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A new species of Neotropical *Drosophila* (Diptera, Drosophilidae) belonging to the *guarani* group



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ABSTRACT

Drosophila butantan sp. nov., a species belonging to the *guarani* group and closely related to *Drosophila nigrifemur* from Bolivia, is described based on a female, and some of its offspring, collected at the forest reserve of the Instituto de Biociências da Universidade de São Paulo, Cidade Universitária “Armando de Salles Oliveira”, São Paulo City, state of São Paulo, Brazil. Although externally similar, the two apparently forest-dwelling species can be told apart by having distinct oviscapt valves and spermathecal introverts and tips. Accordingly, a proposal is made to also include *D. nigrifemur*, a previously unassigned species, in the *guarani* group. The two species seem to be also related to *Drosophila alexandrei* and *Drosophila guaraja* as indicated by their external morphology, their elongate spermathecae and the not so sharply pointed oviscapt valves. The karyotypes of the new species differ from those described for *D. alexandrei* and *D. guaraja*, while those of *D. nigrifemur* remain still unknown. Photomicrographs of the male and female imagines, in addition to drawings and photos of their terminalia, are also included.

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Introduction

Most species of *Drosophila* belonging to the Neotropical *guarani* group are readily recognized by their dark brown body and by having wings with clouded main crossveins. The *guarani* species group was proposed by Dobzhansky and Pavan (1943: 66) and is currently (Bächli, 2017) composed by 16 species: *Drosophila alexandrei* Cordeiro, 1951, *D. araucana* Brncic, 1957, *D. cuscungu Vela and Rafael, 2005*, *D. ecuatoriana Vela and Rafael, 2004*, *D. griseolineata Duda, 1927*, *D. guaraja King, 1947*, *D. guaru Dobzhansky and Pavan, 1943*, *D. huilliche Brncic, 1957*, *D. limbinervis Duda, 1925*, *D. maculifrons Duda, 1927*, *D. ornatifrons Duda, 1927*, *D. pichinchana Vela and Rafael, 2004*, *D. quitensis Vela and Rafael, 2004*, *D. subbadia Patterson and Mainland in Patterson, 1943*, *D. tucumana Vilela and Pereira, 1985*, and *D. urubamba Vilela and Pereira, 1993*.

During a two-year Drosophilid fauna survey conducted from September, 1995 through August, 1997 (Ratcov, 2002 unpublished thesis) in an urban forest reserve in São Paulo City, we collected some small unknown dark brown male and female imagines belonging to the *guarani* group. Although we were unable to

identify them to the species level, we suspected they were conspecific. After having established an isofemale line (coded I48F71) we could confirm the previous male/female association and analyze their male terminalia, especially the aedeagus and the inner spermathecal capsules. At first sight, based on the shape of the latter structures, we suspected they could belong to *Drosophila alexandrei*, a species collected in Rio Grande do Sul state (Cordeiro, 1951), only known from its original description and whose type specimens are thought to be lost. The analysis of the male terminalia was of no help as they were neither depicted nor illustrated in the original description of the candidate species.

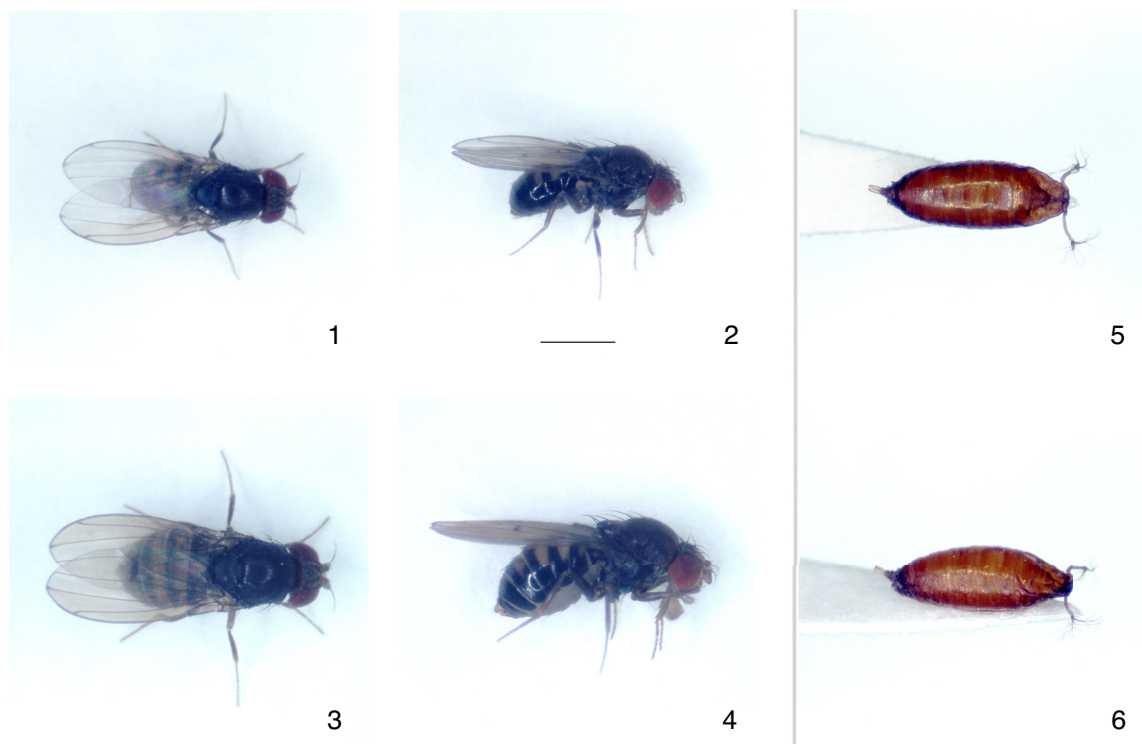
Ten years later, in December 2006, 10 specimens (4 males: 6 females) of this undescribed species were identified among 495 drosophilids aspirated from living inflorescences of *Goepertia monophylla* (Vell.) Borchs. & S. Suárez (Borchsenius et al., 2012) (Marantaceae), in the same forest reserve (Vaz et al., 2014).

