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Identities revealed: female of *Anastrepha cruzi* Lima, 1934 and *Anastrepha caballeroi* Norrbom, 2015 (Diptera, Tephritidae) found in the Brazilian Amazon Rainforest

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Abstract

Anastrepha cruzi was described in 1934, from a specimen without an abdomen. The holotype is the only known record for this species, collected in the Brazilian Amazon Rainforest (location unknown). However, during fruit fly surveys with McPhail-type traps on the campus of the Federal University of Amazonas in Manaus (state of Amazonas), two females were identified as *A. cruzi*, based on thoracic and wing patterns. These females are described in detail here, including the aculeus tip, to clarify the identity and facilitate recognition of *A. cruzi*. A single female of *A. caballeroi* Norrbom was also collected in these surveys, and this species is recorded here for the first time in Brazil.

Key words: Taxonomy, Trypetinae, Fruit flies, Morphological characterization, New record, State of Amazonas

Introduction

The genus *Anastrepha* Schiner comprises around 330 known species in the American tropics and subtropics, although numerous additional species that have been collected remain to be described and formally named (Norrbom *et al*. 2021). Approximately 40% (128) of the valid species of *Anastrepha* occur in Brazil (Zucchi & Moraes 2023), of which about 42% (54) are found in the Brazilian Amazon Rainforest (Zucchi *et al.* 2011).

Some species of *Anastrepha* have been described based on a single specimen, and identification is sometimes problematic. One example is *A. cruzi* Lima, described from a specimen without an abdomen, collected near the "Rio Amazonas", state of Amazonas (Lima 1934). Recognition of this species has been based exclusively on thoracic and wing patterns that distinguish *A. cruzi* from other species of *Anastrepha*. However, its characterization is incomplete, as the female terminalia were not described. Since the original description, no other specimens of *A. cruzi* have been recorded in several surveys in the state (Silva *et al*. 2023).

Both species discussed here, *A. cruzi* and *A. caballeroi* Norrbom, were collected in McPhail-type traps during fruit fly surveys on the campus of the Federal University of Amazonas. The traps were situated in a fragment of Amazon Rainforest in the city of Manaus. In this same location, 19 species of *Anastrepha* were previously collected (Costa-Silva 2012), four of which were new records for the state (Costa-Silva *et al*. 2020).

Here, we provide a detailed description and illustrations of the female of *A. cruzi*, based on the Manaus females which were identified from their external morphology. Description of these females is important to clarify the identity of *A. cruzi* and diagnose it accurately from other species, since the identification of *Anastrepha* species is

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based mainly on the female terminalia. Our record of *A. cruzi* is the first following the original description, about 80 years ago.

The new record of *A. caballeroi* increases the number of *Anastrepha* species known to occur in Brazil to 129, and in the state of Amazonas to 45. This state harbors the highest known diversity of *Anastrepha* species, despite the meagre fruit fly surveys carried out in the Amazon.

The data from the description of the female of *A. cruzi* will allow a more appropriate treatment of this species in the interactive system for *Anastrepha* species (Norrbom *et al.* 2012).

Material and methods

Anastrepha fruit flies were collected in an urban fragment of the Amazon Rainforest on the campus of the Federal University of Amazonas (UFAM), Manaus, state of Amazonas, Brazil. This 694 ha area contains mainly Ombrophilous Dense Forest vegetation. The collections were carried out from September 2010 to September 2011, with McPhail-type traps baited with 10% sugar cane molasses + borax. The traps were hung on the trees approximately 1.80 m above ground level, and monitored weekly (Costa-Silva 2012). Both specimens were pinned, labelled and kept at MELQ collection.

Identification of *A. cruzi* was based on keys developed by Zucchi (2000) and Norrbom *et al*. (2012), and on its descriptions (Lima, 1934; Norrbom *et al*. 2012). The identification of *A. caballeroi* was based on Norrbom *et al*. (2015).

The wings were detached from the thorax with microforceps, submerged in Cellosolve™ for one to three days, and mounted on permanent microscope slides with Euparal. The slides were dried for seven days at 25 °C. For study of the aculeus morphology, the abdomen was removed and cleared in heated 10% potassium hydroxide for five to seven min and then washed in water to extrovert the aculeus. The abdomen and aculeus were placed in microvials with glycerin and attached to the pinned specimen.

