta la emergencia del adulto y luego identificadas como *S.* (*E.*) *elegantula*. La pupa descrita se compara con las especies neotropicales *Stilobezzia* (*Stilobezzia*) *punctulata* y *S.* (*S.*) *rabelloi*.

PALABRAS CLAVE: Inmaduros, Mosquitas depredadoras, taxonomía

INTRODUCTION

The predaceous midge genus *Stilobezzia* Kieffer, 1911 is a large and diverse group of Ceratopogonidae with about 361 species, occurring throughout the zoogeographical regions described worldwide (Borkent and Dominiak 2020; Borkent *et al.* 2022; Cazorla and Da Silva, 2023; Da Silva *et al.* 2023), except Antarctica (Borkent 2014).

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, and feed on microorganisms (De Meillon and Wirth 1991; Cazorla *et al.* 2006). Four subgenera are recognized within the genus, the cosmopolitan *S.* (*Acanthohelea*) Kieffer, 1917 and *S.* (*Stilobezzia*) sensu strictu with 338 extant species described, *S.* (*Debenhamia*) Wirth and Grogan 1988, known by two species from Australia, and *S.* (*Eukraiohelea*) Ingram and Macfie 1921, with 14 species from Africa, America and southeastern Asia (Borkent and Dominiak 2020; Borkent *et al.* 2022; Cazorla and Da Silva 2023; Da Silva *et al.* 2023). Borkent (2014) listed 29 species of *Stilobezzia* for which the pupae are known worldwide, six of them in the subgenus *S.* (*Acanthohelea*) and 23 in the subgenus *S.* (*Stilobezzia*), and subsequently Anjos-Santos *et al.* (2017) described the pupa of *S.* (*A.*) *megatheca* Cazorla and Spinelli from Patagonia.

Currently, the subgenus *S.* (*Eukraiohelea*) is represented in the Neotropical region by five species recently reviewed by Cazorla *et al.* (2017): *S. amnigena* (Macfie) and *S. elegantula* (Johannsen) from southern and eastern USA to northeastern Argentina, and *S. dorsofasciata* (Lutz), *S. proxima* Cazorla and Felippe-Bauer and *S. quasielegantula* Cazorla and Felippe-Bauer, restricted to Rio de Janeiro, Brazil. The only described immature within the subgenus is the weak, very superficial description of the egg of *S. dorsofasciata* by Lutz (1914).

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During a field sampling program in the vicinity of Manaus, Brazil, pupae of *Stilobezzia* sp. were collected. They were isolated and observed in the laboratory until the adult emergence and then identified as *S*. (*E*.) *elegantula*. The pupa of this species is herein described and illustrated, being this the first description for one species in this subgenus.

MATERIAL AND METHODS

Study area

The present study was conducted at Camaleão Lake on the Marchantaria Island, in the municipality of Iranduba, Amazonas, Brazil. This island at 15 km from the city of Manaus is surrounded by the white water of the Solimões river. The area is directly exposed to the flood-pulse by the water level fluctuations of the Amazon and its tributaries (Wittmann *et al*. 2010). The climate is equatorial-neotropical, with monthly mean minimum temperatures of 24-26°C and maximums of 28°C (Salati and Vose 1984).

Specimens were collected between April and June 2011 during the high-water period of the Rio Solimões (Junk 1989) in large banks of floating aquatic macrophytes (Figure 1), with a predominance of *Eichhornia crassipes* (Mart.) Solms (03°15'20.4''S, 59°57'55.9''W) or *Pistia stratiotes* L. (03°14'27.5''S, 59°57'30.4''W). In these environments, the water temperatura at the plant root level was 29.8°C, the conductivity 243.8 µS/cm and the pH 9.76.

The pupae were collected with a metallic ladle and separated manually in white plastic trays using plastic pipettes, into smaller glass cups with river-water and transported to the Laboratory of Cytotaxonomy of Aquatic Insects- LACIA in a box thermal plastic container. 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, pupal exuviae and adults were mounted in Canada balsam following the technique described by Borkent and Spinelli (2007). Photomicrographs were taken with a digital camera Micrometrics SE Premium, through a Nikon Eclipse E200 microscope. For terminology see Borkent (2014) and Anjos-Santos *et al*. (2017).

