

Hence, *D. nasuta* subgroup offers an excellent model system to understand the evolutionary divergence in reproductive behavior in closely related species.

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**The importance of identification of the *willistoni* subgroup of *Drosophila* at the species level: the first evidence of *D. equinoxialis* in the Northeast region of Brazil.**

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## Introduction

A problem inherent in the ecological study of drosophilids is that parts of these organisms are composed of various sibling species, and this renders the identification of the specimens more difficult. Mayr (1963) defines sibling species as "natural populations which, although morphologically identical or very similar, are isolated reproductively". This is one of the most serious restrictions to the morphological concept of species. While the sibling species represent a considerable problem to many taxonomists, particularly those who hold that species should be

identified in preserved material, the correct identification of these species in evolutionary and ecological studies is of the greatest importance. Dobzhansky (1970) reinforces this idea emphasizing that the species are natural phenomena that exist independently of our ability to identify them.

Within the *Drosophila* genus, *Sophophora* sub-genus, the *willistoni* subgroup is formed of six sibling species of which four are widely distributed in the Neotropical region and overlap in extensive areas. These are: *Drosophila willistoni*, *D. paulistorum*, *D. tropicalis*, and *D. equinoxialis*. The other two, *D. insularis* and *D. pavlovskiana*, are endemic in the Lesser Antilles and Guiana, respectively. *Drosophila willistoni* is the most widely distributed geographically and is found from the South of Florida/USA and the center-north of Mexico down to the north of Argentina. Second in geographical coverage is the species *D. paulistorum*, which occurs from the Tikal locality of the north of Guatemala to Porto Alegre in the State of Rio Grande do Sul, Brazil. *Drosophila equinoxialis* is distributed from central Mexico through Central America, the Greater Antilles, and the northern half of continental South America. Finally, *D. tropicalis* extends from its farthest northern extension in Tikal, Guatemala as far south as the city of Santa Cruz de la Sierra in Bolivia. The geographic distribution of that *willistoni* subgroup is revised in Spassky *et al.* (1971), Dobzhansky and Powell (1975), Ehrman and Powell (1982), and Santos and Valente (1990).

In Brazil, four species of the *willistoni* subgroup occur in sympatric association - *D. willistoni* and *D. paulistorum* are the most widely distributed species. *Drosophila willistoni* was collected in the Northern regions (the States of Amazonas and Pará), Northeast (Bahia), Center-west (the Federal District and Mato Grosso State), Southeast (Minas Gerais, Rio de Janeiro and São Paulo), and the South (Paraná, Santa Catarina and Rio Grande do Sul). *Drosophila paulistorum* has been registered in the Northern regions (Amazonas and Pará), the Northeast (Bahia, Ceará, Maranhão and Pernambuco), the Center-west (Goiás and Mato Grosso), the Southeast (Minas Gerais, Rio de Janeiro and São Paulo), and the South (Santa Catarina and Rio Grande do Sul). *Drosophila tropicalis* and *D. equinoxialis* were registered only in the Northern regions (both are found in Amazonas and Pará, but only *D. tropicalis* was registered in Acre) and the Center-west (Goiás and Mato Grosso). A revision of the occurrence of these species in Brazilian territory may be found in Gottschalk *et al.* (2008).

In many of the ecological studies carried out in the Neotropical regions, the sibling species of the *willistoni* subgroup were found to be approximately 80% of the collected individuals (Martins, 2001; De Toni *et al.*, 2007). Notwithstanding the growing number of published studies on the ecology of the *Drosophila* Neotropical species, the individuals of *willistoni* subgroup are rarely identified at the species level. As a result of this omission, relatively few studies of the distribution and ecology of the species of the *willistoni* subgroup of *Drosophila* exist. In view of this, Garcia *et al.* (2006) presented a rapid and efficient method for the identification of the *willistoni* subgroup based on the electrophoretic migration of acid phosphatase-1 (*AcpH-1*), which reveals alleles for each one of the species and permits correct diagnosis.

In this study, we realized collections of drosophilids in the State of Pernambuco (in the Brazilian northeast) to enable us to evaluate the presence of the species of the *willistoni* subgroup in different phytophysognomies.