Morphological terminology was based on White *et al*. (1999), Cumming & Wood (2017) for wing venation, and Stone (1942) for wing bands. Wing length was measured from the base of the costa to the wing apex in cell r_{4+5}; wing width was measured from the apex of vein R_1 to the posterior margin of cell m_4. The width of cell r_{4+5} at the level of dm-m was measured on a line from the junction of dm-m and M_1. The maximum width of cell r_{4+5} was measured perpendicular to vein M_1 at the widest subapical part. The apical width of cell r_{4+5} was measured from the apex of vein R_{4+5} to the junction of M_1 and the costa. The width of the distal part of the S-band was measured from the outer (anteroapical) margin of the costa to the inner (posterobasal) margin of the band perpendicular to the band at the apex of vein R_{2+3}. The width of cell r_{2+3} was measured on the same straight perpendicular line from the apex of vein R_{2+3} to R_{4+5}. Oviscape length was measured medially on the ventral side, from the ventromedial indentation to the apex. The aculeus tip length was measured ventrally from the sclerotized margin distal to the cloacal opening to the extreme apex. Illustrations of these measurements and the morphological characters described here can be seen in Norrbom *et al*. (2012).

The measurements were made using a micrometer in a Leica Wild M10 stereomicroscope. Wings were photographed with a Leica DFC 450 camera coupled to an M205C stereomicroscope. The aculeus and aculeus tip of both species were photographed with a Zeiss Axio Imager 2 light microscope. The program Corel Draw 2020 was used to adjust the photograph exposure, brightness, and contrast and to remove debris.

Acronyms for institutions where specimens are deposited: FIOC, Fundação Instituto Oswaldo Cruz; MHNJP, Museo de Historia Natural “Javier Prado”; and MELQ, Museu de Entomologia “Luiz de Queiroz”, Departamento de Entomologia e Acarologia da Escola Superior de Agricultura “Luiz de Queiroz”.

Results

Taxonomy

***Anastrepha cruzi* Lima, 1934**
(Figs. 1–3, 5–7)

Lima, 1934: 513. Type locality: Brazil, Amazonas, "Rio Amazonas" (exact location unknown). HT (without abdomen), vial nr. 1815 (FIOC) (examined by RAZ). Stone, 1942: 40 (taxonomic revision). Foote, 1967: 57.9 (catalogue). Zucchi, 1978: 41 (taxonomic revision). Norrbom *et al*. 1999: 78 (catalogue). Zucchi 1999: 260 (checklist). Zucchi 2000: 41 (key). Norrbom *et al*. 2012 (male description, key).

Diagnosis. *Anastrepha cruzi* can be recognized by the following combination of characters: V-band proximal arm connected anteriorly to S-band along vein R_{4+5} and in cell r_{2+3}, V-band distal arm not reaching vein R_{4+5}, separated from proximal arm or connected at mid-height of cell r_{4+5}; aculeus tip 0.28–0.30 mm long, slightly constricted, then elongate triangular, distal 0.73–0.74 triangular with moderate sized widely spaced serrations. In the key of Norrbom *et al*. (2012*), A. cruzi* runs to *Anastrepha zernyi* Lima, but differs in having a shorter aculeus (2.03–2.33 vs 4.00 mm in *A. zernyi*), and a quite different shape of the aculeus tip.

Description. Mostly orange. Setae reddish brown.

Head. Yellow to orange except brown ocellar tubercle. 4 frontal setae; 2 orbital setae. Ocellar seta weak, at most 1.5 times as long as ocellar tubercle. Facial carina, in profile, straight in dorsal two-thirds. Antenna not extended to ventral facial margin. Palpus in lateral view dorsally curved, evenly setulose. Face with ventral part gradually tapering laterally.

Thorax. Mostly orange; without brown marking, following areas white to pale yellow: postpronotal lobe and lateral margin of scutum bordering it; medial scutal vitta, with posterior end ovoid; sublateral scutal vitta from transverse suture to posterior margin, including base of intra-alar seta; scutellum; dorsal margins of anepisternum and katepisternum; katepimeron; and most of anatergite and katatergite. Subscutellum and mediotergite entirely yellow to orange. Mesonotum 2.90–3.70 mm long (holotype 4.0 mm, Zucchi 1978). Postpronotal lobe, notopleuron, scutum, and scutellum entirely microtrichose; scutal setulae yellow to orange. Chaetotaxy typical for genus. Katepisternal seta orange, much weaker and less than half as long as anepisternal seta.

Legs. Entirely yellow to orange.

