Annals of the Entomological
Society of America
[Arthropod Biology]

S. C. Vaz
Departamento de Genética e Biologia Evolutiva
Instituto de Biociências
Universidade de São Paulo
Rua do Matão 277, salas 246 e 253
Butantã, 05508-090, Caixa-Postal: 11461
São Paulo, SP, Brazil
Phone: +55-11-3091-7585
Fax: +55-11-3091-7553
E-mail: vazsuz@gmail.com

Breeding Sites of Neotropical Drosophilidae (Diptera)

V. Inflorescences of *Calathea cylindrica* and *Calathea monophylla* (Marantaceae)

S. C. Vaz,[1,2] C. R. Vilela,[1] F. J. Krsticevic,[2] and A. B. Carvalho[2]

[1] Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil

[2] Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

ABSTRACT

In December 2006, a total of 495 drosophilids belonging to 19 species were aspirated from inflorescences of *Calathea monophylla* (Vell.) Körn at a forest reserve in the city of São Paulo, state of São Paulo, and a total of 42 specimens belonging to three species of *Drosophila* were aspirated from those of *Calathea cylindrica* (Roscoe) K. Schum. in an urban forest in the city of Rio de Janeiro, state of Rio de Janeiro, Brazil. Additionally, 20 inflorescences of *C. monophylla* and 14 inflorescences of *C. cylindrica* were collected and observed for the emergence of flies in the laboratories. A total of 137 drosophilids belonging to *Zygothrica dispar* (Wiedemann) plus five species of *Drosophila* (*D. griseolineata* Duda and four undescribed species) emerged from *C. monophylla* inflorescences, and a total of 22 specimens, all belonging to two undescribed species of *Drosophila*, emerged from those of *C. cylindrica*. *Drosophila calatheae* sp. nov., ungrouped but related to both the *xanthopallescens* Pipkin and the *bromeliae* Patterson & Stone species groups, is described based on both aspirated and emerged flies from *C. monophylla* inflorescences from São Paulo city. This new species was also reared in the laboratory with a new medium recipe, thus providing larvae for chromosomal studies.

RESUMO

Em dezembro de 2006, um total de 495 drosofilídeos pertencentes a 19 espécies foram aspirados de inflorescências de *Calathea monophylla* (Vell.) Körn numa reserva florestal na cidade de São Paulo, estado de São Paulo, e 42 espécimes pertencentes a três espécies de *Drosophila* foram aspirados de inflorescências de *Calathea cylindrica* (Roscoe) K. Schum. em uma floresta urbana na cidade do Rio de Janeiro, estado do Rio de Janeiro, Brasil. Adicionalmente, 20 inflorescências de *C. monophylla* e 14 inflorescências de *C. cylindrica* foram coletadas e observadas quanto à emergência de moscas adultas em laboratório. Um total de 137 drosofilídeos pertencentes a *Zygothrica dispar* (Wiedemann) e a cinco espécies de *Drosophila* (*D. griseolineata* Duda e quatro espécies não descritas) emergiram de inflorescências de *C. monophylla*, e 22 espécimes, todos pertencentes a duas espécies de *Drosophila* não descritas, emergiram das inflorescências de *C. cylindrica.* *Drosophila calatheae* sp. nov., espécie não agrupada, porém relacionada aos grupos *xanthopallescens* Pipkin e *bromeliae* Patterson & Stone, é descrita a partir de espécimes aspirados e emergidos de inflorescências de *C. monophylla* da cidade de São Paulo. Esta nova espécie também foi criada em laboratório com uma nova receita de meio de cultura, fornecendo larvas para estudos cromossômicos.

KEY WORDS

Drosophila spp.*,* flower-breeder, new species, mitotic chromosomes, Brazil

74 The Neotropical plant genus *Calathea* G.F.W. Meyer, occurring throughout the
75 humid American tropics, comprises about 300 species (Andersson 1998). There is an
76 estimated 70 to 90 species inhabiting Brazil (Braga 2005), where they are vernacularly
77 known as "caeté" or "caetê". According to Andersson (1998: 286), numerous species of
78 *Calathea* occur primarily in swamp forests and riverine inundation forests and, although
79 needing special arrangements for indoor cultivation, are rapidly increasing in importance as
80 an ornamental, especially in tropical and subtropical horticulture. Lorenzi and Souza (2008)
81 display remarkable color photographs of 27 species of *Calathea* being cultivated as an
82 ornamental in Brazil, which represent around 9% of the known species belonging to the
83 genus. The flowers of *Calathea* species have a corolla tube 5 to 25 times longer than wide
84 and are grouped in roughly spiciform to capitate inflorescences (Andersson 1998).
85 Pipkin (1964) described species of *Drosophila* from Panama that depend on
86 *Calathea* spp. flowers to complete their development. Obligatory flower breeding
87 *Drosophila* spp. belonging to several species groups within the subgenus *Drosophila*, such
88 as the *bromeliae*, *flavopilosa*, *xanthopallescens*, and *onychophora* groups, as well as to
89 other entire subgenera, such as *Phloridosa* and *Siphlodora*, have been reported from the
90 Neotropical Region (reviewed in Brncic 1983). It seems that the ability to explore such
91 resources has been independently acquired several times in different lineages (Markow and
92 O'Grady 2008). The imagoes of the above cited flies depend on living flowers for
93 displaying sexual behavior, feeding and ovipositing, whereas their larval development and
94 metamorphosis can occur on both living and fallen (decaying) flowers.
95 The purpose of this paper is twofold: (1) to identify drosophilids associated to two
96 species of *Calathea* in southeastern Brazil (by aspirating adult flies directly from
97 inflorescences in the field and by observing imagoes emergence from infested flowers

taken to laboratory), and (2) to describe the most abundant of the four new species of *Drosophila* that were collected. This paper is the fifth of a series (Vilela and Pereira 1992, Vilela and Selivon 2000, Vilela 2001, Santos and Vilela, 2005) aiming to discover the breeding sites of Neotropical drosophilids.

Materials and Methods

Collection and Handling of Flies and Floral Resources. The collections were made in the cities of São Paulo and Rio de Janeiro (hereafter shortly referred to as São Paulo and Rio de Janeiro respectively). During the summer blooming season (December 2006 and January 2007), plants of *Calathea monophylla* (Vell.) Körn (non ornamental) from São Paulo (forest reserve [urban] of the Instituto de Biociências da Universidade de São Paulo [IB-USP], Cidade Universitária "Armando de Salles Oliveira" [CUASO]) and of *Calathea cylindrica* (Roscoe) K. Schum. (ornamental) from Rio de Janeiro (Jardim Botânico district, adjacent to Parque Nacional da Tijuca, an urban reserve) were inspected for the presence of adults. Imagoes swarming around and/or perching on the inflorescences were aspirated, followed by the collection of 20 inflorescences of the former species and 14 of the latter.

For the São Paulo collection, the inflorescences were taken to the laboratory in screen-capped plastic boxes, where they were longitudinally cut and each half part kept individually in 250 ml vials containing wet sand and plugged with synthetic foam stoppers. The vials were kept in an incubator with constant temperature (22 ± 1° C) and photoperiod (13 h: 11 h, L:D) to await emergence of adult drosophilids and other insects. To prevent desiccation, and whenever necessary, water was added to the vials by a plant sprayer. All emerged insects were removed daily, late in the afternoon, by an aspirator until no further

emergence took place (ca. 4 weeks later). The emerged specimens were kept on the conventional banana-agar culture media to await hardening of the exoskeleton (ca. 20 days) then preserved in 70% ethanol. Drosophilids were later dried and double-mounted gluing them to cardboard tips on their right side, according to the technique detailed by Bächli et al. (2004: 3, alternative b), sexed and, whenever possible, identified to species. For detailed information regarding the forest reserve of the Instituto de Biociências da Universidade de São Paulo (formerly known as forest reserve of the CUASO [an acronym for Cidade Universitária "Armando de Salles Oliveira"]) refer to Rossi (1994) and Groppo and Pirani (2005). According to the latter paper, two species of *Calathea*, *Calathea lietzeii* E. Morren and *Calathea communis* Wanderley and S. Vieira, are present in the forest reserve. The first one is an introduced species and the latter species, which is native in the area, was later (Braga 2005) considered to be a junior synonym of *Calathea monophylla* (Vell.) Körn. No voucher specimens of *C. monophylla* were collected during this project as three dried specimens (two of them being paratypes [M. Groppo Jr. 70 and M. Groppo Jr. 246] of *C. communis*) from the collection site had been previously deposited (Groppo and Pirani, 2005:201) in the herbarium of the Departamento de Botânica do Instituto de Biociências da USP (coded SPF).

For the Rio de Janeiro collection, the inflorescences were treated in much the same way as those sampled in São Paulo, except for the following details: the inflorescences were kept individually in larger vials (1000 ml) in natural summer photoperiod (circa 14 h: 10 h, L:D) and there was no daily systematic observation. The emerging flies were collected at three times: 2, 5 and 17 days after the collection of inflorescences. The emerged specimens were kept in ethanol 95%. Formerly, the primary vegetation of Rio de Janeiro was a coastal Atlantic rainforest. However, most of it was supplanted by coffee farms

146 especially in the beginning of the 19th century. Today the main forest remnant in the city is
147 the Parque Nacional da Tijuca, which covers some 30 km^2. The park originated with a
148 reforestation project in the second half of the 19th century and is now considered the
149 world's largest urban forest. The collection sites (locally known as Jardim Botânico or
150 Horto Florestal at ca. 22º57.95′S, 43º14.31′ W) are adjacent to the Parque Nacional da
151 Tijuca.

152 At the São Paulo collection site, the non ornamental and native host-plant species
153 *Calathea monophylla* blooms from October to December (Groppo and Pirani, 2005) and
154 occurs along the swampy banks of a brooklet, whereas at the Rio de Janeiro collection site,
155 the ornamental and invasive host-plant species *Calathea cylindrica* blooms year-round and
156 has special affinity for well-lit forest edges (Barreto and Freitas 2007).