More recently, analyses of the mitotic metaphase chromosomes obtained from the larval brain sampled from isofemale line I48F71 and described below suggest the unknown species we have collected and *Drosophila alexandrei* belong to different species.

The purpose of the present paper is to describe this unknown forest-dwelling Neotropical species of the *Drosophila guarani* group, which is attracted to but apparently does not breed on inflorescences of *Goepertia monophylla*, a Marantaceae species locally known as caeté.

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Figs. 1–6. *Drosophila butantan* sp. nov., paratypes and pupa from isofemale line I48F71: (1, 2) male paratype, and (3, 4) female paratype, in dorsal and right lateral views, respectively; (5, 6) ordinary puparium + pupa, dorsal and oblique dorsal views. Scale bar = 1 mm.

Material and methods

One female collected with an entomological net over a banana-baited trap in late August 1996, including her first-generation offspring (5 males, 8 females), plus two specimens (1 male, 1 female) of a much later generation of the same isofemale line (I48F71) yielded in the laboratory in a modified banana-agar medium (Goñi and Vilela, 2016) were used in the description of the new species. The trap was set in the forest reserve of the *Instituto de Biociências da Universidade de São Paulo, Cidade Universitária “Armando de Salles Oliveira”*, São Paulo city, state of São Paulo, an urban fragment of the montane Atlantic forest of south-eastern Brazil. Label data accompanying each type specimen are recorded as given; a comma indicates a line change, and a slash, a label change. Our own notes or interpretations are included in brackets. All specimens are deposited in the *Museu de Zoologia da Universidade de São Paulo*, São Paulo, Brazil (MZUSP). Dissections of reproductive structures follow Wheeler and Kambyssellis (1966), as modified by Kaneshiro (1969) and Bächli et al. (2004). For morphological terminology, measurements, indices, preparations of microscope slides as well as illustrations refer to Vilela and Bächli (1990, 2000) and Bächli et al. (2004). Whenever in the same plate, all line drawings were drawn to the same scale, and all photomicrographs were taken and enlarged to the same magnification.

Photomicrographs of imagoes and pupa (4× objective) were taken with an Olympus camera (PM2) loaded with an analog 35 mm Fujichrome Professional 64T film and attached to an Olympus stereomicroscope (SZ11) with a ring illuminator. The analog images were later digitized with an Epson scanner (Perfection 4180 photo). Photomicrographs of wing (4× objective, optovar 1×) and terminalia structures (10× objective, optovar 1×) were taken with an Olympus Q-Color 5 digital camera attached to an Olympus BX60 microscope. A set of 20–50 pictures was taken by manually focusing each structure at different depths. This set of photomicrographs was then digitally stacked to create an all-in-focus composite using

the Combine ZP software (open source software CombineZP at <http://combinezp.software.informer.com>).

Initially, the isofemale line I48F71 was kept in a short-lasting powdered milk-agar medium (Bächli et al., 2000) for about 10 years. However, during the past 11 years, the cited strain has been also successfully maintained in a long-lasting modified banana-agar culture medium (Goñi and Vilela, 2016). The imagoes are kept in conventional cylindrical glass tubes (20 × 100 mm), containing ca. 3 mL of modified banana-agar medium for 3 weeks, at $18 \pm 1^\circ\text{C}$ and 13:11 h (L:D) photoperiod. During this period, they are transferred once a week to new vials to reach sexual maturation. A tiny ball (ca. 1 mm diameter) of solid and fresh baker’s yeast is added after transferring the flies. The flies are discarded three weeks after emergence and, one week later, two V-shaped filter paper strips are inserted into the medium and the vials are placed inside one ¼ l flask containing wet sand at the bottom. Emerged flies of the new generation are then aspirated once a week and transferred again to a modified banana-agar medium, restarting the cycle. This method has also been successfully used in our laboratory in São Paulo city to keep strains of some species belonging to the *cardini* and *tripunctata* groups. Since early February 2017 the wet sand has successfully been replaced by exfoliated vermiculite. It is worth mentioning the calm behavior of the imagoes of the new species inside the culture flasks containing sand or vermiculite, facilitating the aspiration and transfer of emerged flies to new vials.

