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Neotropical Entomology

ISSN 1519-566X
Volume 42
Number 5

Neotrop Entomol (2013) 42:492-497
DOI 10.1007/s13744-013-0142-4

Springer

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SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Description of the Pupa of *Culicoides crucifer* Clastrier (Diptera: Ceratopogonidae)

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Keywords

Amazonas, chaetotaxy, immature stage, taxonomy

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Edited by Neusa Hamada – INPA

Received 15 April 2013 and accepted 13 May 2013
Published online: 12 July 2013

Abstract

The pupa of *Culicoides crucifer* Clastrier is described, illustrated and photomicrographed by using binocular microscope and phase-contrast microscopy from material collected in an artificial container in Manaus, Brazil. The pupa shows features typical of pupae occurring in calm and clean waters, and it is compared with its similar congeners of the subgenus *Haematomyiidium*, *Culicoides annuliductus* Wirth and *Culicoides debilipalpis* Lutz.

Introduction

Species in the genus *Culicoides* Latreille are by far the most notorious members of the 109 currently recognized extant genera of Ceratopogonidae. In spite of their economic, medical, and veterinary importance, it is surprising that their preimaginal stages, which breed in aquatic or semi-aquatic environments, are relatively poorly known (Ronderos *et al* 2008). Borkent & Spinelli (2007), in their catalog of Neotropical Ceratopogonidae, listed 266 species of *Culicoides*, and since then, 13 species were subsequently described for the region. Of these, 38 belong to the subgenus *Haematomyiidium* Goeldi, but only six of them have their pupae known: *Culicoides debilipalpis* Lutz was described by Barbosa (1953), Forattini (1957), Ronderos *et al* (2010), and Lamberson *et al* (1992), the latter as *Culicoides lahillei* (Iches); *Culicoides annuliductus* Wirth, *Culicoides bayano* Wirth, and *Culicoides filiductus* Wirth by Vitale *et al* (1981); *Culicoides hoffmani* Fox by Linley & Kettle (1964); and *Culicoides paraensis* (Goeldi) by Murphree & Mullen (1991) and Lamberson *et al* (1992).

The purpose of this paper is to fully describe the pupa of the anthropophilic species of the subgenus *Haematomyiidium*, *Culicoides crucifer* Clastrier, from material recently collected in Manaus, Brazil.

Material and Methods

The pupae of *Culicoides crucifer* were collected using pipette from white plastic trays placed in the CAMPUS II/ INPA, Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil. They were carried to the laboratory and placed individually in vials with a drop of water. Observations were done daily until adult emergence. In order to observe the ultrastructural characters, pupae were examined using binocular microscope and phase-contrast microscopy. Measurements and ratios of the pupa were taken using binocular microscope (BCM). For observation with BCM, specimens were slide mounted in Canada balsam following the technique described by Borkent & Spinelli (2007). Pupal exuviae were mounted dorsally, ventrally, and laterally to examine cuticular processes of the cephalothorax, respiratory organs, and abdominal segments. Ink illustrations were made with camera lucida. Photomicrographs were taken with a digital camera Micrometrics SE Premiun, through a Nikon Eclipse E200 microscope. The map was traced from Google Earth and the track was kept in KLM format; afterwards, the format was turned into plt through GPS Visualizer (http://www.gpsvisualizer.com/gpsbabel/gpsbabel_convert) and also drawn on OziExplorer 3.95.4 version.

For terminology, see Ronderos *et al* (2008). Studied specimens are deposited in the collections of Invertebrates, Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA), and División Entomología, Museo de La Plata, Argentina (MLP).

