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Bromeliad-inhabiting mosquitoes in an urban botanical garden of dengue endemic Rio de Janeiro. Are bromeliads productive habitats for the invasive vectors *Aedes aegypti* and *Aedes albopictus*?

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Abstract

Immatures of both *Aedes aegypti* and *Aedes albopictus* have been found in water-holding bromeliad axils in Brazil. Removal of these plants or their treatment with insecticides in public and private gardens have been undertaken during dengue outbreaks in Brazil despite uncertainty as to their importance as productive habitats for dengue vectors. From March 2005-February 2006, we sampled 120 randomly selected bromeliads belonging to 10 species in a public garden less than 200 m from houses in a dengue-endemic neighborhood in Rio de Janeiro. A total of 2,816 mosquito larvae and pupae was collected, with an average of 5.87 immatures per plant per collection. *Culex (Microculex) pleuristriatus* and *Culex* spp of the Ocellatus Group were the most abundant culicid species, found in all species of bromeliads; next in relative abundance were species of the genus *Wyeomyia*. Only two individuals of *Ae. aegypti* (0.07%) and five of *Ae. albopictus* (0.18%) were collected from bromeliads. By contrast, immatures of *Ae. aegypti* were found in manmade containers in nearly 5% of nearby houses. These results demonstrate that bromeliads are not important producers of *Ae. aegypti* and *Ae. albopictus* and, hence, should not be a focus for dengue control. However, the results of this study of only one year in a single area may not represent outcomes in other urban localities where bromeliads, *Ae. aegypti* and dengue coincide in more disturbed habitats.

Keywords

Aedes aegypti; *Aedes albopictus*; dengue; bromeliad mosquitoes; Bromeliaceae

Although domestic *Aedes aegypti* has a clear preference for man-made larval habitats, its immatures may be found in natural containers such as water-containing axils of bromeliads growing in both modified and semi-modified environments of the Americas (Peryassú 1908,

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Forattini & Marques 2000, Marques et al. 2001, Cunha et al. 2002, O'Meara et al. 2003, Fouque et al. 2004, Varejão et al. 2005, Maciel-de-Freitas et al. 2007). Bromeliads are popular ornamental plants and often used in public and private gardens. The abundance of bromeliads where dengue is endemic has been construed to represent a threat to dengue vector control (Natal et al. 1997, Forattini et al. 1998, Forattini & Marques 2000). Even though *Ae. aegypti* immatures have been found in such plants only occasionally (Cunha et al. 2002, O'Meara et al. 2003, Maciel-de-Freitas et al. 2007, David et al. 2009) and few studies have evaluated the productivity of ornamental bromeliads for *Ae. aegypti* (Frank et al. 1988) compared to other larval habitats, bromeliads have been arbitrarily insecticide-treated, removed or incinerated during some dengue epidemics, such as in outbreaks in Rio de Janeiro (RJ) in 2001–2002 and 2008 (Lourenço-de-Oliveira 2008, JR Duarte, unpublished observations).

Herein we describe results of field surveys of the identity and frequency of bromeliad-inhabiting mosquitoes in bromeliads in a public garden in a dengue-endemic area of RJ and demonstrate that native species of *Culex* and *Wyeomyia* mosquitoes were the most abundant culicids and that the invasive species *Ae. aegypti* and *Aedes albopictus* were recovered rarely from these plants, which should not be of concern for dengue control.

MATERIALS AND METHODS

One hundred and twenty bromeliads belonging to 10 species cultivated in the ground at a public garden [Jardim Botânico do Rio de Janeiro (JBRJ); 22°58′04″S 43°13′44″W] were randomly selected, labeled and numbered. The selected bromeliads were growing in the open, less than 200 m from houses in the dengue-endemic neighborhood of Gávea. One of the borders of JBRJ is an ecotone between the garden environment and secondary rain forest, but the sampled bromeliads were located at the other edge, in a modified environment where the garden is contiguous to houses. The bromeliads were exposed to natural rainfall and were not treated with insecticide during the sampling period nor in the previous 10 months. Labeled bromeliads were divided into three groups, each composed of an equal number of specimens belonging to a single species. The number of specimens per group (Table I) varied according to the population density of the bromeliad species at JBRJ. From March 2005-February 2006, bromeliads of all groups were sampled every three months. For instance, bromeliads from group one were sampled in March, June, September and December 2005, while sampling from those of group two began in April 2005, and so on. At each sampling, all the water impounded by all the leaf axils of a labeled bromeliad was aspirated according to Lozovei and Silva (1999), measured (mL) and taken to the laboratory for mosquito identifications using keys for morphological characters of immature stages or emerged adults provided by Lane and Whitman (1951), Corrêa and Ramalho (1956), Coutinho and Forattini (1962), Forattini and Toda (1966), Cotrim and Galati (1977), Consoli and Lourenço-de-Oliveira (1994) and Motta and Lourenço-de-Oliveira (2005). The number of axils holding water and whether the sampled plant was in the sun or partial or total shade was recorded at every sampling. Daily rainfall was obtained from the meteorological station at JBRJ.