Male and female mitotic chromosomes were obtained from cytological preparations of cerebral ganglia of third instar larvae of both sexes of the new species sampled from the isofemale line I48F71, according to the method of Imai et al. (1977, 1988). All cytological preparations were made from single individuals cultured with banana-agar-yeast media at 18–20 °C. Cytological preparations were observed in an Olympus BX60[®] microscope equipped with an Olympus U-MAD-3[®] camera. Suitable mitotic cells were selected and then digitalized using the Image-Pro Plus[®] version 5.1 software. Furthermore, mitotic chromosomes from

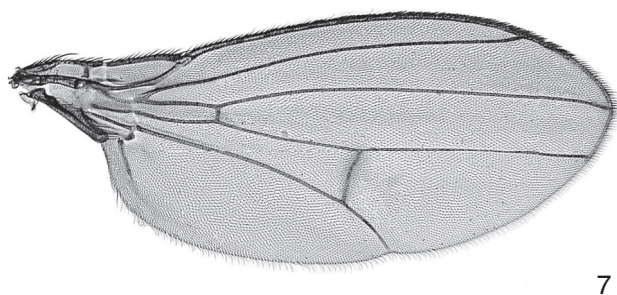


Fig. 7. *Drosophila butantan* sp. nov.: male paratype, right wing, dorsal view. Scale bar = 1 mm.

individuals of both sexes of *Drosophila guaraja* from the isofemale line coded M28F1, established from a single gravid female collected at downtown Ubatuba at the North coast of the state of São Paulo in July 2007, were obtained and analyzed as described above. In this case, the mitotic cells were observed in a Nikon Eclipse 80i microscope with a Digital Sight camera, and selected cells were digitized using NIS-Elements AR software. All cytological images were processed in GIMP 2.8.14 (GNU Image Manipulation Program).

Drosophila butantan sp. nov.

(Figs. 1–28)

Drosophila sp. W3, Vaz et al., 2014: 608+ Supp. Key S1.

Material examined (6 males, 10 females, deposited in MZUSP). Holotype male [first-generation offspring of an isofemale line, coded I48F71, emerged 10–22.X.1996, dissected] labeled: “Brasil – SP, São Paulo, Reserva Florestal [forest reserve] do IB-USP [Instituto de Biociências – Universidade de São Paulo], Cidade Universitária, 28–30.VIII.1996, Ratcov & Vilela coll./*Drosophila butantan* [male symbol] Ratcov & Vilela/terminalia ilustrada [illustrated terminalia]/HOLOTYPE/[glass microvial with terminalia in glycerin]”. Paratypes = one male and one female (photomicrographed) from an undetermined generation of the cited isofemale line, one wild-caught female [mother of the cited isofemale line] plus its first-generation offspring but the holotype: 4 males [one dissected] and 8 females [two dissected]; same data as holotype.

Type locality. Forest Reserve of IB-USP, *Cidade Universitária “Armando de Salles Oliveira”*, district of Butantã, São Paulo city, state of São Paulo, Brazil.

Diagnosis. Small coffee-brown fly; eye wine-red; head, legs, scutum and scutellum coffee-brown, dull; pleura blackish brown, semi-dull; abdomen conspicuously shiny, tergites 1–4 anteriorly light brown, with broad, well delimited, blackish brown distal bands, tergites 5–7 usually entirely blackish brown; halter, cercus, epiproct and hypoproct light brown; anterior scutellars divergent; wing light brown, crossvein dM-Cu and apices of R_{2+3} , R_{4+5} and M veins slightly clouded, tip of M vein slightly turned posterad, C index 2.84–3.29; valve of oviscapt apically somewhat pointed but not sharply.

Description. Male ($n=6$) (Figs. 1 and 2). Head brown. Frontal length 0.28 (0.27–0.29) mm, frontal index = 0.89 (0.85–0.92), top to bottom width ratio = 1.72 (1.64–1.77). Frontal triangle subshining, about 83–100% of frontal length; ocellar triangle dark brown, about 33–36% of frontal length. Orbital plates subshining, narrower adjacent to or2, anteriorly diverging from eye margin, about 92–100% of frontal length. Orbital setae black, or2 outside or1 and or3; distance of or3 to or1 = 80% of or3 to vtm, or1/or3 ratio = 0.67, or2/or1 ratio = 0.33, postocellar setae = 63 (58–73)%, ocellar setae = 82 (75–83)% of frontal length; vt index 1.00 (0.90–1.11); vibrissal index 0.75 (0.67–0.71). Face and cheek shiny light brown. Carina light brown, prominent, straight, not

sulcate. Cheek index about 7–10. Eye wine-red; eye index = 1.17 (1.11–1.25). Scape light brown, pedicel mostly dark brown, apically lighter, first flagellomere brown; length to width ratio 1.8. Arista with 5 dorsal, 2 ventral long branches, and about 7 inner branches relatively long, plus terminal fork. Proboscis and palpus brown.