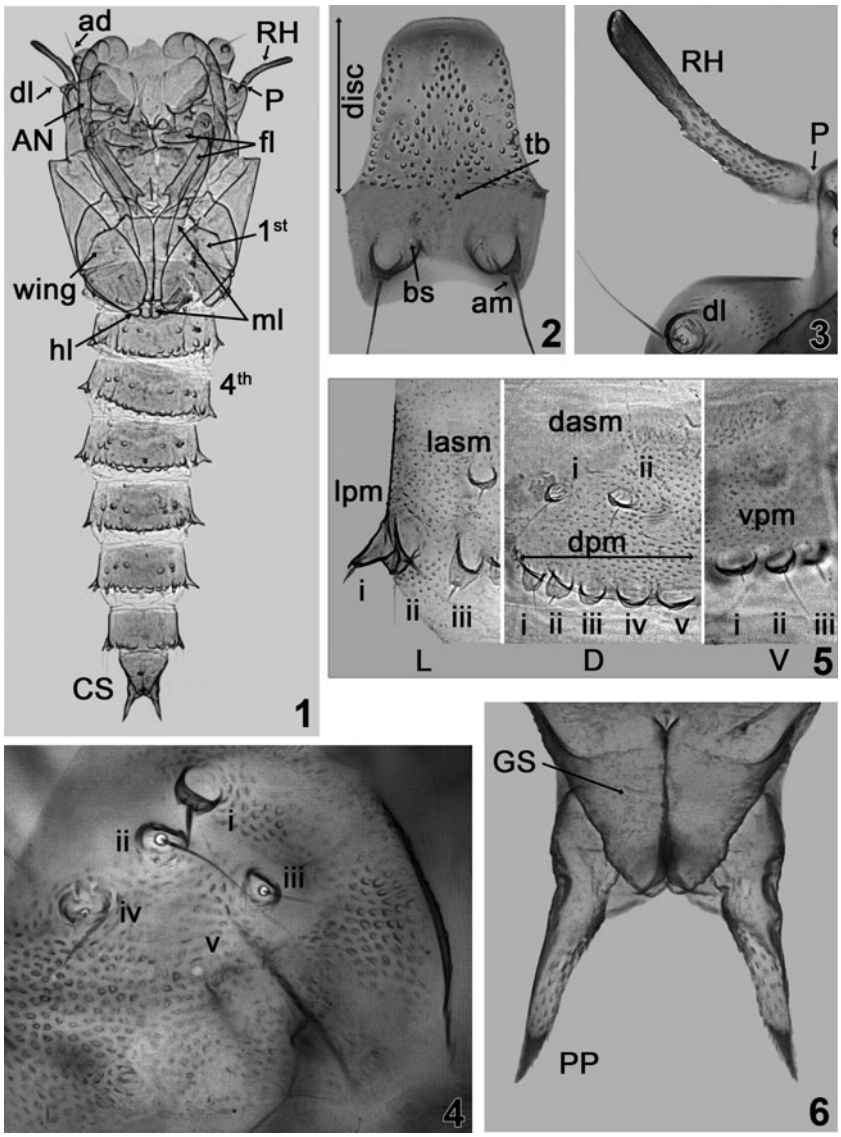
Results and Discussion

Culicoides crucifer Clastrier (Figs 1–6, 7–9, 10–12, 13)

Culicoides crucifer Clastrier 1968, 85 (male, female; French Guiana); Wirth 1974, 29 (in catalog species south of USA); Aitken *et al* 1975, 118 (Trinidad records); Vitale *et al* 1981, 147 (in key of the *C. debilipalpis* group); Wirth *et al* 1988, 46 (in Atlas species south of USA); Borkent & Wirth 1997, 66 (in World catalog); Borkent & Spinelli 2000, 31 (in catalog species south of USA); Trindade & Gorayeb 2005, 67 (Brazil, Para records); Borkent & Spinelli 2007, 66 (in Neotropical catalog); Silva *et al* 2010, 71 (Brazil, Para records); Borkent 2013, 79 (in world catalog).