157 **Identification and Imaging of Fly and Plant Taxa.** Plant species were identified using
158 Wanderley and Vieira (2002), Braga (2005), Groppo and Pirani (2005) and Lorenzi and
159 Souza (2008). The collected drosophilid flies, belonging to previously described species,
160 were identified using the papers of Malogolowkin (1953), Burla (1954), Breuer and Rocha
161 (1971), Val (1982), Grimaldi (1987), Vilela and Bächli (1990), and Bächli et al. (2000). In
162 addition to the analyses of their external morphology, male flies were also identified to
163 species by examination of the terminalia, especially the aedeagus, which can be extruded
164 by gently squeezing the abdomen distal end of anesthetized live specimens, as detailed by
165 Spassky (1957: 51). Dissections were performed according to Wheeler and Kambysellis
166 (1966), as modified by Kaneshiro (1969) and Bächli et al. (2004), whenever necessary.
167 Samples of the aspirated and/or emerged flies, including non drosophilids, were pinned and
168 labeled and will be deposited in the Museu de Zoologia, Universidade de São Paulo, São

Paulo, SP (MZSP). No attempt was made to identify Diptera species belonging to other families.

The photomicrographs of the different terminalia structures were taken with an Olympus BX60 microscope and an Olympus Q-Color 5 digital camera. A 10x objective was used to generate a set of 20-50 pictures by manually focusing a given structure at different depths. This set of photomicrographs was then digitally stacked to create an all-in-focus composite (open-source software CombineZP at http://www.hadleyweb.pwp.blueyonder.co.uk/CZP/Installation.htm). This technique works especially well for heavily sclerotized structures in oblique posterior view (e.g Fig. 21) where minor internal details do not show up clearly. Photomicrographs of polytene and metaphase chromosomes were taken under 40x and 100x oil immersion objectives, respectively. Outdoor photographs were taken with either analog (for *Calathea monophylla*) or digital (for *Calathea cylindrica*) portable cameras. Close views of flies, eggs, pupae and wing were obtained on a stereomicroscope with integrated digital camera, except for types specimens which were photographed with analog equipment.

Rearing Flies in Laboratory. It is noteworthy that, although being apparently a specialized flower feeding and breeding fly, *Drosophila calatheae* sp. nov. collected in Rio de Janeiro was reared for about 4 years (2007-2011) in laboratory conditions using a new flower enriched recipe medium, as follows: agar (1.9 g), mold inhibitor Methylparaben (0.7 g), Fleischmann® dry yeast (6.6 g), malt extract (7.2 g), banana fruits of the prata cultivar (112.5 g), Karo® corn syrup (11.4 g) and inflorescences of Calathea cylindrica (34 g). The inflorescences were collected especially during the rainy season, from October to March, placed inside zipper storage plastic bags and kept in the freezer at -20 ° C until use. The ingredients are placed directly into a blender jar with 240 ml of deionized tap water and

193 homogenized for 4 minutes, without dissolving the molt inhibitor in etanol. The soft
194 mixture is then heated to boiling, with continuously stirring. After boiling, 0.53 ml of
195 proprionic acid (an additional mold inhibitor) is added to cooled medium (below 70 °C).
196 The medium can then be poured into 300 ml glass bottles. To simulate the oviposition sites
197 a tip of thawed inflorescence is placed on the surface of the medium. The flies were kept at
198 22 °C in a rearing chamber and replicated once a week by transferring them to fresh
199 medium.

200 **Chromosomes.** Preparations of mitotic metaphase and polytene chromosomes were done
201 as follows. Larvae were sexed as proposed by Cooper (1950) and dissected. For each
202 sample, cerebral ganglia, imaginal discs and salivary glands were simultaneously extracted.
203 The first two structures were used for preparation of metaphase chromosomes, whereas the
204 salivary glands were used for preparation of polytene chromosomes. We followed the
205 protocol of Pimpinelli et al. (2000) for preparing mitotic chromosomes using a hypotonic
206 solution of sodium citrate (0.5%) and potassium chloride (0.28%). Orcein (2%) Riedel stain
207 was dissolved in lactic acid (85%) and acetic acid (50%). Polytene chromosomes were
208 prepared using the squash technique followed by staining with lacto-acetic orcein
209 (Ashburner 1989).

210 **Description Model.** Description of the new species follows Vilela and Bächli (1990, 2000)
211 and Bächli et al. (2004, 2005). Measurements of paired structures were taken from the flies'
212 left side. The right wing of one female paratype was removed and treated with the same
213 procedure used for preparing the terminalia; including staining with diluted Gage´s stain.
214 Label data attached to each type specimen are cited in full with slashes indicating a label
215 change; clarifying notes are included in brackets.

216

Results and Discussion

Drosophilids aspirated and emerged from inflorescences of *Calathea monophylla* and *C. cylindrica*, collected in São Paulo and Rio de Janeiro respectively, are listed in Tables 1-4. The 11 most abundant (more than 1 individual) aspirated species of *Drosophila* and all emerged Drosophilids are included in separate identification keys (Supp. Key S1 and Supp. Key S2, respectively). As stressed by Carson (1971), although most species of *Drosophila* are attracted and able to feed upon a large variety of substrates, oviposition and larval development are more specialized activities. Examples of adaptation to feeding resources in *Drosophila* are: resistance to plant toxins (Jones 1998), tolerance to ethanol and acetic acid resulting from fermentation of sweet substrates such as fallen fruits (Chakir et al. 1996), and specialized ovipositor structure (Pipkin et al. 1966).

The close association between this most abundant new species of *Drosophila*, described below, and its putative primary host plants (inflorescences of *Calathea monophylla* and *C. cylindrica*) is shown in Figs. 1-12.

Aspirated Flies

A total of 495 drosophilids belonging to 19 species were aspirated in the field from inflorescences of *C. monophylla* (Table 1) and 42 specimens belonging to three species of *Drosophila* were aspirated from those of *C. cylindrica* (Table 2). As it happens over fleshy fungi, it seems that only a small part of the species feeding and/or resting and/or even displaying sexual courtship on the inflorescences uses them as breeding sites.

It is noteworthy that five undescribed species of *Drosophila*, coded with a capital letter followed by a number, were aspirated from the inflorescences of *Calathea monophylla* in an urban secondary rain forest reserve located in the west region of São

Paulo city (Table 1). One species, described below as *Drosophila calatheae*, has thus far been successfully reared in lab conditions, allowing for very detailed further analysis. *Drosophila* sp. G4 could also be reared for some generations using this new recipe. This species plus the other three undescribed species are currently being studied and will be the subjects of forthcoming papers.

Females of *Drosophila fuscolineata* and *Drosophila mediopunctata*, for which just one female specimen was aspirated from the inflorescences, were identified using their male offspring.

Emerged Flies

A total of 137 drosophilids belonging to one species, *Zygothrica dispar* (Wiedemann 1830), and five species of *Drosophila, D. griseolineata* plus four putatively undescribed species, emerged in the laboratory from the inflorescences of *C. monophylla* collected in São Paulo (Table 3), whereas 22 specimens, all belonging to two undescribed species of *Drosophila*, emerged from the inflorescences of *C. cylindrica* collected in Rio de Janeiro (Table 4).

A broad-headed male of *Zygothrica dispar* that emerged from an inflorescence of *Calathea monophylla* was photomicrographed (Fig. 13), dissected, and had its identification confirmed through the analysis of the terminalia (also photomicrographed; Figs. 14 and 15). Unfortunately, part of the anterior region of the aedeagal apodeme was accidentally cut and lost during dissection, thus not showing its complete extension well beyond the anterior margin of hypandrium, as stated by Grimaldi (1998) to be an important diagnostic feature. However, its extension could be deduced from the remaining part. Like most if not all of its congeners, *Zygothrica dispar* is often seen in relatively large numbers over several fleshy fungi species. However, after surveying the literature on more than a

hundred described species of Neotropical *Zygothrica,* Grimaldi (1987) noted that only about three out of ca. 16 species, for which the breeding sites are known, were found to have fungi species as larval breeding sites. So far, *Zygothrica dispar* has been bred from decaying flowers of *Brunfelsia grandiflora* D.Don (according to Frota-Pessoa 1952) and *Brunfelsia excelsa* (according to Malogolowkin, 1953 and Grimaldi 1987; however this binomial has not been found in the botanical literature), belonging to the Solanaceae family, in the Rio de Janeiro Botanical Garden, Brazil. Yet, according to Frota-Pessoa (1952), it has also been bred from living flowers of two ornamental and exotic species *Hedychium coronarium* J.Koenig (Zingiberaceae) and *Thumbergia alata* Bojer ex Sims (Acanthaceae), collected in the same Botanical Garden. Santos and Vilela (2005) bred this species from a fallen flower of *Cestrum amictum* Schltdl. (Solanaceae) and based on this datum and the literature they wrote that *Zygothrica dispar* "seems to be an opportunistic, polyphagous and ground-feeding species that could oviposit in any decaying flower".

The smaller diversity of species that emerged from the inflorescences in the Rio de Janeiro collection, compared to that of the São Paulo collection, may be due to different plant samples, 14 and 20 inflorescences collected, respectively. It could also be due to the host plant species itself, ornamental and invasive in the collection site (*Calathea cylindrica*) versus native (*Calathea monophylla*).

Four out of five putatively undescribed species of *Drosophila* aspirated from inflorescences of *Calathea monophylla*, including the new species described below, also emerged from them. It is most likely that this species of "caeté" is one, or even the main, of many host plants used by those *Drosophila* species during their larval development.