## Materials and Methods

During 2008 and 2010 drosophilids were collected in different phytophysognomies in the State of Pernambuco, Brazil. Within the Atlantic Forest regions, four localities in the municipalities of Tamandaré, Recife, Pombos and Bezerros were sampled and three other municipalities were investigated in the Caatinga: Buíque, Serra Talhada, and Triunfo. Finally, the collections were

completed in three mangroves areas in Tamandaré, Recife, and Cabo de Santo Agostinho. All insects were collected with the model traps proposed by Tidon and Sene (1988) using banana bait.

All the flies we collected were identified at the species level. In the case of the representatives of the *willistoni* subgroup, each sample of 100 individuals was identified at the species level through the method of electrophoresis with the *AcpH-1* enzyme (Garcia *et al.*, 2006) and by the analysis of the male hypandrium (Malogolowkin, 1952; Spassky, 1957). For this latter analysis the final portion of the abdomens of the individuals of the *willistoni* subgroup was prepared with potassium hydroxide (KOH) 10% stained with fuchsine acid and dissected in glycerol in accordance with the methodology proposed by Wheeler and Kambysellis (1966). The hypandrium were photographed in a NIKON 80i microscope coupled to a digital video microscope model DS-Fil-UC.

Table 1. Drosophilids sampling areas in the State of Pernambuco with the numbers obtained at each sampling point of both the drosophilids and the *willistoni* subgroup. The collections were made between the years of 2008 and 2010. D.w = *D. willistoni*, D.p = *D. paulistorum* and D.e = *D. equinoxialis*.

Environment	Location	Coordinates	N	subgroup <i>willistoni</i> of <i>Drosophila</i>		
				D.w	D.p	D.e
Atlantic Forest	Saltinho Biological Reserve, Tamandaré municipality	08°44'S; 35°11'W	4,089	1,876	36	5
	Dois Irmãos Park, Municipality of Recife	08°00'S; 34°57'W	5,460	936	1,814	0
	Ronda Forest, Municipality of Pombos	08°08'S; 35°23' W	2,948	1,036	1,024	0
	Serra Negra District, Municipality of Bezerros	08°13'S; 35°46'W	4,546	117	55	0
Caatinga	Municipality of Buíque	08°36'S; 37°14'W	4,243	403	131	0
	Municipality of Serra Talhada	07°57'S; 38°17'W	7,155	2,555	593	0
	Municipality of Triunfo	07°49'S; 38°06'W	2,030	790	297	0
Mangrove	Formoso River, Municipality of Tamandaré	08°41'S; 35°06'W	10,985	22	9	0
	Pina Basin, Municipality of Recife	08°05'S; 34°54'W	2,507	5	3	0
	Suape Beach, Municipality of Cabo de Santo Agostinho	08°17'S; 25°02'W	23,216	103	10	3
Total			67,179	7,843	3,972	8

## Results and Discussion

A total of 67,179 drosophilids were collected from the different sampling points. Table 1 details the locations in the State of Pernambuco where the collections were made and indicates the total number of individuals obtained in each location and the absolute abundance of the species of the *willistoni* subgroup. Three species of the *willistoni* subgroup were observed: *D. willistoni*, *D. paulistorum*, and *D. equinoxialis*. Through this extensive collection of drosophilids, with the identification of a sample of the *willistoni* subgroup at the species level, we obtained the first registration of the presence of *D. equinoxialis* in the Northeast region of Brazil. Although few individuals were found – only 0.01% of the collected drosophilids – this species was obtained both from the Atlantic Forest (Saltinho Biological Reserve) areas and from the mangrove (Suape). Of the three species of the *willistoni* subgroup, *D. willistoni* was the most abundant and represented 11.67%

of the sampled individuals. Our finding also represents the first register of this species in the State of Pernambuco. *Drosophila paulistorum* was the second most abundant species of the *willistoni* subgroup with 5.91% of the collected drosophilids.

Amongst the environments studied, the *willistoni* subgroup species were most abundant in the Atlantic Forest (40.48%), followed by the Caatinga (35.52%) and by the Mangroves (0.42%). More complete data on this sampling is being prepared for publication.

