

Three new species of *Zygothrica* (Diptera: Drosophilidae) from the Neotropical region, Brazil

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([urn:isid:zoobank.org:pub:37B52B2E-936D-4FDB-BE36-C52E4F3D932D](https://urn.isid:zoobank.org/pub:37B52B2E-936D-4FDB-BE36-C52E4F3D932D))

ARTICLE INFO

Article history:

Received 29 May 2021

Accepted 20 September 2021

Available online 25 October 2021

Associate Editor: Marcia Couri

Keywords:

Neotropical biodiversity

Mycophagy

Drosophilinae

Taxonomy

ABSTRACT

Zygothrica (Diptera: Drosophilidae) is an almost entirely Neotropical genus, with thirteen species found in other biogeographical regions. The genus includes around 130 valid species, of which 54 occur in Brazil. There have been no new descriptions of species of this genus since the 1987s, although a large number of undescribed species are recognized in entomological collections. Here, three species of *Zygothrica* Wiedemann (1830) are described from Brazil, and figures are presented for external morphology and terminalia.

Introduction

Zygothrica Wiedemann (1830) was first proposed as a subgenus of *Achias* (Platystomatidae: Diptera), and then elevated to genus by Loew (1873). Later, a pioneering study was carried out by Sturtevant (1920), where he reviewed all previously described Drosophilidae species and proposed a new species of *Zygothrica*. Subsequently, Duda (1925) and Burla (1956) studied and described species from Costa Rica and Brazil, respectively, increasing the number of species in the genus. Despite these studies, until the 1980s there were inconsistencies in genus delimitation. Burla (1956) proposed some diagnostic characters for *Zygothrica*, which were later, refuted by others taxonomists because they overlap with some *Hirtodrosophila* Duda 1923 characters. In 1987, a more accurate diagnosis for *Zygothrica* was proposed by Grimaldi, together with a phylogenetic hypothesis and ecological aspects of some species, in his monographic paper on the systematics and phylogeny of the genus.

To date, the genus is predominantly Neotropical and has around 130 valid species (Bächli, 2020), of which 54 occur in Brazil (Grimaldi, 1990; Gottschalk et al., 2008; Robe et al., 2014; Tidon et al., 2017). No new species descriptions of this genus have been reported to the Neotropical region since 1987, when Grimaldi described 49 new species, representing the most substantial contribution of his study. However, a large number of unidentified specimens have been deposited in entomological collections. In this paper, we describe three new species from Brazil, and present figures for external morphology and terminalia.

Materials and methods

Individuals were identified based on external morphology and the analysis of male or female terminalia, which were prepared following a protocol adapted from (Bächli et al., 2004; Mendes and Gottschalk, 2019). The terminalia were disarticulated in glycerin and mounted

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on temporary microscope slides with glycerin jelly (aqueous solution of 2% gelatin and 50% glycerin) (Grimaldi, 1987) for illustration and photographic records. Subsequently, the microscope slides were dismantled and the genital sclerites were placed in microtubes with glycerin and attached to the specimen.

In five individuals of *Zygothrica duovittata* sp. nov., the terminalia had previously been mounted on permanent slides with natural Canada balsam. In this case, we dismantled the slides following the protocol proposed by Vilela and Bächli (2000). As the Canada balsam in the microscope slides did not stiffen, the coverslips covering the male terminalia were easily removed without the use of xylol. The genital sclerites were transferred to a depression slide with a drop of creosote, and were observed hourly until the sclerites seemed clear. Subsequently, the sclerites were placed in 100% ethanol for 2 minutes and then in glycerine to be disarticulated and mounted on a slide with glycerine jelly (as described above) for illustration and photographic records.

The terminalia were drawn using an Olympus CL 6000 optical microscope with an attached camera lucida (1.8x magnification for the camera lucida, 20x for the objective lens and 10x for the eyepiece lens). One individual of each species were selected and photographed with a Zeiss Discovery V.20 photomicroscope, in lateral, dorsal and frontal views. The wing was also photographed in detail. The images were then stacked using the software AxioVision 4.9.1.

The morphological nomenclature followed Cumming and Wood (2010). Measurements of body structures and color descriptions followed Grimaldi (1987) and Vilela and Bächli (2000). Complementary information added to the original labels was obtained from the database of the Instituto Brasileiro de Geografia e Estatística and presented between brackets in the type series characterization. Species distribution information was obtained from TaxoDros v.1.04 (2019/02) (Bächli, 2020).

The three species described were already dry and pinned, except to the paratypes of *Zygothrica duovittata* sp. nov. which were dried with the hexamethyldisilazane protocol (Brown, 1993) and then pinned. Here, species were morphologically compared with photographs of known *Zygothrica* species, which are mainly type series deposited in collections. Finally, all specimens studied still are preserved in dry way in a paper triangle on entomological pins, except to abdominal sclerites and terminalia attached in glycerine and pinned with the respective specimens. All holotypes and paratypes are deposited in the Entomological Collection of the Instituto Oswaldo Cruz (CEIOC), Rio de Janeiro, RJ, Brazil.

Results

Taxonomy

The species groups arrangement followed Grimaldi (1987) and Burla (1956).

Zygothrica Wiedemann

Zygothrica Wiedemann, 1830: 16 (subgenus of *Achias* Fabricius). Type species *Achias dispar* Wiedemann, 1830 (original designation). Subsequent references: Sturtevant, 1920: 156; Burla, 1956: 215; Grimaldi, 1987: 147.

Drosophilura Hendel, 1913: 389. Type species *Drosophilura caudata* Hendel, 1913 (original designation).

Tanyglossa Duda, 1925: 189 (subgenus of *Zygothrica*). Type species *Zygothrica tenuirostris* Duda, 1925. Preoccupied by Meigen, 1803: 267 (Diptera: Tabanidae).

Diagnosis. See Grimaldi (1987).

dispar group

aldrichi subgroup

Zygothrica japunibaensis sp. nov.