***Drosophila calatheae* Vaz, Vilela, Krsticevic et Carvalho sp. nov.**

(Figs. 3-6 [lower], 8-12, 16-41)

Type Material. (26 imagines [13 ♂♂ and 13♀♀], deposited in MZSP). HOLOTYPE: 1 male (Figs. 16 and 17) [photomicrographed, double-mounted, dissected, terminalia illustrated] labelled: "Brasil - SP - São Paulo, Reserva Florestal do IB-USP, Cidade Universitária (M18), 12 to 28-XII-2006, C.R. Vilela *coll.* / aspirada de inflorescência de [aspirated from inflorescence of] *Calathea monophylla* / *Drosophila calatheae* ♂ Vaz *et al.* / HOLOTYPE / [microvial with terminalia in glycerin]". PARATYPES: Twenty-five (12 ♂♂, 13 ♀♀), same data as holotype, except for 3 ♂♂ and 4 ♀♀, coded M20, emerged from inflorescences collected on 29-XII-2006 and bearing the label: “emergida de inflorescência de [emerged from inflorescence of] *Calathea monophylla*”. Two of the female paratypes were dissected, and one was previously photomicrographed (Figs. 18 and 19) and other had its right wing removed.

Type Locality. BRAZIL: State of São Paulo, São Paulo, Cidade Universitária “Armando de Salles Oliveira”, Forest Reserve of the Instituto de Biociências da Universidade de São Paulo (23º33.96′S; 46º43.72′ W, ca. 750 m elevation; urban secondary Atlantic Forest fragment, stream border, aspirating over or emerging from inflorescences of *Calathea monophylla*).

Diagnosis. Body color dull brownish yellow, covered with brown setae and setulae; orange eyes (in live specimens); frons brownish yellow, medially and laterally light brown pollinose; antennae and palpi brownish yellow, the latter sexually dimorphic; scutum brownish yellow; h index 0.7 - 1.2; mid katepisternal about 33 - 67 % of the anterior one; two large prescutellars; abdomen dull, uniformly yellow; wings light brown, slightly darker anteriorly, conspicuously spotted (Fig. 20); C index 1.1 - 1.4.

Male (Figs. 16 and 17). Head brownish yellow, relatively large, slightly laterally enlarged somewhat embracing the anterior border of the thorax. Frons brownish yellow, dull, frontal length 0.36 mm (0.32 - 0.39 mm); frontal index = 1.01 (0.93 - 1.14), top to bottom width ratio = 1.50 (1.31 - 1.71). Frontal triangle light brown pollinose, about 100 % of frontal length; ocellar triangle shiny light brown, about 31 - 40 % of frontal length. Or3 to or1 = 67 - 120% of that to inner vertical; or1 / or3 ratio = 0.99; or2 / or1 ratio = 0.68; poc 35% - 54%, oc 67% - 80% of frontal length; vt index = 1.12 (1.00 - 1.22), vibrissal index = 0.44 (0.22 - 0.56), facial carina light brown, nose-like, slightly sulcate. Gena light brown, cheek index about 12.54 (9.33 - 15.00). Eye conspicuously large, orange (in live specimens, somewhat similar to the mutant *eosin* of *Drosophila melanogaster*). Eye index = 1.42 (1.32 - 1.56). Pedicel brown, laterally lighter, first flagellomere light brown, length to width ratio 1.58 (1.20 - 2.00). Arista with 4-5 dorsal, 2-3 ventral and 2-7 tiny inner branches, plus terminal fork. Proboscis brown. Palpus brownish yellow, globose, densely haired, brush-like.

Thorax brownish yellow; length 1.25 mm (1.19 – 1.32 mm). Scutum pollinose, brownish-yellow, 8-9 rows of acrostichal setae. h index = 0.92 (0.73 - 1.12). Transverse distance of dorsocentral setae 309 % of longitudinal distance; dc index = 0.64 (0.59 - 0.71); One pair of large prescutellars, ps index = 0.48 (0.38 – 0.56). Scutellum pollinose, laterally light brown, medially darker; distance between apical scutellar setae about 80% of that of apical to basal one, basal setae convergent; apical setae cruciate, scut index = 0.90 (0.84 – 1.00). Halters yellow. Pleura brownish yellow, sterno index = 0.74 (0.67 - 0.88), mid katepisternal seta about 31-67 % of the anterior one. Legs brownish yellow.

Wing (Fig. 20) light brown, anteriorly slightly darker, lappet dark brown, bearing five conspicuous dark brown spots along distal border, decreasing in size from first through

fifth, both crossveins strongly clouded, length 2.38 mm (2.29 – 2.49 mm), length to width ratio = 2.05 (1.98 - 2.11). Indices: C = 1.26 (1.17 - 1.36), ac = 2.94 (2.54 - 3.36), hb = 0.64 (0.60 - 0.66), 4C = 1.42 (1.32 - 1.56), 4v = 1.51 (1.39 - 1.62), 5x = 0.84 (0.73 - 1.00), M = 0.37 (0.31 - 0.46), prox. x = 0.70 (0.64 - 0.75).

Abdomen uniformly yellow, tergites devoid of distal marginal bands. Male sternite 5 wider (1.7x) than long.

Terminalia (Figs. 21-28). Epandrium mostly microtrichose, with 17 lower, and no upper setae; ventral lobe microtrichose ventrally, distally slightly concave, dorsoposteriorly partially fused to surstylus and slightly covering it. Cerci anteriorly and medially fused to epandrium, mostly microtrichose and devoid of ventral lobe. Surstylus dorsally fused to distal one-quarter of epandrium lobe, ventrally microtrichose, bearing ca. 10 dorso-positioned, cone-shaped prensisetae, 28 outer and mostly over ventral area, and 2 inner setae. Decasternum as in Fig. 21. Hypandrium (Figs. 22 and 23) U-shaped in ventral view (flattened in lateral view), anterior and posterior margins somewhat concave, shorter than epandrium; posterior hypandrial process and dorsal arch absent; gonopod fused to hypandrium (fusion line apparent), linked to paraphysis by membranous tissue, apparently bearing one tiny seta near the anterior inner margin. Aedeagus (Figs. 24-28) short, anteriorly expanded laterally, somewhat hut-shaped, fused to aedeagal apodeme, dorsally convex, ventrally bearing a pair of short, anteriorly projected spurs, which reach the paraphysis; dorsal cleft reduced to a small opening adjacent to the fusion aedeagus-aedeagal apodeme. Aedeagal apodeme shorter than aedeagus, posteriorly expanded, laterally flattened. Ventral rod entirely fused to aedeagal apodeme. Paraphysis square-shaped, not microtrichose, distally with 1 tiny seta, connected to distal margin of aedeagal apodeme by membranous tissue.

Female (Figs. 18 and 19). Measurements: frontal length 0.38 mm (0.34 - 0.42) mm; frontal index = 0.99 (0.88 - 1.07), top to bottom width ratio = 1.46 (1.07 - 1.67). Frontal triangle about 100 % of frontal length; ocellar about 31%-40% of frontal length. Or3 to or1 = 83 - 120% of that to inner vertical; or1 / or3 ratio = 1.01; or2 / or1 ratio = 0.66; poc 35 - 47%, oc 59 - 80% of frontal length; vt index = 1.10 (1.00 - 1.22); vibrissal index = 0.44 (0.22 - 0.62). Cheek index about 11.97 (7.50 - 16.00). Eye index = 1.32 (1.27 - 1.39), first flagellomere length to width ratio 1.70 (1.33-2.25). Arista with 4-5 dorsal, 2 ventral and 2-4 tiny inner branches, plus terminal fork. Palpus brownish yellow, boomerang-shaped, sparsely haired. Thorax length 1.36 (1.20 - 1.46) mm. 8-9 rows of acrostichal setae. h index = 1.00 (0.90 - 1.22). Transverse distance of dorsocentral setae 307 % of longitudinal distance; dc index = 0.62 (0.50 - 0.71); ps index = 0.47 (0.44 – 0.53). Distance between apical scutellar setae about 79% of that of apical to basal one, scut index = 0.90 (0.84 - 0.95). Sterno index = 0.78 (0.61 - 0.88), mid katepisternal seta about 33 - 64 % of the anterior one. Wing length 2.55 (2.44 – 2.68), length to width ratio = 2.04 (1.94 - 2.15). Indices: C = 1.28 (1.12 - 1.40), ac = 2.99 (2.80 - 3.08), hb = 0.63 (0.58 - 0.67), 4C = 1.46 (1.38 - 1.60), 4v = 1.56 (1.48 - 1.68), 5x = 0.84 (0.75 - 0.92), M = 0.37 (0.33 - 0.44), prox. x = 0.73 (0.65 - 0.88).

Terminalia (Figs. 29 and 30). Tergite VIII dorsoposteriorly microtrichose, strongly scletorized in its upper 2/3, weakly sclerotized in its lower 1/3, ventrally bearing 6 setulae. Oviscapt valve apically rounded with ca. 12 marginal and 6 discal peg-like ovisensilla. Spermathecal capsule spherical, sclerotized, proximally sulcate, distally papilate (Fig. 30); basal introvert ca. 5/6 of capsule length; apical introvert absent.

Eggs. Whitish, bearing 4 filaments of equal length; posterior ones twice as wide as anterior ones (Figs. 4 and 12).

