



Three new Neotropical species of *Hirtodrosophila* Duda, 1923 (Diptera: Drosophilidae)

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ABSTRACT

Hirtodrosophila Duda, 1923 is a worldwide genus that encompasses about 170 species. In the Neotropical Region there are records of 38 species. In this paper, we describe three new species of Neotropical *Hirtodrosophila* - *H. pilosa* n. sp., *H. fluminensis* n. sp. and *H. brasiliensis* n. sp. -, which are not assigned to any species group. The three new species can be easily distinguished from any known American species by their external morphological traits. Furthermore, we describe a female specimen, refraining from designating it as a new species because of the lack of characteristics to adequately delimit the species.

Introduction

When Duda (1923) first proposed *Hirtodrosophila*, its taxonomic status was unclear (Frota-Pessoa, 1945). In the following year, Duda (1924) presented it as a subgenus of *Drosophila* Fallén, 1823. Later, Grimaldi (1990) elevated it to the generic rank. The type species, after some confusion [see Frota-Pessoa (1945) and Brake and Bächli (2008)], is *D. carinata* Duda, 1923 (= *D. latifrontata* Frota-Pessoa, 1945). This genus is distributed worldwide and comprises approximately 170 described species classified into 10 species groups (Bächli, 2022). The most recent molecular phylogenetic analyses showed that *Hirtodrosophila* is non-monophyletic and separated into a few lineages each including some species of other genera of the *Zygothrica* genus group (Gautério et al., 2020; Zhang et al., 2021).

From the Neotropical Region a total of 38 *Hirtodrosophila* species have been recorded to date (Vilela and Bächli, 2004, 2005; Grimaldi, 2018; Junges et al., 2019; Peñafiel-Vinueza and Rafael, 2019): two species in the *glabrifrons* group (Burla, 1956), eight in the *hirticornis* group (Burla, 1956),

two in the *magnarcus* group (Frota-Pessoa, 1951), four in the *thoracis* group (Grimaldi, 1987); and 22 ungrouped species (Bächli, 2022).

In this paper, we describe three new species of Neotropical *Hirtodrosophila*, which are not assigned to any species group.

Materials and methods

Specimens were dried using a Hexamethyldisilazane (HMDS) protocol and then point-mounted (Kirk-Spriggs, 2017) with white glue. The entomological triangles used in the point-mounting were extracted from 120g/m² bond paper. The mounted specimens were photomicrographed; if the wing covered the abdomen, it was detached. Once photomicrographs were taken, the left wing was detached with a pair of fine tweezers. The wings were placed on temporary slides with water and then photomicrographed. To examine and photomicrograph the terminalia, the dried specimens were placed in a humid chamber with synthetic camphor at 7°C, where they were kept for about four weeks so as to moisturize and soften them.

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Once the specimens were softened, the distal part of abdomen was detached. Soft tissues of the detached abdomen were dissolved using a proteinase K solution. The sclerites were, then, placed in the glycerol and dissected with the aid of minute pins under a stereomicroscope. The dissected parts were placed on a temporary slide with a drop of glycerin jelly (a solution of 48.5% water, 48.5% glycerin, and 3% jelly) so as to appropriately position them. After the glycerin jelly slightly hardened, a glass cover was placed on the droplet and photographs were taken. The remaining body part of dissected specimen was dried again in an oven at 40°C to 50°C for 24 h.

The morphological terminology followed primarily McEvey and Grimaldi (2021) and Cumming and Wood (2017) and secondarily Bächli et al. (2004) if the structures were not contemplated on the first two works. Longitudinal vittae on the scutum are encoded as v-1, 2, ..., 6 from the medial to the most lateral one after Grimaldi (1987), though the homology is uncertain. The abdominal tergites and sternites were encoded from the anterior to the posterior ones as "T1, T2, T3, ..." and "S1, S2, S3, ...", respectively. The apical portion of phallus is termed as distiphallus or acrophallus with some synonyms for various structures among dipteran taxa (Cumming and Wood, 2017). However, since the homology of these structures is uncertain, the posterior section of the phallus near the phallotrema is here called "phallotrema section".

The following measurements were taken with the $\pm 10 \mu\text{m}$ precision for the holotypes and paratypes, according to Vilela (1983) and Correa et al. (2021): body length = in lateral view, the summed length of head, thorax and abdomen; wing length = the distance from near the intersection of the first (M_1) and fourth (M_4) medial vein branches to the apex of third branch of radial vein (R_{4+5}); wing width = the smaller distance between near the middle of the Costal vein to near the apex of the M_4 ; the distance between proclinate fronto-orbital seta (*pc frorb s*) and anterior reclinate fronto-orbital seta (*a rc frorb s*); the distance between *a rc frorb s* and posterior reclinate fronto-orbital seta (*p rc frorb s*); CII = distance between the subcostal break and the apex of the second branch of radial vein (R_{2+3}); CIII = distance between the apex of R_{2+3} and the apex of the R_{4+5} ; CIV = distance between the apexes of R_{4+5} and M_1 ; CIII' = section of CIII with hard bristles; MIII = distance between the intersection of the M_1 and radial-medial crossvein (r-m) and the intersection between M_1 and discal medial crossvein (dm-m); MIV = distance between the intersection of the M_1 and dm-m and the apex of the M_1 ; M_4' = distance between the apex of M_4 and the intersection of the M_4 and dm-m; dm-m' = dm-m length; and R_{4+5}' = distance between the intersection of R_{2+3} and R_{4+5} and the intersection of R_{4+5} and r-m. Then, the following indices were calculated: C (CII/CIII), ac (CIII/CIV), hb (CIII'/CIII), 4c (CIII/MIII), 4v (MIV/MIII), 5x (M_4' /dm-m'), M (M_4' /MIII), and prox. x (R_{4+5}' /MIII).

The external morphology was photographed at several depths of focus using a Zeiss Discovery V.20 stereomicroscope equipped with the Axiohome system Software, which provided the magnification scales. Wing pictures were taken using a Samsung Galaxy A8 smartphone model SM-A530F, with ProCam X Lite V.1.10 software, attached to an Olympus BX51 microscope with 4 \times and 10 \times objective lens. An objective micrometer was photographed at the same magnification as wing pictures to calibrate the wing measurements. The terminalia structures were microphotographed at different magnifications depending on the structure size and at several depths of focus, using a Canon EOS REBEL T3 camera attached to an Olympus BX51 microscope with 10x, 20x, and 40x objective lens. To indicate the scale bar on each picture, an objective micrometer was also photographed at the same magnification. All the pictures were saved at the maximum resolution in JPEG format. The pictures taken at different depths of focus were stacked into an all-in-focus composite image by the rendering method "C" (pyramid)

with the minimal smoothing setting of the Helicon Focus 7.7.5 Pro lifetime software (www.heliconsoft.com).

All specimens' conditions are as follows: "specimen point-mounted; left wing removed and set between coverslips attached to the specimen; abdomen dissected; detached sclerites stored in a microtube with glycerin". In addition, certain aspects are noted for each studied specimen when deemed necessary.

The holotypes and paratypes of the described species are to be deposited in the "Museu de Zoologia da Universidade de São Paulo" - "MZUSP".

The map was produced using QGIS v3.20 Odense Software (<http://www.qgis.org>), the available records of the specimens, satellite images extracted from google maps (<http://www.google.com/maps>) using SAS.Planet software (<http://www.sasgis.org>), a shapefile of the World Political Boundaries ([dataset] Pope, 2017), and a shapefile of the Federation Units of Brazil (<https://www.ibge.gov.br>).

Results

Hirtodrosophila Duda, 1923

Diagnosis.

See Grimaldi, 2018.

Hirtodrosophila pilosa n. sp.

urn:lsid:zoobank.org:act:D66D90A1-0514-4416-88AB-FC4D05695BF8 (Figs. 1, 2, 9a, 10)

Material examined.

Holotype: male, labelled "BRA [=Brazil] - RJ [=State of Rio de Janeiro] - PARNA [=National Park] de Itatiaia (22°25'35.57"S, 44°37'14.6"W) Data: 21.xii.2020-04.i.2021 Col.: Telles MHF; Vicenzi N OBS.: Armadilha [=Trap] Malaise" (Fig. 10), "*Hirtodrosophila pilosa* n. sp. Frech-Telles, Valente-Gaiesky, Gottschalk, ♂ Holotype".

Diagnosis.

Flies yellow with brown marks. Thick acrostichal setulae in eight irregular rows anteriorly and six at the height of the anterior dorsocentral setae. Wings with a modest apically pointy darkened costal lappet. Phallus tubular, projected ventrally, slim; phallotrema section oblong, narrowing near apex in lateral view.

Description.