(urn:lsid:zoobank.org:act:DABA9659-236C-4C35-8C6E-D56405244409)
(Figures 1-3; 10A)

Type-series. Holotype ♂ labeled "*Zygothrica japunibaensis* sp. nov. Mendes *et al.* HOLOTYPE ♂", "[Brazil], E[stado] do Rio [de Janeiro], Angra [dos Reis], Japun[í]ba, L. Trav. F; VII.[1]936; Col: L. Trav.". Abdominal sclerites and terminalia in vials with glycerine; wing was removed and mounted on a permanent microscope slide and all material was attached to the specimen.

Type locality. Japuníba, city of Angra dos Reis, State of Rio de Janeiro, Brazil.

Diagnosis. Body color mainly brown; mesonotum brown with two yellow narrow stripes in the line of the dorsocentrals, extending up to 2/3 of the length of mesonotum; acrostichal setulae in 6 lines; whitish-yellow pleurae; abdomen brown pollinate, tergites III and VI lighter; arista with 5 dorsal branches, 1 ventral and 3 short inner branches, plus terminal fork; first flagellomere ochre, hairy, length about 2.5x the length of the pedicel; facial carina prominent, ochre; legs yellow, apex of tibia darker; wings with apical spot, dM-Cu infuscated, mid-radial spot reaching R₂₊₃ and proximal radial-medial spots.

Description. ♂

Head. Brown; head width = 0.9mm, slightly wider than thorax (hypercephalic). Eyes dark red, bare. First flagellomere ochre, hairy, length about 2.5x the length of the pedicel, and about 2x the width. Aristae



Figure 1 *Zygothrica japunibaensis* sp. nov., Holotype ♂. Lateral view. Scale bar: 1.0 mm.

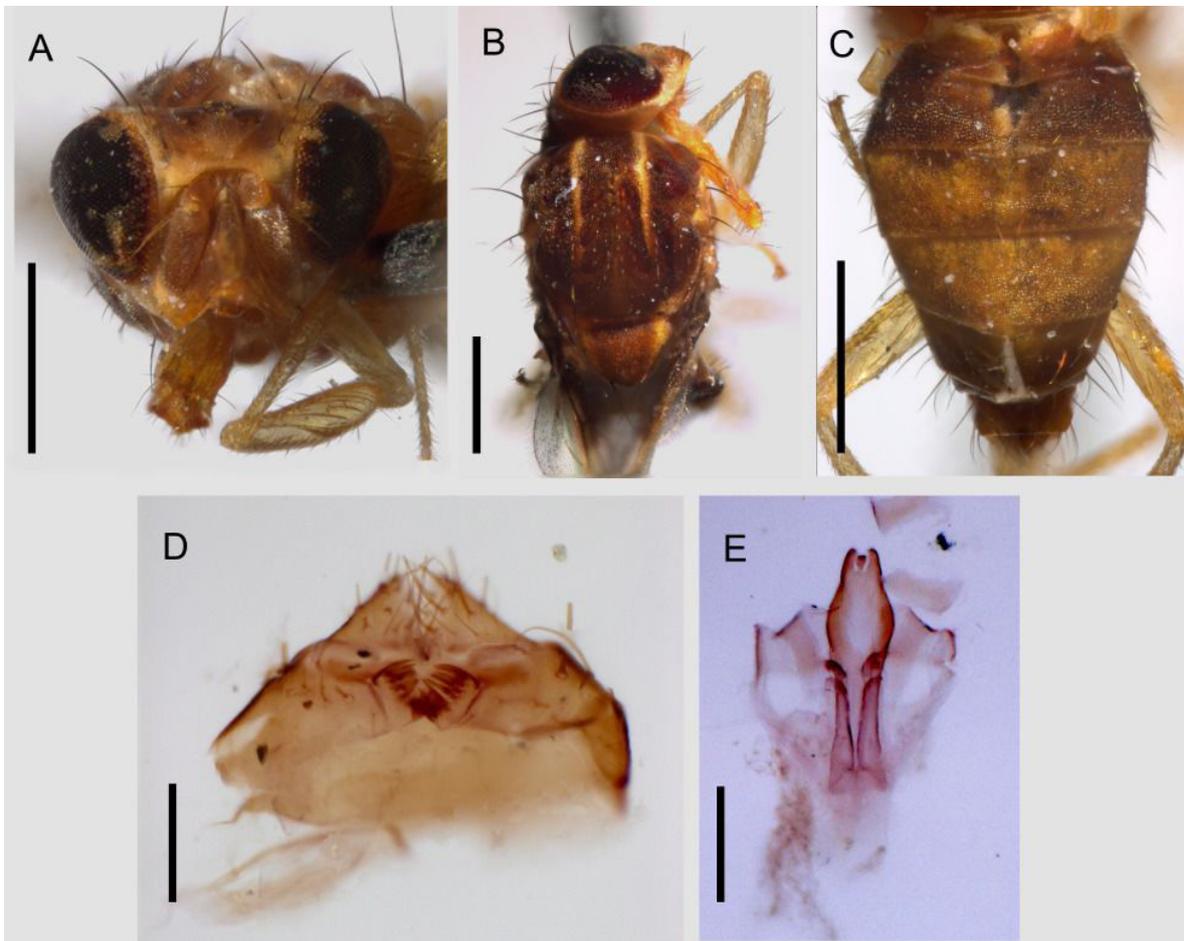


Figure 2 *Zythrica japonibaensis* sp. nov., Holotype ♂. a: head, front view; b: thorax, dorsal view; c: abdomen, dorsal view; d: epandrium, cerci, surstyli, and subepandrial sclerite, posteroventral view; e: phallus, phallapodeme, hypandrium, pregonites, and postgonites, ventral view. Scale bars: a, b, c: 0.5 mm; d, e: 0.1 mm.

