

REVISTA BRASILEIRA DE Entomologia

www.rbentomologia.com

Systematics, Morphology and Biogeography

Two new species of *Drosophila* (Diptera, Drosophilidae) associated with inflorescences of *Goeppertia monophylla* (Marantaceae) in the city of São Paulo, state of São Paulo, Brazil



Suzana Casaccia Vaz^{a,b}, Carlos Ribeiro Vilela^{b,*}, Antonio Bernardo Carvalho^a

^a Universidade Federal do Rio de Janeiro, Instituto de Biologia, Departamento de Genética, Rio de Janeiro, RJ, Brazil ^b Universidade de São Paulo, Instituto de Biociências, Departamento de Genética e Biologia Evolutiva, São Paulo, SP, Brazil

ARTICLE INFO

Article history: Received 8 February 2018 Accepted 26 March 2018 Available online 7 April 2018 Associate Editor: Andrzej Grzywacz

Keywords: Breeding site Caeté Drosophilinae Feeding site Taxonomy

ABSTRACT

Two new Brazilian species of *Drosophila* (subgenus *Drosophila*) are described and illustrated: *Drosophila* asymmetrica sp. nov. and *Drosophila peixotoi* sp. nov. Both species were collected, and emerged, from inflorescences of *Goeppertia monophylla* (Marantaceae) in the urban Forest Reserve of the Instituto de Biociências da Universidade de São Paulo and their types will be deposited in the Museu de Zoologia da USP. The former species, which could not be assigned to any known group, has a conspicuously asymmetric aedeagus and a narrow oviscapt valve. The latter species belongs to the *guarani* group and is closely related to *D. guaru*, *D. ornatifrons* and *D. subbadia*, from which it can be distinguished by the presence of just one conspicuous large black spine at inner lower tip of cercus instead of two spines.

© 2018 Sociedade Brasileira de Entomologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Flower-associated species of *Drosophila* belonging to several groups within the subgenus *Drosophila*, such as the *bromeliae*, *flavopilosa*, *onychophora* and *xanthopallescens*, as well as to other subgenera, such as *Phloridosa* and *Siphlodora*, have been reported from the Neotropical Region (Brncic, 1983; Santos and Vilela, 2005). The Drosophilidae genera *Palmomyia*, *Palmophila*, *Scaptomyza* and *Zygothrica* have also been known to include flower-associated, Neotropical species (Malogolowkin, 1952; Brncic, 1983; Vilela, 1984; Grimaldi, 1987; Grimaldi et al., 2003; Santos and Vilela, 2005). Adaptations to exploit such resources have evolved independently several times in different lineages (Markow and O'Grady, 2008).

In a recently published article (Vaz et al., 2014) we described *Drosophila calatheae*, a flower-breeding species associated with inflorescences of *Calathea monophylla* and *C. cylindrica*, vernacularly known as caeté in Brazil. The genus *Calathea* was shown (Borchsenius et al., 2012) to be polyphyletic and the genus *Goeppertia* was resurrected and redefined to include several species of a *Calathea* clade including *C. monophylla* and *C. cylindrica*. Therefore, we should now refer to our previously studied breeding

sites as Goeppertia monophylla and G. cylindrica. Besides Drosophila calatheae we also recognized four additional unknown Drosophila species associated to these inflorescences and less abundant regarding emergence (Vaz et al., 2014; see Tables 1-4). Drosophila calatheae and Drosophila asymmetrica sp. nov. (the latter cited by Vaz et al., 2014 as Drosophila sp. I4) were the two most common species emerging from collected inflorescences while Drosophila peixotoi sp. nov. (cited by Vaz et al., 2014 as Drosophila sp. G4) was their most common visitor. Together, these three species accounted for 120 out of 137 drosophilids flies (~88%) that emerged from 20 inflorescences of G. monophylla. The fourth most common visitor (cited by Vaz et al., 2014 as Drosophila sp. W3), belonging to the guarani group, was recently described (Ratcov et al., 2017) as Drosophila butantan Ratcov, Vilela and Goñi. It should be pointed out that no specimen of the latter species emerged from the inflorescences of Goeppertia monophylla.

The purpose of this article is to describe two new species bred from *G. monophylla* (Figs. 1–4) inflorescences: *Drosophila asymmetrica* sp. nov. (ungrouped) and *Drosophila peixotoi* sp. nov. (*guarani* group).

Materials and methods

* Corresponding author.

Methodology regarding collection, handling and identification of flies and floral resources are detailed in Vaz et al. (2014).

https://doi.org/10.1016/j.rbe.2018.03.003

0085-5626/© 2018 Sociedade Brasileira de Entomologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

E-mail: crvilela@ib.usp.br (C.R. Vilela).



Figs. 1–4. *Goeppertia monophylla* in the Forest Reserve of the Instituto de Biociências da Universidade de São Paulo, São Paulo, SP, Brazil, on 26-27.XII.2006: (1) clumps of caeté on the banks of the stream; (2) two adjacent individuals bearing old inflorescences; (3) young inflorescence with buds; (4) old inflorescence with opened flowers and decaying flowers.

The aspirated and emerged flies are summarized and updated in Table 1. Dissections of reproductive structures were performed according to Wheeler and Kambysellis (1966), as modified by Kaneshiro (1969) and Bächli et al. (2004).

Table 1

Drosophilids aspirated in nature between 12 and 28.XII.2006 from several inflorescences of *Goeppertia monophylla* and emerged in the laboratory between 30.XII.2006 and 12.I.2007 from 20 inflorescences of the same species collected in the Forest Reserve of IB-USP, Cidade Universitária "Armando de Salles Oliveira", São Paulo, state of São Paulo, Brazil.^a

Drosophilid species	Aspirated (in a 5-day collection	Emerged /)
Drosophila calatheae	177	68
Drosophila peixotoi sp. nov. (cited as Drosophila sp. G4)	226	22
Drosophila asymmetrica sp. nov.(cited as Drosophila sp. 14) 24	30
Drosophila butantan (cited as Drosophila sp. W3)	10	0
Other drosophilids	58	17
Total	495	137

^a Data summarized and updated from Vaz et al. (2014).

