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Short Communication

Is *Drosophila nasuta* Lamb (Diptera, Drosophilidae) currently reaching the status of a cosmopolitan species?

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ABSTRACT

In early March 2015, three males and two females of one unknown species of *Drosophila* were collected from a compost pile and some garbage cans in the west region of the city of São Paulo, state of São Paulo, Brazil. Morphologically it is easily identified by the presence of the following conspicuous features: a brownish dorsal stripe along pleura, an entirely iridescent silvery-whitish frons when seen directly from the front, and a row of cuneiform setae on anteroventral side of femur of foreleg; the former two traits being more evident in males. The species was easily reared in a modified banana-agar medium and two isofemale lines were established allowing to obtain mitotic cells showing a diploid chromosome number of $2n = 8$. Based both on morphological and chromosomal features, in addition to the geographical distribution, we concluded that the unknown flies belong to *Drosophila nasuta* Lamb, 1914, a tropical species of the *nasuta* subgroup of the *Drosophila immigrans* species group. Photomicrographs of male imagines, terminalia, mitotic and meiotic metaphase plates, as well as of female mitotic metaphase, are included.

On March 3rd 2015, while looking for specimens belonging to *Drosophila ananassae* Doleschall, 1858, one of us (BG) collected a total of 64 flies by net sweeping over garbage cans (coded M59) (23°33′52.66″ S, 46°43′51.25″ W, 781 m) and a compost pile (coded M60) (23°33′53.66″ S, 46°43′51.43″ W, 782 m) located at the *Instituto de Biociências* in the main campus of the *Universidade de São Paulo*, Cidade Universitária "Armando de Salles Oliveira", west São Paulo city, state of São Paulo, Brazil. The species identifications were based on Spassky (1957), Magalhães (1962), Val and Sene (1980), Vilela and Bächli (1990), Vilela et al. (2002) and Bächli et al. (2004). Upon identifying the anesthetized sampled flies, the first author (CRV) noticed an unknown male, which at first sight, appeared similar to *D. ananassae* Doleschall, 1858, but differed by bearing a conspicuously iridescent silvery-white frons when seen directly from the front (Fig. 1), one wide longitudinal brown stripe on the half dorsal area of the pleurae (Fig. 2), one row of cuneiform setae on anteroventral side of profemur (Fig. 3), in addition to a wing Costal index of ca. 3.1 (Fig. 4). Being aware that *D. ananassae* males are devoid of those traits, and have, according to Lin et al. (1973), a much smaller wing Costal index of ca. 1.5, we decided to examine the internal terminalia of the unknown living male. Following the method detailed by Spassky (1957), the first author gently pressed the tip of its abdomen with the aid of a pair of entomological pins. Upon analyzing the extruded aedeagus, CRV noticed it had, on the middle dorsal surface, a notable sea-anemone-like structure (Figs. 8–10) he had never observed on any of the Neotropical species of *Drosophila* known to him. As a roughly similar row of cuneiform setae on anteroventral side of profemur is also present in the cosmopolitan species *Drosophila immigrans* Sturtevant, 1921 (see Bächli et al., 2004), a search was conducted for the literature regarding the mostly South Asian *immigrans* species group.

According to Bächli (2015), the large *D. immigrans* species group, currently containing 105 species, is divided into five subgroups in addition to 13 species unassigned to any of them. Wilson et al. (1969) states that all but one (*Drosophila pallidifrons* Wheeler in Wilson et al., 1969) of the 12 species currently (Bächli, 2015) belonging to the *nasuta* subgroup, bear a conspicuous silvery-white frons. Therefore, we first suspected that the three unknown collected males could belong to one of 11 following candidate species: *Drosophila albomicans* (Duda, 1923), *Drosophila kepulauana* Wheeler in Wilson et al., 1969, *Drosophila kohkoa* Wheeler in Wilson et al., 1969, *D. neonasuta* Sajjan and Krishnamurthy, 1972, *Drosophila niveifrons* Okada and Carson, 1982, *Drosophila nixifrons* Tan, Hsu and Sheng, 1949, *Drosophila pulaua* Wheeler in Wilson et al., 1969, *Drosophila sulfurigaster* (Duda, 1923), *D. taiensis* Kumar and Gupta, 1988, *Drosophila tongpua* Lin and Tseng, 1973, and