For statistical analyses we used the programs SPSS, Minitab and Statistica 6.0. For purposes of correlation analyses, samples from the same plant separated by three months were considered as independent. Spearman's correlations (significance level: $p < 0.05$) were used to evaluate relationships between total mosquito abundance and (i) the number of bromeliad leaf axils holding water and (ii) the total amount of water (mL). The same test was applied to calculate the correlations between abundances of individual mosquito species and (i) the amount of water held in the bromeliad's leaf axils and (ii) rainfall. Nonparametric Kruskal-Wallis tests ($p < 0.05$) were used to analyze variations in mosquito abundance in each bromeliad species, in relation to their location in open-sunlit, shaded or partially shaded sites and amount of water held in each sunlit bromeliad. *Ae. aegypti* and *Ae. albopictus* were not considered in statistical analyses because of their rarity in collections. A Friedman's test was used to analyze effects of bromeliad location (sun light vs. shade) in which bromeliad species was considered a block effect.

RESULTS AND DISCUSSION

A total of 2,816 immature mosquitoes was collected. Species of *Culex* (77.2%) and *Wyeomyia* (21.4%) were the most abundant bromeliad mosquitoes (Table II). *Culex* (*Microculex*) *pleuristriatus* was by far the most common species (42.5%), followed by species of *Culex* belonging to the Ocellatus Group (34.7%).

Only two larvae of *Ae. aegypti* (0.07% of total mosquitoes) and five of *Ae. albopictus* (0.18%) were collected in more than one year of sampling of the bromeliads. This result suggests that larvae of these species are inferior competitors with native bromeliad-inhabiting species or that adult females prefer not to oviposit in bromeliads. Manmade containers (e.g., drains, plant vases, plastic basins, tires, water tanks) in nearby houses were consistently positive for both species of *Aedes*, particularly *Ae. aegypti*. The house infestation index for *Ae. aegypti* nearby was found to be as high as 5% in a survey conducted simultaneous to our sampling of bromeliads in 2006 (Secretaria Municipal de Saúde, unpublished observations). During the 2001 and 2008 dengue epidemics in RJ, it was hypothesized that bromeliads in public gardens and even native bromeliads growing in the ecotone between the natural and modified environments would be productive and persistent *Ae. aegypti* habitats from which emerged adults would invade houses and recolonize manmade larval habitats. Our findings suggest just that this concern is unfounded, as only two larvae and no pupae of *Ae. aegypti* were found in a single bromeliad compared to high infestations in surrounding houses. Varejão et al. (2005) did not detect any relationship between *Ae. aegypti* occurrence in native bromeliads on rock slopes and the *Stegomyia* indices in adjacent areas in the state of Espírito Santo, Brazil. Marques et al. (2001) found more *Ae. albopictus* larvae in exotic urban bromeliads than in native or periurban ones in the state of São Paulo and considered bromeliads to be of secondary importance as larval habitats for *Ae. albopictus* compared to artificial containers.

The number of collected immatures and species composition varied according to bromeliad species (Table I). Around 31% of all mosquitoes were found in *Aechmea blanchetiana*, *Neoregelia compacta*, *Neoregelia cruenta* and *Neoregelia johannis*, which showed the greatest diversity of mosquito species composition in their axils. Species of *Wyeomyia* were the most frequent mosquitoes in *N. compacta* (68.8%) and *Billbergia nana* (50%).

With mosquito species designated as blocks, a Friedman's test indicated a marginally significant effect of bromeliad location on mosquito abundance ($\chi^2 = 5.64$, DF = 2, $p = 0.06$) (Fig. 1). However, Lopez (1997) (*apud* Lopez et al. 1998) have suggested that shade favors faunal abundance, species composition and richness in the leaf axils of bromeliads.