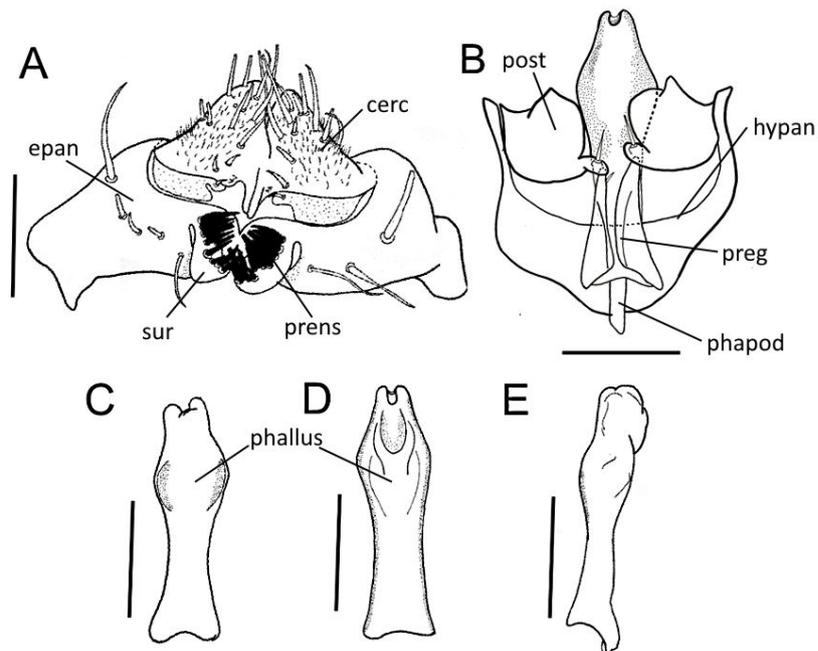


Figure 3 *Zythrica japonibaensis* sp. nov., Holotype ♂. Male terminalia. a: epandrium, cerci, surstyli, and subepandrial sclerite, posterior view; b: phallus, phallapodeme, hypandrium, pregonites, and postgonites, ventral view; c-e: phallus and phallapodeme, dorsal, ventral, and oblique lateral views, respectively. Scale bars: 0.1 mm. *Abbreviations*: cerc, cercus; epan, epandrium; post, postgonite; hypan, hypandrium; phapod, phallapodeme; sur, surstylus; prens, prensisetae.

with 5 dorsal branches, 1 ventral and 3 short inner branches, plus the terminal fork. Orbital plates yellow. Orbital bristles dark brown. Distance between or1 and or2 about 60% of the distance between or1 and or3. Frons brown; ocellar triangle slightly darker, covering 1/3 of the length of the frons; length of the frons about 1.5x the anterior width; posterior width of the frons about 2/3 of the anterior width. Brown median frontal vitta almost reaching the ptilinal fissure; postocellar bristles convergent. Face and gena ochre. Proboscis ochre, with lighter palps.

Thorax. Mesonotum brown, with two narrow stripes in the line of the dorsocentral, extending up to 2/3 of the length of mesonotum; 6 lines of parallel acrostichal setulae. Notopleura, supra-alar area and postalar callus yellow. Scutellum brown, marginally yellow. Basal scutellar setae converging. Pleurae yellow. Legs yellow, apex of tibia darker. Thorax length = 1.04mm. Thorax width = 0.66mm.

Wings. Membrane with color pattern as follows: apical spot, dM-M infuscated, mid-radial spot reaching R_{2+3} and proximal radial-medial spots. R_{2+3} straight toward C, R_{4+5} and M slightly convergent, both slightly curved posteriorly. Halteres yellow. Indices: C = 1.80; ac = 3.87; hb = 0.70; 4c = 0.93; 4v = 1.22; 5x = 1.00; M = 0.25; prox x = 0.43. Wing length = 2.13mm.

Abdomen. Light brown; tergites I, II, V and VI darker than tergites III and IV.

Terminalia. Epandrium rounded not microtrichose, longer than hypandrium; ventral lobes small with 4 bristles. Cerci free, microtrichose, with long bristles. Hipoproctal plate (*sensu* Grimaldi, 1987) present. Ventral cercal lobes (*sensu* Grimaldi, 1987) membranous. Surstyli rounded, with 10-14 prensisetae arranged in crescent format. Subepandrial sclerite previously projected ahead. Postgonites (gonopods *sensu* Grimaldi, 1987) square-shaped, with one prominent bristle and fused to the hypandrium. Pregonites (paraphysis *sensu* Grimaldi, 1987) fused to postgonites and without obvious bristles. Hypandrium V-shaped. Phallus (basiphallus + distiphallus *sensu* Grimaldi, 1987) tubular, with basiphallus narrower than distiphallus, with an indent in the apex, bare; length of the phallus about 3x the width of distiphallus; basiphallus fused to the phallapodeme (aedeagal apodeme *sensu* Grimaldi, 1987). Phallapodeme shorter than phallus.

♀ Unknown.

Geographic distribution. Known only from type locality.

Etymology. The species epithet refers to the type locality of Japuníba, situated in the city of Angra dos Reis, State of Rio de Janeiro, Brazil.

Comments: The phallus is similar to *Z. nigropleura* Grimaldi 1987, but the distiphallal scales are absent and the length of the phallapodeme is smaller in relation to the phallus in *Z. japunibaensis* sp. nov.

orbitalis group

Zygothrica duovittata sp. nov.

(urn:lsid:zoobank.org:act:BA6C2E5B-6378-4C6A-B858-2DAACA3D02D4)
(Figures 4-6; 10B)

Type series. Holotype: ♂ labeled "*Zygothrica duovittata* sp. nov. Mendes *et al.* HOLOTYPE ♂", "Brasil, Paraná, Diamantina do Norte, Est[ação]. Ecol[ógica]. do Caiuá; 22°49'38"S 45°39'45"W; 30.IV.2011; Col: J. P. Junges". Paratypes: 14 ♂ labeled "*Zygothrica duovittata* sp. nov. Mendes *et al.*; PARATYPE 01 ♂" "Brasil, São Paulo, Teodoro Sampaio, P.E. Morro do Diabo; 03.V.2011; Col: J. P. Junges"; 01 ♂ labeled "*Zygothrica duovittata* sp. nov. Mendes *et al.*; PARATYPE ♂", "Brasil, [Pará], Belém do Pará, Utinga; 22.VI.[19]65; Col: H.S. Lopes". Abdominal sclerites and the terminalia were stored in microvials with glycerine, one wing was removed and prepared on a permanent microscope slide and all materials were attached to the specimens. Finally, the material type was fixed in ethanol 70%.