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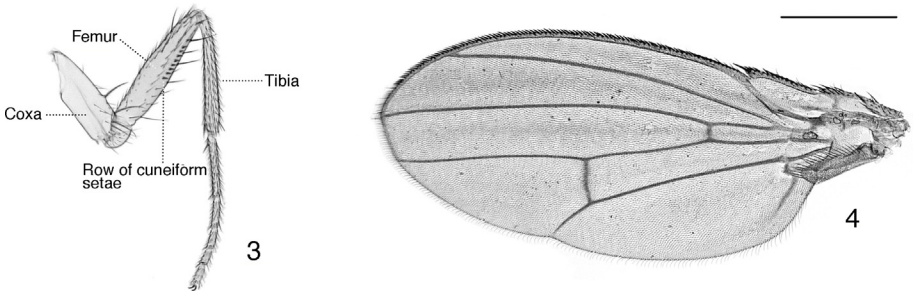
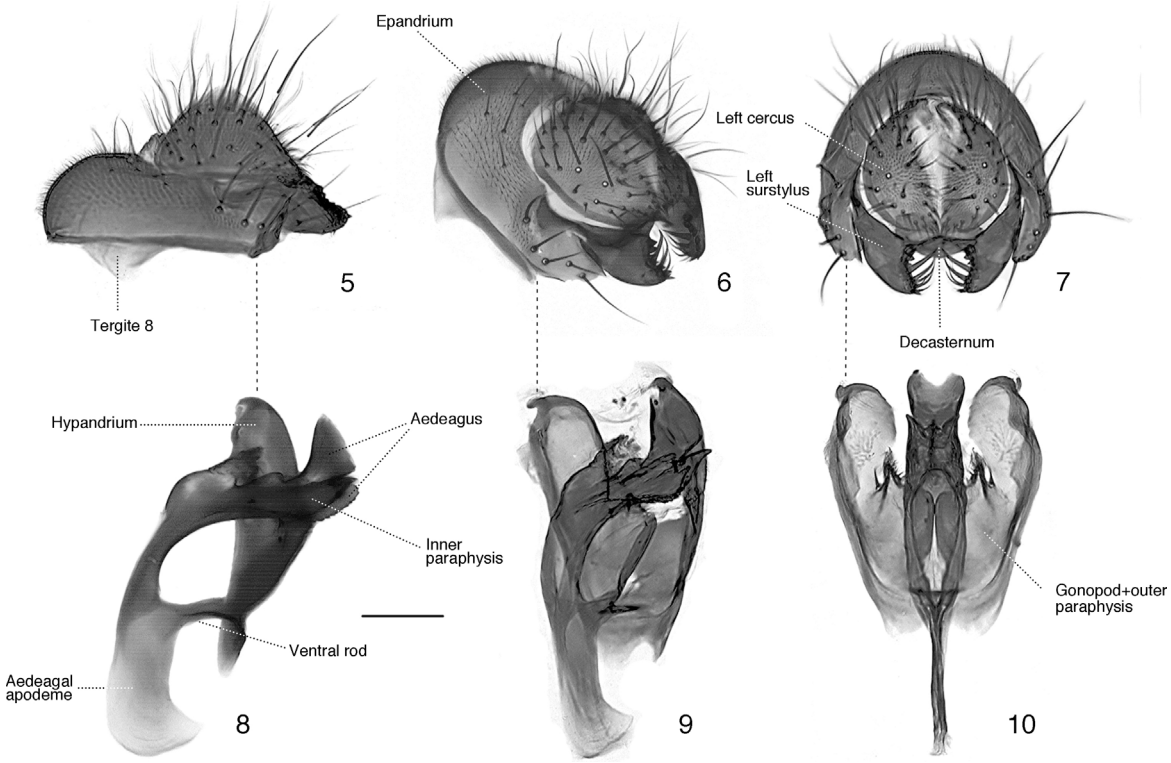
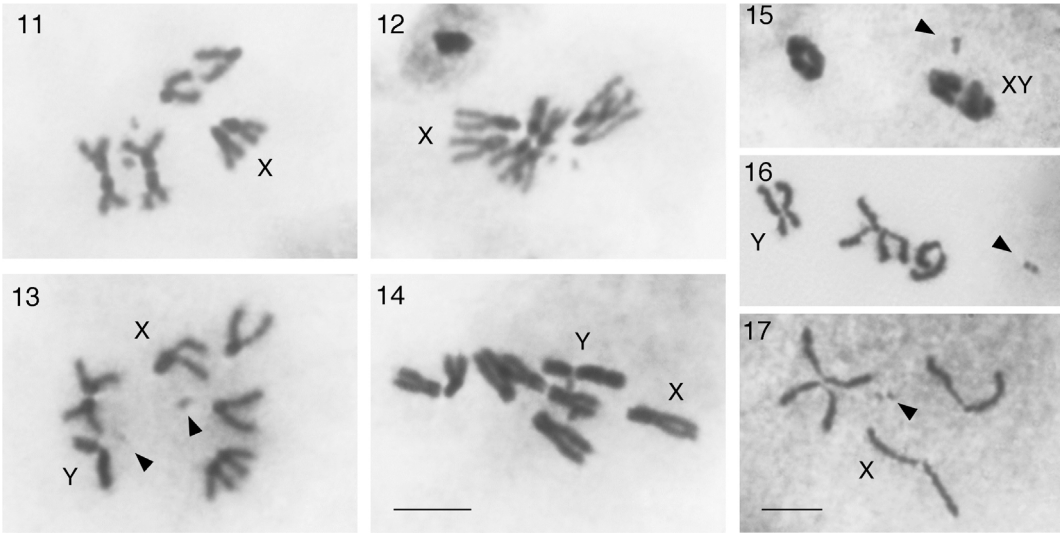
Figs. 1–2. External male morphology of *Drosophila nasuta* (isofemale line M59F1), Cidade Universitária "Armando de Salles Oliveira", São Paulo, state of São Paulo, Brazil. 1. Head, anterodorsal view, showing the conspicuous iridescent white silvery frons. 2. Imago left lateral view, showing the large brownish stripe on half dorsal area of pleura. Scale bars: 1 = 0.2 mm, 2 = 1 mm.