Photomicrographs of adults were taken under $4 \times$ objective with an Olympus camera (PM2) loaded with an analog 35 mm Fujichrome Professional 64T film and attached to an Olympus stereomicroscope (SZ11) bearing a ring illuminator. The analog images were later digitized with an Epson scanner (Perfection 4180 photo). Photomicrographs of terminalia structures were taken with an Olympus BX60 microscope and an Olympus Q-Color 5 digital camera. A 10× objective was used to generate a set of 20–50 pictures by manually focusing on a given structure at different depths. This set of photomicrographs was then digitally stacked to create an all-in-focus composite using the Combine ZP free software (Hadley, 2010). Outdoor photographs were taken with a Nikon FM-10 analog camera loaded with Fujifilm Superia 100 ASA.

Description of the new species follows Vilela and Bächli (1990, 2000) and Bächli et al. (2004, 2005). Measurements of paired structures were taken from the flies' left side. The right wing of one double mounted male of each type series was removed with the tip of an entomological pin, placed in a drop of a solution of 70% ethanol in 5% glycerin on a well slide for at least 30 min, transferred to a drop of pure glycerin on a microscope slide, covered with a coverslip, and photomicrographed under a $4 \times$ objective as detailed for terminalia, except for the fact that they were not stained. Label data attached to each type specimen are cited in full with comma designating a line change, slashes indicating a label change and clarifying notes included in brackets. Type series of both new species will be deposited in the *Museu de Zoologia da Universidade de São Paulo* (MZSP).

D. peixotoi sp. nov. was reared for several generations in the laboratory using a flower enriched medium described in Vaz et al. (2014).

Results

Taxonomy

Genus Drosophila Fállen, 1823:4. Subgenus Drosophila Sturtevant, 1942:30.

Drosophila (Drosophila) asymmetrica sp. nov. (Figs. 5-33)

Drosophila sp. I4, Vaz et al., 2014:608 (Table 1, feeding site), 609 (Fig. 6, puparium [upper one]), 611 (Tables 3 and 4, breeding site), suppl. key S1 (feeding site, key), suppl. key S2 (breeding site, key).

Diagnosis. Medium-sized dull grayish yellow fly, body length (excluding wing) about 2.7 mm (male) and 3.0 mm (female); h index 1.1–1.8; mid katepisternal about 13–40% of the anterior one; abdomen dull, grayish yellow; tergites 2–5 with medially interrupted posterior darker bands; tergites 1 and 6 devoid of bands; wings uniformly slightly darkened; C index 2.0–3.0; larvae and pupae with very long conspicuous posterior and anterior horns, respectively; aedeagus distally asymmetric; conspicuous narrow oviscapt valve.

Material examined (10 males, 7 females; deposited in MZSP). Holotype male [wild-caught, coded M18, double-mounted, photomicrographed (Figs. 5–7), right wing removed (Fig. 11), dissected (Figs. 12–26)] labeled: "Brasil – SP – São Paulo, Reserva Florestal



Figs. 5–10. *Drosophila asymmetrica* sp. nov., type specimens: (5–7) male paratype, left lateral, oblique dorsal and dorsal views, respectively; (8–10) female paratype, left lateral, oblique dorsal and dorsal views, respectively. Scale bar = 1 mm.



Fig. 11. Drosophila asymmetrica sp. nov.: male holotype, right wing, dorsal view. Scale bar = 1 mm.

do Instituto de Biociências, 28.XII.2006, Vilela *coll./Drosophila asymmetrica* [male symbol], Vaz, Vilela & Carvalho/fotomicrografado [photomicrographed]/terminália ilustrada [illustrated terminalia]/HOLOTYPE/[microvial with terminalia and right wing in glycerin]". Seven paratypes ($5 \, c^3 \sigma$, $2 \, q q$), same data as holotype, plus 9 paratypes ($4 \, c^3 \sigma^3$ [one photomicrographed (Figs. 5–7)], $5 \, q q$ [one photomicrographed (Figs. 8–10), one dissected (Figs. 27–33)]), same data as holotype except collection date (18, 22, 26.XII.2006).

Type locality. Forest Reserve of the Instituto de Biociências da Universidade de São Paulo (IB-USP) (23°33.96′ S; 46°43.72′ W), Cidade Universitária "Armando de Salles Oliveira", São Paulo city, state of São Paulo, Brazil.

Description. Male (n = 10). Head and frons dull grayish yellow, frontal length 0.37 mm (0.34–0.39) mm; frontal index = 1.12 (1.00–1.23), top to bottom width ratio = 1.56 (1.46–1.69). Frontal triangle light gray pollinose, about 100% of frontal length; ocellar triangle shiny light brown, 31–43% of frontal length. Or3 to or1 = 67–83% of that to inner vertical; or1/or3 ratio = 0.95 (0.78–1.22); or2/or1 ratio = 0.50 (0.36–0.71); poc 50–60%, oc 56–73% of frontal length; vibrissal index = 0.14–0.40, vt index = 1.12 (1.00–1.33), facial carina light brown, nose-like, slightly sulcate. Gena light brown, cheek index about 5.50–8.00. Eye dark red. Eye index = 1.10–1.40. Pedicel brown, laterally lighter, first flagellomere light brown, length to width ratio 1.20–2.00. Arista with 3 dorsal, 1–2 ventral and 3–4 tiny inner branches, plus terminal fork. Proboscis brown.