Thorax mostly dark brown; length ca. 1.00 mm. Scutum pollinose, anteriorly light brown, laterally and posteriorly dark brown; 6 rows of acrostichals. Setae and setulae dark brown with golden sheen. Transverse distance of dorsocentral setae 200% of longitudinal distance; dc index 0.70. Scutellum pollinose dark brown, apically blunt; distance between apical scutellar setae equidistant to that between apical and basal one; basal setae strongly divergent, apical ones cruciate; scut index = 1.04. Pleura shiny brown, sterno index = 0.54, median katepisternal seta about 50–63% of anterior one. Halter light brown basally, dark brown distally. Legs uniformly light brown; apical seta on protibia and mesotibia; preapical seta on all tibiae.

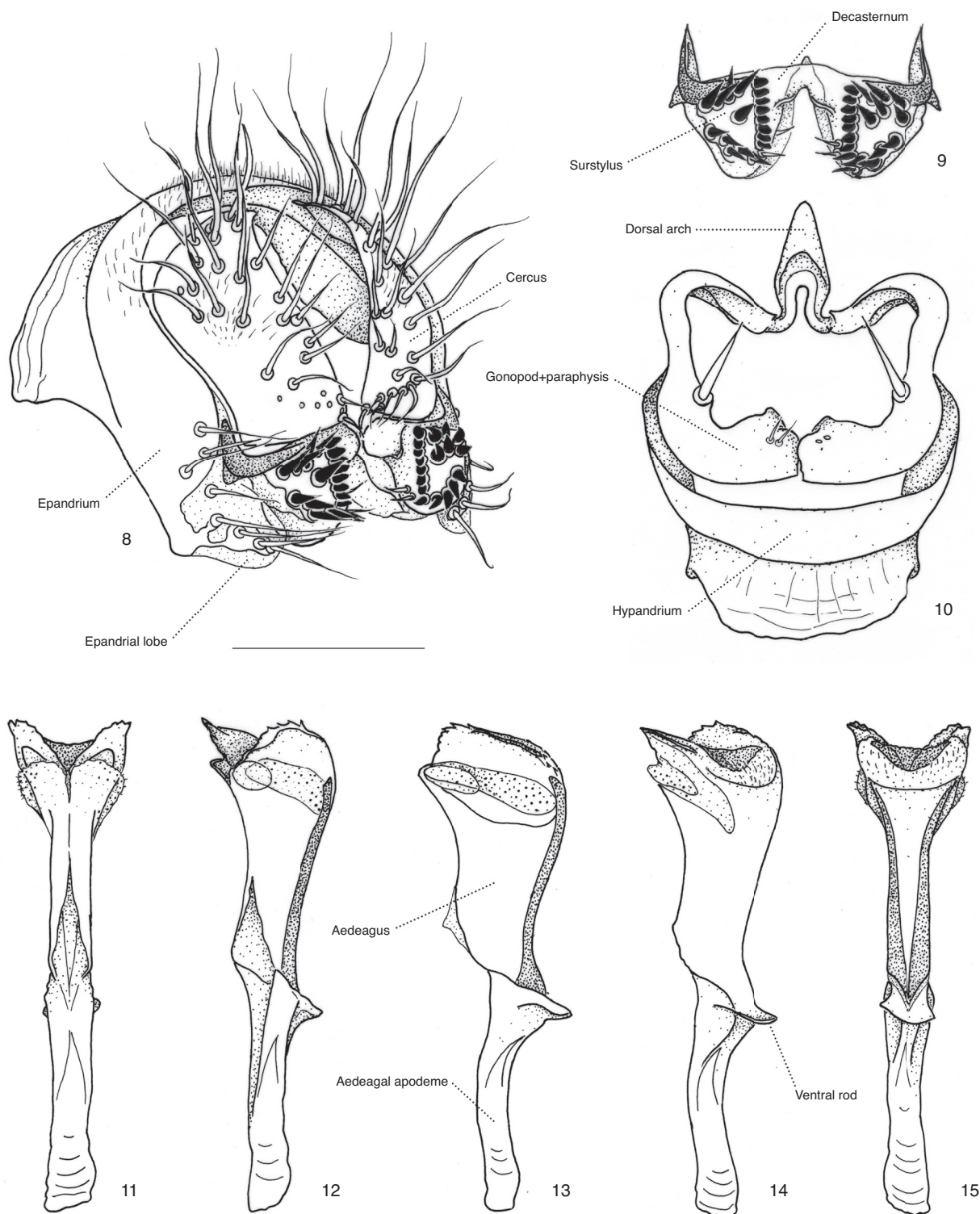
Wing (Fig. 7) light brown hyaline, crossveins R-M and dM-Cu, and apices of veins R_{2+3} , R_{4+5} and M slightly clouded, tip of vein R_{4+5} slightly curved posterad; length 1.90 (1.80–1.93) mm, length to width ratio = 2.02 (1.95–2.08). Indices: C = 3.06 (2.84–3.29), ac = 2.15 (2.00–2.38), hb = 0.49 (0.44–0.50), 4C = 0.79 (0.75–0.86), 4v = 1.67 (1.62–1.73), 5x = 1.14 (1.11–1.22), M = 0.45 (0.42–0.46), prox. X = 0.55 (0.50–0.59).