385 **Puparia**. Remarkably long (ca. 5.6 mm, without spiracles) relative to body length of
386 imagoes (ca. 4 mm without wings); light brown; long horns (Fig. 6 [lower]); horn index
387 about 3.8; stalk of anterior spiracle conspicuously black, with about 22 light brownish
388 yellow tracheal branches.
389 **Etymology**. The epithet *calatheae* is a noun in the genitive case, referring to the putative
390 main host plants genus (*Calathea* spp.).
391 **Distribution**. So far this species is only known from the cities of São Paulo (type locality)
392 and Rio de Janeiro, Brazil. However, it probably also occurs in the states of Minas Gerais,
393 Paraná, Santa Catarina, and Rio Grande do Sul, following the distribution range of
394 *Calathea monophylla*. This species of "caeté" occurs from the state of Rio de Janeiro
395 [southeastern Brazil] to the state of Rio Grande do Sul [southern Brazil] (Wanderley and
396 Vieira 2002: 117 [cited as *Catathea communis*] and Braga 2005). The fact that *Drosophila*
397 *calatheae* sp. nov. has also been aspirated and emerged from inflorescences of *Calathea*
398 *cylindrica*, a species commonly cultivated as ornamental in Rio de Janeiro but also endemic
399 to the Atlantic Forest biome, is an indication that it has a potential of breeding in species of
400 *Calathea* other than *C. monophylla* and accordingly being a more widespread species.
401 According to a personal communication of JMA Braga to Barreto and Freitas (2007: 422),
402 *C. cylindrica* is endemic to the middle part of the cited biome (from the state of Bahia
403 through the state of São Paulo). Based on their statement, we predict *D. calatheae* sp. nov.
404 might also occur in the eastern parts of the states of Bahia, Minas Gerais and Espírito
405 Santo, in association with flowers of *C. cylindrica*. Although *C. cylindrica* is not present in
406 the forest reserve of the IB-USP (Groppo and Pirani 2005: 201), located in the western part
407 of São Paulo, it does occur naturally in a much larger area (527 ha) named "Parque

Estadual das Fontes do Ipiranga", a state park of natural Atlantic Forest located in the southern part of the city (Wanderley 1991).

Relationship. So far this species cannot be adequately included within any known group of Neotropical species of *Drosophila.* It shares features, however, with both the *bromeliae* and the *xanthopallescens* species groups (Table 5). These two poorly known groups need to be revised to clarify their relationships.

Chromosomes. *Drosophila calatheae* sp. nov. has a diploid chromosome number of 2n = 10 (Fig. 31-38). Female mitotic metaphase consists of four pairs of V-shaped and one pair of rod-shaped chromosomes (Figs. 31-34). Male mitotic metaphase differs from the female in having a J-shaped Y chromosome (Figs. 35-38). The largest V-shaped pair is almost completely heterochromatic and presents a secondary constriction in the less heterochromatic arm. This pair probably corresponds to the dot chromosome of the *Drosophila* spp. supposedly plesiomorphic karyotype (2n= 12 [5 pairs of rod, 1 pair of dots]). Unexpectedly it is not dot like, being about twice as long as the second largest chromosome (the X). A similar condition, although not presenting such a remarkable size difference, has been described for *Drosophila dreyfusi* Dobzhansky and Pavan (*dreyfusi* group) by Dobzhansky and Pavan (1943) and for *Drosophila arauna* Pavan and Nacrur (*annulimana* group) by Tosi and Pereira (1993). Heterochromatic blocks were also detected adjacent to the centromere of one arm of a medium-sized V-shaped pair (Fig. 36, arrow). Apparently, there is a tendency for the heterochromatic chromosomes (largest V-shaped and Y) to remain close to each other at the mitotic metaphase plates. Fig. 39 summarizes our interpretation of *D. calatheae* sp. nov. karyotype. Only six arms of chromosomes, corresponding to euchromatic regions, were evidenced in the polytene preparations (Figs. 40 and 41). A chromocenter (Fig. 40, arrow) strongly unites the two X chromosome arms to

the arm of the rod-shaped chromosome, the remaining pairs apparently stand unattached or detach frequently during the squashing process. No karyotypic variation was detected between the samples from São Paulo and Rio de Janeiro.

Ecology. Breeds from inflorescences of *Calathea monophylla*, *C. cylindrica* (Marantaceae) and *Heliconia bihai* (Heliconiaceae). We predict its larvae could be pollen and/or nectar and/or petal feeding, most probably after the pollinator has triggered the explosive pollination mechanism while opening the flowers.

Laboratory Rearing. Aiming to establish isofemale lines, we have at first made an additional selective collection of *Drosophila calatheae* sp. nov. at the forest reserve of the IB-USP, São Paulo (SP), on 17 and 19 November 2009 (coded M39). Thirty-one specimens (17 ♂♂: 14 ♀♀) were aspirated from several inflorescences of *Calathea monophylla*, distributed randomly in seven vials (at least a couple per vial) containing usual banana-agar medium in addition to two fresh open flowers per vial, and observed under a stereomicroscope. Occasionally some rapid back and forth movements of the proboscis over the wet outer surface of the darker and grayish petals and staminodes were observed for several specimens, mainly females, suggesting they were feeding over there. Soon after, the gut of females usually turned grayish and became somewhat visible through the semi-transparent abdominal pleura, reinforcing our suspicion. On 24 November 2009, the flies were transferred to seven new vials containing fresh culture medium plus fresh flowers collected early in the morning. Late in the afternoon, dozens of four-filamented eggs were observed mainly on the basal region of the outer surface of petals and petal-like staminodes of most flowers, especially those that were wetter and darker. Larvae were also seen moving around the pollinated curved stigma and within the corolla tube. Even though it is likely the observed eggs and larvae belong to *Drosophila calatheae* sp. nov., the most

456 abundant species, other less abundant species of Drosophilidae could also have oviposited
457 before the flowers were collected. During transference of the flies, three larvae were seen
458 on the banana-medium of one of the seven original vials (then kept at uncontrolled room
459 temperature) where they were able to develop and pupariate on different dates. Two
460 puparia, clearly distinct from each other, were detected on 2 December inside the culture
461 medium, with their opercula and extruded horns appearing on the surface. One puparium
462 was more roundish and reddish brown in color, bearing rather conspicuous black branches
463 emerging from reddish brown horns, and the other was more elongate, light brown in color
464 and bore inconspicuous brown-colored branches emerging from their horns. They were
465 washed in distilled water and transferred individually to new vials, aiming to correctly
466 associate each specimen with its respective puparium. Upon emerging from the darker
467 puparium, one male imago proved to belong to *Zygothrica dispar*; however no imago
468 emerged from the lighter puparium because its pupa had mummified inside it. It clearly
469 does not belong to *Drosophila calatheae* sp. nov. because of its relatively short puparial
470 horn. The remaining larva pupariated on 3 December on the inner surface of the vial, and
471 was then also individualized, and from which one female imago of *Zygothrica dispar*
472 emerged. Similar to one of the two puparia that was previously observed inside the culture
473 medium, it had the conspicuously black branches emerging from reddish brown horns.
474 Later a modified culture medium enriched with previously frozen inflorescences of
475 *Calathea cylindrica* was successfully used in Rio de Janeiro to maintain an isofemale line
476 of *Drosophila calatheae* sp.n. for about 4 years.
477 **Remarks**. An additional and preliminary experiment took place in an island named “Ilha
478 Grande” (a forest reserve in Angra dos Reis, state of Rio de Janeiro) to verify if
479 inflorescences of *Heliconia bihai* (L.) L. (Heliconiaceae) are used as breeding sites by

480 drosophilids. In 6 non-consecutive days between October and November, 2008,
481 drosophilids were aspirated from the bracts of this plant. Nine out of the 23 collected flies
482 belonged to *D. calatheae* sp. nov. On 11 December 2008, 10 inflorescences were collected
483 and kept in plastic vials and observed until 22 December 2008. From a total of 33 emerged
484 drosophilids, 14 belonged to this new species. We have also aspirated *D. calatheae* sp. nov.
485 from *Heliconia bihai* bracts in "Quinta da Boa Vista" (Rio de Janeiro) ~100 km apart from
486 the first cited collection site.

487 **Putative Pollinators and/or Robbers**

488 Large Apidae bees were sometimes seen flying around inflorescences while the flies
489 were being aspirated at the São Paulo collection site. On 22 December 2009, three large
490 bees belonging to two different species were net swept while landing on inflorescences of
491 *Calathea monophylla*: two females belonging to *Bombus (Fervidobombus) morio* (Fig. 42),
492 and a male belonging to *Euglossa (Euglossella) mandibularis* (Fig. 43). The bumblebee
493 *Bombus morio* is abundant in southern Brazil where it has been collected on almost one
494 fourth of the plant species in urban and natural biomes (Cortopassi-Laurino et al., 2003),
495 and is most probably a nectar (or pollen) robber of *Calathea* spp. flowers. *Euglossa* is a
496 genus of Apidae bees (tribe Euglossini) that includes pollinators of several species of
497 *Calathea* (Barreto and Freitas 2007; Kennedy 1978). The three collected specimens,
498 labelled "Brasil - SP - São Paulo, Reserva Florestal do IB-USP, Cidade Universitária
499 (M40), 22.XII.2009, C.R. Vilela *coll*.", were kindly identified by Dr. Isabel Alves do Santos
500 and are deposited in Cepann (Coleção Entomológica Paulo Nogueira Neto) at Instituto de
501 Biociências, Universidade de São Paulo.

Acknowledgements

We are indebted to José Mariano Amabis and Beatriz Goñi for advise on chromosomes, to Denise S. Sheepmaker for allowing the use of her stereophotomicroscope, to Giovana Vaz for preparing the plates, to Janaína Brito for help with field work, to Beto Campos for taking most of the outdoor pictures, to Eduardo Dupim and Dulci Pereira da Rocha for help with rearing of flies, to Isabel Alves dos Santos for identifying Apidae species, to Augusto dos Santos Rampasso for testing and revising the keys, and to Wayne N. Mathis for reviewing the English. Flies were collected with authorization from IBAMA/SISBIO (number 31434-1). This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and NIH grant GM64590.

References Cited

Andersson, L. 1998. Marantaceae, pp. 278-293. In K. Kubitzki (ed.), The Families and Genera of Vascular Plants, vol. 4. Flowering plants, Monocotyledons: Alismatanae and Commelinanae (except Gramineae). Springer-Verlag, Berlin, x +511 pp.

Ashburner, M. 1989. *Drosophila:* a laboratory manual. Spring Harbor Laboratory Press, Cold Spring Harbor, NY, xxiii, 434 pp.