Type locality. Estação Ecológica do Caiuá, city of Diamantina do Norte, State of Paraná, Brazil (22°49'38"S, 45°39'45").

Diagnosis. Predominantly yellow body; scutum light brown, with four dark brown bands between dorsocentral bristle lines, the central pair darker and wider (the intensity of the coloration could vary); 8-9 irregular lines of acrostichal setulae between dc; scutellum light brown, slightly lighter margin; pleura yellow; abdomen yellow, with medial black bands on tergites I, II, III and IV; arista with 5 dorsal, 1 ventral and 4 short inner branches, plus the terminal fork; first flagellomere light brown, length about 2.5x the width, and about 2x the length of the pedicel; carina facial light brown; legs yellow; wings hyaline, without spots; R_{2+3} straight, R_{4+5} slightly convergent toward M.

Description. ♂

Head. Brown; width = 1.10 (0.96-1.13) mm. Eyes red, bare. Pedicel and scape brown, flagellomere brownish yellow, length about 2.5x the width, and about 2x the length of the pedicel. Arista with 5 dorsal branches, 1 ventral and 4 short inner branches, plus the terminal fork. Orbital plates brown. Orbital bristles brown. Distance between or1 and or2 = 0.06 (0.05-0.08) mm, between and1 to or3 = 0.10 (0.9-0.11) mm and between or2 and or3 = 0.04 (0.04-0.07) mm. Front ochre. Ocellar triangle brown, occupying approximately 1/2 of the frontal length; anterior frontal width equal to length, posterior width slightly smaller than anterior. Face ochre. Gena yellow. Proboscis, palps and labellum yellow.

Thorax. Brown, with four dark brown bands, the two central are more intense and wider; 8-9 irregular lines of acrostichal setulae between the dorsocentral bristles; scutellum brown and slightly lighter in the margins; yellow pleura; 2 katepisternal setae, posterior about 2x the length of the anterior. Legs yellow. Thorax length = 1.07 (1.07-1.29) mm, width = 0.75 (0.84-0.98) mm.

Wings. Hyalines, without distinct spots; veins R_{2+3} straight, R_{4+5} slightly curved towards M; bM-Cu absent; halteres yellow. Indices: C = 2.63 (1.87-3.20); ac = 3.15 (2.20-3.76); hb = 0.58 (0.52-0.63); 4c = 0.82 (0.60-0.88); 4v = 1.39 (1.24-1.48); 5x = 1.44 (1.18-1.82); M = 0.35 (0.29-0.42); prox. x = 0.34 (0.32-0.40). Wing length = 2.74 (2.17-2.46) mm.

Abdomen. Yellow, with medium black bands on tergites II, III and V, extending from anterior to posterior margin and not extending laterally. Note: The color intensity of the tergites may vary.



Figure 4 *Zygothrica duovittata* sp. nov., Holotype ♂. Lateral view. Scale bars: 1.0 mm.