The number of immature mosquitoes collected per sampled bromeliad was positively correlated with the number of axils per bromeliad (Spearman's $\rho = 0.234$, $p < 0.0001$, $n = 480$) (Fig. 2) as well as with the amount of water held by the plant ($\rho = 0.529$, $p < 0.0001$, $n = 468$) (Fig. 3). When considering mosquito species separately, positive correlations were found between the amount of water in the plant and the number immatures of: *Wyeomyia incaudata* ($\rho = 0.495$, $p < 0.0001$, $n = 480$), *Cx. pleuristriatus* ($\rho = 0.438$, $p < 0.0001$, $n = 480$), *Wyeomyia pilicauda* ($\rho = 0.280$, $p < 0.0001$, $n = 480$), *Culex ocellatus* ($\rho = 0.238$, $p < 0.0001$, $n = 480$), *Culex nigrimacula* ($\rho = 0.193$, $p < 0.0001$, $n = 480$), *Wyeomyia theobaldi* ($\rho = 0.149$, $p = 0.001$, $n = 480$) and *Wyeomyia forcipenis* ($\rho = 0.136$, $p = 0.003$, $n = 480$).

The number of mosquitoes found in the huge bromeliad *Alcantarea imperialis* was not considered for these analyses, because mosquito numbers differed from those in the other plants and the amount of water held by *A. imperialis* was usually 10–20 folds greater than that held by the other species (Table I). The median number of mosquitoes collected in *A. imperialis* was among the highest (Fig. 4) reported among bromeliad species and the number of mosquitoes collected per sample varied much less in this huge bromeliad species than in most of other plants, such as *A. blanchetiana* and species of genus *Neoregelia* (Fig. 4).

Although rainfall reported in the first two trimesters (Mar–Aug) of the sampling period was lower than in the third and fourth trimesters (Sept-Feb), the number of mosquitoes per collection in all bromeliads did not differ significantly among trimesters (Kruskal-Wallis $H = 3.17$, $p = 0.366$) (Fig. 5). Correlations between lagged rainfall and mosquito abundances were analyzed, following Lourenço-de-Oliveira et al. (2004). Significant correlations ($n = 480$, $p \leq 0.05$) were observed between rainfall in the first week before collections for abundances of *Cx. nigrimacula* ($\rho = 0.630$) and *Wy. pilicauda* ($\rho = 0.585$).

The five most common mosquito species occurred together in the same sample significantly more often than predicted by chance (Table III). The absence of significant negative correlations in the associations of species suggests that females of these species are using similar clues for oviposition in favorable bromeliads. Species of the genus *Wyeomyia* and *Culex* have frequently been found together in bromeliads (Marques et al. 2001, Yanoviak et al. 2006). Interestingly, the frequency of these *Culex* species in the cultivated bromeliads at JBRJ is similar to values reported in native plants in the natural environment elsewhere in Brazil (Corrêa & Ramalho 1956, Lourenço-de-Oliveira et al. 1986, Marques et al. 2001, Silva et al. 2004).

Some mosquitoes, such as *Culex* (*Microculex*) and *Culex* belonging to the Ocellatus Group and some *Wyeomyia* (*Phoniomyia*) species seem to be intimately associated with bromeliads in the Neotropical region. Bromeliad-specific *Wyeomyia* spp have been shown in Florida to inhibit the growth and survivorship of *Ae. albopictus* co-occurring in axils of *Bilbergia pyramidalis* (Lounibos et al. 2003). Interspecific competition with bromeliad-specialist species may make this habitat less suitable for the container generalist species *Ae. albopictus* and *Ae. aegypti* in JBRJ.

Our results and previous works (Marques et al. 2001, Cunha et al. 2002, Maciel-de-Freitas et al. 2007) lead to the conclusion that these bromeliads are not important or even productive microhabitats for *Ae. aegypti* and, hence, do not need to be treated for dengue control. Additionally, we hypothesized that the occasional findings and frequency of *Ae. aegypti* in bromeliads in the modified environment may be correlated to high infestation levels in such an area as result of competition for ovipositing habitats. However the mosquito fauna in bromeliads maintained domestically in yards or houses, where *Culex* spp and *Wyeomyia* spp may be less common than in JBRJ, deserves to be investigated, because bromeliads have became popular houseplants in dengue endemic areas. Indeed, the results of this study of only one year in a single area may not represent outcomes in other urban localities where bromeliads, *Ae. aegypti* and dengue coincide in more disturbed habitats.