Holotype. **Head** (Fig. 1a, 1c): yellow, with brown marks. Eyes reddish, pubescent. Face yellow, dark brown near facial carina; facial carina white, prominent. Antennal scape brown; pedicel basally brown, apically lighter; flagellomere 1 brown, basally yellow, compressed, mango-shaped; arista with five (left) or four (right) dorsal branches, one ventral branch, and terminal fork. Frons light yellow and brown; fronto-orbital plate brown, with yellow band adjacent to eye; fronto-orbital setae black; *a rc frorb s* situated anteriorly to *pc frorb s*; distance between *pc frorb s* and *a rc frorb s* = 0.03 mm, between *pc frorb s* and *p rc frorb s* = 0.08 mm, and between *a rc frorb s* and *p rc frorb s* = 0.10 mm; frontal vitta brown, lighter near ptilinal suture; frontal triangle brown; ocellar triangle dark brown, prominent; postocellar setae crossing. Genae light yellow, black at bases of vibrissae, with small, black spot under eye. Palpus dark brown to black, large; labellum light brown to dark brown. **Thorax** (Fig. 1b, 1c): yellow, with brown marks. Scutum yellow with brown marks; v-1 greyish; v-2 yellow, fine; v-3 brown; v-4 yellow; v-5 brown; v-6 yellow; brown spots present at bases of dorsocentral setae and

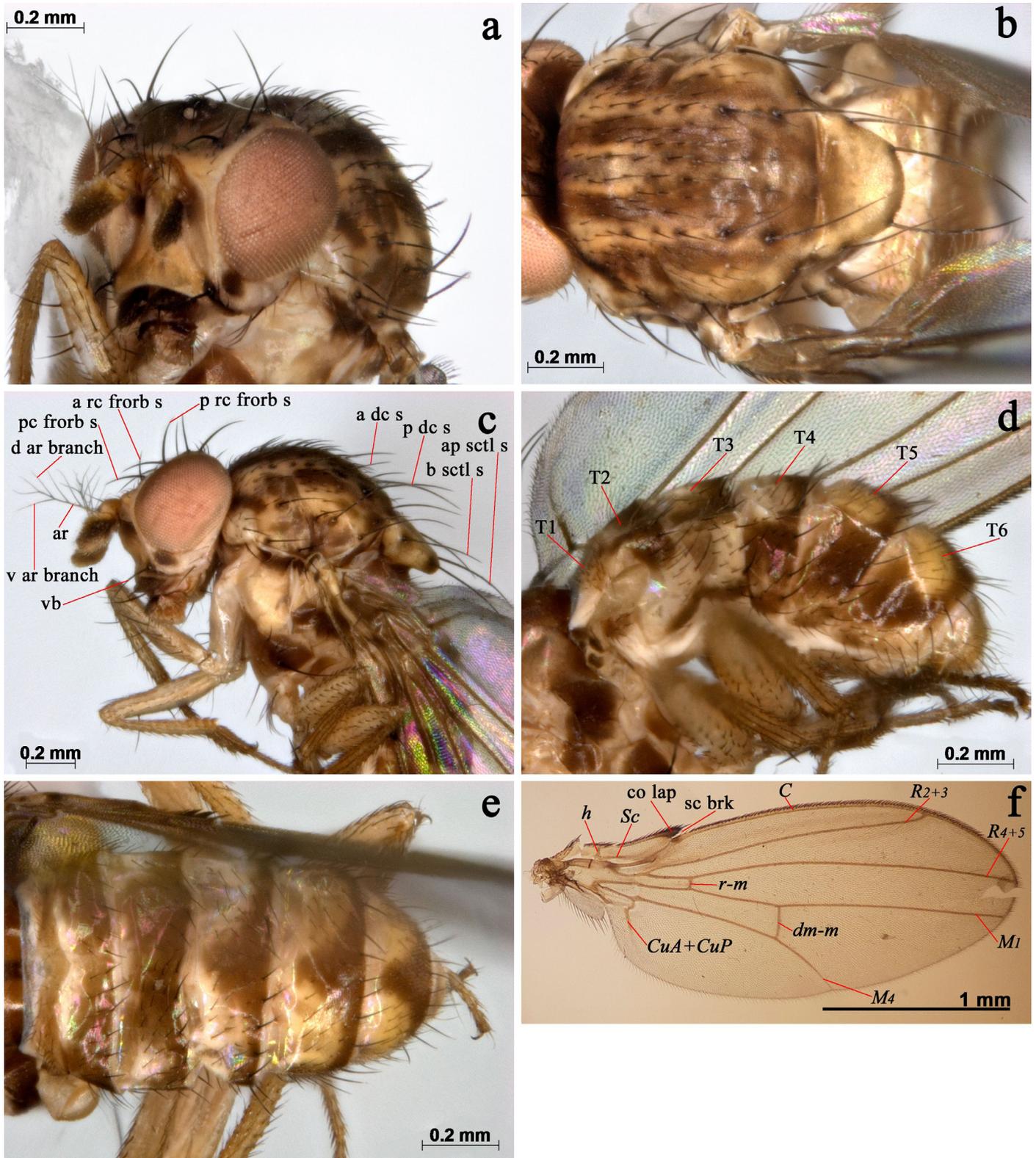


Figure 1 Holotype of *Hirtodrosophila pilosa* n. sp.: a, head (anterolateral view); b, thorax (dorsal view); c, head and thorax (lateral view); d, abdomen (lateral view); e, abdomen (dorsal view); f, wing. Abbreviations: a dc s, anterior dorsocentral seta; a rc frorb s, anterior reclinate fronto-orbital seta; ap sctl s, apical scutellar seta; ar, arista; b sctl s, basal scutellar seta; C, costal vein; co lap, costal lappet; *CuA+CuP*, anterior branch of cubital vein + posterior branch of cubital vein; d ar branch, dorsal arista branch; *dm-m*, discal medial crossvein; *h*, humeral crossvein; *M*₁, first branch of medial vein; *M*₄, fourth branch of medial vein; p dc s, posterior dorsocentral setae; p rc frorb s, posterior reclinate fronto-orbital seta; pc frorb s, proclinate fronto-orbital seta; *R*₂₊₃, second branch of radial vein; *R*₄₊₅, third branch of radial vein; *r-m*, radial-medial crossvein; sc brk, subcostal break; Sc, subcostal vein; T1, tergite 1; T2, tergite 2; T3, tergite 3; T4, tergite 4; T5, tergite 5; T6, tergite 6; v ar branch, ventral arista branch; vb, vibrissa.