The material examined is deposited in the following collections: INPA- Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil; MLPA- División Entomología, Museo de La Plata, Argentina.

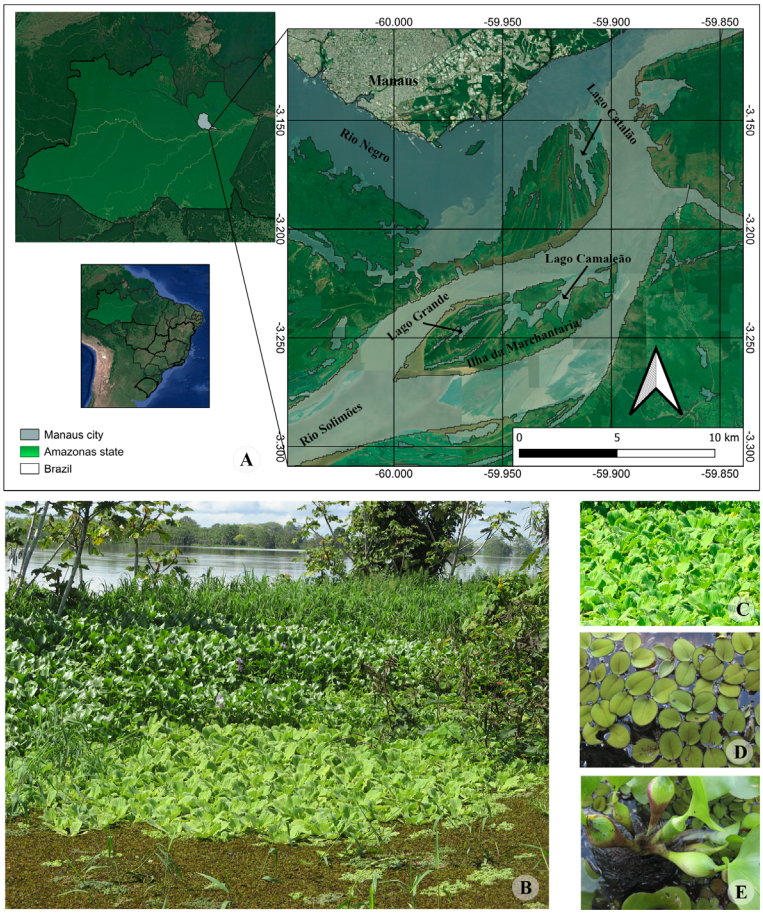

Figure 1. Geographical location of Lago Camaleão in wetlands at Ilha da Marchantaria, Iranduba, Amazonas, Brasil. B-E show the collection sites of the immature stages of *Stilobezzia*.

RESULTS

Stilobezzia (Eukraiohelea) elegantula (Johannsen)

Zoobank identifier: urn:lsid:zoobank.org:pub:85069F97-F8AC-42C1-9116-4CF002492721

Material examined

BRAZIL, AMAZONAS, Iranduba, Ilha da Marchantaria, Lago Camaleão, 03°15'20.4''S; 59°57'55.9''W, 18-iv-2011, Torreias & Ferreira-Keppler lgt., 2 ♂ (with 'ex pupa'), (1 in INPA; 1 in MLPA); 1 ♀ (with 'ex pupa') (INPA); same date except 03°14'27.5''S; 59°57'30.4''W 27-vi-2011, 1 ♀ (with 'ex pupa') (MLPA).