Thorax dull grayish yellow; length 1.11 (1.00-1.22) mm. Scutum pollinose, brownish-gray, 8 rows of acrostichal setae. h index = 1.32 (1.11-1.80). Transverse distance of dorsocentral setae 251% of longitudinal distance; dc index = 0.66 (0.58-0.72). Scutellum pollinose, laterally light brown, medially darker; distance between apical scutellar setae about 71% of that of apical to basal one, basal setae convergent; apical setae cruciate, scut index = 0.81 (0.68-0.89). Halter gray. Pleura brownish gray, sterno index = 0.78 (0.59-0.88), mid katepisternal seta about 13–40% of the anterior one. Legs brownish gray.

Wing (Fig. 11) uniformly slightly darkened; length 2.06 (1.78–2.67) mm, length to width ratio = 2.06 (1.93–2.21). Indices: C = 2.60 (1.95–2.84), ac = 2.46 (2.11–2.71), hb = 0.49 (0.40–0.63), 4C = 0.90 (0.79–1.16), 4v = 1.69 (1.50–1.91), 5x = 1.75 (1.67–1.86), M = 0.54 (0.46–0.58), prox. x = 0.59 (0.52–0.68).

Abdomen (Figs. 5–7) dull grayish yellow; tergites 2–5 with medially interrupted posterior darker bands; tergites 1 and 6 devoid of bands. Male Terminalia (Figs. 12–26). Epandrium microtrichose on posterior median and dorsal areas; ca. 2 upper and 12 lower setae; ventral lobe weakly sclerotized, slightly covering surstylus, devoid of microtrichiae, posterior margin noticeably concave. Cerci mediodorsally microtrichose, widely fused to epandrium. Surstylus bearing a small patch of microtrichiae on mediodorsal area, with ca. 7 cone-shaped prensisetae, ca. 4 long, strong outer setae and ca. 11 long, thin, mostly inner setae. Decasternum as in Figs. 13, 14, 23; anteriorly turned dorsalwards, anteromedial tip area slightly sclerotized (not seen



Figs. 12–26. Drosophila asymmetrica sp. nov., male holotype, terminalia: (12–14) epandrium, cerci, surstyli, and decasternum, left lateral, oblique posterior and posterior views, respectively; (15–17) hypandrium, gonopods and paraphyses, left lateral, oblique posterior and posterior views, respectively; (18–22) aedeagus + aedeagal apodeme, several views from dorsal through ventral; (23) epandrium, cerci, surstyli, and decasternum; ventral posterior view; (24–26) ejaculatory apodeme, dorsal, oblique dorsal and left lateral views, respectively.

in Figs. 13, 23), sharply carinate on posterior mediodorsal half (Fig. 14). Hypandrium (Figs. 15–17) shorter than epandrium, anterior margin convex; dorsal arch and posterior hypandrial process absent; gonopod bare, linked to paraphysis by membranous tissue (Fig. 17). Aedeagus (Figs. 18-22) medially and distally asymmetric, anteriorly bearing a pair of tiny, acute spines pointed basalwards, subdistally bearing pair of dorsal, small, triangle-shaped spines (Fig. 18); right side medially and distally pleated leftwards over most dorsal cleft, partially covering left side, hiding left subdistal spine. Aedeagus in dorsal (Fig. 18) and ventral (Fig. 22) views depicting only the right subdistal spine on the left side; apically blunt in lateral view (Fig. 20). Aedeagal apodeme, rod-shaped, curved, laterally flattened, slightly shorter than aedeagus. Ventral rod completely fused to aedeagal apodeme, distally expanded laterally, relatively long (Fig. 20). Paraphysis right-angled triangleshaped in lateral view (Fig. 16), bearing a small setula at very distal tip. Ejaculatory apodeme as long as aedeagal apodeme, dorsodistally expanded laterally and crescent-shaped (Figs. 24, 25), laterally L-shaped and proximally expanded dorsoventrally (Fig. 26).

Female (n=7). Head and frons dull gray, frontal length 0.40 mm (0.37–0.41) mm; frontal index=1.12 (1.00–1.21), top to bottom width ratio=1.65 (1.47–1.79). Frontal triangle light gray pollinose, about 100% of frontal length; ocellar triangle shiny light brown, 35–40% of frontal length. Or3 to or1=67–100% of that to inner vertical; or1/or3 ratio=0.88 (0.82–0.91); or2/or1 ratio=0.53 (0.44–0.60); poc 53–67%, oc 71–81% of frontal length; vibrissal index=0.21–0.42; vt index=1.07 (1.00–1.18); facial carina light brown, nose-like, slightly sulcate. Gena light brown, cheek index about 5.75–8.33. Eye dark red. Eye index=1.14–1.42. Pedicel brown, laterally lighter, first flagellomere light brown, length to width ratio 1.40–2.00. Arista with 3 dorsal, 2 ventral and 2–5 tiny inner branches, plus terminal fork. Proboscis brown.



Figs. 27–32. Drosophila asymmetrica sp. nov., female paratype (not the specimen in Figs. 8–10), terminalia and spermathecae: (27–30) tergite 8, epiproct and hypoproct, left lateral, oblique posterior, posterior and ventral posterior views, respectively; (31) left oviscapt valve, outer lateral view; (32) spermathecae inner capsules, lateral view. Scale bar = 0.1 mm.



Fig. 33. Drosophila asymmetrica sp. nov., female paratype, terminalia: left oviscapt valve, outer lateral view. Scale bar = 0.1 mm.