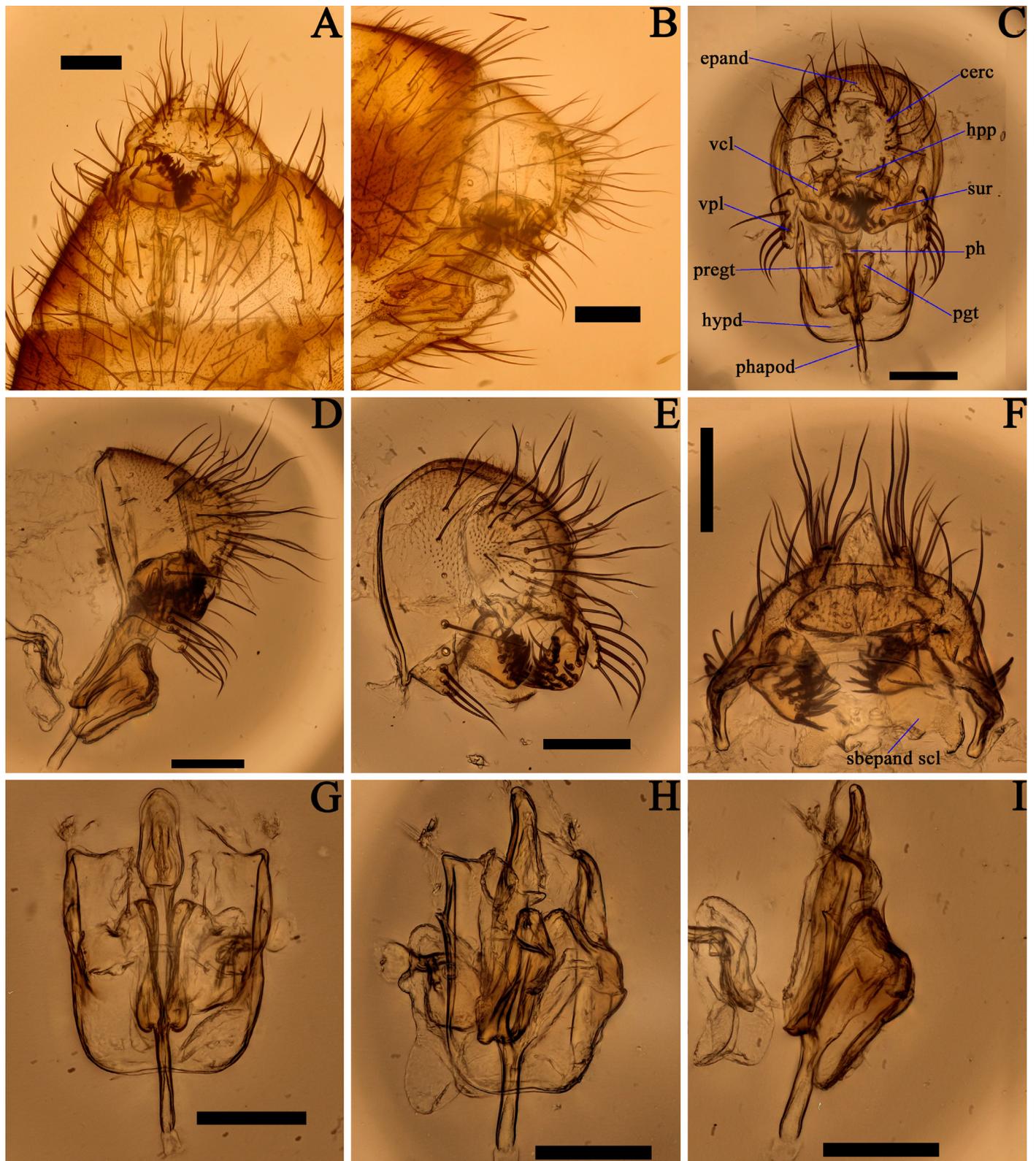


Figure 2 Holotype of *Hirtodrosophila pilosa* n. sp.: a, posterior end of abdomen (ventral view); b, posterior end of abdomen (lateral view); c, articulated periphallallic and phallic organs (posteroventral view); d, articulated periphallallic and phallic organs (lateral view); e, epandrium and associated sclerites (posterolateral view); f, epandrium and associated sclerites (ventral view); g, hypandrium, phallus and associated sclerites (ventral view); h, hypandrium, phallus and associated sclerites (lateroventral view); i, hypandrium, phallus and associated sclerites (lateral view). Scale bars: 0.1 mm. Abbreviations: cerc, cercus; epand, epandrium; hpp, hypoproctal plate; hypd, hypandrium; pgt, postgonite; ph, phallus; phapod, phallapodeme; pregt, pregonite; sur, surstylus; sbepand scl, subepandrial sclerite; vcl, ventral cecal lobe; vpl, ventral epandrial lobe.

setulae, except for the anterior fifth of scutum, sometimes partially fused to each other; postpronotum light yellow; thick acrostichal setulae in eight irregular rows on the first third of scutum and six on the posterior two thirds; acrostichal setulae larger and thicker in mid and dorsocentral rows; anterior dorsocentral setae about half as long as posterior dorsocentral setae; longitudinal distance between ipsilateral dorsocentral setae about half of cross distance; anterior dorsocentral setae placed slightly closer to transversal suture than to posterior end of scutum. Scutellum yellow, darker at bases of setae and medial portion near anterior margin; basal scutellar setae parallel, curved inwards; apical scutellar setae curved inwards. Pleura yellow and brown; proepisternum brown; anepisternum yellow, with brown band on dorsal margin; anepimeron mostly brown, with some small, yellow areas near margins; katepisternum brown; meron brown. Legs yellow. **Wings** (Fig. 1f): hyaline; faint, darker patch present below costal lappet and along R_{4+5} , R_{4+5} and M_1 parallel; R_{2+3} slightly curved to costal at its tip. Halter light, with darker stem. Indices: $C = 2.16$, $ac = 3.05$, $hb = 0.67$, $4c = 1.27$, $4v = 2.52$, $5x = 2.35$, $M = 0.83$, $prox. x = 0.50$; measurements: length = 2.25 mm, width = 0.99 mm. **Abdomen** (Fig. 1d, 1e): tergites glossy; T1 brown; T2 brown, with a pair of yellow patches sub-laterally; T3 brown, with two pairs of yellow spots sub-laterally; T4 yellow, with caudal brown band, which is interrupted sub-laterally and triangularly enlarged medially; T5 and T6 similar to T4. Sternites tan. Intersegmental membranes light. **Terminalia** (Fig. 2): epandrium as wide as long, with microtrichia and eight upper and two lateral setae; ventral lobe large, broad without microtrichia, with approximately 5–6 long setae. Cerci not fused to epandrium, with microtrichia and very long setae. Subepandrial sclerite squarish. Surstyli wide, with conspicuous setation: 5 prenisetae in line, 3 setae in the same line as prenisetae, and 10 inner and 7 outer setae. Hypandrium squarish, longer than wide, almost as large as epandrium. Pregonite large, fused to hypandrium, medially not fused to each other, with a seta near postgonite. Postgonites thick, with setae near the fusion with pregonite. Phallapodeme straight, slim and short, 1/2 as long as phallus. **Body length**: 2.29 mm.

Etymology.

Named in reference to the hairy aspect of the fly.

Hirtodrosophila fluminensis n. sp.

urn:lsid:zoobank.org:act:0C38B9FA-D84E-45D5-8EC4-32ADCC1C709D (Figs. 3, 4, 9b, 10)

Material examined.

Holotype: male, labelled “BRA [=Brazil] – RJ [State of Rio de Janeiro] – PARNA [=National Park] de Itatiaia (22°26'09.58”S, 44°36'28.1”W) Data: 21.xii.2020-04.i.2021 Col.: Telles MHF; Vicenzi N OBS.: Armadilha [=Trap] Malaise” (Fig. 10), “*Hirtodrosophila fluminensis* n. sp. Frech-Telles, Valente-Gaiesky, Gottschalk, ♂ Holotype”. Holotype condition: Right fore leg (except for the coxa) glued on the triangle.

Diagnosis.

Flies tan and yellow with brown marks. Frons silvery pollinose. Abdomen yellow with brown marks. Wings with black, apically pointy, costal lappet. Phallus thick in lateral view, ornamented with scale-like micro-structures dorsally; phallotrema section diamond-shaped, twice as wide as long in ventral view, concave on lateral margins of phallotrema in lateral view.

Description.

Holotype. **Head** (Fig. 3a, 3c): yellow, with brown marks. Eyes reddish, pubescent. Face yellow, dark under the antennae with a narrow prominent light yellow facial carina. Antennal scape covered; pedicel light yellow, basally darker; flagellomere 1 yellow, compressed; arista with seven to eight dorsal branches, one ventral branch, and terminal fork. Fronto-orbital plate yellow, darker posterior of *prc frorb s*; fronto-orbital setae black; *pc frorb s* situated laterally and slightly anterior of *arc frorb s*; distance between *pc frorb s* and *arc frorb s* = 0.02 mm, between *pc frorb s* and *prc frorb s* = 0.10 mm, and between *arc frorb s* and *prc frorb s* = 0.09 mm; frontal vitta brown, lighter near the ptilinal suture; frontal triangle brown, small; ocellar triangle black, prominent; postocellar setae black, crossing. Genae yellow, darker at the ventral margin and under the eye near the vibrissae, dark spot at the base of vibrissae. Palpus tan, large; labrum light yellow, with dark marks; labellum brown. **Thorax** (Fig. 2b, 2c): yellow, with brown marks; v-1 greyish, fine; v-2 light brown; v-3 brown; v-4 light brown; v-5 brown, faint; v-6 light brown; v-3 are convergent at the height of anterior dorsocentral setae and parallel at the height of posterior dorsocentral setae; v-5 are convergent at the posterior dorsocentral setae and fused with v-3 near the posterior margin; transverse suture brown; six semi-regular rows of acrostichal setulae that are irregular and scarcer progressively posterior of the height of the anterior dorsocentral setae; anterior dorsocentral setae about 1/2 the length of the posterior dorsocentral setae; longitudinal dorsocentral setae distance about half the length of transversal dorsocentral setae distance; anterior dorsocentral setae placed slightly closer to the transversal suture than to the posterior end of scutum. Postpronotum light yellow with a darker spot at its center. Scutellum yellow, with brown mark at the anterior margin not reaching the lateral margin, slightly darker at setae base; basal scutellar setae converging, almost parallel, slightly curved inwards; apical scutellar setae curved inwards, crossing. Pleura yellow with three dark brown bands, one at the dorsal margin of proepisternum and anepisternum, one medially on the anepimeron, and one formed at the dorsal half of katepisternum advancing posteriorly. Legs yellow; mid and hind femora with slightly darker ring medially. **Wings** (Fig. 3f): hyaline; faint darker patch present below costal lappet. R_{4+5} and M_1 parallel; R_{2+3} slightly curved to costal at its tip. Halter light yellow. Indices: $C = 2.06$, $ac = 3.24$, $hb = 0.76$, $4c = 1.48$, $4v = 2.76$, $5x = 2.61$, $M = 1.02$, $prox. x = 0.63$; measurements: length = 2.38 mm, width = 1.10 mm. **Abdomen** (Fig. 3d, 3e): T1 faint brown, with sub-lateral yellow patches, laterally brown; T2 brown, with sub-lateral yellow patches, a small yellow spot at the anterior margin sub-lateral of the central brown area, yellow spot medially at the anterior margin; T3 brown, with sub-lateral yellow patches, round yellow mark at the anterior margin sub-lateral of the central brown area; T4 same as T3 but the round yellow marks are larger; T5 same as T6 but the round yellow marks are larger; T6 yellow, with round faint brown caudal mark medially, laterally brown; sternites light yellow. Intersegmental membranes light. **Terminalia** (Fig. 4): epandrium as wide as long, with microtrichia and 12 upper setae and eight lateral setae; ventral lobe short, broad, without microtrichia, with approximately six long setae. Cerci not fused to epandrium, with microtrichia and with very long setae, hypoproctal plate present, ventral cecal plate large with at least three setae. Subepandrial sclerite sclerotized. Surstyli wide, with a small inward projection; two lines of 3 prenisetae, one of them in the previously mentioned projection; about 15 inner conspicuous setae. Hypandrium almost as wide as it is long, smaller than epandrium. Pregonites fused to the hypandrium, fused to each other, with a seta near postgonite. Postgonites thick, with three setae near the fusion with pregonite. Phallapodeme straight, shorter than phallus. **Body length**: 2.43mm.