Figs. 3–4. Wild-caught male of *Drosophila nasuta* (specimen M60C1), Cidade Universitária "Armando de Salles Oliveira", São Paulo, state of São Paulo, Brazil. 3. Left foreleg, anterior view. 4. Left wing, dorsal view. Scale bar = 0.5 mm.



Figs. 5–10. Wild-caught male of *Drosophila nasuta* (specimen M60C1), Cidade Universitária "Armando de Salles Oliveira", São Paulo, state of São Paulo, Brazil. 5–7. External male terminalia: tergite 8+epandrium, cerci, surstyli, and decasternum. 5. Left lateral view. 6. Oblique posterior view. 7. Posterior view. 8–10. Internal male terminalia: aedeagus, aedeagal apodeme, ventral rod, paraphyses, hypandrium + gonopods. 8. Left lateral view. 9. Oblique posterior view. 10. Posterior view. Scale bar 0.1 mm.



Figs. 11–17. Chromosomes from larvae and young male imagoes of the invasive *Drosophila nasuta* (isofemale lines M59F1 and M60F1), Cidade Universitária "Armando de Salles Oliveira", São Paulo, state of São Paulo, Brazil. 11–14. C-band mitotic metaphase with $2n = 8$ in neuroblast cells of females (11, 12) and males (13, 14), note the rod-X chromosomes and the J-shaped heterochromatic Y chromosome. 15–17. Male meiotic configurations. 15. Metaphase I, note the XY heterologous association. 16–17. Metaphase II cells, the sex chromosomes are indicated. The dot chromosomes are indicated by arrowheads. All photomicrographs of the mitotic or the meiotic cells are to the same scale. Scale bars = 10 μm.

Drosophila nasuta Lamb, 1914. While most of the 11 candidate species are apparently restricted to relatively small areas, *D. albomicans* and *D. nasuta* are more widely distributed. The former species occurs in the Oriental, Palaearctic and Australian Regions (Asia [India to Japan], New Guinea and some pacific Islands) and the latter has been collected in the Afrotropical and Oriental Regions (central to southern Africa and south Asia) (Brake and Bächli, 2008). *D. nasuta* is an invasive and common species in most of the Hawaiian Islands and has also been recorded in western North America (Hardy, 1965). It should be stressed that the collected specimens and/or references to the latter record were omitted by Hardy (op. cit.) and we were unable to recover it from the literature.

Aiming to analyze the foreleg, wing and terminalia at a large magnification, one of the collected males was dissected. For preparing leg and terminalia microscope slides we followed Wheeler and Kambysellis (1966) as modified by Kaneshiro (1969) and Bächli et al. (2004). Leg and wing were not stained, but the former was treated with KOH 10% and the latter was temporarily slide mounted in 70% ethanol plus 5% of glycerin. A set of photomicrographs taken at different depths of focus with an Olympus U-MAD-3 camera attached to an Olympus BX60 microscope were digitally stacked to create an all-in-focus composite using the open-source software *CombineZP* (Hadley, 2010) as shown in Figs. 3–10. The methods used to combine the images were: stack, weighted average and finally, a combination of both (by combining them using weighted average) as previously tested by Vaz (personal communication) and applied to the images published in Vaz et al. (2014). The same software was also used to stack four photomicrographs of the head of one F_1 male taken in frontal view (Fig. 1) on a stereomicroscope Zeiss (STEMI DV4) equipped with a 10 Megapixel Opton digital camera and the images were acquired with the ISCapture 2.5 imaging software (Scienon). However, in the latter case, before stacking the frames, an alignment of the original four photomicrographs, two by two, had to be applied using the same software. Voucher specimens will be housed in the *Museu de Zoologia da Universidade de São Paulo*.

