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Short Communication

Diversity of yellow fever mosquito vectors in the Atlantic Forest of Rio de Janeiro, Brazil

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Abstract

Introduction: Environmental modifications caused by human activities have led to changes in mosquito vector populations, and sylvatic species have adapted to breeding in urban areas. **Methods:** Mosquitoes were collected using ovitraps in three sampling sites in the Atlantic Forest in the State of Rio de Janeiro, Brazil. **Results:** We collected 2,162 Culicidae specimens. *Haemagogus janthinomys* and *Haemagogus leucocelaenus*, both sylvatic yellow fever virus vectors, were the most common species found. **Conclusion:** There is a potential for the transmission of arboviruses in and around these natural reserves. Therefore, it is necessary to maintain entomological surveillance programs in the region.

Keywords: *Haemagogus*. Yellow fever. Ovitraps.

Understanding the biodiversity of mosquito species in the Atlantic Forest and their response to both human disturbance and forest recovery is important for predicting changes in mosquito populations, especially those commonly associated with sylvatic habitats. Although the mosquito fauna of the Atlantic Forest is diverse and includes potential vectors for yellow fever virus as well as other arboviruses, from an epidemiological point of view, the *Haemagogus* and *Sabethes* spp. are the most important in the transmission of yellow fever virus because they are the primary vectors in the forest areas of the Americas(1). *Haemagogus* spp., in particular, are sylvatic, active during the warmest hours of the day, and found primarily in the tree canopy of tropical forests. Nonetheless, they will take blood meals at ground level in deforested areas and some of these species also show a tendency toward domiciliation(2). However, behavioral tendencies may vary across regions and seasons. Therefore, we collected mosquito eggs in order to evaluate the mosquito diversity in environmental preservation areas in the Southeastern Brazilian State of Rio de Janeiro.

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Mosquito eggs were collected from the Itatiaia National Park [*Parque Nacional de Itatiaia* (PARNA-Itatiaia)], the Poço das Antas Biological Reserve [*Reserva Biológica de Poço das Antas* (RBioPA)], and the Bom Retiro Private Natural Heritage Reserve [*Reserva Particular do Patrimônio Natural do Bom Retiro* (RPPNBR)] (**Figure 1**). PARNA-Itatiaia, situated 176km from the City of Rio de Janeiro, was the first national reserve in Brazil. It covers an area of 28,155ha and is heavily affected by anthropogenic activities, including housing development and palm cabbage harvesting. The reserve includes two ecologically distinct areas between 400 and 2,791m above sea level: one with rock formations at higher elevations and one lower with numerous waterfalls and small lakes. Rainfall in PARNA-Itatiaia is heavy and occurs mainly in the summer. Annual precipitation averages 2,400mm with the heaviest rainfall in January (27 rainy days and 388mm of rainfall on average). The collection site was located at 22º25'52.1" S and 44º37'16.7" W.

RBioPA is situated in the municipality of Silva Jardim and encompasses an area of 5,000ha. Constituted in 1914, the reserve includes several areas that were previously orchards, houses, or pastures; however, the forest has gradually recovered, and primary forest fragments with original vegetation can be found on the alluvial plains and in the lower areas of the reservation. The climate is hot and wet with most rainfall occurring in the summer (total rainfall = 1,000mm concentrated between October