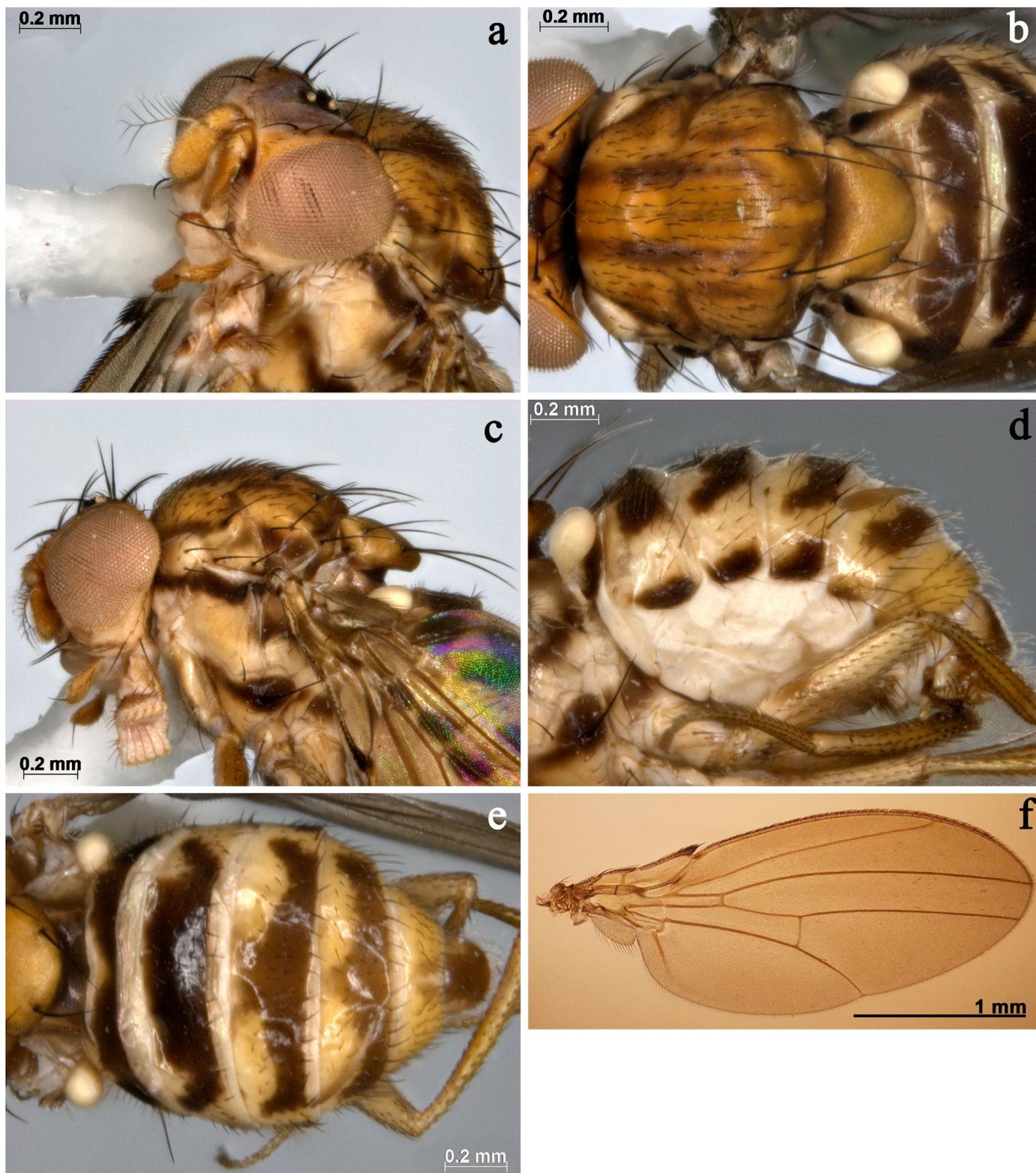


Figure 3 Holotype of *Hirtodrosophila fluminensis* n. sp.: a, head (anterolateral view); b, thorax (dorsal view); c, head and thorax (lateral view); d, abdomen (lateral view); e, abdomen (dorsal view); f, wing.

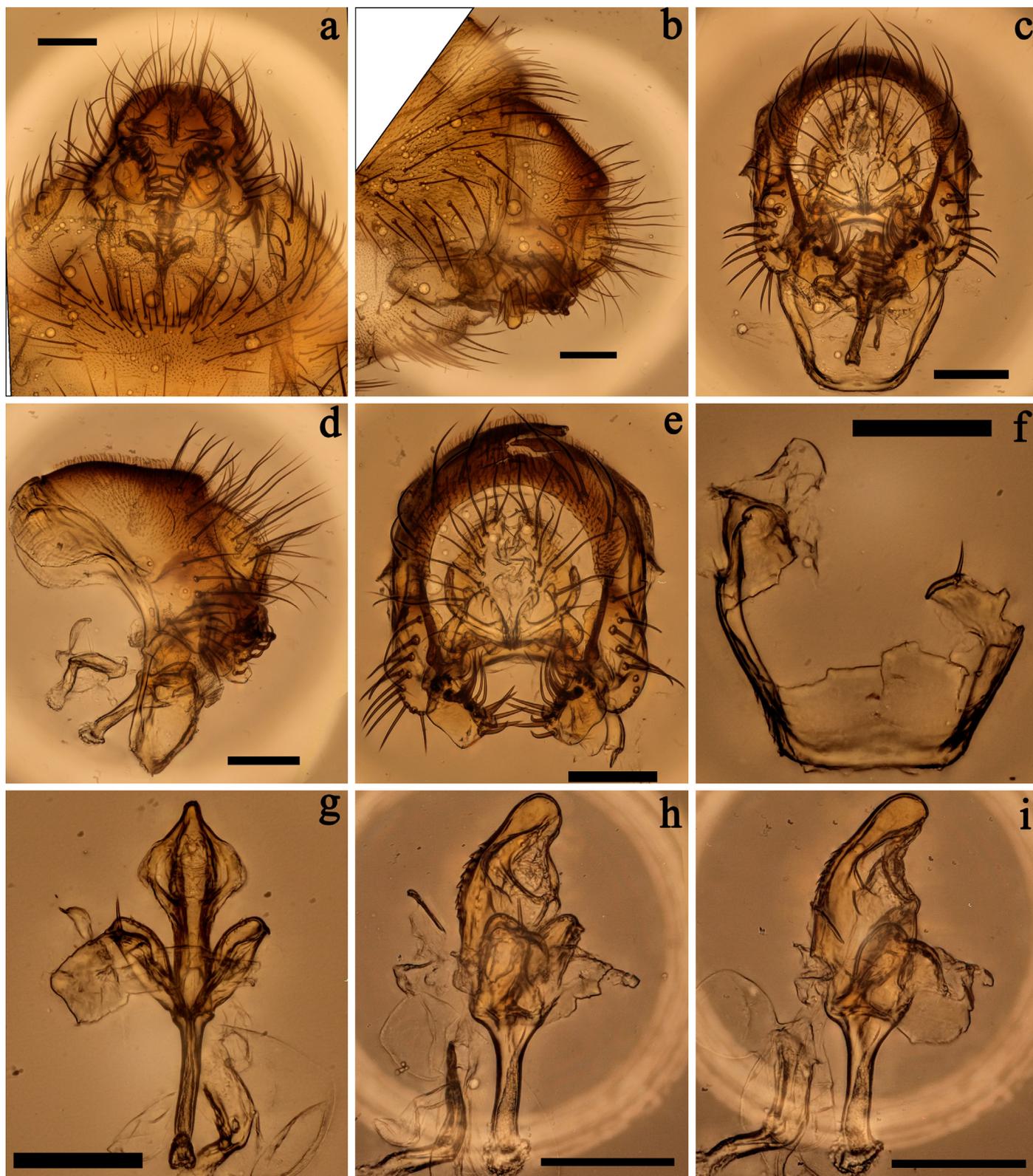


Figure 4 Holotype of *Hirtodrosophila fluminensis* n. sp.: a, posterior end of abdomen (ventral view); b, posterior end of abdomen (lateral view); c, articulated peripheral and phallic organs (posteroventral view); d, articulated peripheral and phallic organs (lateral view); e, epandrium and associated sclerites (posterior view); f, hypandrium (ventral view); g, phallus and associated sclerites (ventral view); h, phallus and associated sclerites (lateroventral view); i, phallus and associated sclerites (lateral view). Scale bars: 0.1 mm.

Etymology.

Named in reference to the State of Rio de Janeiro, where the holotype was collected.

Hirtodrosophila brasiliensis n. sp.

urn:lsid:zoobank.org:act:F9DEF68B-0C5A-4562-8F8E-5E9C3F15C3FB (Figs. 5, 6, 9c-9e, 10)

Material examined.