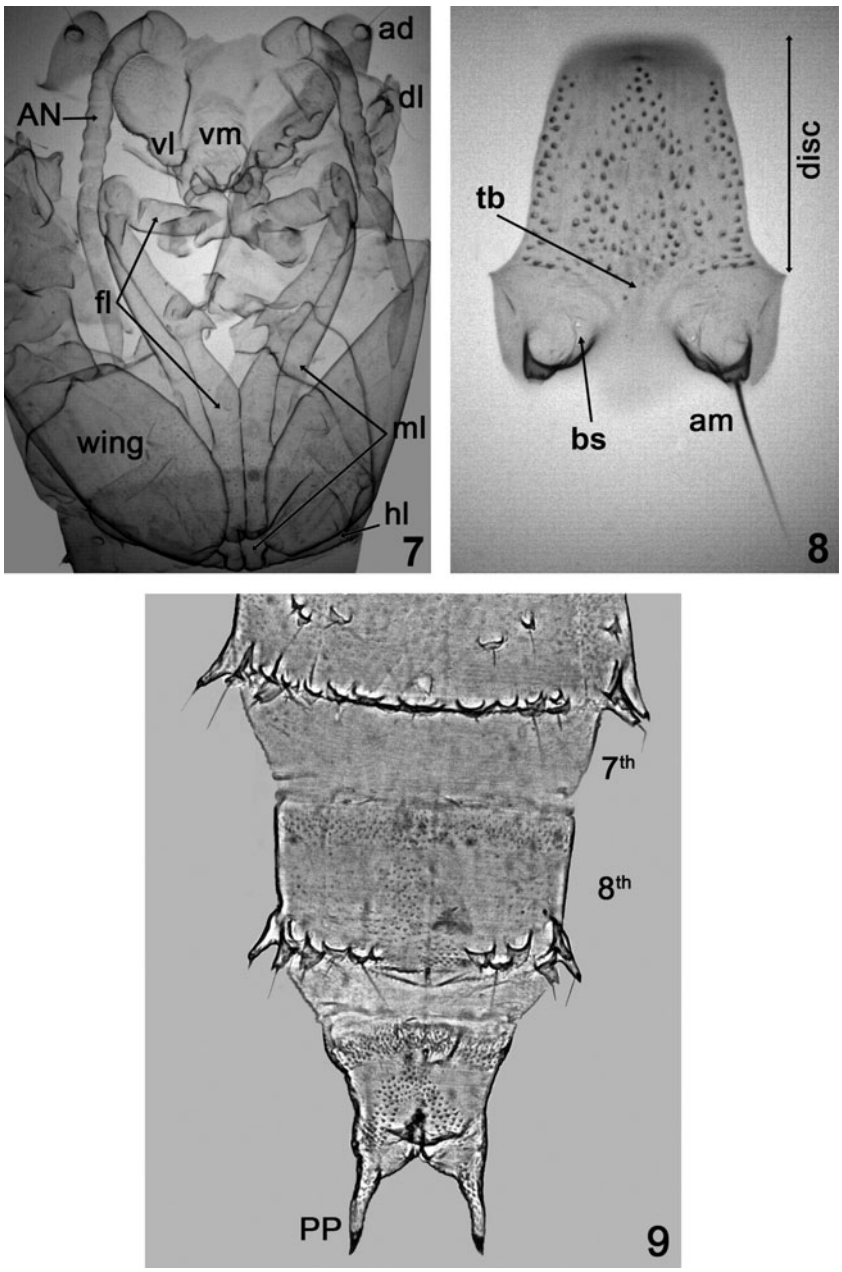
Description of male pupa (Figs 1–6, 10–12)

Entire pupa as in Fig 1. Exuviae pale yellowish. Total length 2.20–2.45 mm (2.34, *n*=5). Length of cephalothorax 1.00–1.25 mm (1.08, *n*=5), width 0.52–0.60 mm (0.56, *n*=4). Operculum (Fig 2) slightly wider than long, distal margin blunt; disk surface covered by stout rounded spinules; anteromarginal tubercle (am) well-developed, 0.040 mm, base rounded, wide, with one elongate, stout, pointed seta inserted in conical-shaped area; basal sensillum present; posterior margin slightly convex, smooth except for 2–4 small rounded spinules immediately posterior to transverse