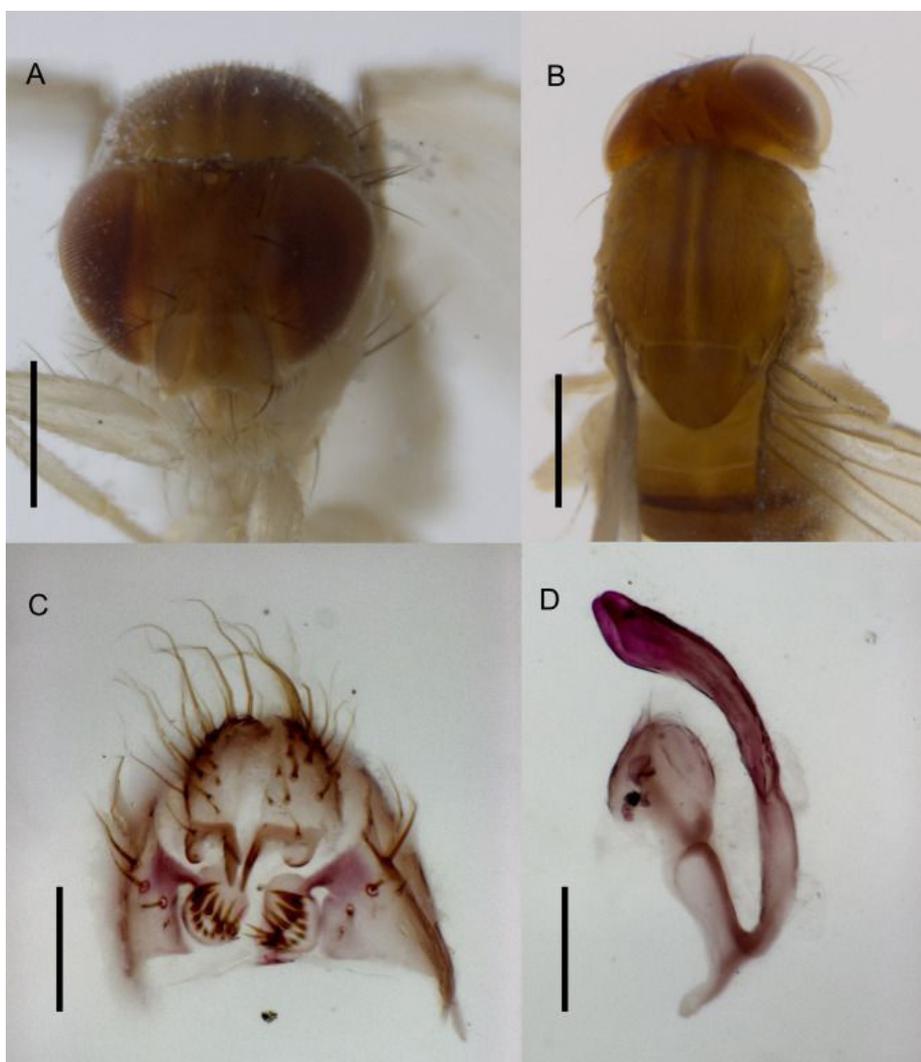


Figure 5 *Zythrica duovittata* sp. nov., Holotype ♂. a: head, frontal view; b: thorax, dorsal view; c: epandrium, cerci, surstyli and subepandrial sclerite, ventroposterior view; d: phallus, phallapodeme, and postgonites, lateral view. Scale bars: a-b: 0.5 mm; c-d: 0.1 mm.

Terminalia. Epandrium with U-shaped form, ventral lobes small. Cerci elongated posteriorly, with long bristles and microtrichose (except in the inner margin). Hipoproctal plate present, with two median-ventral elongated processes and one pair of bristles. Surstyli rounded, with 10-16 prenisetae arranged in two rows. Prominent and previously designed subepandrial sclerite. Large postgonites (gonopods *sensu* Grimaldi, 1987), attached to the hypandrium and containing a median bristle on the inner margin. Large pregonites (paraphysis *sensu* Grimaldi, 1987) fused to gonopods and with three obvious bristles. Hypandrium V-shaped, as long as the epandrium and with many growth lines. Phallus (aedeagus *sensu* Grimaldi, 1987) with margin ornamented by scales fused to the phallapodeme (aedeagal apodeme *sensu* Grimaldi, 1987) with irregular/wavy margin, with two apical projections, broad in the apex, with scales in the ventral portion and small bristles laterally. Phallapodeme shorter than phallus and tapered in lateral view.

♀ Unknown.

Geographic distribution. The holotype and paratype specimens were collected in the Estação Ecológica do Caiuá, Diamantina do Norte, Paraná, Brazil (22°49'38"S 45°39'45"W); Teodoro Sampaio, São Paulo, Brazil; and Utinga, Belém, Pará, Brazil.

Etymology. The specific epithet derives from the Latin “duo” which means two and “vitta” which means stripes or bands, and was proposed in reference to two darker longitudinal stripes in the middle of the thorax of the specimens.

vittatifrons group

Zythrica grajau sp. nov.

(urn:lsid:zoobank.org:act:348F7C30-2441-4CC0-A74A-0E05D8F2E690) (Figures 7-9; 10C)

Type. Holotype ♂ labeled with the information “*Zythrica grajau* sp. nov. Mendes *et al.* HOLOTYPE ♂”, “Brasil, [Rio de Janeiro], Rio de Janeiro, Grajau; 21.II.1965; Col: H.S. Lopes”. Abdominal sclerites and the terminalia were stored in a microvial with glycerine, the wing was removed and prepared on a permanent slide with Canada balsam. The material was attached to the specimen.

Type locality. Grajáú, Rio de Janeiro, Rio de Janeiro, Brazil.

Diagnosis. Body color predominantly yellow. Thorax yellow, with three pairs of dark brown longitudinal stripes in the scutum, a pair of stripes between dorsocentral bristles, a pair along the dorsocentral and a pair laterally to the dorsocentral, interrupted in the transverse

