

SHORT COMMUNICATION

The invasive spotted-wing *Drosophila* (Diptera, Drosophilidae) has been found in the city of São Paulo (Brazil)

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ABSTRACT. The invasive spotted-wing *Drosophila* (Diptera, Drosophilidae) has been found in the city of São Paulo (Brazil). *Drosophila suzukii* (Matsumura, 1931), the cherry fly or spotted-wing *Drosophila*, a pest species from the Oriental and southeastern Palaearctic regions belonging to the *melanogaster* group, invaded the Nearctic and western countries of the Palaearctic regions late last decade (2008) and, more recently (2013), the southern Brazilian states of Rio Grande do Sul and Santa Catarina. Early in 2014 it was reared from blueberries produced in São Joaquim, state of Santa Catarina, that were bought at a São Paulo city grocery store. Despite being a cold-adapted species, after having arrived to the southeastern state of São Paulo, this invasive fly will probably expand its territory to other Brazilian states and South American countries through trade of cultivated soft skin small fruits, such as blueberries and strawberries, as well as naturally through the use of small wild fruits as breeding sites.

KEYWORDS. Blueberries; *Drosophila suzukii*; karyotypes; *melanogaster* group; terminalia.

The *Drosophila melanogaster* species group, currently including 184 species (Bächli 2014) inhabiting the Afrotropical, Oriental and southeastern Palaearctic Regions, is by far the most diverse group of its genus. By the end of the 20th century five species belonging to this group had reportedly invaded and become established in the Nearctic and Neotropical regions: *D. ananassae* Doleschall, 1858, *D. kikkawai* Burla, 1954, *D. malerkotliana* Parshad & Paika, 1965, *D. melanogaster* Meigen, 1830 (found indoors all over the world), and *D. simulans* Sturtevant, 1919. Except for *D. malerkotliana*, which was collected for the first time in the American continent by F.M. Sene on 19.IV.1976 (collector's notebook, unpublished data) in Barra do Maxaranguape, state of Rio Grande do Norte, Brazil (Sene *et al.* 1980; Val & Sene 1980), the arrival dates of the remaining four species into the New World are unknown. The sixth and most recent invader of this group, *D. suzukii*, a species apparently native to the southeastern Palaearctic region and a serious pest of healthy, small cultivated fruits is, since August 2008 (California, USA; Lee *et al.* 2011), on its way to becoming widespread in the New World. It was first detected in the state of Florida (USA) in August 2009 (Steck *et al.* 2009). It belongs to an Oriental subgroup (*suzukii* subgroup) of 18 cryptic species. According to Deprá *et al.* (2014), the first collection of this new invader in Brazil was represented by a single male sampled at the *Reserva Biológica Estadual de Aguai*, Nova Veneza, state of Santa Catarina on 27.II.2013 (collector not stated).