Also, it is known that the suitability of tank bromeliads for *Ae. albopictus* depends on bromeliad shape, e.g., *Neoregelia* spp being more suitable for development than *Billbergia* spp (Raban 2006). Moreover, our results may not apply to areas where bromeliads are not endemic yet are grown as ornamental plants in private gardens, such as Hawaii, where *Ae. albopictus* flourishes in bromeliad axils in the absence of competing native mosquito species (Yang et al. 2003).

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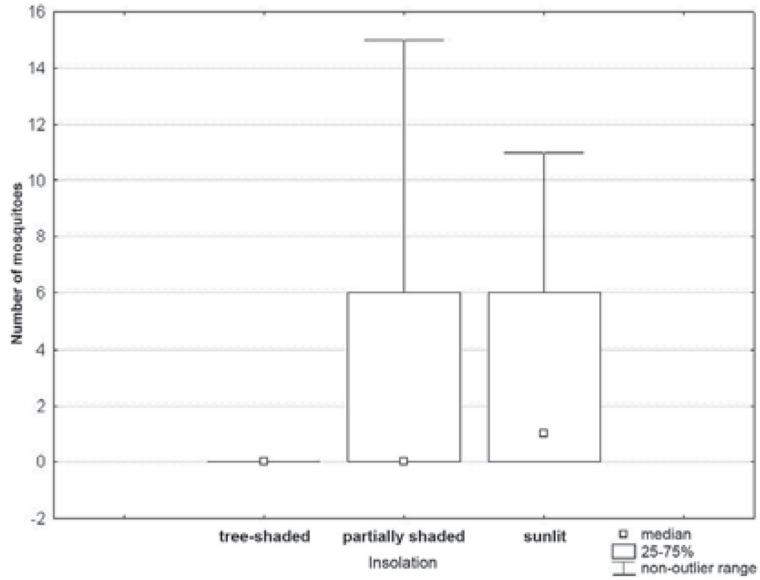

Fig. 1.
box-plot of numbers of immature mosquitoes collected in tree-shaded, partially shaded and sunlit bromeliads at Jardim Botânico do Rio de Janeiro from March 2005–February 2006.

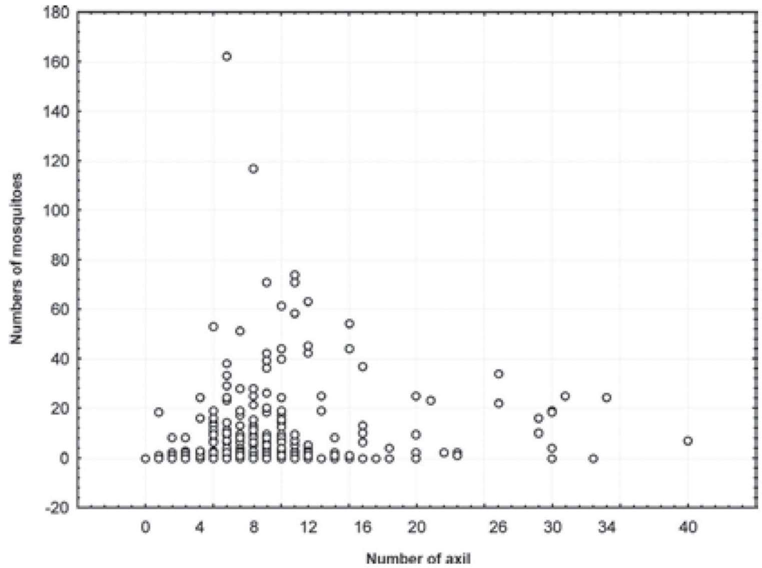

Fig. 2.
dispersion analysis of number of immature mosquitoes and leaf axils in bromeliads at Jardim Botânico do Rio de Janeiro from March 2005–February 2006.