Based on the photomicrographs the male terminalia of some of the sibling species belonging to the *D. nasuta* subgroup presented by Wilson et al. (1969) one can deduce that they are virtually identical. However, as we believe that subtle differences might exist among them, we are describing below, with more details, the male terminalia of one of males we have collected. The external male terminalia of the wild-caught specimen M60C1 (Figs. 5–7), consist of a pair of dorsally microthricose cerci not fused to a mostly microthricose epandrium; a pair of surstyli bearing a curved row ca. seven short cone-shaped, sharp-tipped and spaced prensisetae and some inner setae gradually decreasing in size from dorsalmost to ventralmost, devoid of outer setae, and connected to an upper-positioned decasternum (Figs. 6, 7). The internal male terminalia (Figs. 8–10) consist of a curved, apically upwards bent aedeagus, dorsally bearing a conspicuous and mostly membranous sea-anemone-like structure on the middle dorsal region and embraced by two pairs of paraphyses; inner one anteriorly fused to anterior half of aedeagus, distally free, sharply pointed and marginally serrated ventrally; outer one reduced, distally-positioned, fused with hypandrium + gonopods; ventral rod dorsoventrally flatten, fused to subanterior margin of hypandrium. Aedeagal apodeme wide, anteriorly turned ventralwards (Fig. 8), laterally flattened (Fig. 10). Gonopods mostly fused to hypandrium arms and bearing, in the median area, a small seta adjacent to an inner positioned finger-like projection covered with many setulae (Fig. 10). The sclerites comprising the internal and external male terminalia are individually identified in Figs. 5–10 following Bächli et al. (2004). Additionally, we propose that the inner paraphyses of the wild-caught male are only proximally fused to aedeagus and not completely fused as it may have occurred in most of the remaining species belonging to the subgenus *Drosophila*.

As reported by Wilson et al. (1969), the male basic chromosome configuration of the *D. immigrans* group consists of a X rod and Y rod, a pair of dots (chromosome 4), a pair of V's (chromosome 2), and the double length rod (chromosome 3). They propose that the double length rod is composed of two of the five long rods of the primitive *Drosophila* karyotype, which consists of five pairs of rods plus a pair of dots. It arose by pericentric inversion of a two-element V or by a total translocation of a rod to the end of another. All species of the *D. nasuta* subgroup show a karyotype of $2n = 8$, with the basic configuration of 2R + 1V + 1D (or 1 short rod), with the exception of *D. albomicans*, where a fusion between the sex chromosome and chromosome 3 resulted in a diploid number $2n = 6$ (Wakahama et al., 1983). As *D. nasuta* is morphologically apparently

Table 1
Species richness, absolute and relative abundance of *Drosophila* spp. collected at the main campus of the Universidade de São Paulo, Cidade Universitária "Armando de Salles Oliveira", São Paulo, SP, Brazil, March 3rd 2015.

Species group	Species	M59		M60		Total	Relative
		♂	♀	♂	♀		Abundance %
cardini	*D. cardinoides* Dobzhansky and Pavan, 1943	0	2	1	1	4	6.25
immigrans	*D. nasuta* Lamb, 1914	1	1	2	1	5	7.81
repleta	*D. hydei* Sturtevant, 1921	2	2	0	0	4	6.25
	D. mercatorum Patterson and Wheeler, 1942	7	2	0	0	9	14.06
melanogaster	*D. ananassae* Doleschall, 1858	3	[a]	0	0	3	4.69
	D. kikkawai Burla, 1954	0	3	1	1	5	7.81
	D. malerkotliana Parshad and Paika, 1964	10	[a]	0	0	10	15.63
	D. ananassae + *D. malerkotliana* females	0	5	0	0	5	7.81
	D. melanogaster Meigen, 1830	3	1	0	0	4	6.25
	D. simulans Sturtevant, 1919	3	2	0	0	5	7.81
saltans	*D. sturtevanti* Duda, 1927	3	3	0	0	6	9.38
willistoni	*D. fumipennis* Duda, 1925	0	0	1	0	1	1.56
	D. paulistorum Dobzhansky and Pavan in Burla et al., 1949	1	0	0	0	1	1.56
	D. willistoni Sturtevant, 1916	1	0	1	0	2	3.13
Total		34	21	6	3	64	100

[a] Females unidentified to species.

indistinguishable from *D. albomicans*, chromosome configuration of the species in question must be crucial to identify the invasive species. Fortunately, we were successfully able to breed the invasive species in the laboratory as detailed below.