Holotype: male, labelled "BRA [=Brazil] – MG [=State of Minas Gerais] – Bocaina de Minas (22°19'54.02"S, 44°32'36.19"W) Data: 05.xii.2020-20.xii.2020 Col.: Telles MHF; Vicenzi N OBS.: Armadilha [=Trap] Malaise" (Fig. 10), "*Hirtodrosophila brasiliensis* n. sp. Frech-Telles, Valente-Gaiesky, Gottschalk, ♂ Holotype".

Paratypes: 1 male, labelled "Brasil, RS [=State of Rio Grande do Sul], Rio Grande, ESEC [=Ecological Station] Taim, Mata da Sede - R3 Ponto 62 S32.53781 W52.53425 07-17.xii.2011 - Malaise Krüger RF col." (Fig. 10), "*Hirtodrosophila brasiliensis* n. sp. Frech-Telles, Valente-Gaiesky, Gottschalk, ♂ Paratype". Specimen condition: Scutum damaged; 1 male, labelled "Brasil, RS [=State of Rio Grande do Sul], Rio Grande, ESEC [=Ecological Station] Taim, Mata do Nicola -R3 Ponto 54 S32.56269 W52.50985, 07-17.xii.2011 - Malaise Krüger RF col." (Fig. 10), "*Hirtodrosophila brasiliensis* n. sp. Frech-Telles, Valente-Gaiesky, Gottschalk, ♂ Paratype". Specimen condition: Wrinkled.

Diagnosis.

Flies, in lateral view, with a seemingly continual longitudinal dark brown band from head to T5, along the dorsal margin of the pleura and lateral margins of T1-T5. Wings infuscated, with large, black, apically roundish, costal lappet. Phallus ornamented with tiny lumps and scale-like micro-structures except for phallotrema margins; phallotrema section round, widened, less sclerotized near phallotrema margins.

Description.

Holotype (Figs. 5, 6, 9c). **Head** (Fig. 5a, 5c): dorsal half brown and ventral half light yellow. Eyes red, pubescent. Face dark brown, dorsal margin lighter, ventral half light yellow; facial carina mostly dark brown, with dorsal end lighter, narrow, prominent. Antennal scape brown; pedicel basally brown and apically lighter; flagellomere 1 dark brown, lighter basally, compressed, mango-shaped; arista with seven dorsal and one ventral branches, and terminal fork. Frons brown; fronto-orbital plate yellow, brown spot from the base of *p rc frob s* to the base of medial vertical setae; fronto-orbital setae black; long *pc frob s* and *p rc frob s*; distance between *pc frob s* and *a rc frob s* = 0.03 mm, between *pc frob s* and *p rc frob s* = 0.07 mm, and between *a rc frob s* and *p rc frob s* = 0.06 mm; frontal vitta brown, yellow at the margin of ptilinal suture; frontal triangle small; ocellar triangle darker and flat; postocellar setae black, crossing. Genae light yellow. Clypeus brown; palpus brown, darker apically, large; labrum brown, labellum dark brown. **Thorax** (Fig. 5b, 5c): brown and light yellow. Scutum mostly brown, fine faint medial and sub-medial darker longitudinal bands, slightly lighter in the supra-alar region; anterior dorsocentral setae smaller than posterior dorsocentral setae; longitudinal dorsocentral setae distance about half the length of transversal dorsocentral setae distance; anterior dorsocentral setae placed about halfway between the transversal suture to the posterior end of scutum; six rows of acrostichal setulae. Postpronotum yellow. Scutellum brown, yellow laterally near the anterior margin; basal scutellar setae parallel, curved downwards, apical setae missing. Pleura light yellow, with brown longitudinal bands; proepisternum,

anepimeron and anepisternum light yellow with marks forming a continuous dark brown band at the dorsal margin; katepisternum brown forming a band that advances posteriorly. Legs tan; fore coxae light brown; femora with dark brown bands on basal half; tibiae slightly darker. **Wings** (Fig. 5f): faint darker patch present bellow costal lappet. R_{4+5} and M_1 slightly divergent. Halter brown, apically yellow. Indices: $C = 1.82$, $ac = 2.75$, $hb = 0.94$, $4c = 1.62$, $4v = 2.94$, $5x = 3.15$, $M = 1.21$, $prox. x = 0.68$; measurements: length = 1.87 mm, width = 0.84 mm. **Abdomen** (Fig. 5d, 5e): T1 brownish; T2 dark brown, with sub-lateral yellow patches and a medial triangle-shaped yellow mark pointing posteriorly, the yellow mark is posteriorly faint; T3 black, with sub-lateral yellow patches and anterior small faint yellow spots sub-laterally on the brown area, medial faint yellow patch; T4 yellow, with a caudal black band enlarged medially resembling a hillock, sub-lateral yellow patches, laterally black, the black band is medially fainter at anterior margin; T5 yellow, with a caudal black band enlarged medially, laterally brown; T6 yellow. Sternites light. Intersegmental membranes light. **Terminalia** (Fig. 6): epandrium as wide as long, with microtrichia and 10 upper setae; ventral lobe small, without microtrichia, with 1 apical seta. Cerci projected, not fused to epandrium, with microtrichia and long setae. Subepandrial sclerite sclerotized and articulated with hypandrium. Surstylus round in ventral view, with approximately 5 prenisetae, 2 inner and 4 outer setae. Hypandrium longer than wide, larger than epandrium. Pregonite fused to the hypandrium, fused to each other, with a seta near postgonite. Postgonites sinuous in ventral view; broader in lateral view, with setae near pregonite. Phallotrema is roundish, resembling a 90°-turned eyelid. The overall shape of the phallus resembles a pitcher plant. Phallapodeme slightly curved ventrally, slim, approximately the same size as phallus. **Body length**: 2.04 mm.

Paratypes. 1 male (Fig. 9d), **Wing** indices: $C = 1.78$, $ac = 2.95$, $hb = 0.84$, $4c = 1.71$, $4v = 3.11$, $5x = 3.13$, $M = 1.24$, $prox. x = 0.63$; measurements: length = 2.01 mm, width = 0.97 mm; **Body length**: 2.28 mm. 1 male (Fig. 9e), **Wing** indices: $C = 1.87$, $ac = 3.00$, $hb = 0.97$, $4c = 1.71$, $4v = 3.11$, $5x = 3.07$, $M = 1.23$, $prox. x = 0.69$; measurements: length = 1.97 mm, width = 0.91 mm; **Body length**: 1.64 mm. Color and roundness of scutum varied on the paratypes, probably due to fixation artefacts.

Etymology.

Named in reference to Brazil, where the types were collected.

The following description is of a female specimen of which species status could not be determined yet.

Hirtodrosophila sp.1

(Figs. 7, 8, 9f, 10)

Material examined.

1 female, labelled "Brasil, Amazonas, Brasil, ZF-2 [Experimental station], Km-14, 2°35'21"S; 60°06'55"W, 9-24/ii/2018, Malaise [Trap] grande, Solo [the trap was placed on the ground], Nascente [=spring], JA Rafael - Rede BIA [=Thematic network "Biodiversity of Insects in the Amazon"]" (Fig. 10). Specimen condition: Right wing removed, scutellar apical setae missing.

Diagnosis.

Head with its ventral half entirely light yellow except for the labellum; abdomen with yellow, black and brown tergites.