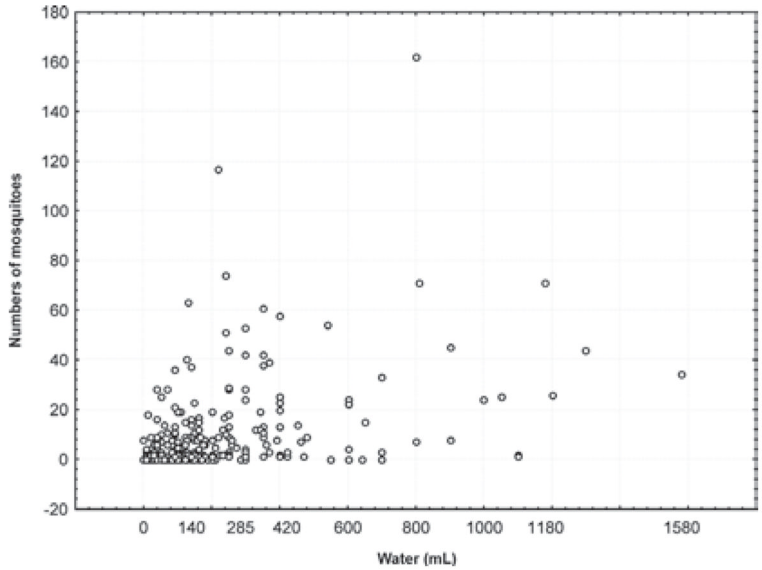

Fig. 3.
dispersion analysis of number of immature mosquitoes and water held in bromeliads at Jardim Botânico do Rio de Janeiro from March 2005–February 2006. Data from the huge bromeliad *Alcantarea imperiali*s were not considered.

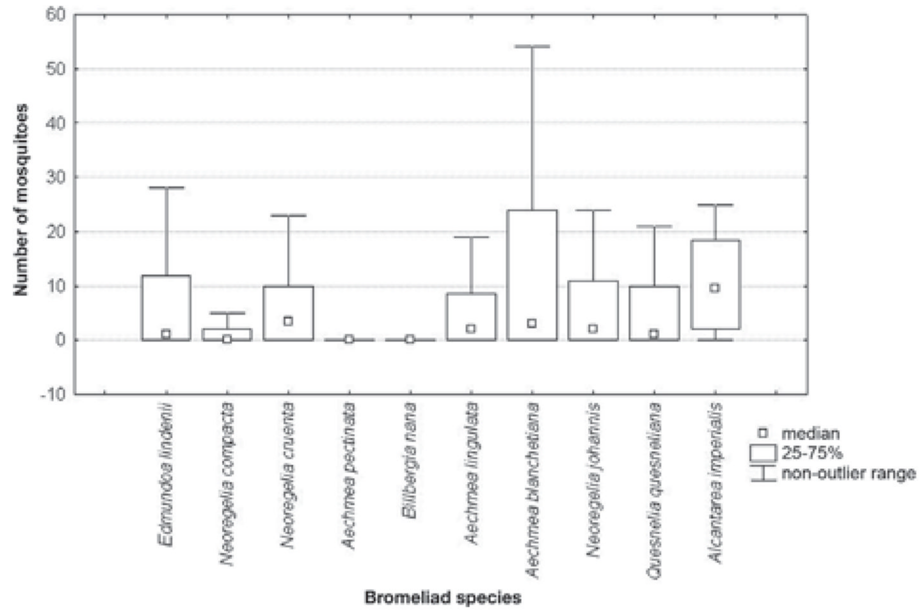

Fig. 4.
box-plot of numbers of immature mosquitoes collected in bromeliad species at Jardim Botânico do Rio de Janeiro from March 2005–February 2006.

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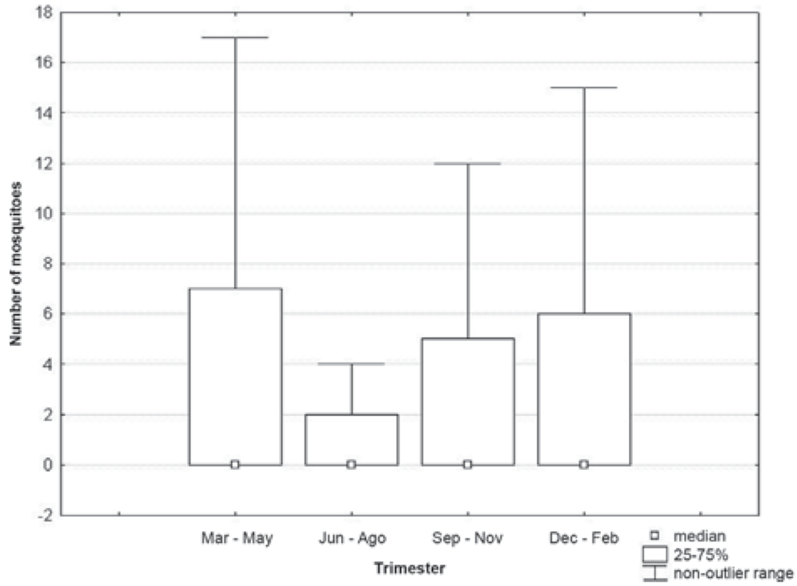

Fig 5.
box-plot of numbers of immature mosquitoes collected in bromeliads by trimester, from March 2005 to February 2006, at Jardim Botânico do Rio de Janeiro.