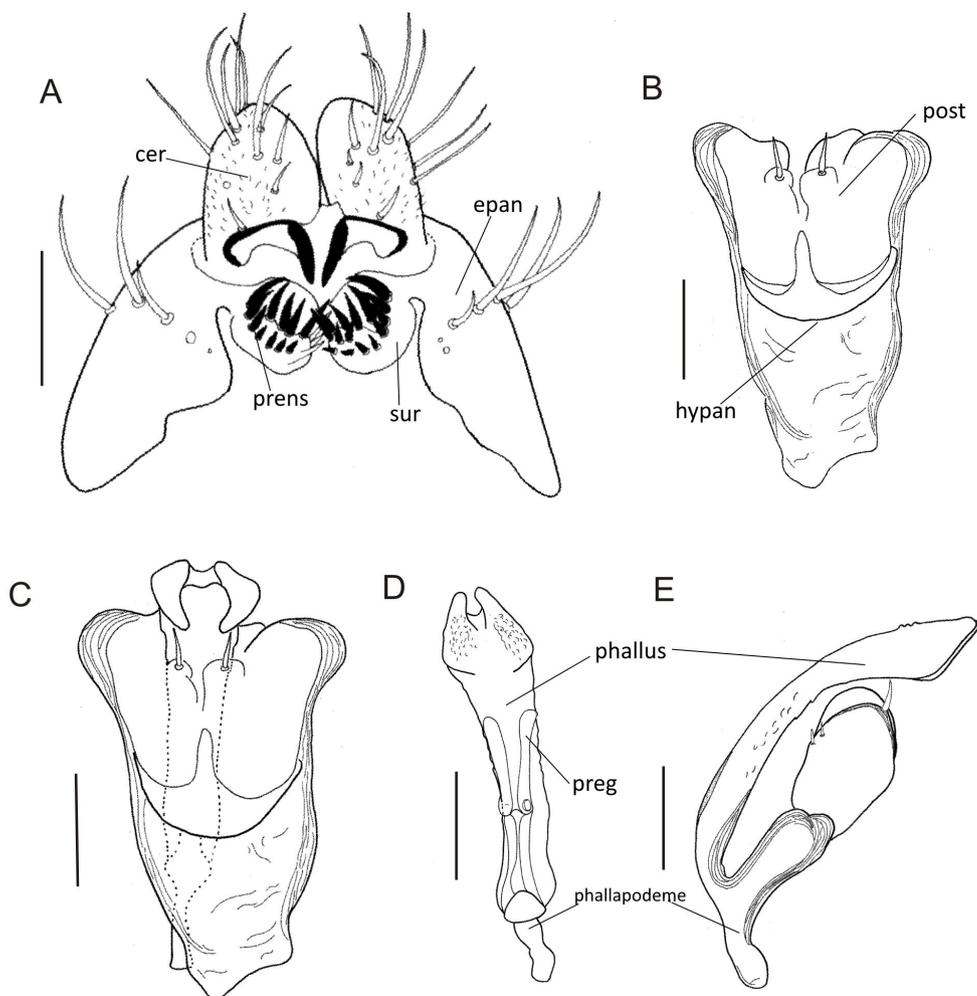


Figure 6 *Zygothrica duovittata* sp. nov., Holotype ♂. Male terminalia. a: eandrium, cerci, surstyli, and subepandrial sclerite, posterior view; b-c: phallus, phallapodeme, hypandrium, pregonites, and postgonites, ventral view; d-e: phallus and phallapodeme, ventral and lateral views, respectively. Scale bars: 0.1 mm. *Abbreviations*: cerc, cercus; epan, eandrium; post, postgonite; preg, pregonite; hypan, hypandrium; sur, surstylus; prens, prenisetae.

suture; scutellum almost entirely dark brown with three yellow bands, one median and two laterally; convergent basal scutellar bristles; pleura yellow. Abdomen yellow; tergites I yellow, II and III with dark brown bands extending laterally without reaching the margin, tergites IV to VI with brown rounded spots in the median region; aristae with 5 dorsal, 2 ventral and 3-4 short inner branches, plus terminal fork; flagellomere yellow; prominent facial carina, slightly whitish-yellow; legs yellow. Wings hyaline; lappet and bM-Cu absent; brown spot on the apical portion of the wing and extending from the final portion of C to just before M. R_{4+5} slightly curved. Halteres yellow.

Description. ♂

Head. Yellow. Width = 0.68 mm. Eyes dark red, with interfacetal setulae. Escape yellow, pedicel brown and flagellomere yellow, slightly whitish; length of the flagellomere approximately 2x the length of the pedicel. Aristae with 5 dorsal, 2 ventral and 3-4 short inner branches, plus terminal fork. Orbital plates yellow. Orbital bristles dark brown. Distance between or1 and or2 = 0.06mm, between or1 and or3 = 0.13mm and between or2 and or3 = 0.07mm. Frons with dark brown frontal vittae, and orbital plates yellow. Ocellar triangle yellow, corresponding to more than 2/3 of the length of the forehead; region between ocelli dark brown; convergent postocellar bristles; anterior width of the frons equivalent to the length, posterior width slightly smaller than



Figure 7 *Zygothrica grajau* sp. nov., Holotype ♂. Lateral view. Scale bars: 1.0 mm.

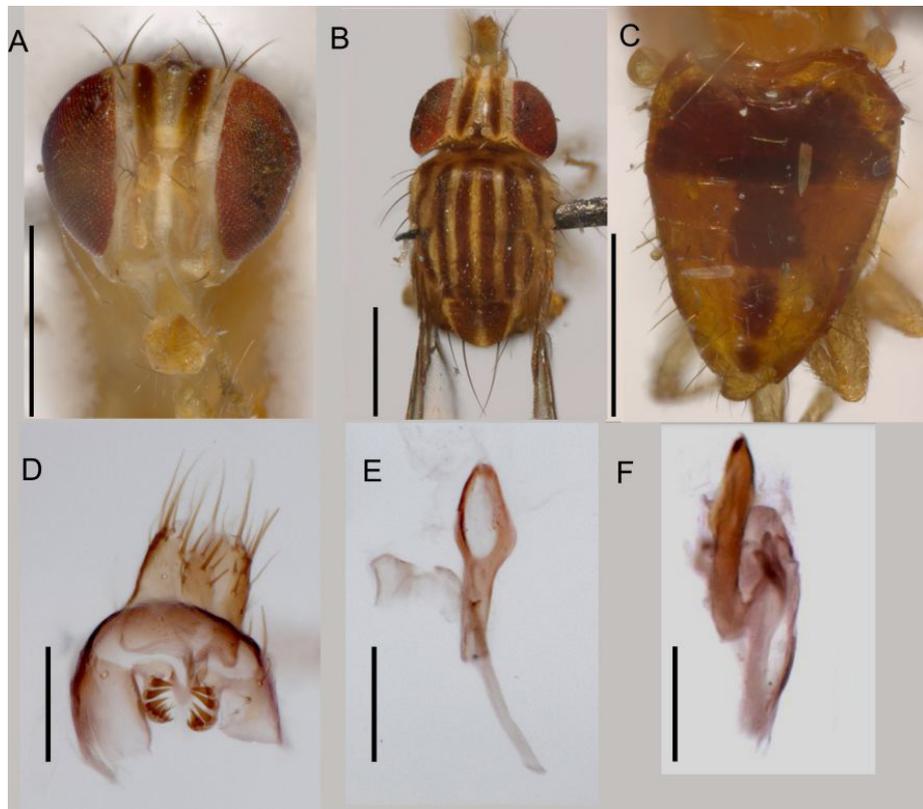


Figure 8 *Zygothrica grajau* sp. nov., Holotype ♂. a: head, frontal view; b: thorax, dorsal view; c: abdomen, dorsal view; d: epandrium, cerci, surstyli, and subepandrial sclerite, posteroventral view; e-f: phallus, phallapodeme, and postgonites, ventral view; f: phallus, phallapodeme, and postgonites, ventral view. Scale bars: a-c: 0.5 mm; d-f: 0.1 mm.