Thorax dull grayish yellow; length 1.27 (1.20–1.39) mm. Scutum pollinose, brownish-gray, 8 rows of acrostichal setae. h index = 1.27 (1.11–1.50). Transverse distance of dorsocentral setae 242% of lon-gitudinal distance; dc index = 0.66 (0.58-0.74). Scutellum pollinose, laterally light brown, medially darker; distance between apical scutellar setae about 78% of that of apical to basal one, basal setae convergent; apical setae cruciate, scut index = 0.85 (0.81-0.90). Halter grayish yellow. Pleura grayish yellow, sterno index = 0.80 (0.74-0.83), mid katepisternal seta about 21–33% of the anterior one. Legs dull grayish yellow.

Wing uniformly slightly darkened; length 2.34 (2.20–2.54) mm, length to width ratio=2.08 (2.02–2.14). Indices: C=2.70 (2.42–2.95), ac=2.42 (2.20–2.67), hb=0.53 (0.40–0.64), 4C=0.89 (0.79–1.00), 4v=1.66 (1.52–1.96), 5x=1.51 (1.33–1.75), M=0.52 (0.44–0.61), prox. x=0.58 (0.52–0.63).

Female terminalia (Figs. 27-33). Tergite 8 narrow, devoid of setae (Fig. 27), anterodorsally microtrichose, mediodistally weakly sclerotized (Fig. 28); ventral half curved frontwards, distally bearing a row of ca. 8 thin, long setulae adjacent to posterior margin (Figs. 27-30); epiproct mostly microtrichose, setose, anterolaterally embraced by basal area of larger hypoproct (Fig. 29); hypoproct mostly microtrichose, setose, conspicuously bearing two pairs of stronger and longer setae (Fig. 27). Valve of oviscapt relatively narrow, distally rounded, subdistally slightly expanded dorsoventrally and ventrally slightly sinuate in lateral view (Figs. 31, 33), bearing ca. 5 discal and about 20 marginal, peg-like, mostly roundishtipped, outer ovisensilla (Fig. 33); trichoid-like inner ovisensilla: 3 thin distally positioned, and 1 long, slightly curved, subterminal (Fig. 33). Spermathecal inner capsule cylinder-shaped, sclerotized. enlarged at base, distally flattened; basal introvert deeply invaginated (Fig. 32).

Puparium. One puparium of *Drosophila asymmetrica*, sp. nov. collected from an inflorescence of *G. cylindrica*, in Rio de Janeiro city was photomicrographed (Fig. 6 [upper one] of Vaz et al., 2014 [cited the figure caption as *Drosophila* sp. 14]; host plant unintentionally omitted) together with one puparium of *Drosophila calatheae* [lower one], obtained from the from the same host plant. Puparia of both species conspicuously bear long horns and a wide air-filled space on their anterior end, probably related to floatation inside bracts phytotelmata.

Ecology. Breeds on inflorescences of *Goeppertia monophylla* (Table 1).

Etymology. Named to indicate its conspicuously asymmetric aedeagus.

Note. A remarkably similar oviscapt valve and similar spermathecae were depicted and photomicrographed by Vilela & Bächli (1990:215, Fig. 58E Fig. 58; 323, Fig. 166B) for the noticeably smaller female paralectotype of *Drosophila boliviana* Duda, 1927, collected from "Yungas von Coroico", Bolivia. The cited authors considered the two female paralectotypes of the type series of *D. boliviana* as belonging to distinct species. As we suspect the smaller female paralectotype of *Drosophila boliviana* and *Drosophila asymmetrica* could be conspecific, the eventual capture and identification of a similar male from "Yungas von Coroico" would be desirable to confirm our suspicion.

Drosophila (Drosophila) peixotoi sp. nov. (Figs. 34-56).

Drosophila sp. G4, Vaz et al., 2014:608 (Table 1, feeding site), 610 (Table 2, feeding site), 611 (Table 3, breeding site), suppl. key S1 (feeding site, key), suppl. key S2 (breeding site, key).



Figs. 34–39. Drosophila peixotoi sp. nov., type specimens: (34–36) male holotype, left lateral, oblique dorsal and dorsal views, respectively; (37–39) female paratype, left lateral, oblique dorsal and dorsal views, respectively. Scale bar = 1 mm.



Fig. 40. Drosophila peixotoi sp. nov.: male paratype, right wing, dorsal view. Scale bar = 1 mm.

Diagnosis. Small-sized dull brown fly, with a relatively large head in lateral view, body length (excluding wing) about 2.1 mm (male) and 2.2 mm (female), front brown, ocellar triangle dark brown; scutum pollinose, light brown anteriorly and between dorsocentrals, gradually darkening toward lateral and distal regions; scutellum dull dark brown; setae and setulae dark brown with a remarkable golden sheen; wings light brown hyaline, tips of veins R_{2+3} , R_{4+5} and M slightly clouded, crossveins slightly clouded (dM-Cu darker), lappet bearing one thick and one thin seta at tip; abdominal tergites shiny; male terminalia conspicuously bearing a large black spine at inner lower tip of cercus; oviscapt valve distally short and blunt, submarine-shaped.

Material examined (10 males, 10 females, deposited in MZSP). Holotype male (wild-caught, coded M18, double-mounted, right wing removed, dissected [Figs. 41–53]) labeled: "Brasil – SP – São Paulo, Reserva Florestal do [Forest Reserve of] IB-USP, Cidade Universitária, 26.XII.2006, Vilela *coll./Drosophila peixotoi* [male symbol] Vaz, Vilela & Carvalho/fotomicrografado [photomicrographed]/terminália ilustrada [illustrated termina-lia]/HOLOTYPE/[glass microvial with terminalia and right wing in glycerin]". Paratypes (9 ♂♂ [one with right wing removed (Fig. 40)], 10 ♀♀ [one photomicrographed (Figs. 37-39) and dissected (Figs. 54–56)]), same data as holotype.