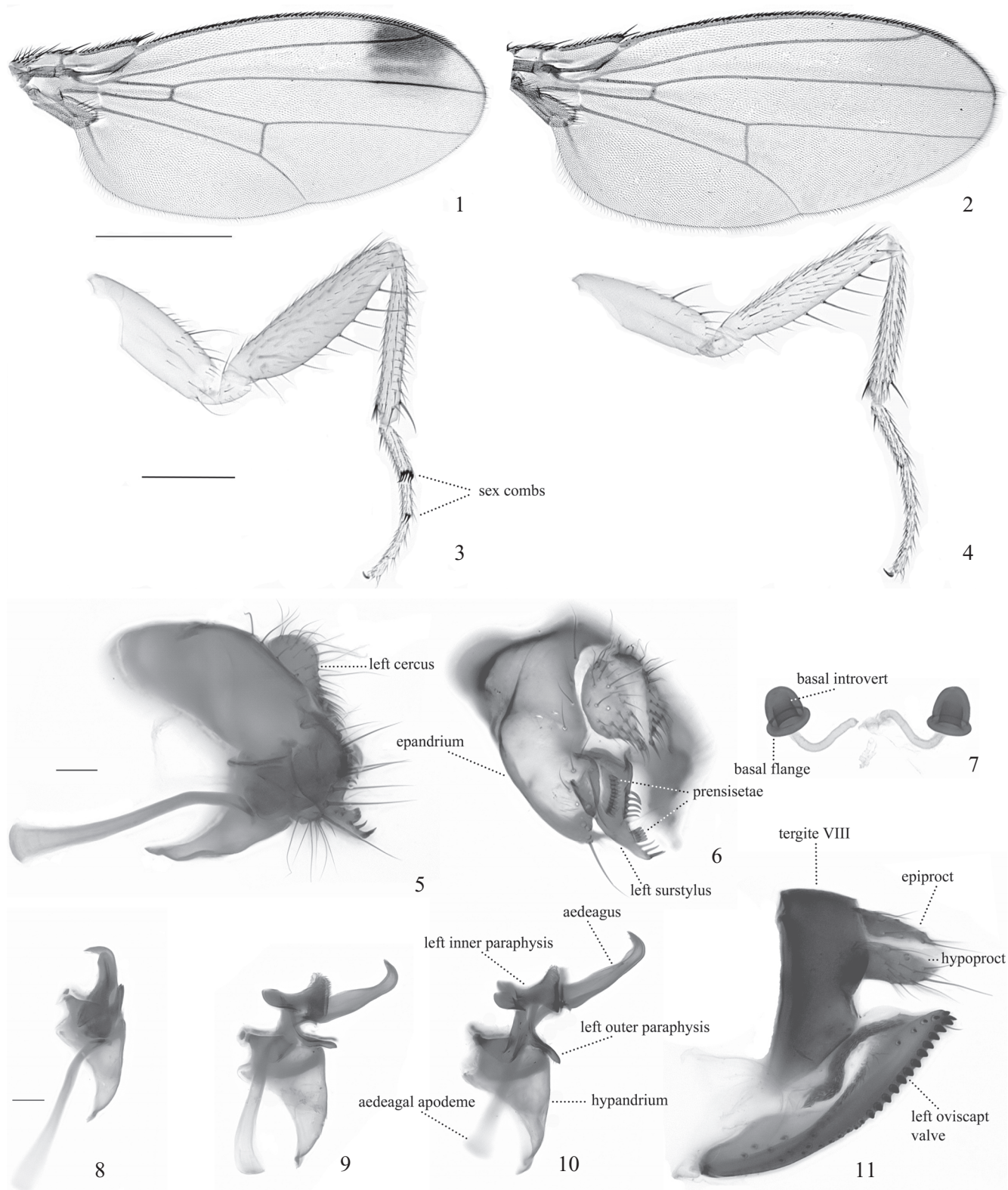
However, according to Calabria *et al.* (2012), it is worthwhile to note that *D. suzukii* had already been previously collected in the Neotropical Region, in Costa Rica (March 1997) and Ecuador (August 1998) (personal communication of P. O'Grady to J. Máca). Whether or not this fruit pest had already been established and spread naturally in the American continent since those first collections and/or new invasions from the Old World have occurred in California in 2008 remains to be clarified. A female from a Californian isofemale line established in 2009 was used to sequence for the first time the genome of this pest fly (Chiu *et al.* 2013). Although the preferred host fruits seem to be cherries and many species of berries (mainly blueberries, raspberries and strawberries), several other commercial and backyard soft skin fruits, including table and wine grapes, peaches, pears, and plums (Kanzawa 1939), have been reported to host the spotted-wing *Drosophila*. Among the host cultivated fruits, the greatest concern for the state of São Paulo would be strawberries because it has, together with the state of Espírito Santo, the highest average production (34 t/ha) of this crop per Brazilian State (Antunes & Duarte 2005). Strawberries have recently been reported by a producer as being seriously attacked by the spotted-wing *Drosophila* in the southernmost Brazilian state of Rio Grande do Sul (Santos 2014). A greater range of fruits belonging to additional plant families has also been reported to be attacked by *Drosophila suzukii* but only when they are overripe, decaying or previously damaged. According to an official report published by the Australia Govern-

ment (2013), they include apples, loquats, persimmons and tomatoes (all originally cited by Kanzawa 1939), bananas, figs, and kiwis. However, checking one of the original references (Price *et al.* (2009), mistakenly cited as Price & Nagle 2009) cited by the report, we were not able to confirm the data regarding bananas. It clearly seems that the Australian report misinterpreted the work of Price *et al.* (2009), which refer to species of *Drosophila* other than *D. suzukii*. Although cryptic with several species of its subgroup, the polyphagous *Drosophila suzukii* can be distinguished from its closest relatives by the number, size and relative position of sexual combs on the tarsomeres of male forelegs in addition to many other details of the male and female terminalia sclerites.