Abdomen (Fig. 2) shiny, brownish black; tergites 1–4 light brown with a characteristic distal dark brown band, well delimited, medially interrupted and laterally broadened, reaching anterior margin of tergite; tergite 5 entirely shiny brownish black, except for a thin median longitudinal light brown stripe in some specimens, and tergite 6 entirely shiny dark brown.

Male terminalia (Figs. 8–18). Epandrium (Figs. 8, 9 and 16) almost bare, slightly microtrichose on posterior dorsal area; upper setae absent; ca. 8 lower setae; ventral lobe mostly membranous (Figs. 8 and 16), not covering surstylus. Cercus slightly microtrichose on dorsocentral area, linked to epandrium by membranous tissue (Fig. 8). Surstylus not microtrichose, with about 7–8 cone-shaped prensisetae, about 10 long, strong outer setae and about 2 short, thin, mostly inner setae (Figs. 8, 9 and 16). Decasternum as in Figs. 8 and 9. Hypandrium (Figs. 10 and 17) as long as epandrium, anterior margin convex; posterior hypandrial process absent; dorsal arch medially pointed posterad, strongly sclerotized; gonopod not microtrichose, fused to paraphysis, bearing one long seta on median inner margin. Aedeagus (Figs. 11–15 and 18) distally bifid (in dorsal and ventral views, Figs. 11 and 15), blunt and slightly expanded dorsoventrally (in lateral view, Figs. 13 and 18); subapically bearing a dorsoventral membranous area covered with tiny spines; dorsal cleft ca. 1/3 length of aedeagus (Fig. 12); paraphysis not microtrichose, fused to gonopod, submedially bearing two setulae adjacent to proximal margin (Figs. 10 and 17). Aedeagal apodeme as long as aedeagus and fused to it, rod-shaped, dorso-distally bifid (Figs. 11–15 and 18). Ventral rod triangle-shaped, completely fused to aedeagal apodeme (Figs. 12–15, 18).

Female ($n=10$) (Figs. 3 and 4). Color difference from male: in some specimens, color pattern of tergite 5 is similar to that of male tergite 4 (compare Figs. 2 and 4) with dark brown distal band interrupted by a large light brown triangle-shaped longitudinal stripe running from tergite 4 to 5, and color pattern of tergite 6 of some specimens not being entirely dark brown but bearing a thin median light brown longitudinal stripe.

Measurements: Frontal length 0.30 mm; frontal index = 0.87, top to bottom width ratio = 0.61. Frontal triangle about 75–92% of frontal length. Ocellar triangle about 33–42% of frontal length. Orbital plates about 92–100% of frontal length. Distance of or3 to or1 = 67–125% of or3 to vtm, or1/or3 ratio = 0.65 (0.60–0.70), postocellar setae = 67 (54–75)%, ocellar setae = 91 (85–100)% of frontal length; vt index = 1.04 (1.00–1.10); vibrissal index = 0.61

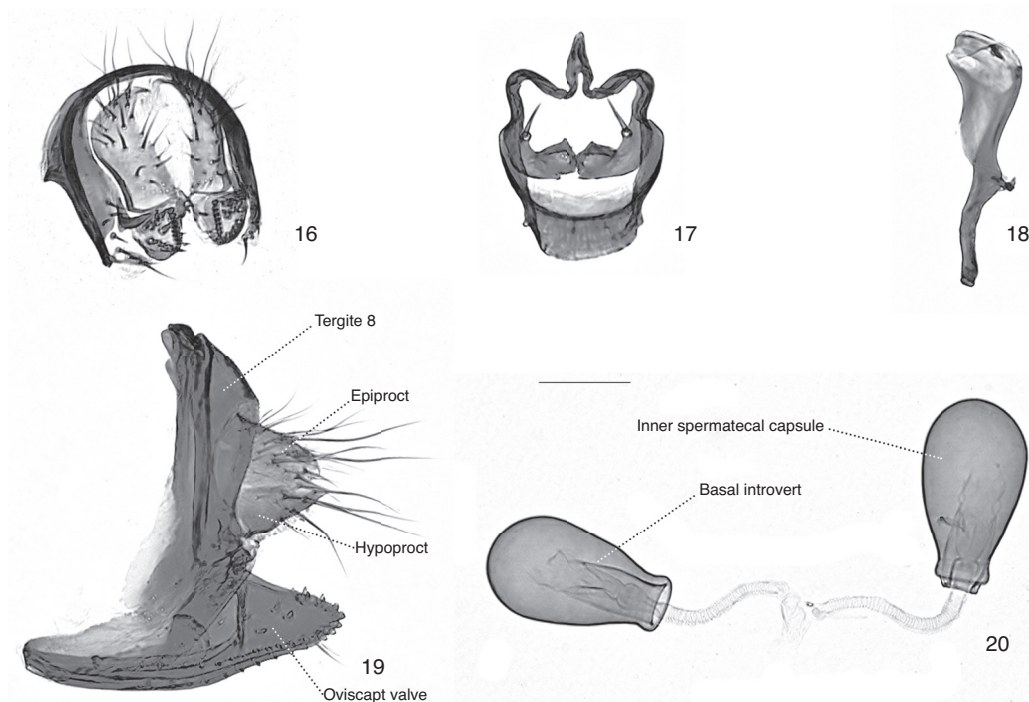


Figs. 8–15. *Drosophila butantan* sp. nov., male holotype, terminalia: (8) epandrium, cerci, surstyli, and decasternum, oblique posterior view; (9) surstyli and decasternum, posterior view; (10) hypandrium, gonopods and paraphyses, posterior view; (11–15) aedeagus + aedeagal apodeme, several views from dorsal through ventral. Sale bar = 0.1 mm.