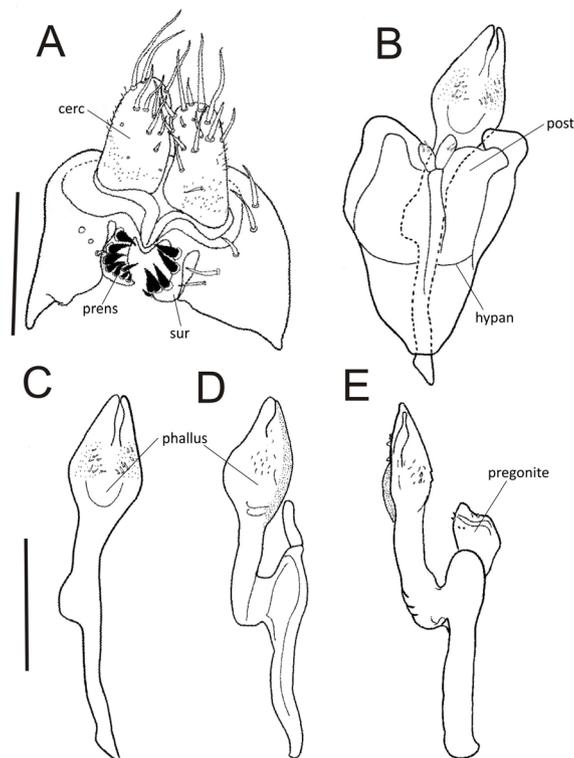


Figure 9 *Zygothrica grajau* sp. nov., Holotype ♂. Male terminalia. a: epandrium, cerci, surstyli, and subepandrial sclerite, posteroventral view; b: hypandrium, postgonites, pregonites, phallus and phallapodeme, ventral view; c-e: phallus, phallapodeme, and postgonites in frontal, lateral, and oblique lateral view. Scale bars: 0.1 mm. *Abbreviations*: cerc, cercus; post, postgonite; hypan, hypandrium; sur, surstylus; prens, prensisetae.

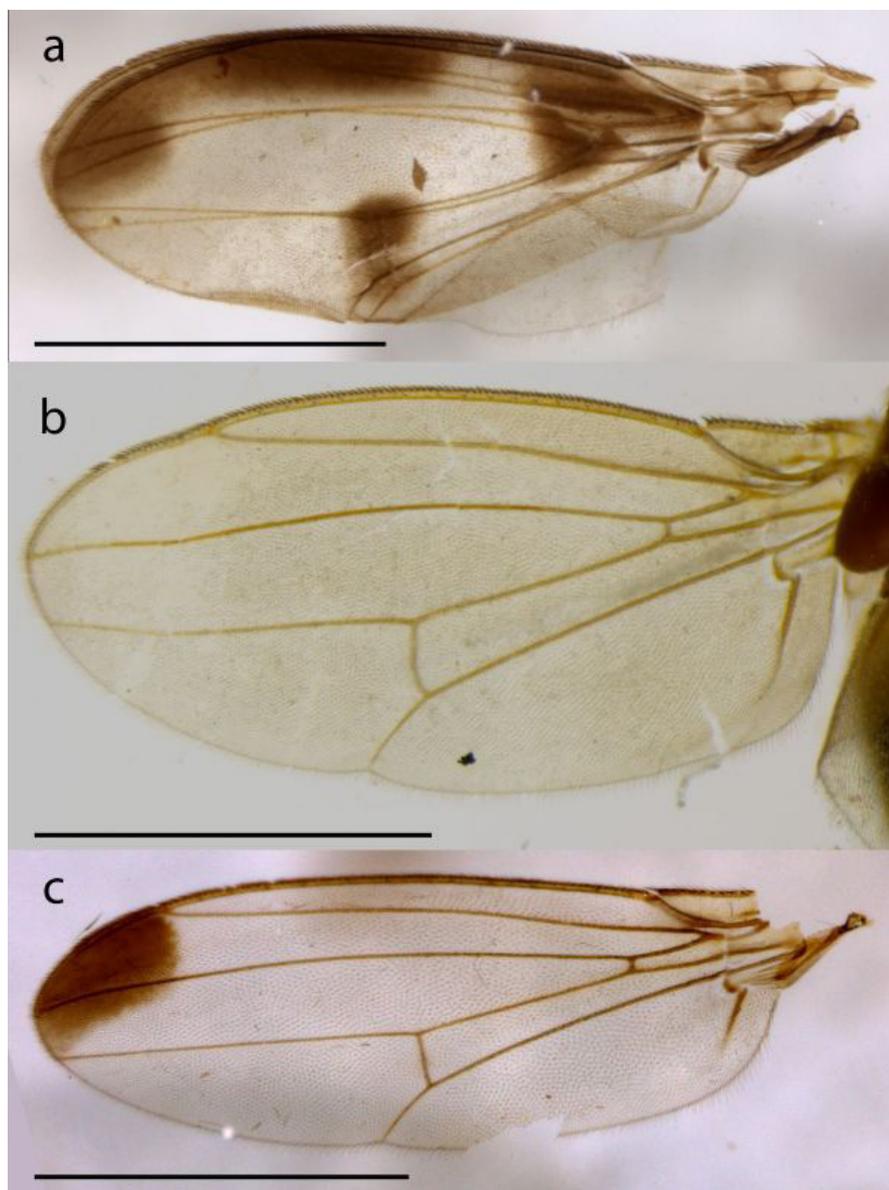


Figure 10 Wings of the new *Zygothrica* species described. a: *Zygothrica japonibaensis* sp. nov. Holotype ♂; b: *Zygothrica duovittata* sp. nov. Holotype ♂; c: *Zygothrica grajau* sp. nov. Holotype ♂. Scale bars: 1.0 mm.

the anterior. Facial carina large, yellow. Gena yellow, slightly whitish. Proboscis, palpus, and labellum yellow.

Thorax. Yellow, with three pairs of dark brown dorsal longitudinal bands; a pair between dorsocentral bristles, a pair along the dorsocentral bristle lines and a pair laterally of the dorsocentral bristle lines and interrupted in the transverse suture; scutellum dark brown with three yellow longitudinal stripes, one median and two lateral; basal scutellar bristles convergent; pleura yellow, 2 katepisternal bristles, of which the second is 1.5x longer than the first. Length = 0.88mm, width = 0.70mm. Legs yellow.