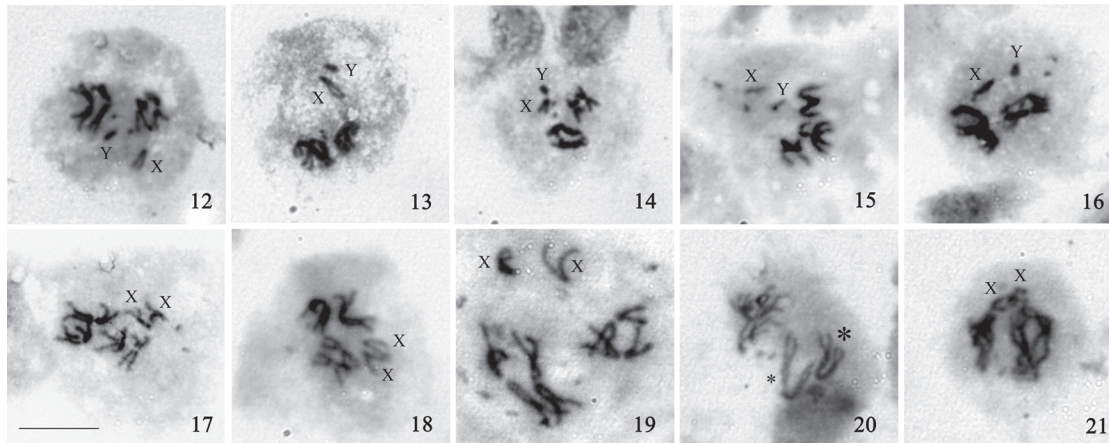
Worried about the potential invasion of southeastern Brazilian states by *Drosophila suzukii* mainly through national and international fruit trade we decided to buy samples of its most commonly reported host fruits to detect eventual infestations. A large fruit store near the main campus of the Universidade de São Paulo at the Cidade Universitária "Armando de Salles Oliveira", west São Paulo city, was chosen as a place to be sampled. On January 28th, 2014, the following three species of fruits were bought by one of us (LM) at a grocery store located at avenida Corifeu de Azevedo Marques 1000 (23°34'22.08"S, 46°43'27.32"W; 744 m altitude), Vila Indiana: cherries (505 g) imported from Chile, raspberries (125 g) imported from Mexico, and blueberries, from both São Joaquim (125 g), state of Santa Catarina, and Vacaria (100 g), state of Rio Grande do Sul. Once in the laboratory, the samples were coded M48, M49, M50 and M51 respectively and the fruits, varying in number according to fruit size and species, were placed in 250 ml vials containing wet sand and plugged with synthetic foam stoppers, as follows: 10 fruits per vial for cherries (seven vials), and raspberries (three vials), and 20 fruits per vial for blueberries from São Joaquim (four vials plus one vial with 10 fruits) and Vacaria (five vials plus one vial with 16 fruits). The vials were kept in an incubator with constant temperature (22 ± 1°C) and photoperiod (13 h: 11 h, L:D) until emergence of adult insects. When necessary, water was added to the vials with a plant sprayer to prevent desiccation. Emerged adults were maintained on banana-agar culture media for some days and then double-mounted by gluing them to cardboard tips on their right side (Bächli *et al.* 2004: 3, alternative b), sexed and identified to species. Only two species emerged from this first purchase: *Drosophila suzukii* (one female on 8–9.II.2014, one male on 13.II.2014) from blueberries produced in São Joaquim and *Zaprionus indianus* (one female on 11.II.2014, two males on 12 and 15–16.II.2014) from cherries imported from Chile. The *Drosophila suzukii* male + female were kept individually in vials with banana-agar culture medium plus a tiny ball of fresh baker's yeast (*Saccharomyces cerevisiae*) until 18.II.2014, when they were crossed early in the morning at uncontrolled room temperature. Precopula lasted 27 min and copulation, exactly 1 h. The resulting isofemale line (coded M50F1) is currently being maintained at 22 ± 1°C and was used to obtain information on life cycle,