(0.44–0.75). Cheek index about 7.43 (6.67–10.50). Eye index = 1.16 (1.10–1.24). Thorax length 1.12 (1.10–1.17) mm. h index = 1.00 (0.90–1.14). Transverse distance of dorsocentral setae 229–267% of longitudinal distance; dc index = 0.63 (0.53–0.72). Distance between apical scutellar setae about 88–114% of that between apical and basal one; scut index = 1.01 (0.94–1.12), sterno index = 0.54 (0.44–0.62), median katepisternal seta about 44–67% of

anterior one. Wing length 2.18 (1.95–2.46) mm, length to width ratio = 2.13 (1.95–2.28). Indices: C = 3.21 (3.05–3.44), ac = 2.26 (2.00–2.57), hb = 0.47 (0.42–0.50), 4C = 0.78 (0.74–0.83), 4v = 1.70 (1.60–1.96), 5x = 1.26 (1.10–1.44), M = 0.49 (0.42–0.54), prox. X = 0.56 (0.48–0.71).

Terminalia (Figs. 19–22). Oviscapt valve double-walled, apically roundish, submedially slightly expanded dorsad, ventrally



Figs. 16–20. *Drosophila butantan* sp. nov., male holotype (16–18) and female paratype (19–20), terminalia: (16) epandrium, cerci, surstylus and decasternum, oblique posterior view; (17) hypandrium, gonopods + paraphyses, posterior view; (18) aedeagus + aedeagal apodeme, left lateral view; (19) tergite 8, epiproct and hypoproct, oviscapt valves, left lateral view; (20) inner spermathecal capsules, lateral view. Scale bar = 0.1 mm.

slightly undulate, with ca. 16 discal and about 6 marginal, peg-like, mostly roundish-tipped (neither sharply pointed nor bearing a mediodorsal, pointed triangle-shaped process, as it occurs in *D. nigrifemur*), outer ovisensilla; trichoid-like inner ovisensilla: 3 thin distally positioned, and 1 long, slightly curved, subterminal; inner wall (Fig. 21, dashed line) proximally narrow, distally as wide as outer wall. Spermathecal inner capsule elongate, light bulb-shaped, sclerotized, distally not flatten (somewhat flatten in *D. nigrifemur*), slightly waisted subbasally, furrowed at basal 1/7; basal introvert deeply invaginated (but neither reaching the tip nor distally expanded, as it occurs in *D. nigrifemur*) and subproximally dilated.

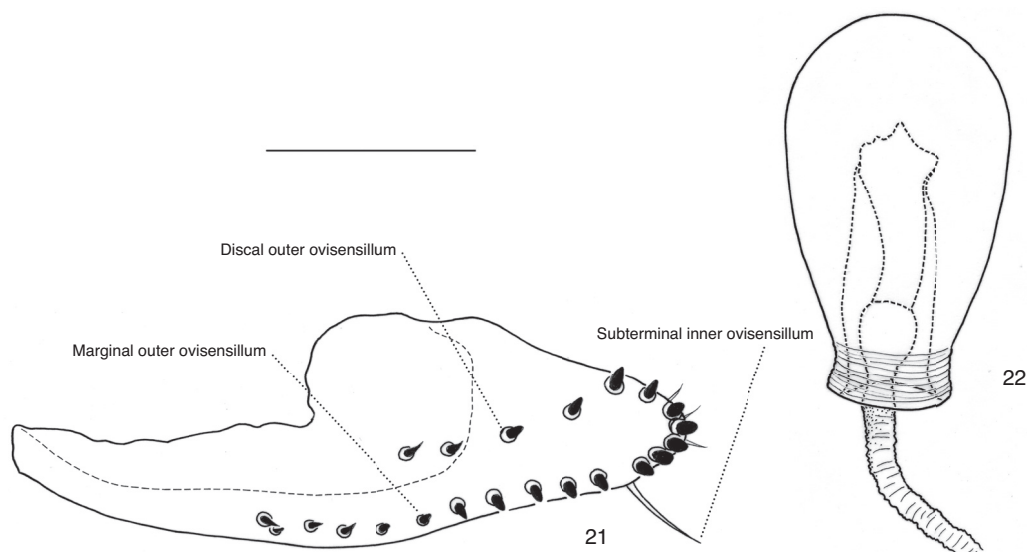
Egg ($n = 1$). Whitish, length ca. 0.51 mm, bearing 4 thin filaments of equal length (0.43 mm), slightly shorter than egg.

Puparium ($n = 1$) (Figs. 5 and 6). Reddish dark brown; horn index about 2.8, with ca. 18 light brown tracheal branches per stalk; stalks of anterior and posterior spiracles light brown.