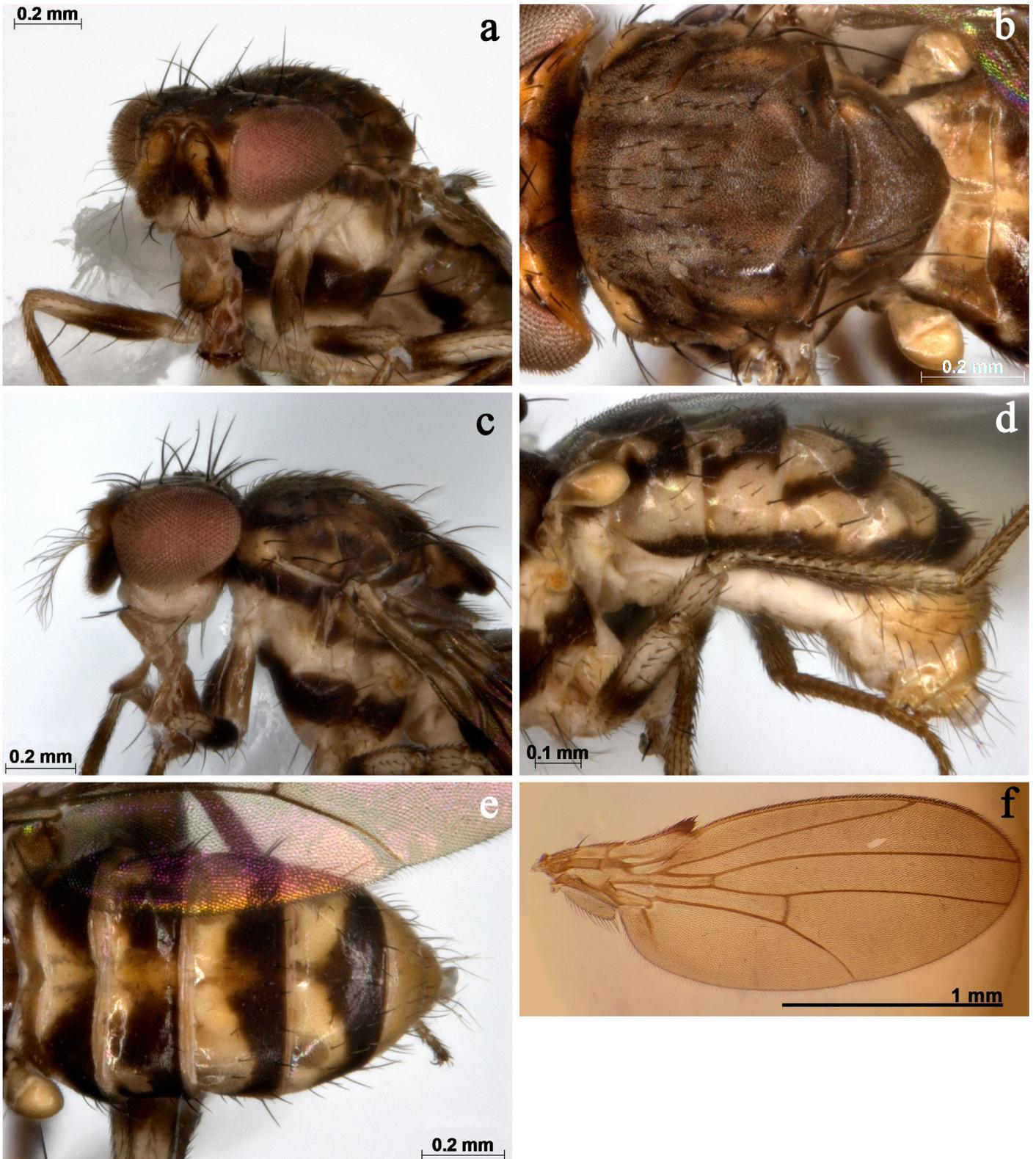


Figure 5 Holotype of *Hirtodrosophila brasiliensis* n. sp.: a, head (anterolateral view); b, thorax (dorsal view); c, head and thorax (lateral view); d, abdomen (lateral view); e, abdomen (dorsal view); f, wing.

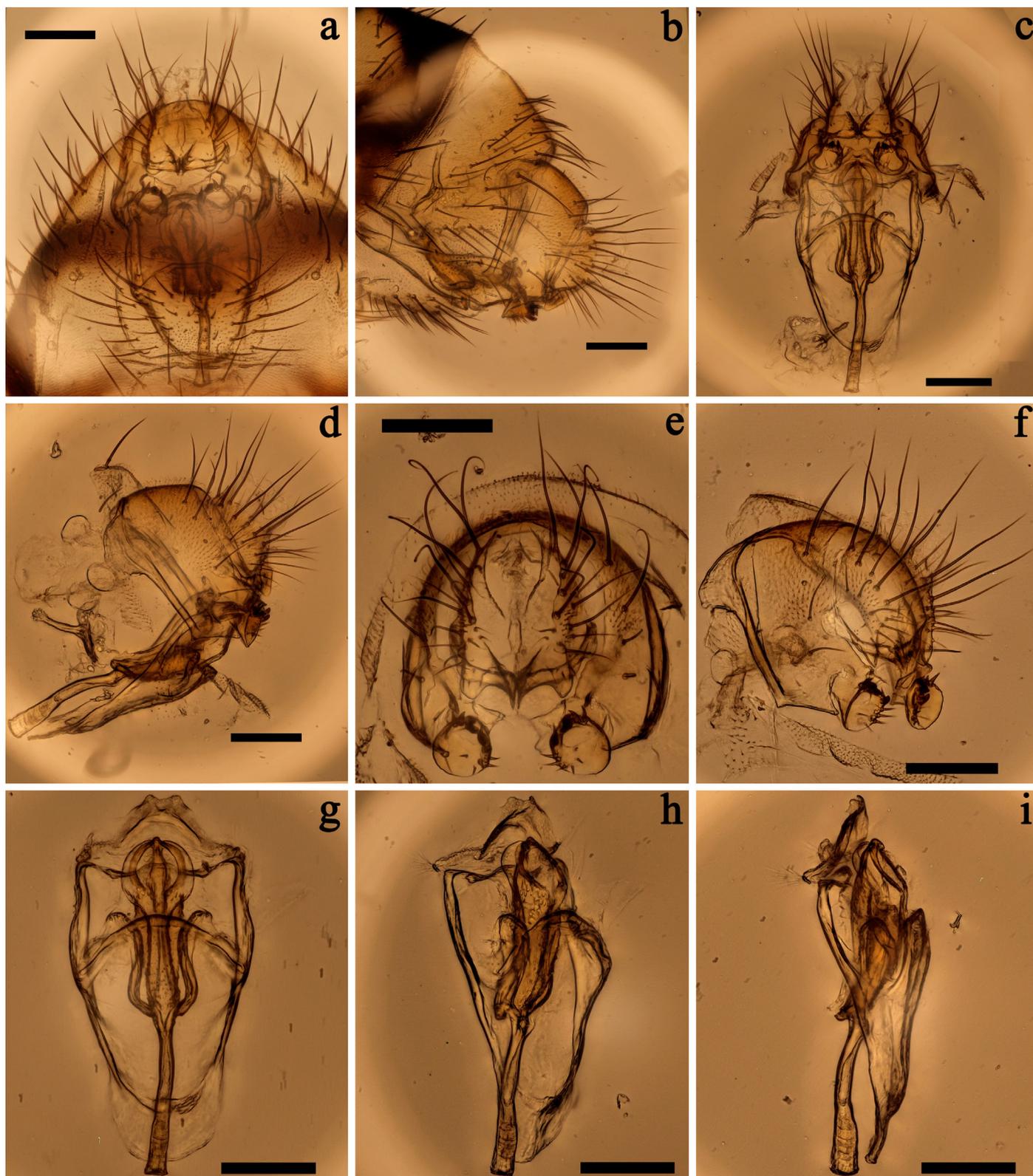


Figure 6 Holotype of *Hirtodrosophila brasiliensis* n. sp.: a, posterior end of abdomen (ventral view); b, posterior end of abdomen (lateral view); c, articulated peripheral and phallic organs (posteroventral view); d, articulated peripheral and phallic organs (lateral view); e, epandrium and associated sclerites (posterior view); f, epandrium and associated sclerites (posterolateral view); g, hypandrium, phallus, associated sclerites and subepandrial sclerite (ventral view); h, hypandrium, phallus, associated sclerites and subepandrial sclerite (lateroventral view); i, hypandrium, phallus, associated sclerites and subepandrial sclerite (lateral view). Scale bar: 0.1 mm.