Type locality. Forest Reserve of the Instituto de Biociências da Universidade de São Paulo (IB-USP) (23°33.96′ S; 46°43.72′ W), Cidade Universitária "Armando de Salles Oliveira", São Paulo city, state of São Paulo, Brazil.

Description. Male (*n* = 10). Head relatively large in lateral view, brown. Frontal length 0.26 (0.24–0.29) mm, frontal index=0.98 (0.83–1.00), top to bottom width ratio = 1.33 (1.17–1.50). Frontal triangle laterally shiny, 60-80% of frontal length; ocellar triangle shiny dark brown, 40-50% of frontal length. Orbital plates shiny, 75-91% of frontal length. Orbital setae black, or2 outside or1 and or3; distance of or3 to or1=75% of or3 to vtm, or1/or3 ratio=0.85, or2/or1 ratio=0.38, postocellar setae=52 (50-73)%, ocellar setae = 78 (67-91)% of frontal length; vt index 0.92 (0.78-1.00); vibrissal index 0.88 (0.83-1.00). Face and cheek light brown. Carina light brown, prominent, narrow, not sulcate. Cheek index 6–10. Eye index = 1.28 (1.18–1.58). Scape and pedicel light brown, first flagellomere brown, length to width ratio = 1.68 (1.50-2.00). Arista with 4-5 dorsal, 2 ventral long branches, and 4-6 inner branches relatively long, plus terminal fork. Proboscis and palpus brown.

Thorax subshining, mostly brown; length 0.87 mm; 6 rows of acrostichals. Transverse distance of dorsocentral setae 213% of longitudinal distance; dc index 0.64. Scutellum pollinose dark brown, apically blunt; distance between apical scutellar setae 100–150% of that between apical and basal one; scut index = 0.94 (0.80-1.07); basal setae divergent, apical ones cruciate. Pleura subshining dark brown, sterno index = 0.48, median katepisternal seta 33–67% of anterior one. Halter light brown. Legs uniformly light brown; apical seta on protibia and mesotibia; preapical seta on all tibiae.

Wing (Fig. 40) light brown, crossveins R-M and dM-Cu, and apices of veins R_{2+3} , R_{4+5} and M slightly clouded; length 1.82



Figs. 41–53. Drosophila peixotoi sp. nov., male holotype, terminalia: (41–43, 52) epandrium, cerci, surstyli, and decasternum, left lateral, oblique posterior, posterior and ventral posterior views, respectively; (44–46, 53) hypandrium and gonopods + paraphyses, left lateral, oblique posterior, posterior and anterior views, respectively; (47–51) aedeagus-aedeagal apodeme, several views from dorsal through ventral. Scale bar = 0.1 mm.



Figs. 54, 55. Drosophila peixotoi sp. nov., female paratype, spermathecae and terminalia: (54) spermathecae inner capsules, lateral view; (55) left oviscapt valve, outer lateral view. Scale bar = 0.1 mm.

(1.71–1.95) mm, length to width ratio=2.10 (2.03–2.19). Indices: C=2.94 (2.72–3.13), ac=2.13 (1.88–2.43), hb=0.30 (0.25–0.38), 4C=0.82 (0.76–0.89), 4v=1.67 (1.59–1.83), 5x=1.48 (1.29–1.67), M=0.49 (0.43–0.55), prox. X=0.53 (0.45–0.67).

Abdomen (Figs. 34–36) shiny brownish, tergite 1 yellow, tergites 2–4 anteromedially yellow with a distal dark brown band,

medially interrupted and laterally broadened, reaching anterior margin of tergite; tergite 4 bearing an irregular coffee brown spot on the median region of the yellow area; tergites 5–6 entirely coffee brown (tergite 5 anteriorly lighter submedially in some specimens).

Male Terminalia (Figs. 41–53). Epandrium (Figs. 41–43, 52) almost bare, slightly microtrichose on posterior dorsal area; upper



Fig. 56. Drosophila peixotoi sp. nov., female paratype, terminalia: left oviscapt valve, outer lateral view. Scale bar = 0.1 mm.

setae absent; 4-5 lower setae in a vertical row; ventral lobe not covering surstylus. Cercus microtrichose on dorsocentral area, conspicuously bearing a strong, long (ca. 2/3 length of cercus), slightly curved spine at inner lower tip, linked to epandrium by membranous tissue (Fig. 43); the curved spine bears a longitudinal line along most of its length that could be an indication that it has originated from the fusion of two spines. Surstylus not microtrichose, with about 8-9 cone-shaped prensisetae, about 10-12 long, strong outer setae and about 9 long, thin, mostly inner setae. Decasternum upper-positioned, as in Fig. 52. Hypandrium (Figs. 44-46, 53) as long as epandrium, anterior margin convex; posterior hypandrial process absent; dorsal arch W-shaped, strongly sclerotized (Figs. 46, 53); gonopod not microtrichose, linked to paraphysis by membranous tissue, bearing one long seta on anterior inner margin. Aedeagus (Figs. 47-51) distally bifid (in dorsal and ventral views, Figs. 47, 51), distal 1/3 laterally covered with many spines, marginally serrated ventrally (Fig. 50), turned abruptly dorsad (Fig. 49) and bearing a dorsoventral lappet, mediodorsally with a pair of membranous, anterodorsally slightly sclerotized, finger-shaped, backwards directed, conspicuous processes (Figs. 48-50), which are strongly expanded laterally as two triangle-shaped structures (in dorsal view, Fig. 47), shorter than aedeagus (ca. 1/3 its length) and lateroventrally covered with tiny spines; dorsal cleft restricted to a tiny opening adjacent to the fusion aedeagus-aedeagal apodeme; paraphysis dorsodistally swollen, not microtrichose, bearing a setula on posterior inner margin just adjacent to swollen area (Figs. 45, 46, 53). Aedeagal apodeme rod-shaped, as long as aedeagus; anteriorly slightly expanded dorsoventrally (Figs. 49, 50). Ventral rod triangle-shaped, completely fused to aedeagal apodeme (Fig. 49).