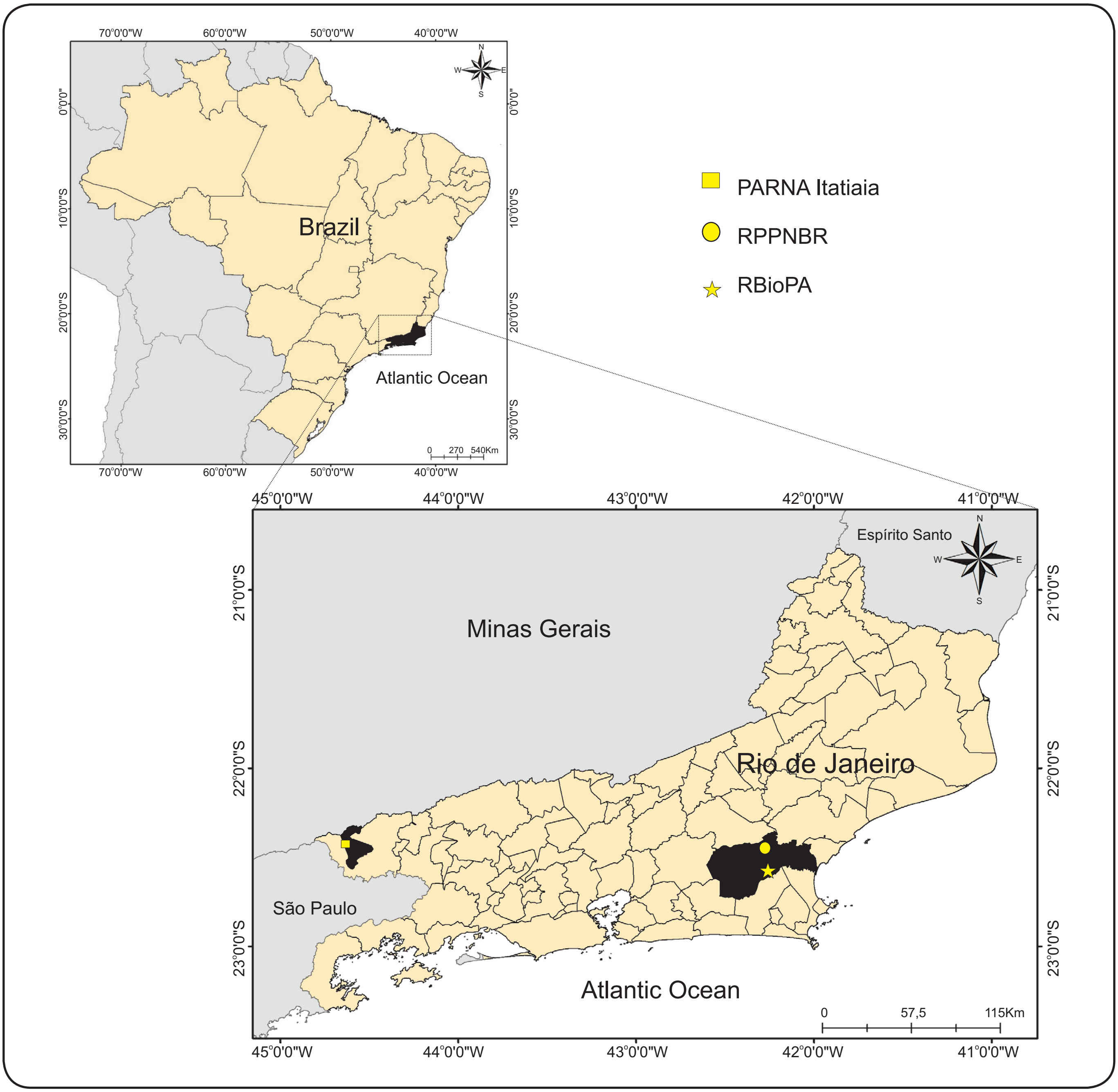

FIGURE 1 - The location of each study area in the State of Rio de Janeiro, Brazil. PARNA-Itatiaia: *Parque Nacional de Itatiaia*; **RBioPA:** *Reserva Biológica de Poço das Antas*; **RPPNBR:** *Reserva Particular do Patrimônio Natural do Bom Retiro***.**

and April). Maximum temperatures range from 30-32ºC and minimum temperatures are always above 18ºC[3]. The collection site was at 22º33'11.4" S and 42º17'49.8" W.

RPPNBR is situated 140km from the City of Rio de Janeiro. It covers an area of approximately 556ha and is almost completely covered by primary Atlantic Forest. The region is heavily influenced by intense solar radiation and Atlantic Ocean humidity producing a tropical wet climate[4]. The collection site was at 22º27'15.3" S and 42º18'02.4" W.