terminalia, sex-combs and karyotypes. On 12.II.2014, upon returning to the same grocery store, we noticed and aspirated flies (coded M52) from overripe bananas aiming to verify which species were present as adults. The following nineteen flies were captured: *Drosophila cardini* Sturtevant, 1916 (three males, two females), *D. malerkotliana* Parshad & Paika, 1965 (one female), *D. melanogaster* (three males), *D. hydei* Sturtevant, 1921 (one male, one female), and *D. willistoni* Sturtevant, 1916 (two males, six females). The identifications of the species other than *Drosophila suzukii* were based on Val & Sene (1980), Vilela & Bächli (1990), Vilela *et al.* (2002) and Bächli *et al.* (2004). It is worthwhile to note that *D. suzukii* imagoes were not sampled and, therefore, the infestation of the blueberries previously purchased most probably occurred at the fruit farms and/or fruit cooperatives located in São Joaquim. If it had occurred in the grocery store it would be expected that the cherries (preferred host) and raspberries exposed in the same display and purchased on the same day would have been infested as well. A second purchase of blueberries was made 18 days later (on February 15th 2014) at the same grocery store. It included 375 g of blueberries from São Joaquim, state of Santa Catarina, Brazil (coded M54) and 250 g of blueberries imported from Curicó, Chile (coded M55). The methods were the same as used in the first purchase, resulting in 19 vials of Brazilian blueberries (18 with 20 fruits plus one with seven) and 11 vials of Chilean blueberries (10 with 20 fruits and one with eleven) kept at 22 ± 1°C. Emerged flies were removed daily, late in the afternoon, with an aspirator until no further emergence took place (ca. 4 weeks). Eleven flies, consisting of one *D. melanogaster* male and ten *D. suzukii* (three males and seven females) emerged, between 27.II and 06.III, from the M54 sample. At 22 ± 1°C, the life cycle of *D. suzukii* is just a little bit longer, taking ca. 13 days, than the 11 days recorded by David & Clavel (1966, 1967, *apud* Ashburner & Thompson 1978) for *D. melanogaster*. On 23.VII.2014 a total of 540 g of strawberries from Jarinu, state of São Paulo, was purchased at the same grocery store (coded M56) and the fruits were distributed in 8 vials as detailed for the other fruits and checked daily until 16.VIII.2014. No flies emerged from the sampled strawberries indicating they were not infested by the invasive species.

The identification of the spotted-wing *Drosophila* was based on the sexually dimorphic wing pattern, male foreleg sex combs, male and female terminalia (Bock & Wheeler 1972), and mitotic prometaphase and metaphase chromosomes obtained from larval brains of both sexes (Lemeunier *et al.* 1986; Deng *et al.* 2007), as illustrated and redescribed below. For preparing legs and terminalia slides we followed Wheeler & Kambyzellis (1966) as modified by Kaneshiro (1969) and Bächli *et al.* (2004); for mitotic chromosomes we followed Baimai (1977). Legs and wings were not stained and the latter were temporarily slide mounted in 70% ethanol plus 5% of glycerin. A set of photomicrographs taken at different depths of focus was digitally stacked to create an all-in-focus composite presented in Figs. 1–11 using the open-