Female (n = 10). Color difference from male: in some specimens, color pattern of tergite 5 is similar to that of male tergite 4.

Measurements: Frontal length 0.26 mm; frontal index = 0.87, top to bottom width ratio = 1.25. Frontal triangle 60–80% of frontal length. Ocellar triangle 33–50% of frontal length. Orbital Plates 80–100% of frontal length. Distance of or3 to or1 = 60–100% of or3 to vtm, or1/or3 ratio = 0.88 (0.75–1.12), or2/or 1 ratio = 0.31 (0.22–043), postocellar setae = 69 (64–75)%, ocellar setae = 75 (64–80)% of frontal length; vt index = 0.99 (0.88–1.12); vibrissal index = 0.78 (0.71–0.86). Cheek index 9.75 (9.00–10.50). Eye index = 1.30 (1.20–1.38). First flagellomere length/width ratio = 1.62 (1.25–1.75).

Thorax length 0.93 (0.88–1.00) mm. h index = 0.90 (0.62–1.00). Transverse distance of dorsocentral setae 171–260% of longitudinal distance; dc index = 0.68 (0.56–0.79). Distance between apical scutellar setae 100–125% of that between apical and basal one; scut index = 0.92 (0.87–1.00), sterno index = 0.57 (0.46–0.69), median katepisternal seta 46–69% of anterior one. Wing length 1.98

(1.82–2.10) mm, length to width ratio=2.16 (2.08–2.22). Indices: C=3.19 (2.89–3.44), ac=2.00 (1.78–2.43), hb=0.29 (0.24–0.32), 4C=0.77 (0.71–0.82), 4v=1.67 (1.50–1.84), 5x=1.49 (1.22–1.83), M=0.49 (0.42–0.55), prox. x=0.52 (0.46–0.59).

Female terminalia (Figs. 54–56). Valve of oviscapt distally relatively short, submarine-shaped in lateral view, double-walled, inner wall (Fig. 56, dotted line) ca. 2/3 narrower than outer wall, apically roundish, submedially slightly expanded dorsad looking like a submarine sail, ventrally strongly convex, with ca. 14 discal and about 11 marginal, peg-like, mostly roundish-tipped, outer ovisensilla; trichoid-like inner ovisensilla: 3 thin distally positioned, and 1 long, slightly curved, subterminal. Spermathecal inner capsule light bulb-shaped, sclerotized, not furrowed at base; basal introvert deeply invaginated.

Ecology. Breeds on inflorescences of *Goeppertia monophylla* (Table 1).

Etymology. Named after Dr. Alexandre Afrânio Peixoto (1963–2013) for his research contributions on *Drosophila* genetics, evolution, and behavior (Laboratório de Biologia Molecular de Insetos, Fiocruz, Rio de Janeiro, Brazil).

Note. Five isofemale lines of *Drosophila peixotoi* sp. nov. were established by Flavia J. Krsticevic from flies aspirated from inflorescences of *Goeppertia cylindrica* of a private backyard garden in Santa Teresa district, Rio de Janeiro. The five isofemale lines (coded STA-KF7, STA-KF8, STA-KF9, STA-KF11, STA-KF12) were reared for several generations on a *G. cylindrica* enriched medium described in Vaz et al. (2014), and samples are preserved in ethanol in the laboratory of one of us (ABC). However, it should be pointed out that no adults of *Drosophila peixotoi* sp. nov. emerged from *G. cylindrica* inflorescences collected in the Jardim Botânico district (22°57.95' S, 43°14.31' W), adjacent to Parque Nacional da Tijuca (Rio de Janeiro city), although they were frequently found feeding on these inflorescences in nature (Vaz et al., 2014:610, 611 [Tables 3, 4]).

Discussion

Asymmetry in male terminalia. Left-right asymmetry in the male terminalia appeared several times independently in the evolutionary history of insects and has been associated with changes in mating positions (Huber, 2010). In drosophilids asymmetry is not common but also appeared independently several times, such as in the *canalinea, flavopilosa* and *nannoptera* species groups (Vilela, 1984; Vilela and Bächli, 1990; Vilela and Pereira, 1992; Vilela et al., 2008; Lang and Orgogozo, 2012). *Drosophila pachea*, belonging to the *nannoptera* group, has a conspicuous asymmetry in its epandrium lobes, with the left lobe being about 50% longer and thinner than the right lobe. Copulation requires a characteristic one-sided mating posture (Lang and Orgogozo, 2012). The asymmetry we describe in *Drosophila asymmetrica* sp. nov. is limited to the aedeagus, similar to two species of the *flavopilosa* group: *Drosophila mariaehelenae* Vilela, 1984 and *Drosophila hollisae* Vilela and Pereira, 1992. We have not made any observation on copulation postures in this species.