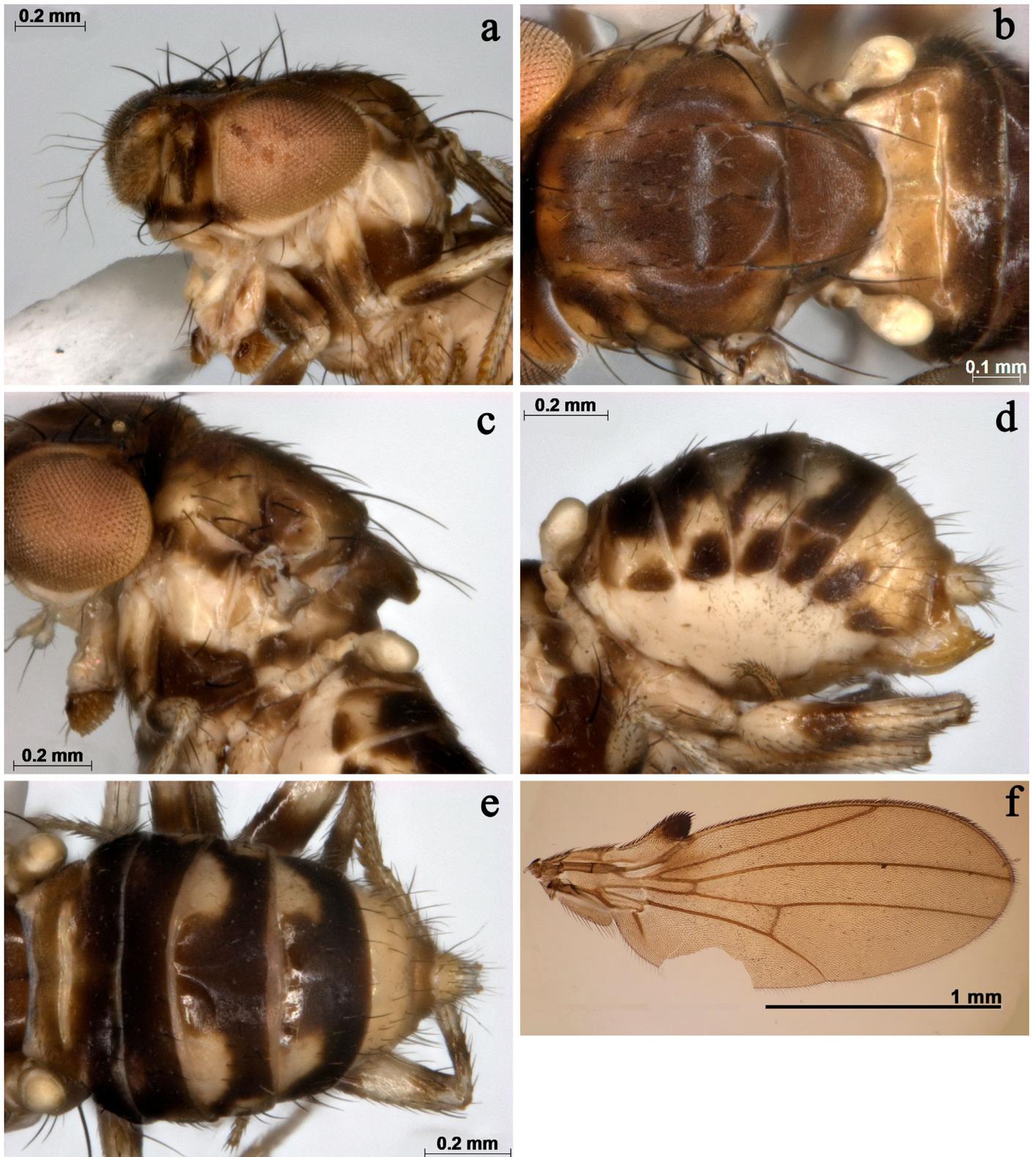


Figure 7 *Hirtodrosophila* sp.1: a, head (anterolateral view); b, thorax (dorsal view); c, thorax (lateral view); d, abdomen (lateral view); e, abdomen (dorsal view); f, wing.

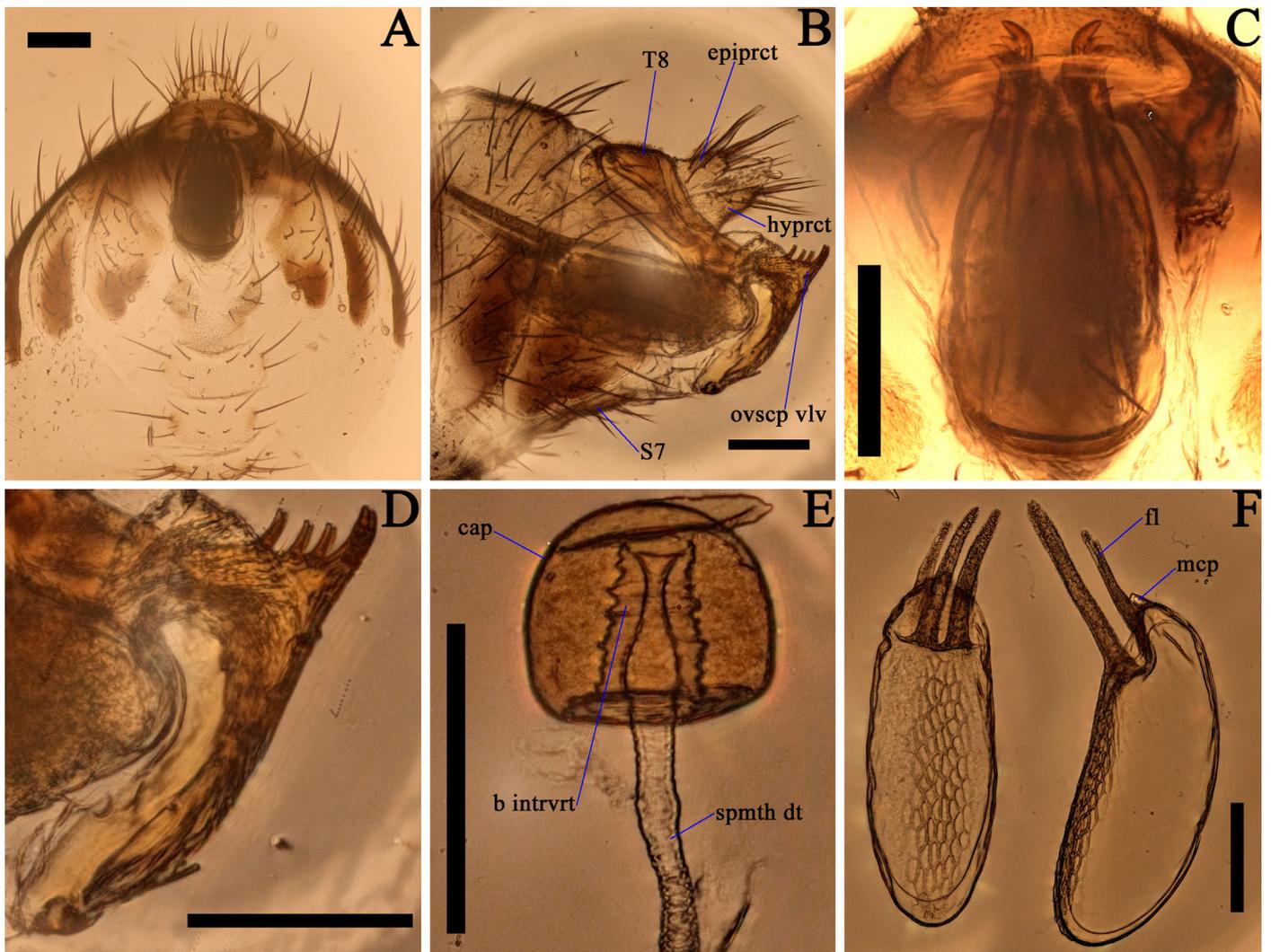


Figure 8 *Hirtodrosophila* sp.1: a, posterior end of abdomen (ventral view); b, posterior end of abdomen (lateral view); c, oviscapt (ventral view); d, oviscapt (lateral view); e, spermatheca (lateral view); f, egg choria (left, dorsal view; right, lateral view). Scale bars: 0.1 mm. Abbreviations: b intrvrt, basal introvert; epiprct, epiproct; fl, filament; hyprct, hypoproct; cap, capsule; mcp, micropyle; ovscp vlv, oviscapt valve; spmth dt, spermathecal duct; S7, seventh sternite; T8, eighth tergite.

Description.

Head (Fig. 7a): dorsal half brown and ventral half light yellow. Eyes red, pubescent. Face dark brown, with a transversal lighter band medially, facial carina brownish. Antennal scape brown; pedicel basally brown and apically lighter; flagellomere 1 dark brown, lighter basally, compressed, mango-shaped; arista with seven dorsal and one ventral branches, and terminal fork. Frons entirely dark brown except for the fronto-orbital plate; fronto-orbital plate yellow, with a brown spot at the base of *pc frorb s*; fronto-orbital setae black; distance between *pc frorb s* and *a rc frorb s* = 0.03 mm, between *pc frorb s* and *p rc frorb s* = 0.07 mm, and between *a rc frorb s* and *p rc frorb s* = 0.04 mm; frontal vitta dark brown, slightly lighter near ptilinal suture; frontal triangle medium; ocellar triangle darker; postocellar setae black, crossing. Genae light yellow below vibrissae. Clypeus light yellow; palpus light yellow; labrum mostly light yellow, labellum brown. **Thorax** (Fig. 7b, 7c): brown and light yellow; scutum mostly brown, with lighter areas on anterior half; anterior dorsocentral setae smaller than posterior dorsocentral setae; four rows of acrostichal setulae. Postpronotum yellow. Scutellum brown; basal scutellar setae curved inwards. Proepisternum, anepimeron and anepisternum light yellow, with marks forming a continuous, dark brown band on dorsal margin;

katapisternum brown, forming a band extending posteriorly. Legs tan; fore coxae light brown; femora with sub-basal dark rings; mid and hind tibiae with two dark rings. **Wings** (Fig. 7f): infuscated, with black, large and broad, apically round costal lappet. Faint darker patch present bellow costal lappet. R_{4+5} and M_1 slightly divergent. Halter brown, apically yellow. Indices: $C = 1.18$, $ac = 3.72$, $hb = 1.14$, $4c = 1.81$, $4v = 2.49$, $5x = 2.31$, $M = 0.81$, $prox. x = 0.46$; measurements: length = 1.76 mm, width = 0.75 mm. **Abdomen** (Fig. 7d, 7e): T1 faint brown; T2 black, with sub-lateral yellow patches; T3 black, with sub-lateral yellow patches, with anterior small faint yellow spots sub-lateral on the brown area, medially faint yellow patch; T4 black, with sub-lateral yellow patches, sub-medial round yellow mark at the anterior margin; T5 black, with sub-lateral faint yellow patches, sub-medial round yellow mark at the anterior margin; T6 and T7 yellow, laterally black. Intersegmental membranes light. Sternites mostly yellow, S7 with microtrichia and with 8 setulae. **Terminalia** (Fig. 8a-8e): T8 with microtrichia on dorsal region. Epiproct and hypoproct with microtrichia and many long or smaller setae. Oviscapt valve sclerotized, long, squarish at tip in lateral view, with 4 large, peg-like ovisensilla on posterior margin, 4 peg-like ovisensilla on apical, ventral margin, and tiny pegs on anterior, ventral margin. Spermathecal capsule sclerotized, dome-shaped; introvert