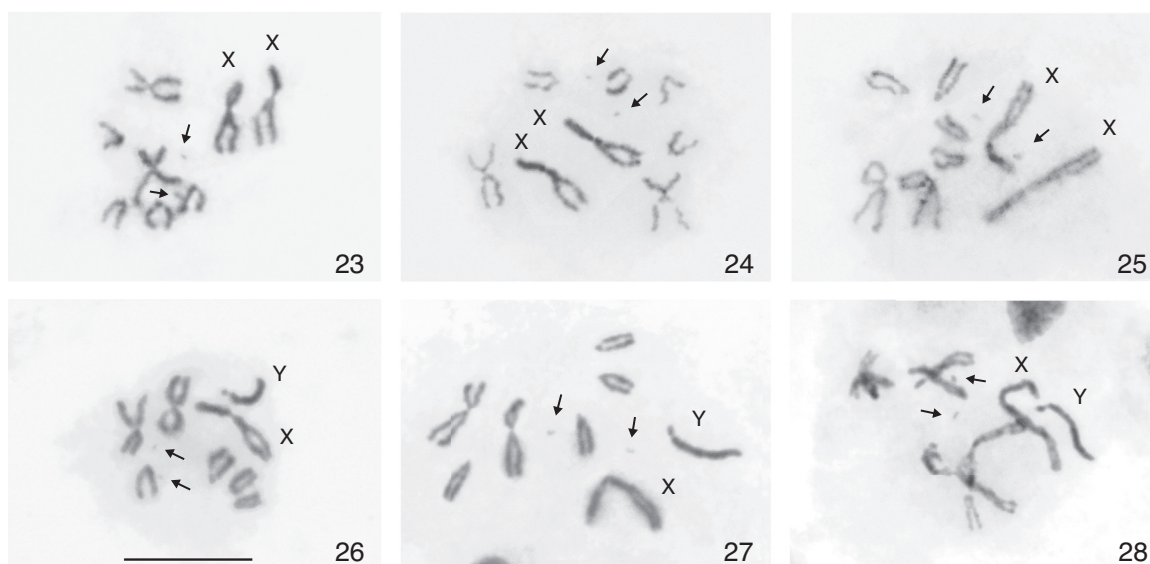
Distribution. This species is known only from the type locality (city of São Paulo, state of São Paulo, Brazil).

Etymology. The epithet *butantan* [alternative spelling of Butantã] is a noun in apposition and an allusion to the district where the type locality is located.

Ecology. Only 9 males (0.07%) of *Drosophila butantan* sp. nov. were identified among 13,057 males of species belonging to the



Figs. 21 and 22. *Drosophila butantan* sp. nov., female paratype, terminalia: (21) left oviscapt valve, outer lateral view; (22) inner spermathecal capsule, lateral view. Scale bar = 0.1 mm.



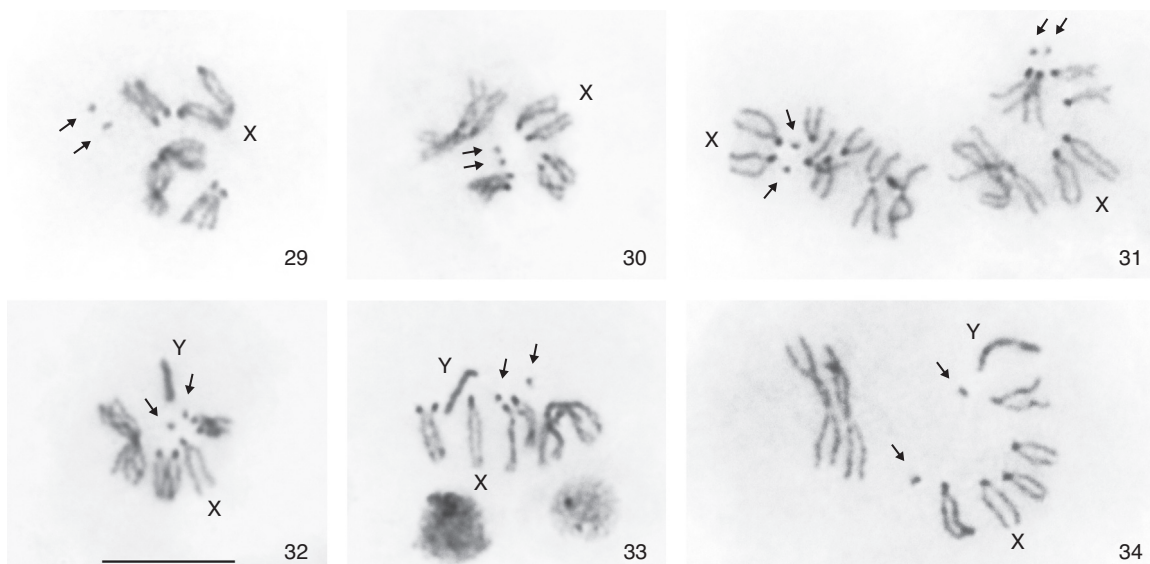
Figs. 23–28. Mitotic chromosomes of *Drosophila butantan* sp. nov., (23–25) female and (26–28) male metaphase plates from larval neuroblasts. The chromosome number is $2n = 10$ (2R, 2V, 1D), with metacentric (V-shaped) X and telocentric (rod-shaped) Y chromosomes. Sex chromosomes are indicated in all cells and arrows indicate the small and faintly stained dot chromosomes. Scale bar = 10 μm .