Taxonomic history of the Drosophila guarani species group. The guarani species group was first recognized by Dobzhansky and Pavan (1943) and included four described species (D. guarani, D. guaru, D. guaramunu and D. griseolineata). King (1947) added D. subbadia and D. guaraja to the group and proposed its division into two subgroups: guaramunu and guarani. He noted that the male cerci of the guarani subgroup (then including D. guarani, D. guaru and D. subbadia) bear "heavily pigmented and peculiarly curved bristles which look like bent spikes". As to guaramunu subgroup (then including D. guaramunu, D. griseolineata and D. guaraja) no clear morphological diagnosis was proposed. Kastritsis (1969) later suggested these two subgroups should be raised to the species group status due to characteristics on polytene chromosome morphology that approximate species of the guaramunu subgroup to some species of the tripunctata species group. In fact, the guaramunu subgroup seems to be an evolutionary transition between tripunctata and guarani regarding not only chromosomal morphology but internal and external anatomy (Throckmorton, 1962; Frota-Pessoa, 1954) and data on molecular phylogeny (Hatadani et al., 2009; Van der Linde et al., 2010). As more species related to the group were described the limits between guarani and guaramunu subdivision became unclear and species were mainly assigned to guarani as a species group. A taxonomic review by Val et al. (1981) discusses the difficulty of placing species in either guarani or guaramunu divisions and treats guarani as a species group comprising a total of 10 species. They also discuss some of the male terminalia heterogeneity of the guaramunu subgroup and its relation to tripunctata. In a more recent review of Neotropical drosophilids (Vilela and Bächli, 1990) D. guaramunu was considered a junior synonym of D. maculifrons and D. guarani a junior synonym of D. ornatifrons, but the group name was maintained as guarani. In that same work, D. neoguaramunu was transferred to the tripunctata group. These authors reinforced the similarity between species from the guarani subgroup and even raised the possibility of synonymy between D. subbadia (described from the state of Michoacán, Mexico) and D. guaru (described from the state of São Paulo, Brazil). They stated "further analysis using strains from all the range of distribution of both species will probably reveal that they indeed belong to the same biological species or species in statu nascendi" (Vilela and Bächli, 1990:79).

New taxonomic proposal. The guarani species group sensu lato is clearly not monophyletic mainly due to heterogeneity of the guaramunu subgroup and its overlapping with the tripunctata species group. However, as discussed previously, the guarani subgroup seems highly homogeneous. Therefore we propose that: (I) the guaramunu and guarani species groups sensu Kastritsis (1969) not to be adopted, and the guarani species group be maintained until more details of its relation to tripunctata and other groups is available; (II) the guaramunu subgroup be dismissed and the guarani subgroup be maintained but recircumscribed in a narrower sense according to King (1947). The guarani subgroup would then include the following species: D. ornatifrons, D. guaru, D. subbadia and D. peixotoi sp. nov, sharing the following presumed synapomorphy: presence of one or two pairs of conspicuous large black spines on ventral cerci. Accordingly, D. tucumana and D. urubamba are excluded from the guarani subgroup. According to this new proposal the guarani species group currently would comprise 19 species (Ratcov et al., 2017; Bächli, 2018; this work) of which 4 belongs to the guarani subgroup and the remaining 15 have uncertain relationships.

Breeding sites in the Drosophila guarani species group. Most species of the group were described based on adult flies attracted to banana-baited traps and data regarding natural larval breeding sites are scarce. D. griseolineata, D. maculifrons (cited as D. guaramunu), and D. ornatifrons (cited as D. guarani) seems to be the most polyphagous species emerging from several types of fruits and infructescences (Valente and Araújo, 1991). D. guaraja was reared from infructescences of Philodendron bipinnatifidum (Araceae; Vilela, 2001). Pipkin et al. (1966) cited one unidentified species of the group (guarani sp.) emerging from flowers of Erythrina berteroana (Fabaceae). Along with D. peixotoi sp. nov. we also identified D. griseolineata emerging from inflorescences of Goeppertia monophylla, although less abundantly (7 vs. 22 D. peixotoi sp. nov. specimens out of 137 drosophilids; Vaz et al., 2014). So far, D. peixotoi sp. nov. seems to be the only exclusive and host-specific flower breeding species of its group. Studies regarding emergence of flies from flowers and inflorescences of different plant species are needed to determine the breeding sites of those species of Drosophila that are rarely aspirated or net swept over fruit-baited traps.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We are indebted to Denise S. Sheepmaker for allowing the use of her stereophotomicroscope and to Flavia Krsticevic for help with lab and field work. We thank an anonymous reviewer for his careful reading of our manuscript and suggestions that improved the submitted version. This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ and Wellcome Trust (grant 207486/Z/17/Z).

References

- Bächli, G., 2018. TaxoDros: The Database on Taxonomy of Drosophilidae, Available at: http://taxodros.unizh.ch/ (accessed 11.01.18).
- Bächli, G., Vilela, C.R., Escher, S.A., Saura, A., 2004. The Drosophilidae (Diptera) of Fennoscandia and Denmark. In: Fauna Entomologica Scandinavica v. 39. Leiden, Brill, 362 pp.
- Bächli, G., Vilela, C.R., McEvey, S.F., 2005. Nine new species of Afrotropical Leucophenga (Diptera, Drosophilidae). Mitt. Schweiz. Entomol. Ges. 78, 23–57.
- Brncic, D., 1983. Ecology of flower breeding *Drosophila*. In: Ashburner, M.A., Carson, H.L., Thompson Jr., J.N. (Eds.), The Genetics and Biology of Drosophila v. 3b. Academic Press, London, pp. 333–382.
- Borchsenius, F., Suárez, L.S.S., Prince, L.M., 2012. Molecular phylogeny and redefined generic limits of *Calathea* (Marantaceae). Syst. Bot. 37, 620–635.
- Dobzhansky, T., Pavan, C., 1943. Studies on Brazilian species of *Drosophila*. Univ. São Paulo, Fac. Filos., Cienc. Let., Bol. 36, Biol. Geral 4, 7–72.
- Fallén, C.F., 1823. Geomyzides Sveciae. Lundae, Berlingianis, 8 pp.
- Frota-Pessoa, O., 1954. Revision of the tripunctata group of Drosophila with description of fifteen new species (Drosophilidae, Diptera). Arq. Museu Paranaense 10, 253–304.
- Grimaldi, D.A., 1987. Phylogenetics and taxonomy of Zygothrica (Diptera: Drosophilidae). Bull. Am. Mus. Nat. Hist. 187, 103–268.
- Grimaldⁱ, D.A., Ervik, F., Bernal, R., 2003. Two new Neotropical Genera of Drosophilidae (Diptera) visiting palm flowers. J. Kansas Entomol. Soc. 76, 109–124.
- Hadley, A., 2010. Best Software by Alan Hadley, http://www.hadleyweb.pwp. blueyonder.co.uk/.
- Hatadani, L.M., McInerney, J.O., de Medeiros, H.F., Martins Junqueira, A.C., de Azeredo-Espin, A.M., Klaczko, L.B., 2009. Molecular phylogeny of the *Drosophila tripunctata* and closely related species groups (Diptera: Drosophilidae). Mol. Phylogenet. Evol. 51, 595–600.
- Huber, B.A., 2010. Mating positions and the evolution of asymmetric insect genitalia. Genetica 138, 19–25.
- Kaneshiro, K.Y., 1969. A Study of the Relationships of Hawaiian Drosophila Species Based on External Male Genitalia. University of Texas Publications 6918, pp. 55–70.
- Kastritsis, C.D., 1969. The chromosomes of some species of the guarani group of Drosophila. J. Hered. 60, 51–57.