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TABLE I

Sampled bromeliads and collected mosquitoes at Jardim Botânico do Rio de Janeiro from March 2005–February 2006

Bromeliads	Number of sampled bromeliad (number examined per sampling)	Collected mosquitoes n	Mean of water (SD)	Species of mosquito[a]
Alcantarea imperialis (Carrière) Harms	3 (1)	130	7600 (2.758)	2, 4, 7, 10, 13, 14
Aechmea blanchetiana (Baker) L. B. Sm.	15 (5)	878	351 (346)	3, 4, 7, 10, 11, 13, 14
Aechmea lingulata (L.) Baker	9 (3)	228	158 (206)	4, 7, 10, 11, 12, 13
Aechmea pectinata Baker	15 (5)	105	31 (45)	3, 10, 11
Billbergia nana E. Pereira	21 (7)	40	11 (17)	10, 11, 13, 14
Edmundoa lindenii (Regel) Leme	9 (3)	286	114 (136)	4, 7, 9, 10, 11, 13, 14
Neoregelia compacta (Mez) L. B. Sm.	21(7)	109	61 (63)	1, 3, 7, 11,12, 13, 14
Neoregelia cruenta (R Graham) L. B. Sm.	9 (3)	247	139 (107)	3, 4, 7, 9, 10, 11, 13,
Neoregelia johannis (Carrière) L. B. Sm.	9 (3)	450	293 (339)	2, 3, 4, 7, 10, 11, 12, 13, 14
Quesnelia quesneliana (Brongniart) L. B. Sm.	9 (3)	341	173 (123)	2, 3, 7, 10, 11, 12, 14
Total	120 (40)	2816	315 (1.250)	

[a] see Table II.

TABLE II

Abundances of mosquito species collected in 10 bromeliad species at Jardim Botânico do Rio de Janeiro from March 2005–February 2006

	Species	**n**	**%**
1	*Ae.* (*Stg.*) *aegypti* (Linnaeus)	2	0.07
2	*Ae.* (*Stg.*) *albopictus* (Skuse)	5	0.18
3	*Cx.* (*Mel.*) *nigrimacula* Lane & Whitman	19	0.67
4	*Cx.* (*Mel.*) *ocellatus* Theobald	20	0.71
5	*Culex* spp Ocellatus Group[a]	939	33.35
6	*Cx.* (*Mcx.*) *carioca* Lane & Whitman	2	0.07
7	*Cx.* (*Mcx.*) *pleuristriatus* Lutz	1,195	42.44
8	*Cx.* (*Mcx.*) *stonei* Lane & Whitman	1	0.04
9	*Runchomyia* sp.	5	0.18
10	*Wy.* (*Pho.*) *incaudata* (Root)	394	13.99
11	*Wy.* (*Pho.*) *pilicauda* (Root)	89	3.16
12	*Wy.* (*Pho.*) *theobaldi* (Lane & Cerqueira)	29	1.03
13	*Wy.* (*Spi.*) *forcipenis* (Lourenço-de-Oliveira & Silva)	91	3.23
14	*Toxorhynchites* sp.	25	0.89
Total		2,816	100

[a]immature forms of *Cx. nigrimacula* or *Cx. ocellatus* could not be separated by keys.

TABLE III

Interspecific tests of association by Spearman's rho (top) and Hurlbert's C8

Species	*Culex* spp Ocellatus Group	*Wyeomyia incaudata*	*Wyeomyia pilicauda*	*Wyeomyia forcipecis*
Cx. (*Microculex*)	0.220[a]	0.277[a]	0.108[a]	0.033
pleuristriatus	0.089[a]	0.343[a]	0.088[a]	0.020
	(< 0.001)[a]	(< 0.001)[a]	(0.017)[a]	(0.471)
Cx. spp		0.208[a]	0.089	−0.050
Ocellatus Group		0.590[a]	0.158	−1
		(< 0.001)[a]	(0.051)	(0.278)
Wy. incaudata			0.283[a]	0.097[a]
			0.167[a]	0.041[a]
			(<0.001)[a]	(0.034)[a]
Wy. pilicauda				0.118[a]
				0.088[a]
				(0.010)[a]

numbers in parentheses are significance levels for the Spearman's test.

[a] association analyses significant at $p < 0.05$.