Bächli, G., C.R. Vilela, and S.F. McEvey. 2005. Nine new species of Afrotropical *Leucophenga* (Diptera, Drosophilidae). Mitt. Schweiz. Entomol. Ges. 78:23-57.

Bächli, G., C.R. Vilela, and V. Ratcov. 2000. Morphological differences among *Drosophila paraguayensis* Duda, 1927 and its close relatives (Diptera, Drosophilidae). Mitt. Schweiz. Entomol. Ges. 73:67-92.

Bächli, G., C.R. Vilela, A.S. Escher, and A. Saura. 2004. The Drosophilidae (Diptera) of Fennoscandia and Denmark. Fauna Entomologica Scandinavica, vol. 39. Brill, Leiden, 362 pp.

Barreto, A.A., and L. Freitas. 2007. Atributos florais de um sistema de polinização especializado: *Calathea cylindrica* (Roscoe) K. Schum. (Marantaceae) e abelhas Euglossini. Rev. Bras. Bot. 30: 421-431.

Braga, J.M.A. 2005. Marantaceae - Novidades taxonômicas e nomenclaturais III: Tipificações, sinonímias e uma nova combinação em *Calathea*. Acta Bot. Brasilica 19: 763-768.

Breuer, M.E., and R.F. Rocha. 1971. Genitália masculina de algumas espécies de *Drosophila* dos grupos “repleta” e “tripunctata” (Diptera, Drosophilidae). Pap. Avulsos Zool. 25: 121-137.

Brncic, D. 1983. Ecology of flower-breeding *Drosophila*, pp. 333-382. In: M. Ashburner, H.L. Carson, and J.R. Thompson (eds.), The Genetics and Biology of *Drosophila*, vol. 3d. Academic, London.

Burla, H. 1954. Study on the polymorphism in *Zygothrica dispar* and *Z. prodispar*, and description of *Z. laticeps* sp. n. (Drosophilidae), Diptera). Arq. Mus. Paranaense 10: 231-252.

Carson, H.L. 1971. The ecology of *Drosophila* breeding sites. Harold L. Lyon Arboretum Lecture 2: 1-28.

Chakir, M., P. Capy, J. Genermont, E. Pla, and J. David. 1996. Adaptation to fermenting resources in *Drosophila melanogaster*: ethanol and acetic acid tolerances share a common genetic basis. Evolution 50: 767-776.

Cooper, K.W. 1950. Normal spermatogenesis in *Drosophila*. In M. Demerec (ed.), Biology of Drosophila. John Wiley, New York, 1-56.

Cortopassi-Laurino, M., F.R.N. Knoll, V.L. Imperatriz-Fonseca. 2003. Nicho trófico e abundância de *Bombus morio* e *Bombus atratus* em diferentes biomas brasileiros. In: G. A. R. Melo and I. Alves-dos-Santos, Apoidea Neotropica: Homenagem aos 90 Anos de Jesus Santiago Moure. Editora UNESC, Criciúma.

Dobzhansky, T., and C. Pavan. 1943. Studies on Brazilian species of *Drosophila*. Univ. Sao Paulo, Fac. Filos., Cienc. Let., Bol. 36, Biologia Geral 04: 7-72.

Frota-Pessoa, O. 1952. Flower-feeding drosophilidae. Drosoph. Inf. Serv. 26: 101-102.

Grimaldi, D.A. 1987. Phylogenetics and taxonomy of *Zygothrica* (Diptera: Drosophilidae). Bull. Am. Mus. Nat. Hist. 186: 103-268.

563 **Groppo, M., and J.R. Pirani. 2005.** Levantamento florístico das espécies de ervas,
564 subarbustos, lianas e hemiepífitas da mata da Reserva da Cidade Universitária "Armando
565 de Salles Oliveira", São Paulo, SP, Brasil. Univ. Sao Paulo, Bol. Bot. 23: 141-233.
566 **Jones, C. 1998. The genetic basis of** *Drosophila sechellia*'s resistance to a host plant toxin.
567 Genetics 149: 1899-1908.
568 **Kaneshiro, K.Y. 1969.** A study of the relationships of Hawaiian *Drosophila* species based
569 on external male genitalia. Univ. Tex. Publ. 6918: 55-70.
570 **Kennedy, H. 1978.** Systematics and pollination of the "closed-flowered" species of
571 *Calathea* (Marantaceae). Univ. Calif. Publ. Bot., vol. 71. University of California, Berkeley,
572 90 pp+20plates.
573 **Lorenzi, H. and H.M. Souza. 2008.** Plantas ornamentais no Brasil: arbustivas, herbáceas e
574 trepadeiras, 4ª ed. Instituto Plantarum, Nova Odessa, Brazil.
575 **Malogolowkin, C. 1953.** Notas sôbre "*Zygothrica dispar*" (Diptera, Drosophilidae). Rev.
576 Bras. Biol. 12(4): 455-457.
577 **Markow, T.A., and P. O'Grady. 2008.** Reproductive ecology of *Drosophila.* Func. Ecol.:
578 1-13. doi: 10.1111/j.1365-2435.2008.01457.x
579 **Pavan, C., and A.B. da Cunha. 1947.** Espécies brasileiras de *Drosophila.* Univ. Sao Paulo,
580 Fac. Filos., Cienc. Let., Bol. 86, Biol. Geral 7: 1-47.
581 **Pimpinelli, S., L. Bonaccorsi, L. Fanti, and M. Gatti. 2000.** Preparation and analysis of
582 *Drosophila* mitotic chromosomes, pp. 3-23. In: Sullivan W, Ashburner M and R.S. Hawley
583 (eds.) *Drosophila* Protocols. Cold Spring Harbor Laboratory Press, New York.
584 **Pipkin, S.B. 1964.** New flower breeding species of *Drosophila* (Diptera: Drosophilidae).
585 Proc. Entomol. Soc. Wash. 66: 217-245.

586 **Pipkin, S.B., R.L. Rodriguez, and J. Leon. 1966.** Plant host specificity among flower-
587 feeding Neotropical *Drosophila* (Diptera: Drosophilidae). Am. Nat. 100: 135-156.
588 **Rossi, L. 1994.** A flora arbóreo-arbustiva da mata da Reserva da Cidade Universitária
589 "Armando de Salles Oliveira" (São Paulo, Brasil). Univ. Sao Paulo, Bol. Bot.. 9: 1-105.
590 **Santos, R.C.O., and C.R. Vilela. 2005.** Breeding sites of Neotropical Drosophilidae
591 (Diptera). IV. Living and fallen flowers of *Sessea brasiliensis* and *Cestrum* spp.
592 (Solanaceae). Rev. Bras. Entomol. 49: 544-551. doi: 10.1590/S0085-56262005000400015.
593 **Spassky, B. 1957.** Morphological differences between sibling species of *Drosophila*. Univ.
594 Tex. Publ. 5721: 48-61.
595 **Sturtevant, A.H. 1921.** The North American species of *Drosophila*. Carnegie Inst.
596 Washington Publ. 301: 1-150.
597 **Throckmorton, L.H. 1962.** The Problem of Phylogeny in the Genus *Drosophila*. Univ.
598 Tex. Publ. 6205: 207-343.
599 **Tosi, D., and M.A.Q.R. Pereira. 1993.** Karyotypes and phylogenetic relationships in
600 *Drosophila* species of the *annulimana* group (Diptera, Drosophilidae). Rev. Bras. Genet.
601 16: 321-333.
602 **Val, F.C. 1982.** The male genitalia of some Neotropical *Drosophila*: notes and illustrations.
603 Pap. Avulsos Zool. 34: 309-347.
604 **Vilela, C.R. 2001.** Breeding sites of Neotropical Drosophilidae (Diptera). III. Rotting
605 infructescences of *Philodendron bipinnatifidum* (Araceae). Rev. Bras. Entomol. 45: 339-
606 344.
607 **Vilela, C.R., and G. Bächli. 1990.** Taxonomic studies on neotropical species of seven
608 genera of Drosophilidae (Diptera). Mitt. Schweiz. Entomol. Ges. 63(suppl.): 1-332.

609 **Vilela, C.R., and G. Bächli. 2000.** Morphological and ecological notes on the two species
610 of *Drosophila* belonging to the subgenus *Siphlodora* Patterson & Mainland, 1944 (Diptera,
611 Drosophilidae). Mitt. Schweiz. Entomol. Ges. 73: 23-47.

612 **Vilela, C.R., and M.A.Q.R. Pereira. 1992.** Breeding sites of Neotropical Drosophilidae
613 (Diptera). I. Living flowers of *Cestrum schlechtendalii* (Solanaceae). Rev. Bras. Entomol.
614 36: 475-482.

615 **Vilela, C.R., and D. Selivon. 2000.** Breeding sites of Neotropical Drosophilidae (Diptera).
616 II. Fallen fruits of *Citharexylum myrianthum* Cham. (Verbenaceae). Drosoph. Inf. Serv. 83:
617 475-482.

618 **Wanderley, M.G.L. 1991.** Flora fanerogâmica da reserva do Parque Estadual das Fontes
619 do Ipiranga (São Paulo Brasil): 183- Marantaceae. Hoehnea 18: 49-51.

620 **Wanderley, M.G.L., and S. Vieira. 2002.** Nova espécie de *Calathea* G. Mey
621 (Marantaceae) para o Brasil. Hoehnea 29: 115-118.

622 **Wheeler, M.R., and M.P. Kambysellis. 1966.** Notes on the Drosophilidae (Diptera) of
623 Samoa. Univ. Tex. Publ. 6615: 533-565.