In the last few years a number of studies focussed on the ecological aspects of drosophilids in the Brazilian territory have been published (De Toni *et al.*, 2001; Silva *et al.*, 2005; Mateus *et al.*, 2006; Tidon, 2006; De Toni *et al.*, 2007; Gottschalk *et al.*, 2007; Mata *et al.*, 2008). These do not, however, identify the individuals of the *willistoni* subgroup at the species level. At a time when the subgroup is found in considerable quantities in the Neotropical region, the lack of information on the presence of each one of the species in different environments makes it more difficult to understand the dynamic of the population of these insects in the nature.

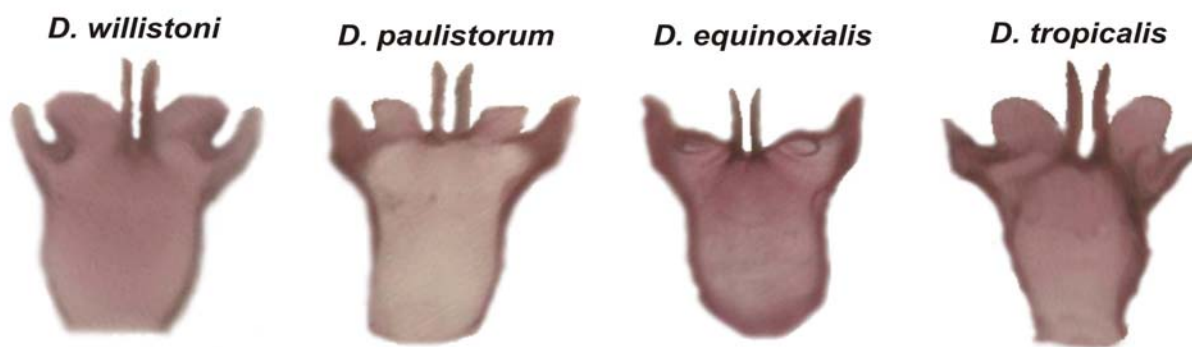


Figure 1. Hypandrium of the species of the *willistoni* subgroup of *Drosophila*: *D. willistoni*, *D. paulistorum*, and *D. equinoxialis* from the Saltinho Biological Reserve in the municipality of Tamandaré, Pernambuco, Brazil, and *D. tropicalis* from the Stock Center, collected in Jalisco, Mexico. Amplification = 1000×

In order to assist researchers seeking to identify representatives of the *willistoni* subgroup, we present in Figure 1 images of the hypandrium of the four species of this subgroup found in overlap in the Neotropical region: *D. willistoni*, *D. paulistorum*, *D. tropicalis*, and *D. equinoxialis*. Although images and photos can be found in the literature in some cases, they usually date from the 1950's and are sometimes difficult to understand for diagnosis purposes.

This present work emphasizes the importance of identifying the species of the *willistoni* subgroup in ecological studies. Only by the identification of these individuals will it be possible to accumulate knowledge of the presence of these insects in different natural environments, as well as their feeding habits and survival strategies.

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### **Absence of recombination in males of *Drosophila mediopunctata*.**

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### **Abstract**

A century ago Morgan reported for the first time that *Drosophila* males do not show recombination. Since then, geneticists have taken advantage of this property to carry out genetic analysis. However, males of a few species of the genus show some recombination. Here, we report the results of an experiment to assess the recombination frequency in *D. mediopunctata* males. We did not find a single recombinant among 1522 flies from the backcross of double heterozygote males for two distant second chromosome mutations. Thus, recombination in males is either absent or very rare (at most 0.2%, upper 95% CI).

### **Introduction**

For the last 25 years we have been working with *Drosophila mediopunctata* to transform this species into a model organism for evolutionary and population genetics (Klaczko, 2006). We have repeatedly obtained scattered data showing that, as in many other *Drosophila* species, there is no recombination in males, as first reported for *D. melanogaster* by Morgan (1912). Assuming this premise, we have carried out experiments of genetic (chromosomal) analysis (e.g., Carvalho and Klaczko, 1993; Hatadani *et al.*, 2004). There are, however, a few species of *Drosophila* showing