Mosquito egg sampling was conducted over 5 months (December 2014 to April 2015) using ovitraps consisting of a 1L black bucket installed 2-3m from the soil and containing water, leaf litter, and four wood plates. These plates were collected twice a month and examined in the laboratory. Plates with mosquito eggs were immersed in transparent trays filled with Milli-Q® water and maintained at 28 ± 1°C. Emerged adults were identified[5] by checking original descriptions and redescriptions when necessary.

We calculated the index of species abundance for each species and then standardized this on a scale from zero to one [standardized index of species abundance (SISA)] as described by Roberts & Hsi according to[6]. This index is determined by the number of specimens collected and the distribution pattern across samples. Species dominance categories were defined as eudominant (>10%), dominant (<10% and >5%), subdominant (<5% and >2%), recessive (<2% and >1%), and rare (<1%)[7].

We also compared the mosquito diversity between sites with the Shannon-Wiener Diversity Index ($H' = \Sigma p_i \ln p_i$, where p_i is the proportional abundance of species i in the collection) using the DivEs Species Diversity program (W.C. Rodrigues; LIZARO Soft). In addition, we calculated the species richness (S) and the Sørensen similarity index (SI). An SI > 0.50 was considered significant. Since collections were not conducted in April 2015 in PARNA-Itatiaia, all comparisons were restricted to the period from December 2014 to March 2015.

Between December 2014 and April 2015, 2,217 specimens from six mosquito species were collected. Since the studied areas were within the distribution of both *Haemagogus janthinomys* and *Haemagogus capricornii* spp. and because the females are very difficult to differentiate[2] and the only male was collected in RPPNBR, the females were only identified as *H. capricornii/janthinomys*. Five specimens could only be identified as *Wyeomyia* spp. (**Table 1**). In addition, *Haemagogus leucocelaenus* (Dyar & Shannon 1924) was the most abundant in all locations, followed by *H. capricornii/janthinomys* in RPPNBR and RBioPA, and by *Limatus durhamii* Theobald, 1901, in PARNA-Itatiaia (**Table 1**). *H. leucocelaenus* was the most dominant in all areas (**Table 2**). There was no significant difference in diversity among the localities (t-test, p > 0.05), and all localities had a similar species richness (more than 50% similarity).

Two species were observed in RPPNBR, whereas five species were found and species richness was higher in PARNA-Itatiaia and RBioPA (**Table 1).** In RPPNBR and RBioPA, the population density was highest in December and April, respectively, and lowest in January. In PARNA-Itatiaia the population density was highest in March and lowest in February.

In PARNA-Itatiaia the species most frequently observed were *H. leucocelaenus* (82.7% in March 2015), *L. durhamii* (11.7% in March 2015), and *Aedes albopictus* (2.4% in August 2014). The least obtained species were *H. capricornii/janthinomys* (0.5% in January 2015), *Culex iridescens* (1.6%), and *Wyeomyia* sp. (0.9%).

In RPPNBR we collected only two mosquito species: *H. leucocelaenus* (> 98.7% in December 2014) and *H. capricornii/janthinomys* (0.6% in February 2015).

Of the three areas studied, the highest Shannon Diversity Index (H' = 0.37) was found for the RBioPA sample site and the greatest species richness (S = 5) was found for the PARNA-Itatiaia site. In addition, at the PARNA-Itatiaia collection site we found three epidemiologically important species: *H. leucocelaenus*, *H. janthinomys*, and *A. albopictus* (**Table 1**). The species diversity comparisons confirmed no significant differences between the different sampling areas (RBioPA x RPPNBR t-test = 22.8851; RBioPA x PARNA-Itatiaia t = 7.0586; RPPNBR x PARNA-Itatiaia t = 10.3493; p > 0.05 for all).