Table 1. Drosophilids aspirated from inflorescences of *Calathea monophylla* (Vell.) Körn. (Marantaceae). Collections made in 5 non-consecutive days in December 2006 at the forest reserve of the Instituto de Biociências da Universidade de São Paulo (23º33.96′S; 46º43.72′ W, ca. 750 m elevation), Cidade Universitária "Armando de Salles Oliveira", São Paulo, state of São Paulo, Brazil.

	collection date: December 2006 ♂ / ♀					
species	12	18	22	26	28	total (♂ + ♀)
Drosophila bromelioides Pavan and Cunha 1947	1/1	6/2	1/1	3/2	1/-	18
Drosophila calatheae sp. nov.	1/1	15/5	21/17	38/26	35/18	177
Drosophila caponei Pavan and Cunha 1947	-	-	-	4/-	-	4
Drosophila cuaso Bächli, Vilela and Ratcov 2000	-	-	4/1	-	-/1	6
Drosophila fuscolineata Duda 1925	-	-	-	-	-/1	1
Drosophila griseolineata Duda 1927	-	2/1	-	-	-	3
Drosophila mediopunctata Dobzhansky and Pavan 1943	-	-	-/1	-	-	1
Drosophila ornatifrons Duda 1927	-	-	-	-/2	-	2
Drosophila paraguayensis Duda 1927	1/-	2/1	3/1	-	1/-	9
Drosophila trifilum Frota-Pessoa 1954	-	1/-	-	-	-	1
Drosophila sp. B3	1/-	3/-	1/-	1/1	1/-	8
Drosophila sp. W3	2/2	-/2	2/2	-	-	10

28

species	collection date: December 2006 ♂ / ♀					
	12	18	22	26	28	total (♂ + ♀)
Drosophila sp. G4	2/1	7/5	23/10	41/29	71/37	226
Drosophila sp. I4	-	3/4	1/1	3/3	6/3	24
Drosophila sp. (*morelia* group)	-	1/-	-	-	-	1
Drosophila sp. ?	-	-/1	-	-	-	1
Diathoneura sp. 1	-	-	1/-	-	-	1
Diathoneura sp. 2	-	-	1/-	-	-	1
Paramycodrosophila costaricana Duda 1925	1/-	-	-	-	-	1
total	9/5	40/21	58/34	90/63	115/60	495

624

625

29

Table 2. *Drosophila* species aspirated from inflorescences of *Calathea cylindrica* (Roscoe) K. Schum. (Marantaceae). Collections made in 5 non-consecutive days in December 2006 in Rio de Janeiro, state of Rio de Janeiro, Brazil (at forest sites adjacent to Parque Nacional da Tijuca; 22º57.95′S; 43º14.31′ W, ca. 90 m elevation).

species	♂	♀	♂ + ♀
Drosophila sp. G4	13	12	25
Drosophila calatheae sp. nov.	7	8	15
Drosophila cardinoides Dobzhansky and Pavan 1943	1	1	2
total	21	21	42

30

637 **Table 3.** Drosophilids emerged in laboratory from twenty inflorescences of *Calathea monophylla* (Vell.) Körn. (Marantaceae). Inflorescences kept
638 under controlled temperature (22 ± 1 °C) after collection on 29-XII-2006 at the forest reserve of the Instituto de Biociências da Universidade de São
639 Paulo (23º33.96′S; 46º43.72′ W, ca. 750 m elevation), Cidade Universitária "Armando de Salles Oliveira", São Paulo, State of São Paulo, Brazil.

	Emergency date: December 2006 / January 2007 ♂/♀																				
species	29	30	31	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	total (♂ + ♀)
Drosophila calatheae sp. nov.	1/2	1/1	7/5	5/5	2/5	1/1	1/1	3/1	-/2	2/4	3/5	2/3	1/-	1/1	-/1	-	-	-	1/-	-	68
Drosophila griseolineata	-	-	-	-	1/-	-	-/1	-	-	1/1	-	1/-	-	-	1/-	-	-	-/1	-	-	7
Drosophila sp. B3 (*tripunctata* group)	-	-	-	-	-	-	-	-	-	-/1	-	-	1/1	-	-	-	-	-	-	-	3
Drosophila sp. G4 (*guarani* group)	-	1/-	-	-	2/-	1/1	-/1	-/1	1/-	-	1/1	1/1	4/2	1/1	-/2	-	-	-	-	-	22
Drosophila sp. I4 (undetermined group)	-	-/2	2/1	2/2	1/5	2/1	-/1	2/1	1/1	-/1	-	-	-	-/1	-	-/3	-	-	-	-/1	30
Zygothrica dispar	-	-	-	-	-	-	-	-	-	-	-	1/-	-	-/1	-/1	1/2	1/-	-	-	-	7
total	1/2	2/3	9/6	7/7	6/10	4/3	1/4	5/3	2/3	3/7	4/6	5/4	6/3	2/4	1/4	1/5	1/-	-/1	1/-	-/1	137

641 **Table 4.** *Drosophila* species emerged in laboratory from fourteen inflorescences of *Calathea*
642 *cylindrica* (Roscoe) K. Schum. (Marantaceae). Inflorescences collected on 22-XII-2006 at Horto
643 Florestal, border of Parque Nacional da Tijuca, Rio de Janeiro, state of Rio de Janeiro, Brazil
644 (22º57.95′S; 43º14.31′ W, ca. 90 m elevation). Emerged flies collected on 24-XII-2006, 27-XII-
645 2006 and 8-I-2007.

646
647

species	♂	♀	♂ + ♀
Drosophila calatheae sp. nov.	9	12	21
Drosophila sp. I4	1	0	1
total	10	12	22

648

649 **Table 5.** Comparison among *Drosophila calatheae* sp. nov. and the *bromeliae* and
650 *xanthopallescens* species groups regarding morphology, chromosomes and breeding sites. Data
651 compiled from Sturtevant (1921: 72-73), Pavan and da Cunha (1947: 7-10), Throckmorton
652 (1962: 254), Pipkin (1964: 231-237), and Brncic (1983: 336-339); morphological terminology
653 and botanical taxonomy updated following Bächli et al. (2004) and Lorenzi and Souza (2008),
654 respectively.

	taxon		
characteristics	*D. calatheae* sp. nov.	*bromeliae* group	*xanthopallescens* group
body color	dull yellow	idem	idem
prescutellars	yes	yes	no
wings	spotted; costal index ~ 1.3	unspotted; costal index > 2	unspotted; costal index > 2
male terminalia	hypandrium without dorsal arch; anal plates fued to epandrium	idem	idem
testes	6 – 7 coils	3 – 6 coils	8 – 11 coils
ventral receptacle	< 30 coils	unknown	> 90 coils
karyotype	2n = 10	2n = 8	unknown
host plant families	Marantaceae and Heliconiaceae	> 10	Marantaceae and Heliconiaceae

655

656 **Figs. 1-6.** *Calathea monophylla*. (1) Clumps of *C. monophylla* (São Paulo). (2) Detail of an
657 inflorescence. (3) *Drosophila calatheae* sp. nov. female ovipositing on a *C. monophylla* petal at
658 lab. (4) Egg of *D. calatheae* sp. nov. on *C. monophylla* petal (note its 4 filaments). (5) Larva of
659 *D. calatheae* sp. nov. from lab culture (note its very long posterior spiracles). (6) Puparia of
660 *Drosophila* sp. I4 (upper) and *D. calatheae* sp. nov. (lower): note the long horn and the wide air-
661 filled space on the anterior end, probably related to floatation inside bracts phytotelmata. Scale
662 bars = 50 cm and 5 cm in 1 and 2, respectively, and 1 mm in 3-6.
663 **Figs. 7-12.** *Calathea cylindrica*. (7) Clump of *C. cylindrica* (Rio de Janeiro). (8) Inflorescence of
664 *C. cylindrica* (arrow points to a *D. calatheae* sp. nov. specimen). (9) A couple in copula on a *C.*
665 *cylindrica* petal. (10) Same as anterior figure with a male voyeur. (11) Close view of *D.*
666 *calatheae* sp. nov. specimen from laboratory culture on a tip of inflorescence bract placed over
667 culture medium. (12) Four-filamented egg on culture medium. Scale bars = 15 cm and 1 cm in 7
668 and 8, respectively, and 1 mm in 9-12.
669 **Figs. 13-15.** *Zygothrica dispar* male. (13) Anesthetized adult emerged from *Calathea*
670 *monophylla* inflorescence, dorsal view. (14) Internal terminalia of the same specimen, ventral
671 view (proximal end of aedeagal apodeme missing; arrow points the accidentally cut tip). (15)
672 Idem, left lateral view. Scale bars = 1 mm and 0.1 mm, respectively.
673 **Figs. 16-19.** *Drosophila calatheae* sp. nov. two type specimens. (16-17) Male holotype in dorsal
674 and left lateral views, respectively. (18-19) Female paratype in dorsal and left lateral views,
675 respectively. Scale bar = 1 mm.

Fig. 20. Left wing of an ordinary *Drosophila calatheae* sp. nov. female aspirated from *Calathea cylindrica* from Rio de Janeiro, ventral view. Scale bar = 1 mm.

Figs. 21-30. *Drosophila calatheae* sp. nov. (21-29) Male holotype and female paratype terminalia. (21) Epandrium, cerci, surstyli and decasternum, oblique posterior view. (22) Hypandrium and gonopods, posterior view. (23) Idem, left lateral view. (24-28) Aedeagus+aedeagal apodeme and paraphyses, several views from dorsal through ventral. (29) Tergite VIII, epiproct, hypoproct and left oviscapt valve, left lateral view. (30) Spermathecae, inner capsules, lateral view. Scale bar = 0.1 mm.

Figs. 31-38. Metaphasic and prometaphasic neuroblast chromosomes of *Drosophila calatheae* sp. nov. (2n=10), from an isofemale line from Rio de Janeiro. (31-34) Females (XX). (35-38) Males (XY). Note the entirely heterochromatic J-shaped Y-chromosome and the remarkably huge and heterochromatic pair which probably corresponds to typical *Drosophila* spp. dot chromosomes. Arrow in 36 points to the partially heterochromatic V-shaped autosome pair. Scale bar = 5 μm.