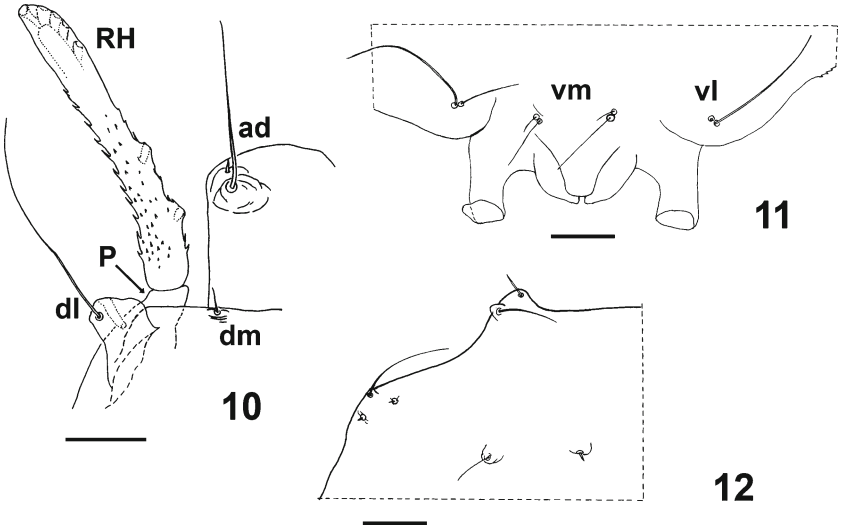


Figs 1–6 Male pupa of *Culicoides crucifer*. *1* Entire pupa (slide-mounted specimen), ventral view; *2* operculum; *3* respiratory horn; *4* dorsal tubercles; *5* fourth abdominal segment; *6* caudal segment. anterodorsal tubercle (*ad*); anteromarginal tubercle (*am*); antenna (*AN*); basal sensillum (*bs*); caudal segment (*CS*); dorsolateral tubercle (*dl*); foreleg (*fl*); genital sac (*GS*); hindleg (*hl*); midleg (*ml*); pedicel (*P*); posterolateral processes (*PP*); respiratory horn (*RH*); transverse band (*tb*). Fourth abdominal segment: dorsal posteromarginal tubercle (*dpm*); dorsal anterosubmarginal tubercle (*dasm*); lateral anterosubmarginal tubercle (*lasm*); lateral posteromarginal tubercle (*lpm*); ventral posteromarginal tubercle (*vpm*)

band; OL 0.14–0.15 mm (0.143, *n*=5); OW 0.15–0.17 mm (0.163, *n*=5); OW/OL 1.10–1.21 (1.15, *n*=6). Respiratory horn (Figs 1, 3, 10) medium-sized, slender, yellowish, distal half dark brown, surface with large scale-like spines; seven to eight apical spiracles, two to three laterally on basal half; pedicel short, stout, P 0.012–0.021 mm (0.016, *n*=5), RH 0.18–0.20 mm (0.19, *n*=5), P/RH 0.06–0.11 (0.083, *n*=3). Cephalothoracic tubercles as follows: anterodorsal tubercle (ad) (Fig 10) blunt, with two setae, one very long, thin, other minute; dorsolateral tubercle (dl) (Figs 1, 3, 10) prominent, with elongate, pointed, stout seta; dorsomedian tubercle (dm) (Fig 10) with short seta; dorsal tubercles (d) (Fig 4) as follows: i with short, stout seta, ii–iii with long, thin seta; iv minute seta, v pore. Two ventrolateral (vl) (Fig 11) thin setae, one long, other medium-sized; two ventromedial (vm) (Fig 11) setae, one medium-sized, other short. Abdominal segments integument with minute spicules spread. First abdominal segment (Figs 1, 12) with setae as follows: three anterolateral thin setae, two minute, one long; two anteromesal thin setae, one long, other short; two posterior setae, mesal one short, thin, other minute. Fourth segment (Figs 1, 5) with all tubercles with small base: two dorsal anterosubmarginal tubercles (dasm), i long, thin seta, ii short seta, i thiner than ii; five dorsal posteromarginal tubercles (dpm), i medium-sized seta, ii short seta, iii–iv without seta, v minute seta; three lateral posteromarginal



Figs 7–9 Female pupa of *Culicoides crucifer*; *7* cephalothorax (ventral view); *8* operculum; *9* seventh, eighth, and ninth (caudal) segments (ventral view). Anterodorsal tubercle (*ad*); anteromarginal tubercle (*am*); antenna (*AN*); basal sensillum (*bs*); dorsolateral tubercle (*dl*); foreleg (*fl*); midleg (*ml*); posterolateral processes (*PP*); transverse band (*tb*); ventrolateral setae (*vl*); ventromedian setae (*vm*); hindleg (*hl*)



Figs 10–12 Male pupa of *Culicoides crucifer*; *10* respitarory organ, anterodorsal, dorsolateral, and dorsomedial tubercles; *11*, ventromedian and ventrolateral setae: *12*, first abdominal segment. *Scale bars* 0.05 mm. anterodorsal tubercle (*ad*); dorsolateral tubercle (*dl*); dorsomedial tubercle (*dm*); pedicel (*P*); respiratory horn (*RH*); ventrolateral setae (*vl*); ventromedian setae (*vm*)