genus *Drosophila* sampled in two years (1996–1997) by Ratcov (unpublished thesis) and Vilela from fermenting banana-yeast baited traps set in the type locality. It was comparatively more abundant in four non-consecutive days of collection conducted in December 2006, in the same forest reserve, by aspirating flies from inflorescences of *Goepertia monophylla*, where four males of *Drosophila butantan*, sp. nov. (1.29%), in addition to six females, were collected among 309 males of species of *Drosophila* sampled. However, none of the 137 drosophilids that emerged from 20 inflorescences of *Goepertia monophylla* collected in the same date and site belonged to *Drosophila butantan* sp. nov. Thus, the larval breeding sites of this species remain unknown.

Chromosomes (Figs. 23–28). *Drosophila butantan* sp. nov. has a karyotype of $2n = 10$ (2R, 2V, 1D), comprising two pairs of rod-shaped telocentric chromosomes of decreasing size, two pairs of large V-shaped metacentrics, and one pair of barely recognizable

dot (D) microchromosomes. The V's are of two kinds, one is approximately equal-armed with both euchromatic arms being observed in pairs in mitotic cells of both sexes, and corresponding to an autosome, while the other, the largest V's with unequal arms, being the shortest arm totally heterochromatic and representing the X chromosome. The sex chromosomes are definitely heteromorphic, while the X is a large V, the Y is a telocentric (rod-shaped) chromosome, shorter than the X chromosome, totally heterochromatic and often curved rather than bent, bearing a small satellite at the proximal region (Figs. 26–28).

Relationship. It belongs to the *Drosophila guarani* species group and is apparently closely related to *Drosophila nigrifemur* from Bolivia (Department of La Paz: Mapiro and San Carlos), redescribed and illustrated by Vilela and Bächli (1990: 116, 293C, 323E). *Drosophila butantan* sp. nov. also shares some similarities with *D. alexandrei* and *D. guaraja* regarding the shape and structure of the



Figs. 29–34. Mitotic chromosomes of *Drosophila guaraja* King, 1947: (29–31) female and (32–34) male metaphase plates from larval neuroblasts. The chromosome composition is $2n = 10$ (3R, 1V, 1D), with telocentric (rod-shaped) X and Y chromosomes. Sex chromosomes are indicated in all cells and arrows indicate the dot chromosomes. Scale bar = 10 μm .

inner spermathecal capsules. The karyotype of *D. butantan* sp. nov. shown here (Figs. 23–28) differs from the karyotype of *D. alexandrei* from Porto Alegre, Brazil, with $2n = 8$ (3R, 1V) reported by Cordeiro (1951), and also from *D. guaraja* from Campos de Jordão, SP, and Rio de Janeiro, Brazil, with $2n = 10$ (3R, 1V, 1D) ($X = R$, $Y = R$) described by King (1947). The mitotic plates of *D. guaraja* from Ubatuba shown in Figs. 29–34 agree, in general, with the chromosome description reported by King (1947) in quotation marks below. This species “shows five pairs of chromosomes, one pair of large equal-armed V’s, 3 pairs of rods of different lengths, and one pair of small dots. The two pairs of longer rods often show satellites and appear to have subterminal centromeres”. The author pointed out that “The sex chromosomes are evidently the smallest rods”, however, our data support that the X clearly corresponds to the largest rod chromosome, as it is shown in Figs. 29–34.

Seven species of the *guarani* group show a diploid number of 12 chromosomes: *D. araucana* (see Brncic, 1957), *D. griseolineata* (see Dobzhansky and Pavan, 1943; King, 1947), *D. guaru* (see Dobzhansky and Pavan, 1943), *D. limbinervis* (see Clayton and Wasserman, 1957), *D. maculifrons* (reported as *D. guaramunu* according to Vilela and Bächli, 1990, in Dobzhansky and Pavan, 1943, King, 1947), *D. ornatifrons* (reported as *D. guarani* according to Vilela and Bächli, 1990, in Dobzhansky and Pavan, 1943, King, 1947), and *D. subbadia* (see King, 1947). The karyotypes of *D. butantan* sp. nov. and *D. guaraja* differ from those of all other species of the group in showing five rather than six pairs of chromosomes (or four pairs of chromosomes, as in *D. alexandrei*). King (1947) proposed an explanation for the reduced chromosome number in *D. guaraja*, and relevant to the hitherto undescribed species shown here. He argues that two autosomal rods have become fused by translocation with resulting loss of one of the centromeres. The sex chromosomes in *D. butantan* sp. nov. are, like those reported for *D. subbadia* by King (1947), definitely heteromorphic ($X = V$, $Y = R$). At present, the cytological observations shown here support the morphological distinctiveness among *D. butantan* sp. nov. and some species of its group. For the time being, *Drosophila butantan* sp. nov. remains unassigned to any of the two subgroups currently recognized within the *guarani* group.

Conflicts of interest

The authors declare no conflicts of interest.

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