Wings. Hyalines; lappet and $bM-Cu$ absent; brown apical spot extending in third and fourth sections of C . Vein R_{4+5} slightly curved posteriorly. Halteres yellow. Indices: $C = 3.04$; $ac = 3.30$; $hb = 0.55$; $4c = 0.74$; $4v = 1.65$; $5x = 1.35$; $M = 0.32$; $prox. x = 0.37$. Length = 1.98mm.

Abdomen. Yellow, tergite I yellow, tergite II yellow with a black band in posterior region not extending laterally, tergite III with black band reaching tergite II and extending laterally, tergite IV with medium

black band touching the previous tergite, tergite V-VI with black rounded median spot not touching the previous tergite.

Terminalia. Epandrium rounded and ventral lobes small. Cerci free, elongated posteriorly; with long setae at the apex and microtrichose at lateral margin and base. Hipoproctal plate present, with two medial ventral elongated processes. Surstylus S-shaped, with 6-8 prenisetae arranged in a half-moon. Postgonites (gonopods *sensu* Grimaldi, 1987) attached to the hypandrium and with two small setae on the inner margin. Small pregonites (paraphysis *sensu* Grimaldi, 1987) fused to gonopods. V-shaped hypandrium, shorter than the epandrium. Phallus (aedeagus *sensu* Grimaldi, 1987), broad in the apical region and with evident setulae in the ventral portion. Phallapodeme (aedeagal apodeme *sensu* Grimaldi, 1987) long and laterally broad.

♀ Unknown.

Geographic distribution: Known only from type locality.

Etymology: The species name refers to the type locality, Grajau, city of Rio de Janeiro, Rio de Janeiro, Brazil.

Discussion

The diagnostic characters we used to place *Zygothrica japunibaensis* sp. nov. in the *aldrichii* subgroup were: the pattern of dark spots on the wings (with an apical spot, spot in the apex of R_{2+3} , and sometimes with this spot joining with the mid-radial spot); brown-red, bare or short-bristled eyes; and a dark brown and bright ocellar triangle (the median frontal vita) extending towards the ptilinal suture. The species presents a medium level of hypercephaly and a distinct pattern of coloration on the abdomen. The abdomen color is similar to *Z. nigropleura*, but *Z. japunibaensis* sp. nov. presents tergites III and VI lighter while in *Z. nigropleura* all tergites are entirely black-brown. The phallus is also similar to *Z. nigropleura*, but the distiphallal scales are absent and the length of the phallapodeme is smaller in relation to the phallus in *Z. japunibaensis* sp. nov.

There are a number of consistent diagnostic characters for the *vittatifrons* group, and these were used to assign *Z. grajau* sp. nov. to this group (Burla, 1956). The characters are: a yellow to brown thorax with 4 longitudinal dark brown to black stripes; the presence of another dark band laterally, which is interrupted by a transverse suture; and slightly brownish wings with dark spots. These later two characters are consistent and were the basis for us to assign *Z. grajau* sp. nov. to this group (Burla, 1956). *Zygothrica grajau* sp. nov. is similar to *Z. vittipoecila* and *Z. vittisecta*, both of which are already registered in Brazil but which have not been assigned to any group. They largely coincide in body morphology, especially in terms of the pattern of coloration of the thorax and wings. Comparing *Z. grajau* sp. nov. with the original descriptions of *Z. vittipoecila* and *Z. vittisecta* (Burla, 1956), we note the following distinctions: (1) differences in the color of the abdomen, as the yellow tergites II and III have a black band on the posterior margin of the tergite, extended laterally but not reaching the margin, and the other tergites have a dark band only in the median region of tergites; (2) slightly straighter and narrower phallapodeme when compared to *Z. vittipoecila*; (3) the phallapodeme is similar in length to the phallus, the angle between the ventral rod and the phallus is small and the surstyli have a smaller number of prensisetae, while in *Z. vittisecta* the cerci are more elongated, the phallapodeme is shorter than the phallus and the angle between the ventral rod and the phallus is bigger, and the prensisetae in the surstyli are more numerous.

Zygothrica duovittata sp. nov. and *Z. orbitalis* (Sturtevant, 1916) are cryptic species within the *orbitalis* group, and the most evident differences are in male terminalia. Both *Z. orbitalis* and *Z. duovittata* sp. nov. have red eyes, acrostichal setulae of between 8-10 lines, brown bristles and dark longitudinal stripes on the thorax. Furthermore, there is no distinction between the color intensity of these bands in the descriptions of the two species (Sturtevant, 1916; Burla, 1956). However, the phallus differs between the species. In *Z. duovittata* sp. nov. it is more rounded distally, with a more pronounced presence of scales and with a more elongated projection of the apex of aedeagus than that of *Z. orbitalis*.

Acknowledgments

We thank Dr. Jane Costa, Dr. Márcio Felix, and MSc. Danielle Cerri from the Entomological Collection of the Instituto Oswaldo Cruz for allowing access to the specimens deposited in the museum; and Dr. Lizandra Jaqueline Robe and MSc. Lucas Baptista Duarte for individuals assigned. We are also grateful to Dr Karen Mustin and Dr Rebeca Zanini for checking this manuscript for English. This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) under grant nº472973/2013-4 and nº141578/2018-1.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Compliance with ethical standards

This research followed the guidelines specified by the research permits from the Entomological Collection of Instituto Oswaldo Cruz (CEIOC), RJ, Brazil.

Author contribution statement

All authors participated in writing the manuscript and wrote the first draft of the manuscript as part of MFM' M.Sc dissertation at the Universidade Federal de Pelotas, RS, Brazil. M.Sc MFM and Dr. MSG identified the insects.

In this study, we identify specimens collected in several localities from Brazil that have been deposited in the Entomological Collection of the Instituto Oswaldo Cruz (CEIOC), RJ, Brazil.

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