Fig. 39. Ideogram of *Drosophila calatheae* sp. nov. male karyotype. Black regions = strong heterochromatin, gray = light heterochromatin, white = euchromatin. The first pair represents the sex chromosomes, the second pair are the partially heterochromatic ones, and the fifth pair represent the aberrantly huge heterochromatic *Drosophila* dot. Interpretation was based on a larger set of metaphasic plates than those shown in Figs. 31-38.

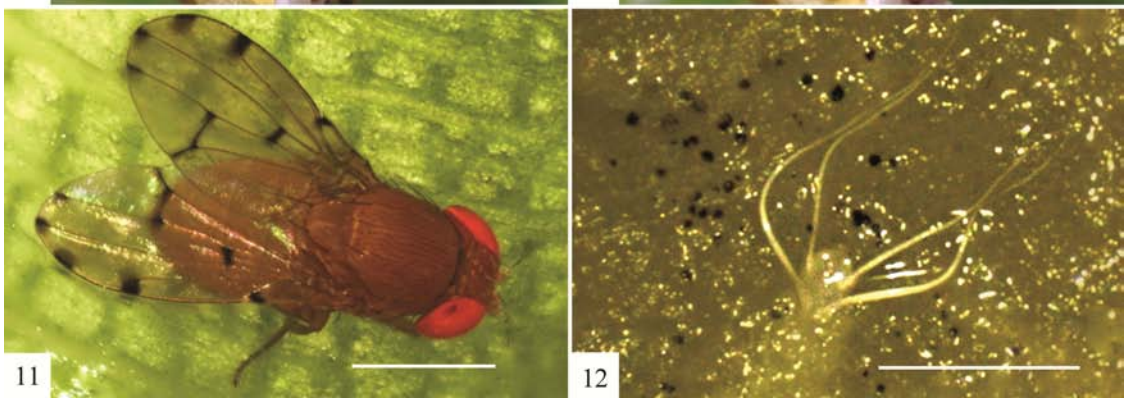
Figs. 40-41. Salivary gland polytene chromosomes of *Drosophila calatheae* sp. nov. third instar larvae. (40) Female. (41) Male. Note the two long X chromosome arms in each sex. Arrow points to chromocenter. Scale bar = 10 μm.

Figs. 42-43. Putative pollinator and/or robber bees (Apidae) of *Calathea monophylla*. (42) *Bombus (Fervidobombus) morio* female. (43) *Euglossa (Euglossella) mandibularis* male. Specimens were collected at the forest reserve of the Instituto de Biociências da Universidade de São Paulo, Cidade Universitária "Armando de Salles Oliveira", São Paulo, SP, by net sweeping over inflorescences. Scale bar = 1 cm.