Wing. Length 7.09–7.81 mm, width 3.00–3.27 mm, ratio 2.36–2.38. Apex of vein R_1 at 0.55 wing length, proximal to level of anterior end of crossvein r-m. Cell c 1.20–1.30 times as long as pterostigma; pterostigma 3.33–3.40 times as long as wide. Vein R_{2+3} not sinuous. Crossvein r-m at 0.64–0.65 distance from bm-m to dm-m on vein M_1. Vein M_1 moderately curved apically; cell r_{4+5} at apex 0.80–0.92 times as wide as at level of dm-m, 0.68–0.78 times as wide as maximum subapical width. Cell cua with distal lobe relatively short, length of cua 1.44–1.46 times as long as anterior margin, lobe 0.58–0.65 times as long as vein CuA+CuP. Wing pattern (Figs. 1–3) mostly orange and medium brown. C-band mostly orange, most of cell c sometimes paler but without subapical hyaline area, most of pterostigma orange-brown, distal margin in cells r_1 and r_{2+3} narrowly brown, fork of vein Rs with small ovoid brown spot, junction of C and crossvein h without brown spot and cell br with small ovoid brown mark on apical margin of band near vein R_{4+5}. C-band and S-band narrowly connected or not connected along vein R_{4+5}. Basal hyaline area in cell dm relatively small, occupying less than one-fifth of the cell. Cell bm hyaline, microtrichose only on subapical fold. Basal half of S-band mostly orange, anterobasal margin narrowly brown except in cells br and dm, posterodistal margin narrowly brown, more broadly in cell m_4, but at most extending to apex of lobe of cell cua, margin with weak or no incision in cell m_4; distal section narrowly brown on most of posterior margin and in cell r_{4+5}; moderately broad at apex of vein R_{2+3} 0.52–0.65 times width of cell r_{2+3}, without marginal hyaline areas; hyaline area proximal to apex of band extended to vein R_{2+3}. V-band with proximal arm brown in cell m_4 and on most of proximal and distal margins; connected anteriorly to S-band along R_{4+5} and in cell r_{2+3}; posterior margin extending in less than two-thirds of distance to vein CuA+CuP; distal arm mostly brown, connected to proximal arm at mid-height of cell r_{4+5} (Fig. 2) or not connected (Fig. 1, 3); hyaline area between arms of V-band (when connected) and vein M_1 less than one-third height of cell r_{4+5}.

Abdomen. Mostly orange, without brown markings.

Female terminalia. Oviscape 2.77–3.14 mm long, 0.98–1.11 times as long as mesonotum, straight in lateral view; entirely orange to pale brown; spiracle at basal 0.36–0.37. Eversible membrane not dissected, pattern of dorsobasal denticles not visible. Aculeus 2.03–2.33 mm long, 0.73–0.74 times oviscape length; in ventral view base expanded (Fig. 5), 0.19–0.25 mm wide, shaft 0.11–0.12 mm wide at midlength; slightly ventrally curved in lateral view (Fig. 6); tip (Fig. 7) 0.28–0.30 mm long, 0.13 times aculeus length, 0.09 mm wide at base, 0.09–0.11 mm wide preapically, 2.75–2.86 times as long as preapical width; in ventral view slightly constricted, then elongate triangular, distal 0.73–0.74 triangular with moderate sized, widely spaced serrations. Spermathecae not dissected.

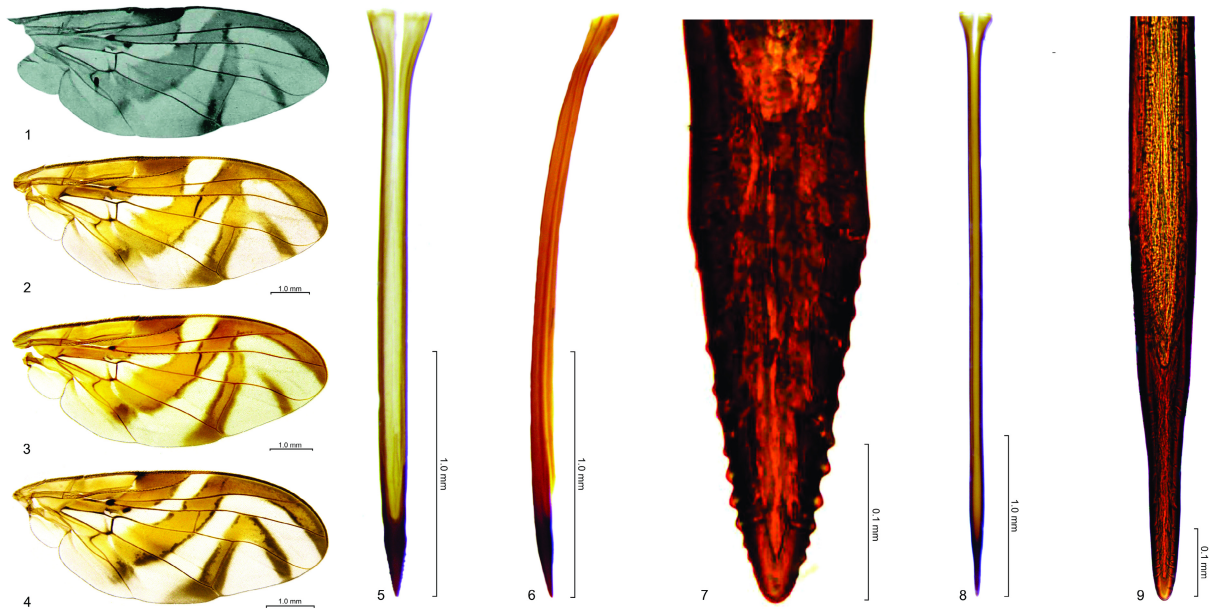
Type data. The holotype (without abdomen) is pinned and kept in vial nr. 1815, and the wing is mounted on microscope slide nr. 1904, both examined by Zucchi (1978) (FIOC collection).