Figs. 1–11. 1–4. Right wing (dorsal view) and front leg (anterior view) of male (1, 3) (specimens M50F1C9, M50F1C3, respectively) and female (2, 4) (M50F1C10, M50F1C5, respectively) of *Drosophila suzukii*, isofemale line M50F1, São Paulo, state of São Paulo, Brazil. Scale bars = 1 mm (1, 2) and 0.2 mm (3, 4). 5–11. Male (5, 6, 8–10) and female terminalia (7, 11) of *Drosophila suzukii*, isofemale line M50F1, São Paulo, state of São Paulo, Brazil. (5) Epandrium, left cercus and left surstylus, left lateral view (M50F1C7). (6) idem, oblique posterior view (specimen M50F1C1). (7) spermathecae, inner capsules, lateral view (M50F1C4). (8–10) aedeagus, aedeagal apodeme, paraphyses, hypandrium+gonopods, left lateral views, showing three subsequent steps of aedeagus extrusion (M50F1C1, M50F1C2, M50F1C3, respectively) (11) tergite VIII, epiproct, hypoproct and left oviscapt valve, left lateral view (M50F1C4). To the same scale: Figs. 5, 6, 7, 11 and 8–10, respectively. Scale bars = 0.1 mm.



Figs. 12–21. Metaphasic and prometaphasic neuroblast chromosomes of *Drosophila suzukii* ($2n = 8$), isofemale line M50F1, São Paulo, state of São Paulo, Brazil. 12–16, male (XY). 17–21, female (XX). Note the entirely heterochromatic rod-shaped Y-chromosome (13) and the large V-shaped pair, composed of one earlier (large asterisk) and one later (small asterisk) condensed homolog (20). All photomicrographs to the same scale. Scale bar = 5 μ m.

source software CombineZP as detailed by Vaz *et al.* (2014). Voucher specimens will be housed in the *Museu de Zoologia da Universidade de São Paulo*. The male wings (Fig. 1) conspicuously differ from the female wings (Fig. 2) in having a large subdistal black spot extending from Costal to R_{4+5} longitudinal veins and clearly seen with the naked eye. Males have two transverse and apically positioned rows of sex combs on each foreleg, a larger one on the first tarsomere and a smaller one on the second (Fig. 3), in agreement with Bock & Wheeler (1972:18, fig. 25), but in disagreement with Lemeunier *et al.* (1986) and Kopp & True (2002:281, fig. 1) (two oblique and basally positioned combs on the first tarsomere, none on the second one). Female forelegs are devoid of modified bristles (Fig. 4).

The external male terminalia (Figs. 5, 6) consist of a pair of cerci not fused to a mostly naked epandrium and a pair of surstyli bearing a unique pattern of two rows of prensisetae (Fig. 6), the larger one slightly concave and medially positioned and the smaller one straight and subapically positioned at the distal margin. The internal male terminalia consist of a long, sinuate aedeagal apodeme (Figs. 8–10), distally articulated with an apically upwards bent aedeagus, embraced by two pair of paraphyses, which in turn articulate with hypandrium+gonopods (Figs. 8–9). Female terminalia: tergite VIII bare, as wide as hypoproct length in lateral view; oviscapt valve apically pointed with strong marginal peg-like ovisensilla (Fig. 11). Inner spermathecal capsule short, bell-shaped, strongly sclerotized, telescoped, and bearing a conspicuous basal flange; basal introvert ca. 9/10 of capsule length; apical introvert absent (Fig. 7).

Drosophila suzukii has a diploid chromosome number of $2n = 8$ (Figs. 12–21). Female mitotic metaphase consists of two pairs of V-shaped, one pair of rod-shaped (X chromosomes) and one pair of dot-shaped chromosomes. The two V-shaped pairs clearly differ in size, the smaller pair being ca. two-thirds the length of the larger one, in agreement with Deng *et al.* (2007:204, figs. 56, 57), but in disagreement with

Lemeunier *et al.* (1986:183, fig. 14; of equal size). Additionally, one arm of the larger pair is mostly heterochromatic. Male mitotic metaphase differs from the female in having a small rod Y chromosome ca. half the length of the X chromosome. It condenses earlier than the remaining chromosomes, and in late metaphase it looks like a large dot, in agreement with Lemeunier *et al.* 1986, or a small rod, in agreement with Deng *et al.* (2007: 199, table 2). We suspect the disagreements cited above are due to misidentified strains and/or wild specimens.

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