The two unknown collected females were kept individually with an unknown collected male in vials containing usual banana-agar culture medium, placed on an incubator with constant temperature (22 ± 1 °C) and photoperiod (13 h:11 h, L:D). It was soon realized that the invasive species was able to breed easily in the laboratory, as two isofemale lines, coded M59F1 and M60F1, were established. The F_1 progeny obtained numbers 92 imagines (44 males and 48 females) from the former line and 63 (26 males and 37 females) from the latter. It is worthwhile to note that, when observed from the front view, the males that are resting over the surface of the culture medium displayed the conspicuous silvery-white iridescent frons, clearly seen with the naked eye. Most of the emerged imagines of the F_1 generation of both lines were preserved either as double-mounted or kept in a solution of 70% ethanol and glycerin 5%. We realized that the two collected females and individuals from the next generations (F_1, F_2), breed well in banana-agar or in cornmeal-yeast-agar medium (Tosi et al., 2007). In banana-agar medium, the wild-caught females or their offspring needed to be transferred into new vials every two days, in order to not overcrowd the medium with larvae. Whenever too many larvae are on the culture medium it soon turns completely liquefied. Whenever it happens, the larvae should be transferred in low numbers into new culture vials and a large amount of absorbent paper stripes should be inserted on medium. As the cornmeal-yeast-agar medium does not become liquid, absorbent paper stripes should be inserted on the medium just to allowing additional surface for the larvae to pupariate.

Analyses of chromosomes involving larvae and adults of both isofemale lines were then performed. Meiotic metaphase from testes of newly emerged males, and mitotic metaphase plates from third instar larvae neuroblast ganglia of both sexes were obtained by applying the air-drying method of Imai et al. (1988), without colchicine treatment. This method produces C-banded extended metaphase chromosomes. All cytological preparations were made from single individuals and data was registered from those cells in which the full chromosome complement was clearly visible. Good-quality photomicrographs of mitotic and meiotic were processed in Adobe Photoshop graphics editor or GIMP 2.8.14 (GNU image Manipulation Program) to create the figure plates.

Female and male mitotic cells showed a chromosome complement of $2n = 8$ and consists of a pair of large V-shaped metacentrics (chromosome 2), two pairs of rod-shaped acrocentric chromosomes (one of these is the X chromosome and the other is the chromosome 3) and a pair of small dots (chromosome 4); the Y is a J-shaped submetacentric chromosome (Figs. 11–14). Male meiotic chromosomes show three bivalents representing the autosomes 2, 3 and the small dot, and the heterologous XY chromosome association (Figs. 15–17). The observed chromosome configuration corresponds to the karyotype of *D. nasuta* as reported by Wakahama and Kitagawa (1972) in cultures collected from Mahé (Seychelles Islands), the type locality according to Lamb (1914). Nirmala and Krishnamurthy (1972) reported a similar karyotype for *D. nasuta* from cultures from Soundatti (Karnataka state) in southern India. Therefore, we concluded that the species currently invading the Neotropical Region is *D. nasuta*.

The high fertility, short life cycle (ca. 12 days at 22 °C) and occurrence of *D. nasuta* in a highly urbanized area located in the transition zone between tropical and temperate climates, allows us to predict that this species has a great potential to change its status from tendency to spread (category 1) to virtually become a cosmopolitan (category 2) species sensu Patterson and Stone (1952) and Carson (1965). According to the former authors category 2 includes *D. ananassae*, *Drosophila busckii* Coquillett, 1901, *Drosophila funebris* (Fabricius, 1787), *Drosophila hydei* Sturtevant, 1921, *D. immigrans*, *Drosophila melanogaster* Meigen, 1830, *Drosophila repleta* Wollaston, 1858, and *Drosophila simulans* Sturtevant, 1919. It is worthwhile to note that, among the 64 collected flies (Table 1), 37 (58%) are represented by invasive species belonging to the *D. immigrans* and *D. melanogaster* species groups.

Conflicts of interest

The authors declare no conflicts of interest.

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