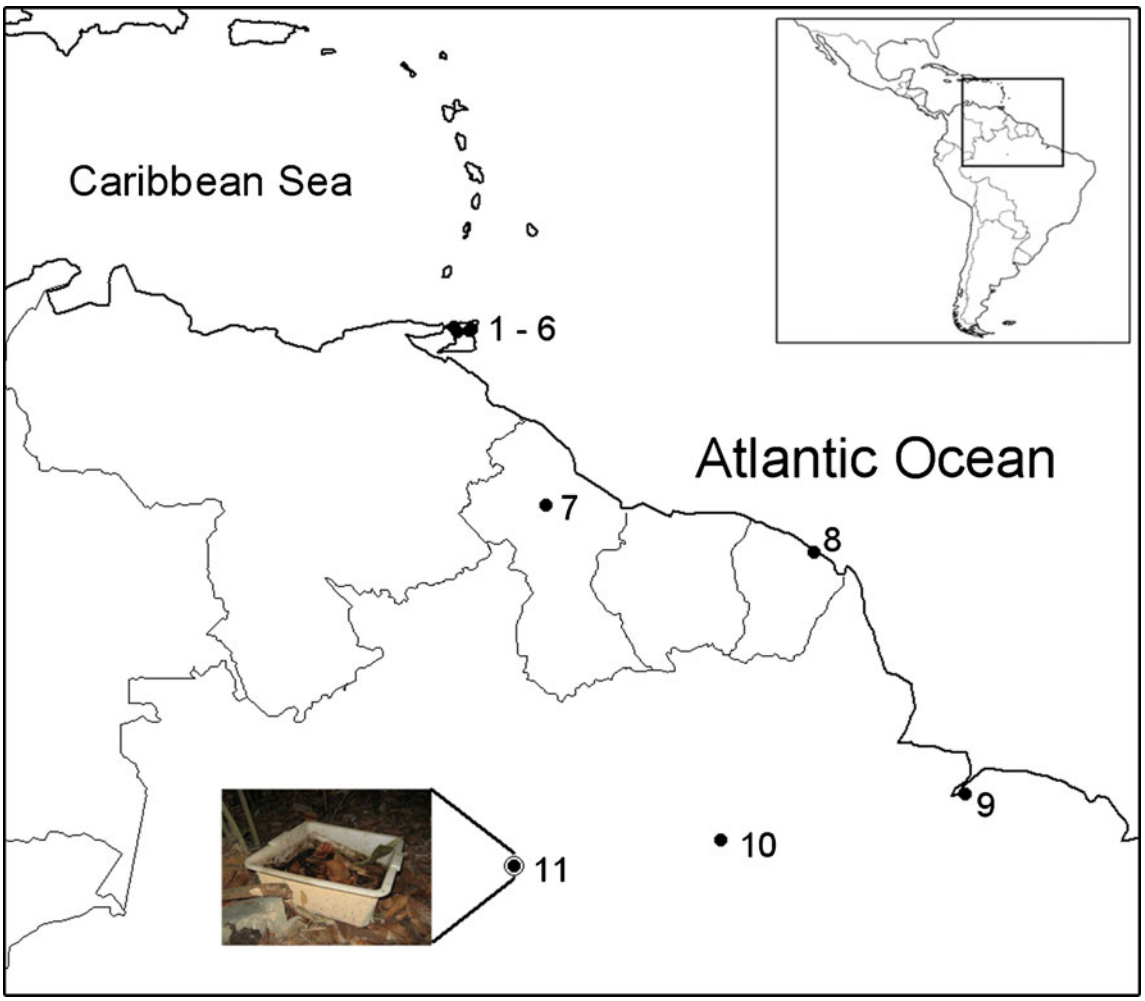


Fig 13 Map of species distribution and collecting site. *1* Cacandee Settlement; *2* Fort Read; *3* Port of Spain; *4* Sangre Grande; *5* U.S. Naval Station; *6* Vega de Oropouche; *7*Guyana; *8* Cayenna; *9* Outeiro; *10* Santarém; *11* Manaus (collecting site)

tubercles (lpm), i, iii spur-like, pointed setae, ii long, thin seta; one lateral anterosubmarginal tubercle (lasm), spur-like seta; three ventral posteromarginal tubercles (vpm), i minute seta, ii long, thin seta, iii short, stout seta. Caudal segment (Figs 1, 6) approximately 1.30 times longer than greatest width, length 0.22–0.24 mm (0.226, *n*=3), width 0.16 mm (*n*=3); with posteriorly directed spicules restricted ventrally to narrow anterior band, dorsally covering the whole segment; posterolateral processes moderately elongated, subparallel with pointed, dark tips; ventral surface of posterolateral processes with spicules present on each side of midline, dorsal surface totally covered by spicules.