1
2
3
4
5
6


7
8

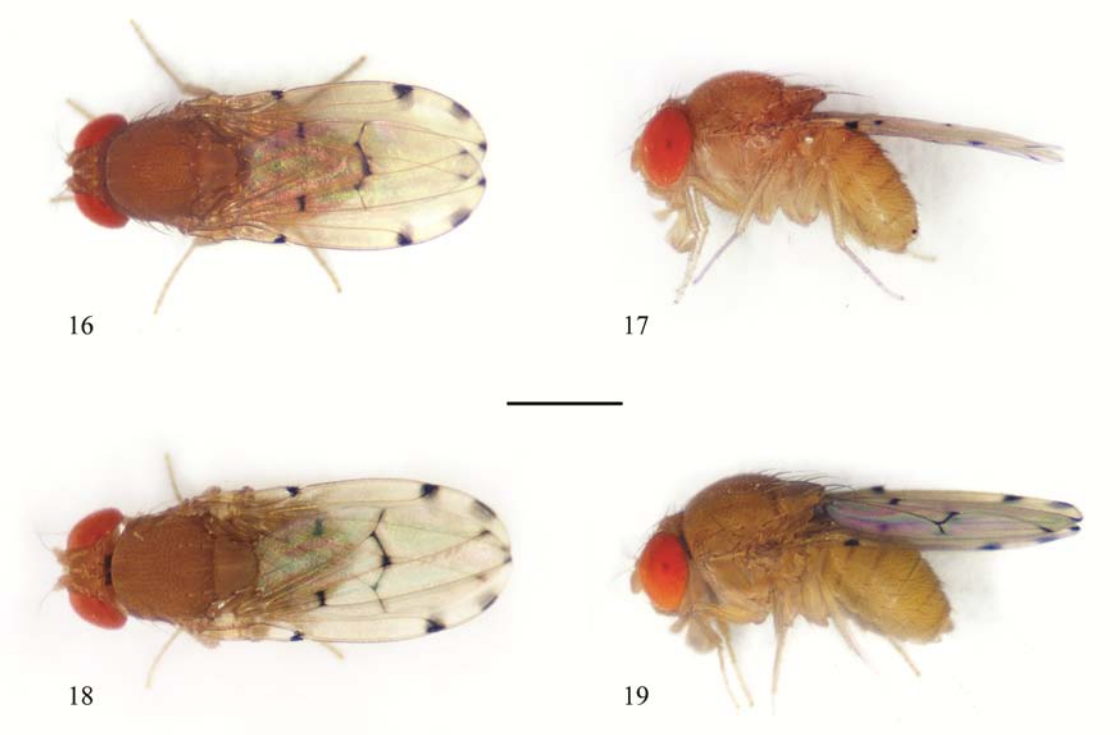

9
10


11
12

13

14

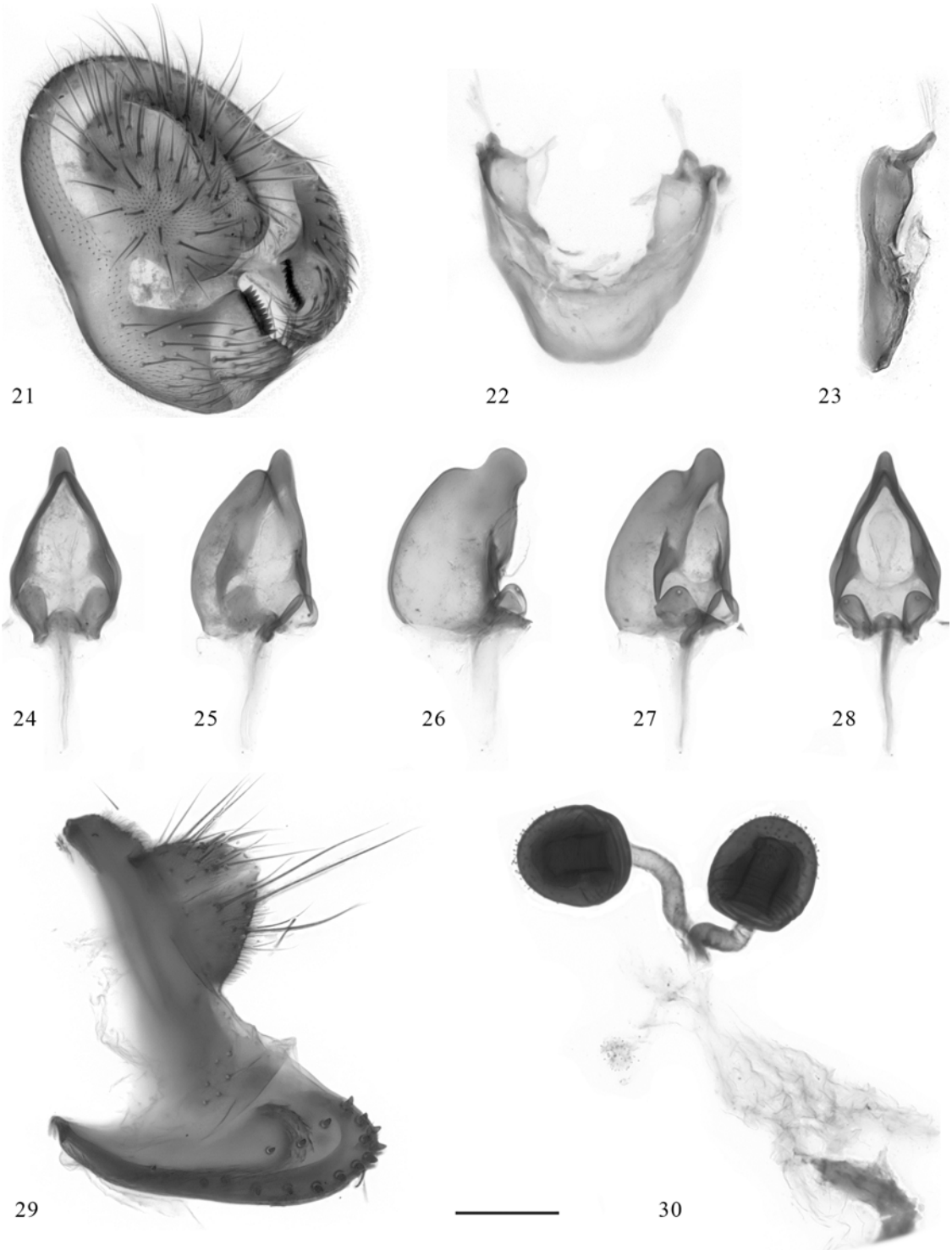
15

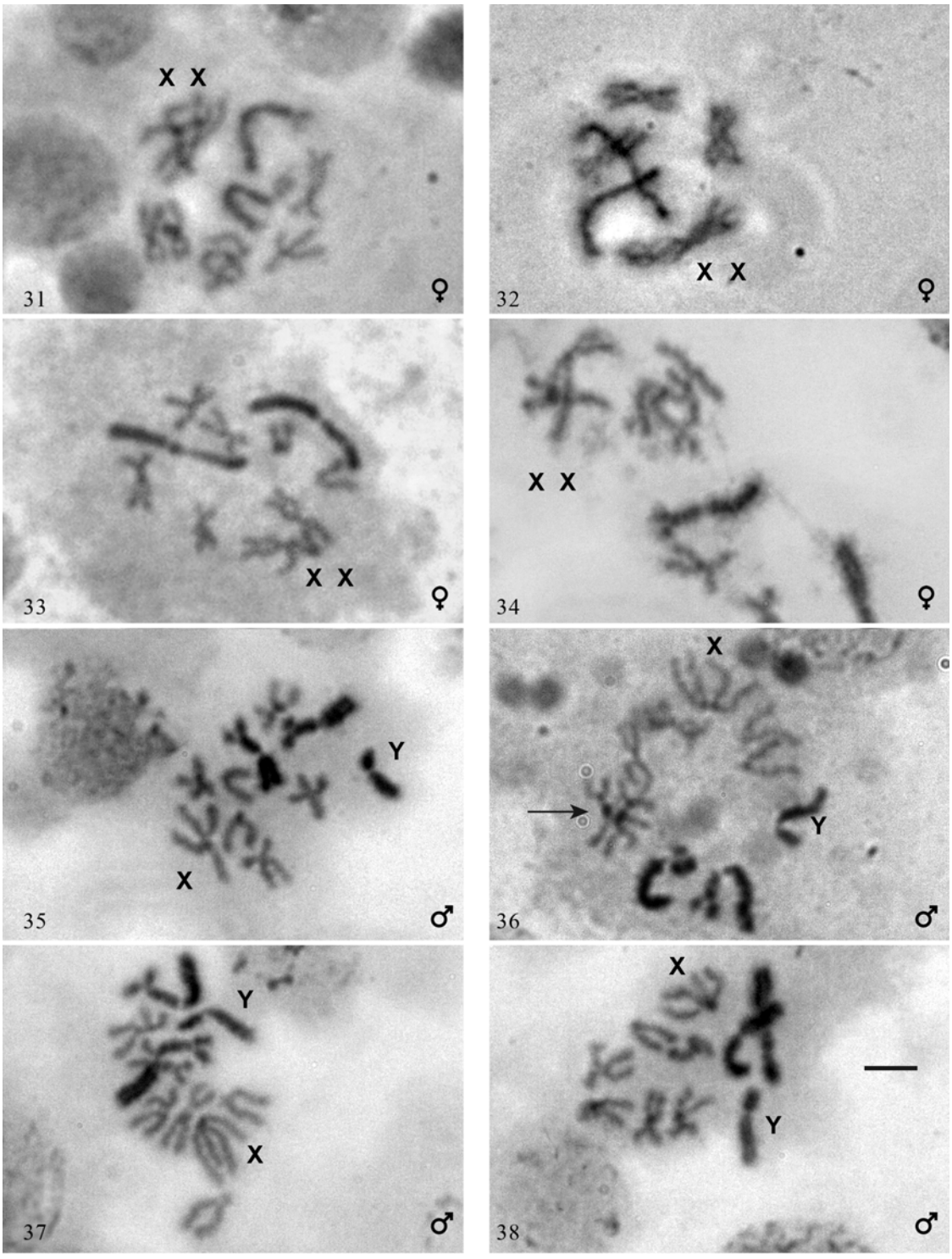


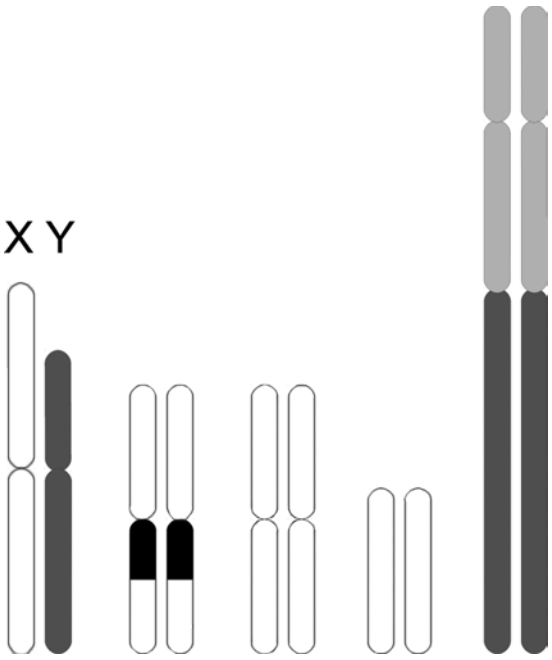
16 17

18 19

20

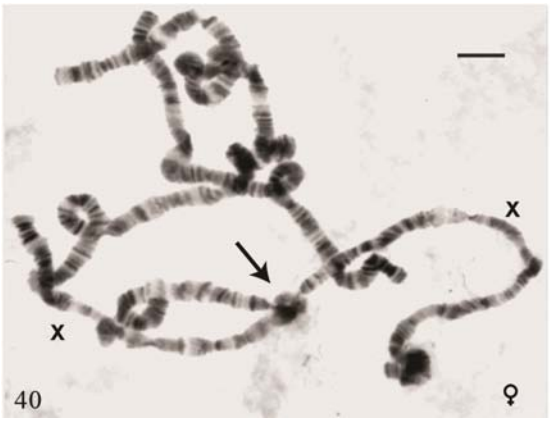

21
22
23
24
25
26
27
28
29
30

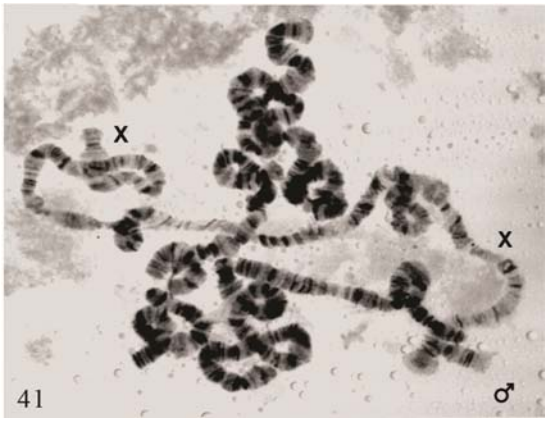

X X
31
♀
X X
32
♀
X X
33
♀
X X
34
♀
Y
X
35
♂
X
Y
36
♂
Y
X
37
♂
X
Y
38
♂



711 39

44


X
X
40
♀


X
X
41
♂

42 43

Gmail by Google

Flavia Krsticevic <fkrsticevic@gmail.com>

Fwd: Manuscript AN-13-148 Version 1

Suzana Vaz <vazsuz@gmail.com> 11 de dezembro de 2013 13:11
Para: "A. Bernardo Carvalho" <bernardo@biologia.ufrj.br>, vilela <crvilela@ib.usp.br>, Flavia Krsticevic <fkrsticevic@gmail.com>

pessoal. so li a primeira frase.. e to compartilhando com vcs. acho que eh meu melhor presente de natal.... e e um presente de niver p be...?

---------- Forwarded message ----------
From:
Date: Tuesday, December 10, 2013
Subject: Manuscript AN-13-148 Version 1
To: vazsuz@gmail.com
Cc: akahan@entsoc.org, dave.taylor@ars.usda.gov

Suzana C. Vaz, Mrs.
Universidade de São Paulo
Departamento de Genética e Biologia Evolutiva
São Paulo, SP

Brazil

RE: AN-13-148 Version 1
Breeding Sites of Neotropical Drosophilidae (Diptera)
V. Inflorescences of Calathea cylindrica and Calathea monophylla (Marantaceae)

Dear Suzana C. Vaz, Mrs.,

The above referenced manuscript has been accepted for publication upon the completion of revisions. Please address the reviewers' comments that are enclosed. If the reviewer included a separate file of the review, it can be accessed by logging onto your My Manuscripts page and clicking the "Download Comments" button. Also note the important information below about submitting high-quality figures for publication.

I apologize for the delay. I never did get a response from several individuals contacted to do the second review. Therefore, I reviewed the manuscript myself (Reviewer 2).

Overall, the manuscript contains interesting information on a new species and larval developmental sites of Neotropical Drosophila. I have indicated many editorial and grammatical suggestions / corrections on the manuscript as did Reviewer 1. Please address those suggestions.

A couple of general points. Subheadings should be indented and in sentence case (capitalize first word, rest in lower case unless a proper noun). Several parts of the manuscript were long and wordy. If possible, please try to make points more concise. Finally, one of my "pet peeves", the use of "breeding" site to refer to "larval developmental" sites. I know everyone does it, but that does not make it correct. Breeding (mating) and larval development are two different phenomena and should not be confused.

I look forward to receiving your revised manuscript.

NOTE: If you have a problem downloading a review from the Rapid Review website, refer to information on the ESA website at http://www.entsoc.org/Pubs/Publish/Policies/Allow_File_Download.pdf on how to change your web browser security settings to allow file downloads from Rapid Review.

We ask that your revised manuscript be returned within 30 days of this letter. Include a response to the reviewers, either in the cover letter or rebuttal, with your manuscript to expedite the final decision on your paper. Please send us the word processing file for your revised manuscript, not a PDF of it. The Rapid Review system will

automatically create the PDF from your file for our use.

We also require that you submit a separate, high-quality digital file for each of your figures with your revised manuscript. Please refer to the ESA web site at http://www.entsoc.org/pubs/publish/style/index.htm#Figures for what software applications and file types are acceptable for digital figures. When you submit these figure files, make sure you check the box that says "For Review/Print Production" when prompted.

When you are ready to return the manuscript, log on to the Rapid Review system at http://www.rapidreview.com/ESA2/CALogon.jsp. As a reminder, your User Name is vazsuz. If you need your password, click "Can't remember your password?" on the logon screen and follow the directions.

Sincerely,

David B. Taylor
Editor
Annals of the Entomological Society of America

REVIEWER 1:
Comments:
I have returned a marked up MS to help the authors improve the language in this MS. I think the work is technically acceptable and the results and methods are fine. Once the writing problems and English language usage issues are fixed, this paper should be acceptable.