Description of female pupa

(Figure 2B, E-F, H; 3A, E). Exuviae overall pale brown. Total length 2.87-3.00 (2.94, n = 2) mm. **Head:** Dorsal apotome (Figure 2B) smooth, anterior margin rounded with small, hyaline notched; distal margin slightly concave with a few wrinkles; DAL 0.11-0.13 (0.205, n = 2) mm; DAW 0.14-0.16 (0.15, n = 2) mm; DAW/DAL 1.07-1.45 (1.26, n = 2); mouthparts with mandible well developed; palpus extending to posterolateral margin of labium; labium separated medially by labrum; apex of labrum rounded. Sensilla: dorsal apotomals (Figure 2B): DA-1-H elongate, thin seta, located on small tubercle, DA-2-H campaniform sensillum at tubercle base; one clypeal/labrals: CL-1-H long, thin seta; oculars: O-2-H campaniform sensillum. **Thorax:** Cephalothorax rectangular, surface smooth with small wrinkles on all surfaces. Length of cephalothorax 0.95-1.02 (0.98, n=2) mm, width 0.85-0.90 (0.97, n = 2) mm. Respiratory organ (Figure 2E) brown, smooth, medium-sized, narrowing gradually to pointed apex, with 28-30 pores in single row; pedicel slender (Figure 2E, H-G), P 0.04-0.05 (0.045, n = 2) mm; RO length 0.27-0.30 (0.28, n = 2) mm, RO width 0.04-0.06(0.05, n = 2) mm; P/RO 0.04-0.06 (0.05, n = 2). Sensilla: two dorsolateral cephalic sclerite (Figure 2F): DL-1-H long, thin seta, DL-2-H campaniform sensillum; two anteromedial setae: AM-1-T, AM-2-T long, thin setae; two anterolaterals: AL-1-T, AL-2-T very long, stout setae, AL-2-T shorter than AL-1-T; dorsals (Figure 2H): D-1-T long, stout seta; D-2-T, D-4-T long, thin setae, D-3-T campaniform sensillum, D-1-T, D-2-T, D-4-T on small tubercle, closely approximated; SA-1-T campaniform sensillum; metathoracics: M-1-T very long, thin seta, M-2-T campaniform sensillum. Abdomen: segments 3-9 covered with very small spicules on anterior margin; tergite 1 (Figure 3A) with two anteromesals: D-2-I, D-3-I long, thin setae; D-4-I, D-7-I campaniform sensilla, D-7-I near to D-3-I, D-8-I, D-9-I long, thin setae, D-8-I stouter than D-9-I; three lateral sensilla L-1-I, L-3-I long, stout setae, L-2–I long, thin seta, all on stout tubercle; segment 4: D-2-IV medium-sized, thin seta, D-4-I, D-7-IV campaniform sensilla, D-7-IV near to D-2-IV, D-5-IV, D-9-IV long, thin setae, D-8-IV long, stout seta; L-1-IV, L-2-IV, L-3-IV, L-4-IV long, stout setae, all on cylindrical tubercles; V-5-IV very long, stout seta, V-6-IV, V-7-IV long, thin setae; segment 9 (Figure 3E) approximately 1.27 times as long as width, length 0.60-0.62 (0.61, n = 2) mm, width 0.15 (n = 2) mm; terminal process very elongate, parallel, length 0.45-0.50 (0.47, n = 2) mm, with D-5-IX campaniform sensillum.

Description of male pupa

(Figure 2A, C-D, G, I-J; 3B-D, F). Habitus as in Figure 2A. Similar to female with usual sexual differences: Total length 2.80-3.00 (2.90, n = 2) mm. Dorsal apotome (Figure 2C) with DAL 0.19-0.22 (0.205, n = 2) mm; DAW 0.09-0.10 (0.095, n = 2) mm; DAW/DAL 1.90-2.44 (2.17, n = 2), clypeal-labrals as in Figure 2D. Respiratory organ, P 0.024-0.045 (n = 2) mm; RO length 0.28-0.30 (0.29, n = 2) mm, RO width 0.05 (n = 2) mm; P/RO 0.16-0.18 (0.17, n = 2) mm; anteromedials and anterolaterals as in Figure 2G. Cephalothorax: length 0.80 mm, width 1.02 mm, metathoracis and tergite 1 as in Figure 2I-J;