Female pupa (Figs 7–9). Similar to male with sexual differences. Total length 2.75–2.90 mm (2.82, *n*=2). Operculum (Fig 8) OL 0.14 mm (*n*=3); OW 0.19 mm (*n*=3); OW/OL 1.35 (*n*=3). Respiratory organ length 0.20 mm (*n*=2); width 0.031–0.033 mm (0.032, *n*=2); pedicel length 0.016–0.019 mm (0.017, *n*=2); P/RO 0.080–0.1 (0.09, *n*=2). Cephalothorax (Fig 7) length 1.25–1.55 mm (1.35, *n*=3), width 0.75–0.87 mm (0.8, *n*=3). Caudal segment (Fig 9) with posteriorly directed spicules restricted to narrow anterior band, not connected to central inverted V-shaped patch of spicules; mesal spicules patch not extending to posterolateral processes, these moderately elongated, subparallel with pointed, dark tips; ventral surface of posterolateral processes with spicules present on each side of midline; CSL 0.23–0.25 mm (0.24, *n*=3), CSW 0.16–0.19 mm (0.176, *n*=3), CPL 0.080–0.12 mm (0.103, *n*=3).

Specimens examined. All the specimens collected as pupa, reared in laboratory (INPA), (03°05′46.97″S, 59°59′23.58″W), Brazil, Amazonas, 3.ii.2009, Ferreira-Keppler, one male (with pupal exuvia) (INPA), same data except 5.ii.2009, three males (with pupal exuviae), two females (with pupal exuviae) (INPA); same data except one male, one female (exuvia lost) (INPA); same data except 2.v.2010, one male (with pupal exuvia), one female (with pupal exuvia) (MLPA).

Distribution **(Fig 13).** Trinidad & Tobago, Guyana, French Guiana, Brazil (Amazonas, Pará). This is the first record of *C. crucifer* from the Amazonas State.

Bionomy. Pupae of *C. crucifer* were captured together with pupae of an undescribed species of *Dasyhelea Kieffer*. The period from pupa to adult lasted from 1 to 3 days in the laboratory.

Fox (1942) commented that the presence of scale-like spines covering the respiratory horn, as it is observed in *C. crucifer*, is typical of species inhabiting phytotelmata. Ronderos & Spinelli (2000) and Huerta *et al* (2001) extend this concept to the species breeding in all kind of calm and clean waters. As it was pointed out by Borkent & Craig (2001), another character related with these environments is the short and stout posterolateral processes of the caudal segment.

Taxonomic discussion

The pupa of *C. crucifer* is nearly identical to *C. annuliductus*, a species inhabiting Panama, except for the respiratory horn entirely pale yellowish and stouter, and for the posterolateral processes of the caudal segment slightly divergent in *C. annuliductus*. The adult of the latter species can be distinguished from *C. crucifer* by the short and swollen third palpal segment, smaller P/H ratio and by the spermathecae poorly sclerotized with necks bearing distinct microscopic annulations, among other slight differences.

The adult of *C. crucifer* is very similar to *Culicoides debilipalpis* Lutz, a widely distributed species from the Nearctic region southward to central Argentina. The morphology of the pupa is also very similar to *C. debilipalpis*, but the latter species shows slight differences mainly in the shape and thickness of setae on tubercles, as follows: the setae of the dorsal tubercles are: i minute seta, ii medium-sized seta, iv long seta, and v minute seta; the two vi setae are long and thin; the first abdominal segment bears one long and one medium-sized anteromesal setae, while the posterior setae are one long and the other minute; the i setae on dasm tubercles are short; and the i setae on the dpm tubercle are short, and the ones in iv are minute setae.

Acknowledgments Our gratitude to Pablo Marino, Nélida Caligaris and Luis Gianbelluca for technical assistance, and Mónica A. Caviglia for the English proofreading. We are grateful to PIP 0381–CONICET, PRJ 12.24/MCTI/ INPA. Also the PRONEX/CNPq/FAPEAM for the Financial support by the Programa de Apoio aos Núcleos de Excelência e Fundação de Amparo à Pesquisa do Estado do Amazonas.

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