Distribution. *Anastrepha cruzi* is recorded only in the state of Amazonas, Brazil. Manaus city is the first known precise location, since the type locality, "Rio Amazonas", is extremely vague because the Amazon River extends for hundreds of kilometers across the state.

Biology. The host plants and other aspects of the biology of this species are unknown.

Material Examined. BRAZIL: 2♀; Amazonas, Manaus, Federal University of Amazonas, 03°05′51.1″S, 59°58′23.8″W, 92 m, collected on 15 July 2011, McPhail traps, F.C. Costa-Silva leg. (MELQ ESALQENT001775 and 001776).

Comments. *Anastrepha cruzi* was originally described by Lima (1934) based on a single specimen without abdomen. To our knowledge, it has not been collected since. In his original description, Lima (1934) wrote that one specimen (without abdomen) with the indication "Rio Amazonas" was brought from Amazonia by the wise master Dr. Oswaldo Cruz, in 1909 (p. 514). However, after almost 80 years, two females that appear to be this species were collected in an urban fragment of Amazon Rainforest in Manaus, capital city of the state of Amazonas. We identified the females as *A. cruzi* based on the following morphological characters: subscutellum and mediotergite entirely yellow, proximal arm of V-band connected anteriorly to S-band, and the distal arm not reaching vein R_{4+5}, connected to proximal arm at mid-height of cell r_{4+5} or isolated. In the key of Norrbom *et al*. (2012), *A. cruzi* runs to *A. zernyi*, but differs in having a shorter aculeus tip (see diagnosis), and a quite different shape of the aculeus tip (see description of *A. zernyi* in Norrbom *et al*. 2012). The above description is based primarily on the Manaus females.



FIGURES 1–4. Wings: 1, *Anastrepha cruzi* (holotype, Lima 1934; Fig. 19); 2–3, *A. cruzi* from Manaus, AM (V-band variation); 4, *A. caballeroi.* **FIGURES 5–9.** Aculei and tips, ventral unless otherwise indicated: 5–7, *A. cruzi* (6, lateral view); 8–9, *A. caballeroi.*

New Record in Brazil

Anastrepha caballeroi Norrbom, 2015

(Figs. 4, 8, 9)

Norrbom *et al*. 2015: 13. Type locality: Peru, Cusco, Estación Biológica Villa Carmen. Holotype ♀ (MHNJP) and paratypes ♀♂ deposited in several institutions (Norrbom *et al*. 2015); Rodriguez *et al*. 2023: 104 (host plants).

Distribution. This species has previously been recorded only from Peru (Cusco and Madre de Dios) (Norrbom *et al*. 2015; Rodriguez *et al*. 2023). Here, we provide the first report from Brazil, in a fragment of Amazon Rainforest in the city of Manaus, state of Amazonas.

Material Examined. BRAZIL: 1♀; Amazonas, Manaus, campus of the Federal University of Amazonas, 03°06′08.3″S, 59°58′31.6″W, 92 m, collected on 15 July 2011, baited McPhail-type trap, F.C. Costa-Silva leg. (MELQ ESALQENT001777).

Host. *Anastrepha caballeroi* larvae were found feeding on fruit pulp of *Quararibea malacocalyx* A. Robyns and S. Nilsson (Malvaceae) (Norrbom *et al*. 2015; Rodriguez *et al*. 2023).

Comments. The identification was based on the morphological diagnostic characters proposed by Norrbom *et al*. (2015), namely, C and S-bands separated, V-band proximal and distal arms connected along vein R_{4+5} (Fig. 4); aculeus 3.39–4.11 mm long (Fig. 8), aculeus tip 0.29–0.36 mm long, non-serrated (Fig. 9) (see Norrbom *et al*. 2015, for details).

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