King, J.C., 1947. Interspecific relationships within the *guarani* group of *Drosophila*. Evolution 1, 143–153.

- Lang, M., Orgogozo, V., 2012. Distinct copulation positions in *Drosophila pachea* males with symmetric or asymmetric external genitalia. Contrib. Zool. 81, 87–94.Malogolowkin, C., 1952. Notas sôbre "Zygothrica dispar" (Diptera, Drosophilidae).
- Rev. Bras. Biol. 12, 455–457. Markow, T.A., O'Grady, P., 2008. Reproductive ecology of *Drosophila*. Funct. Ecol. 22,
- 747–759. Diskis C.B. Bodriguez, B.L. Leon, L. 1066. Diant best specificity among flower.
- Pipkin, S.B., Rodriguez, R.L., Leon, J., 1966. Plant host specificity among flowerfeeding Neotropical Drosophila (Diptera: Drosophilidae). Am. Nat. 100, 135–156.
- Ratcov, V., Vilela, C.R., Goñi, B., 2017. A new species of Neotropical Drosophila (Diptera: Drosophilidae) belonging to the guarani group. Rev. Bras. Entomol. 61, 232–238.
- Santos R.C.O dos, Vilela, C.R., 2005. Breeding sites of Neotropical Drosophilidae (Diptera). IV. Living and fallen flowers of Sessea brasiliensis and Cestrum spp. (Solanaceae). Rev. Bras. Entomol. 49, 544–551.
- Sturtevant, A.H., 1942. The Classification of the Genus Drosophila, With Descriptions of Nine New Species. University of Texas Publications 4213, pp. 5–51.
- Throckmorton, L.H., 1962. The Problem of Phylogeny in the Genus Drosophila. University of Texas Publications 6205, pp. 207–343.
- Val, F.C., Vilela, C.R., Marques, M.D., 1981. Drosophilidae of The Neotropical Region. In: Ashburner, M., Carson, H.L., Thompson, J.N. (Eds.), The Genetics and Biology of Drosophila. V. 3a. Academic Press, Orlando, pp. 123–168.
- Valente, V.L.S., Araújo, A.M., 1991. Ecological aspects of Drosophila species inhabiting wild environments in Southern Brazil (Diptera: Drosophilidae). Rev. Bras. Entomol. 35, 237–253.

- Van der Linde, K., Houle, D., Spicer, G.S., Steppan, S.J., 2010. A supermatrixbased molecular phylogeny of the family Drosophilidae. Genet. Res. 92, 25–38.
- Vaz, S.C., Vilela, C.R., Krsticevic, F.J., Carvalho, A.B., 2014. Developmental Sites of Neotropical Drosophilidae (Diptera). V. Inflorescences of *Calathea cylindrica* and *Calathea monophylla* (Zingiberales: Marantaceae). Ann. Entomol. Soc. Am. 107, 607–620.
- Vilela, C.R., 1984. Occurrence of the Drosophila flavopilosa species group (Diptera, Drosophilidae) in the state of São Paulo (Brazil). Rev. Bras. Zool. 2, 63–69.
- Vilela, C.R., 2001. Breeding sites of Neotropical Drosophilidae (Diptera). III. Rotting infructescences of Philodendron bipinnatifidum (Araceae). Rev. Bras. Entomol. 45, 339–344.
- Vilela, C.R., Bächli, G., 1990. Taxonomic studies on Neotropical species of seven genera of Drosophilidae (Diptera). Mitt. Schweiz. Entomol. Ges. 63 (Suppl.), 1–332.
- Vilela, C.R., Bächli, G., 2000. Morphological and ecological notes on the species of Drosophila belonging to the subgenus *Siphlodora* Patterson & Mainland, 1944 (Diptera, Drosophilidae). Mitt. Schweiz. Entomol. Ges. 73, 23–47.
- Vilela, C.R., Pereira, M.A.Q.R., 1992. Breeding sites of Neotropical Drosophilidae (Diptera). I. Living flowers of *Cestrum schlechtendalii* (Solanaceae). Rev. Bras. Entomol. 36, 475–482.
- Vilela, C.R., Bächli, G., Stensmyr, M.C., 2008. Redescription of Drosophila endobranchia (Diptera, Drosophilidae), an aberrant member of the canalinea species group. Mitt. Schweiz. Entomol. Ges. 81, 199–208.
- Wheeler, M.R., Kambysellis, M.P., 1966. Notes on the Drosophilidae (Diptera) of Samoa. University of Texas Publications 6615, pp. 533–565.