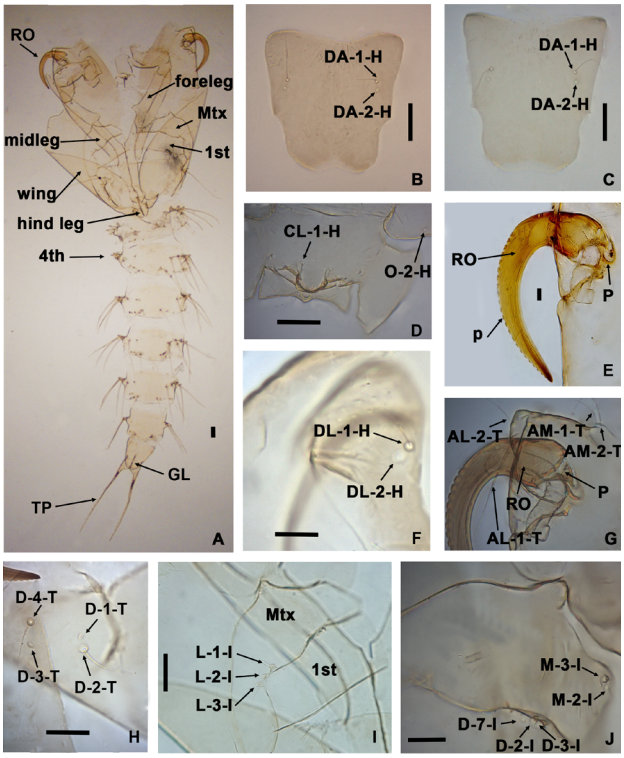

Figure 2. Morphological details pupa of the *Stilobezzia (Eukraiohelea) elegantula* (Johannsen) pupa. **A, C-D, G, I-J:** Male pupa. **B, E-F, H**: Female pupa. **A**- Entire pupa. **B-C** dorsal apotome. **D** - clypeal/labral sensillum and ocular sensillum. **E** - respiratory organ. **F** - dorsolateral cephalic sclerite sensilla. **G** - Anterolateral sensilla and anteromedial sensilla. **H** - dorsal sensilla. **I** - metathorax and tergite 1. **J** - metathoracic sensilla. Scale bars: 0.05 mm. Abbreviations: Anterolateral sensilla (**AL-1-T, AL-2-T**); anteromedial sensilla (**AM-1-T, AM-2-T**); clypeal labral sensillum (**CL-1-H**); dorsal apotome (**DA**); dorsal apotome sensilla (**DA-1-H, DA-2-H**); dorsolateral cephalic sclerite sensilla (**DL-1-H, DL-2-H**); dorsal sensilla (**D-1-T, D-2-T, D-3-T, D-4-T**); genital lobe (**GL**); metathoracic sensilla (**M-2-T, M-3-T**); oculars sensillum **(O-2-H)**; pedicel **(P)**; respiratory organ **(RO);** tergite 1 sensilla **(D-2-I, D-3-1, D-4-I, D-7-I, L-1-I, L-2-I, L-3-I);** terminal process (**TP**). Scale bars = 0.05 mm