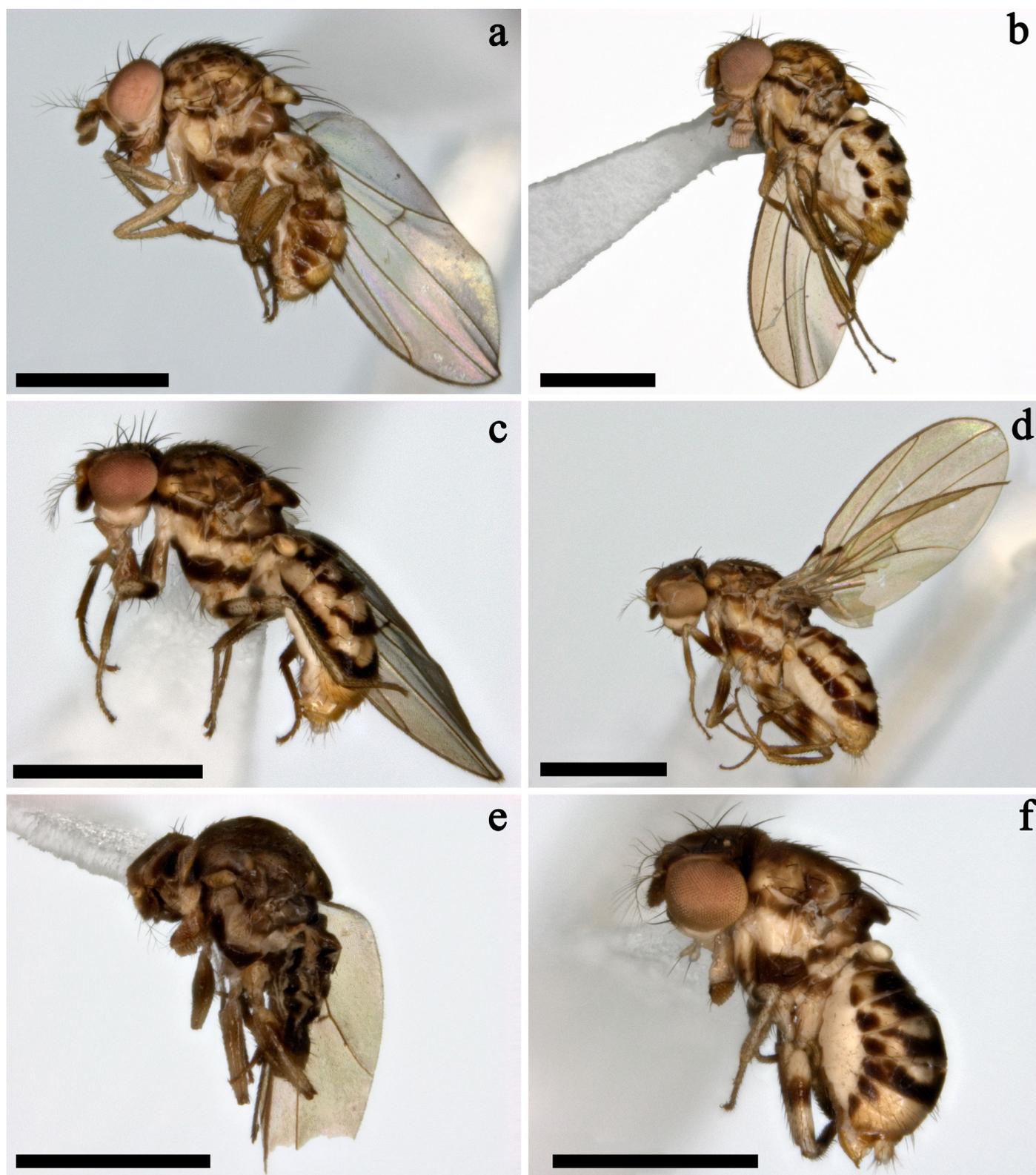


Figure 9 Habitus: a, holotype of *H. pilosa* n. sp.; b, holotype of *H. fluminensis* n. sp.; c, holotype of *H. brasiliensis* n. sp.; d-e, paratypes of *H. brasiliensis* n. sp.; f, *H. sp.1*. Scale bars: 1 mm.

4/5 as deep as capsule height. **Eggs** (Fig. 8f): chorion only dorsally ornated, with four filaments and micropyle; apical filaments about 1/2 as long as sub-apical; sub-apical filaments 1/2 as long as chorion. **Body length**: 1.92 mm.

Discussion

This study has added three new species to the Neotropical *Hirtodrosophila* fauna, increasing the total number of species to 41.

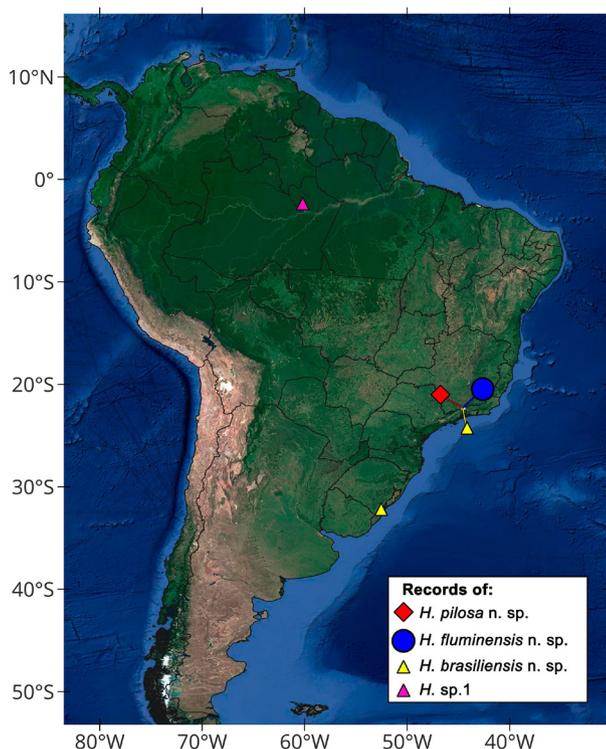


Figure 10 Map showing the collection records of *H. pilosa* n. sp., *H. fluminensis* n. sp., *H. brasiliensis* n. sp., and *H. sp.1*.

Unfortunately, little can be inferred concerning the ecology of the new species as they were collected using Malaise traps. Generally, members of this genus, such as the *hirticornis* group (Junges et al., 2019), are associated with sporocarps (Gautério et al., 2020), but some species of the *melanderi* group (Mei et al., 2019) are associated with fruits. However, the present results corroborate the importance of Malaise traps in uncovering the diversity of some Dipteran groups (Brown, 2005), which might have been overlooked in collections by traditional means.

The species described in this paper differ in the external morphology from the 46 known *Hirtodrosophila* species of the Americas and do not fit any described species group of this genus. At first glance, the studied specimens were thought to be *Paramycodrosophila* Duda, 1924, mainly for the presence of the costal lappet, a key character in the dichotomous key to genera of the Drosophilidae in the Manual of Central American Diptera (Grimaldi, 2010). A darkened costal lappet, however, is present in many genera of Drosophilidae, and might be associated with misplacement of a species in *Paramycodrosophila*: *P. poeciloptera* Duda, 1925, synonymous with *Drosophila schildi* Malloch, 1924. Although *H. pilosa* n. sp. and *H. fluminensis* n. sp. share many diagnostic characters with *Paramycodrosophila*, such as the mentioned above, they are more morphologically similar to some American species *Hirtodrosophila*, such as *H. alabamensis* (Sturtevant, 1981) and *H. pictiventris* (Duda, 1925). However, the key character for their generic assignment is the morphology of the epandrium. The species described here differ in the epandrial morphology largely from *P. costaricana* [see Vilela and Bächli (1990)] and many other yet undescribed Neotropical species of *Paramycodrosophila*, but strongly resemble some known species of *Hirtodrosophila*, such as *H. alabamensis*, *H. florida* Grimaldi, 2018, *H. grisea* (Patterson and Wheeler, 1942), especially in relations to the size and setation of the ventral lobe [see Grimaldi (2018)].

Hirtodrosophila brasiliensis n. sp. is also essentially different in the morphology of male terminalia from *Paramycodrosophila*.

Hirtodrosophila sp.1 seems to be closely related to this species by its overall morphology but is certainly a different species. For the lack of evidence to adequately delimit this species, *H. sp.1* has been refrained from designating as a new species.

To better understand the relationships of the described species within the *Zygothrica* genus group, the phylogenetic information is indispensable. The most recent molecular phylogenetic analyses have revealed that *Hirtodrosophila* is not monophyletic but separated into some lineages, each of which forms a monophyletic group along with another genus of the *Zygothrica* genus group, *Paramycodrosophila*, *Mycodrosophila* Oldenberg, 1914 or *Zygothrica* Wiedemann, 1830 (Gautério et al., 2020; Zhang et al., 2021). Thus, the generic classification within the *Zygothrica* genus group should be reconsidered, based on further phylogenetic analyses with expanded taxon sampling incorporating the three new species described in this study as well.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

MHFT participated in the preparation of the specimens, analyzed the specimens, produced the pictures and wrote the manuscript. VLSVG supervised the study, read and discussed the manuscript. MSG participated in the preparation of the specimens, analyzed the specimens and revised the manuscript. All authors participated in the conceptualization of the study.

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