We also used the dominance index to analyze the species composition in each of the three study areas. In RBioPA, *H. leucocelaenus* and *H. capricornii/janthinomys* were eudominant, *A. albopictus* and *C. iridescens* were subdominant, and *A. terrens* was recessive. In RPPNBR, *H. leucocelaenus* was eudominant and *H. janthinomys* was recessive. In PARNA-Itatiaia, *H. leucocelaenus* was eudominant and *L. durhamii* was dominant (**Table 2**).

Nevertheless, ovitraps have some limitations. For example, they cannot be used to determine absolute population densities, the infusions are not standardized preventing comparison between different traps and occasions, and they are labor intensive[8]. However, the only alternative is to sample eggs from natural habitats; therefore, ovitraps should be complemented by human landing catches and larval surveys. In addition, ovitraps do not capture some species, such as flood mosquitoes (*e.g., A. scapularis* and *A. albifasciatus*); therefore, it is ideal to utilize several sampling methods (such as light traps), baits, and breeding places. However, ovitraps may provide useful data on seasonal fluctuations as well as height and environmental preferences. For example, *H. janthinomys* shows a clear preference for foraging at the highest levels of the forest canopy and lays eggs in tree holes situated in very high and unreachable places[5], indicating preference for egg-laying in higher traps[9].

Except for *A. albopictus*, which has adapted to breeding in bamboo internodes and bromeliads (among other places), all species are adapted to several phytotelmata and some of them have also been found in artificial containers[10]. For example, *Culex* (*Carrollia*) spp. are commonly associated with several different phytotelmata[11], including bamboo internodes, the fungus *Aquascypha hydrophora*, palm spathes, *Heliconia*, Araceae, and artificial containers. However, since immature forms of *C.* (*Carrollia*) *iridescens* (Lutz, 1905) are frequently found in natural habitats in Serra do Mar, São Paulo[12], but remain absent from human landing catches in the same area[13], these mosquitoes seem to have low anthropophily and thus may not be medically important.

Although the studied areas seemed to be quite ecologically different, they were not significantly different in terms of mosquito diversity. However, species dominance was different across sites.

Among the species already identified as potential vectors of yellow fever virus, *H. janthinomys* stands out as the principal vector in the Americas. This species appears to be highly adapted to different biomes and different abiotic conditions (e.g., temperature and humidity). The potential for virus transmission is enhanced by the geographic distribution of this mosquito, which coincides with areas known to be endemic for the disease[2].

Three mosquito species epidemiologically important to the transmission of arboviruses (*H. leucocelaenus*, *H. janthinomys,* and *A. albopictus*) were collected in the present study; however,

TABLE 1 - Mosquitoes captured in the three study areas in Rio de Janeiro, Brazil.

	2014				2015							
	Dec		Jan		Feb		Mar		Apr		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
PARNA-Itatiaia												
Haemagogus (*Conopostegus*) *leucocelaenus* (Dyar & Shannon, 1924)	85	94.4	65	89.0	0	0.0	340	82.7	n.c.	n.c.	490	85.4
Aedes (*Stegomyia*) *albopictus* (Skuse, 1894)	0	0.0	0	0.0	0	0.0	0	0.0	n.c.	n.c.	0	0.0
Wyeomyia sp.	5	5.6	0	0.0	0	0.0	0	0.0	n.c.	n.c.	5	0.9
Haemagogus capricornii/janthinomys	0	0.0	3	4.1	0	0.0	0	0.0	n.c.	n.c.	3	0.5
Limatus durhamii Theobald, 1901	0	0.0	5	6.8	0	0.0	62	15.1	n.c.	n.c.	67	11.7
Culex (*Carrollia*) *iridescens* (Lutz, 1905)	0	0.0	0	0.0	0	0.0	9	2.2	n.c.	n.c.	9	1.6
Subtotal	90	100.0	73	100.0	0	0.0	411	100.0	n.c.	n.c.	574	100.0
RPPNBR												
Haemagogus (*Conopostegus*) *leucocelaenus* (Dyar & Shannon, 1924)	345	100.0	0	0.0	320	97.0	45	100.0	40	100.0	750	98.7
Haemagogus capricornii/janthinomys	0	0.0	0	0.0	8	2.4	0	0.0	0	0.0	8	1.1
Haemagogus (*Haemagogus*) *janthinomys* Dyar, 1921 (Male specimens)	0	0.0	0	0.0	2	0.6	0	0.0	0	0.0	2	0.3
Subtotal	345	100.0	0	0.0	330	100.0	45	100.0	40	100.0	760	100.0
RBioPA												
Haemagogus (*Conopostegus*) *leucocelaenus* (Dyar & Shannon, 1924)	95	95.0	25	71.4	90	81.8	90	46.4	305	68.7	605	27.3
Aedes (*Stegomyia*) *albopictus* (Skuse, 1894)	0	0.0	10	28.6	10	9.1	0	0.0	20	4.5	40	1.8
Aedes (*Protomacleaya*) *terrens* (Walker, 1856)	5	5.0	0	0.0	0	0.0	0	0.0	5	1.1	10	0.5
Haemagogus capricornii/janthinomys	0	0.0	0	0.0	10	9.1	95	49.0	105	23.6	210	9.5
Culex (*Carrollia*) *iridescens* (Lutz 1905)	0	0.0	0	0.0	0	0.0	9	4.6	9	2.0	18	0.8
Subtotal	100	100.0	35	100.0	110	100.0	194	100.0	444	100.0	833	37.6
Total	535	-	108	-	440	-	650	-	484	-	2,217	100.0