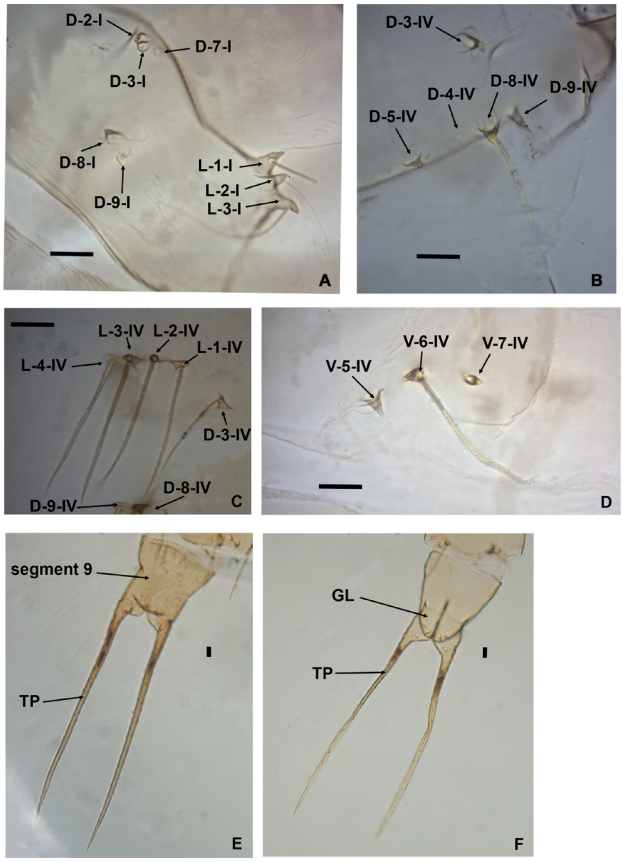

Figure 3. Morphological detail of the *Stilobezzia (Eukraiohelea) elegantula* (Johannsen) pupa. **A**, **E**: Female pupa. **B-D**, **F**: Male pupa. **A** - tergite 1. **B** Segment 4 dorsal sensilla. **C** - Segment 4 lateral sensilla. **D** - Segment 4 ventral sensilla. **E-F** - Segment 9, ventral view. Scale bars: 5 mm. Abbreviations: Genital lobe (**GL**); Segment 4 sensilla (**D-3-IV, D-4-IV, D-5-IV, D-7-IV, L-1-IV, L-2-IV, L-3-IV, L-4-IV, V-5-IV, V-6-IV, V-7-IV**); tergite 1 sensilla (**D-2-I, D-3-I, D-4-I, D-7-I, D-8-I, D-9-I, L-1-I, L-2-I, L-3-I**); terminal processes (**TP**).

segment 4 (Figure 3B-D). Segment 9 (Figure 3F) length 0.57-0.67 (0.62, n = 2) mm, width 0.15-0.20 (0.17, n = 2) mm; genital lobe (Figure 3F) elongate, just reaching to posterior margin of segment; terminal process length 0.42-0.45 (0.43n = 2) mm.

DISCUSSION

Several species of Ceratopogonidae breed in macrophytes, for example the Neotropical *S.* (*S.*) *punctulata* Lane and *S.* (*S.*) *rabelloi* Lane and other genera, such as *Dasyhelea* Kieffer, *Forcipomyia* Kieffer, *Culicoides* Latreille, *Heteromyia* Say and *Bezzia* Kieffer. As pointed out by Borkent and Craig (2001) and Cazorla and Marino (2004), the pupae share characters related with the breeding site, as the respiratory organ narrowing gradually to pointed apex, smooth dorsal apotome, the tubercles of the abdominal segment with simple or branched setae, and very elongated terminal processes.

Stilobezzia (*S.*) *punctulata* is readily distinguished from *S.* (*E.*) *elegantula* by the pale brown cepahalothorax and darker abdomen, the dorsal apotome with DA-1-H stout seta, presence of two clypeal/ labrals and two oculars, respiratory organ with two rows of pores (basal row with 5 pores and apicolateral row with 18 pores), the sensilla of segment 4 with bifurcated setae except D-2-IV and L-1-IV, which are simple setae, and male ninth segment with genital lobe not reaching its posterior margin.

Stilobezzia (*S.*) *rabelloi* can be distinguished from *S.* (*E.*) *elegantula* by the general pale brown coloration except the apical portion of segment 7 and segments 8-9, which are slightly darker the dorsal apotome with DA-1-H represented by a stout seta, DL-1-H stout seta, presence of two clypeal/ labrals and two oculars, respiratory organ with 5 basal and 15 apical pores, AL-1-T and AL-2-T thick setae, dorsals D-1-T; D-2-T and D-4-T with long thin setae, segment 4 with D-2-IV pore, and D-5-IV, D-8-IV, D-9-IV, V-5-IV, V-6-IV are bifurcated with plumose apices.

CONCLUSIONS

Of the 111 extant genera of Ceratopogonidae, the immature stages of only 45 of them are known at present. Therefore, there is still a need for larger collection efforts to document the immature stages of this family and their breeding environments. Because of this, it is necessary to obtain more associated material in order to have a complete idea of the preimagos of the genera of the family and to be able to know the different environments where they breed. Finally, knowing the habitats of the immatures and the precise identification of these immatures is a valuable tool to be able to recommend control measures and avoid outbreaks of the species suspected to be vectors of viruses such as Bluetongue or Oropuche, , which affect both animals and humans.

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AYALA, M.M.; LEGUIZAMÓN, A.: Methodology.
FERREIRA-KEPPLER, R.L.: Conceptualization, Funding acquisition, Writing - original draft.
SPINELLI, G.R.: Conceptualization, Writing - original draft.