PARNA-Itatiaia: *Parque Nacional de Itatiaia*; **RBioPA:** *Reserva Biológica de Poço das Antas*; **RPPNBR:** *Reserva Particular do Patrimônio Natural do Bom Retiro;* **nc:** not collected.

TABLE 2 - Dominance index and standardized index of species abundance for mosquito species in each study area in Rio de Janeiro, Brazil.

Species	Number	D%		SISA	Rank
PARNA-Itatiaia					
Haemagogus (*Conopostegus*) *leucocelaenus* (Dyar & Shannon, 1924)	490	85.4	Eudominant	0.750	1st
Haemagogus capricornii/janthinomys	3	0.5	Rare	0.083	4th
Culex (*Carrollia*) *iridescens* (Lutz, 1905)	9	1.6	Recessive	0.083	4th
Limatus durhamii Theobald, 1901	67	11.7	Eudominant	0.333	2nd
Wyeomyia sp.	5	0.9	Rare	0.167	3rd
Total	574	100.0	-	-	-
Species richness (S)	5	-	-	-	-
Shannon-Wiener Diversity Index (H')	0.2483	-	-	-	-
RPPNBR					
Haemagogus (*Conopostegus*) *leucocelaenus* (Dyar & Shannon, 1924)	750	98.7	Eudominant	0.800	1st
Haemagogus capricornii/janthinomys	8	1.1	Recessive	0.133	2nd
Haemagogus (*Haemagogus*) *janthinomys* Dyar, 1921 (male specimens)	2	0.3	Rare	0.067	3rd
Total	760	100.0	-	-	-
Species richness (S)	2	-	-	-	-
Shannon-Wiener Diversity Index (H')	0.0304	-	-	-	-
RBioPA					
Haemagogus (*Conopostegus*) *leucocelaenus* (Dyar & Shannon, 1924)	605	68.5	Eudominant	0.960	1st
Aedes (*Stegomyia*) *albopictus* (Skuse, 1894)	40	4.5	Subdominant	0.420	3rd
Aedes (*Protomacleaya*) *terrens* (Walker, 1856)	10	1.1	Recessive	0.200	4th
Haemagogus capricornii/janthinomys	210	23.8	Eudominant	0.500	2nd
Culex (*Carrollia*) *iridescens* (Lutz, 1905)	18	2.0	Subdominant	0.200	4th
Total	883	100.0	-	-	-
Species richness (S)	5	-	-	-	-
Shannon-Wiener Diversity Index (H')	0.3782	-	-	-	-

PARNA-Itatiaia: *Parque Nacional de Itatiaia*; **RBioPA:** *Reserva Biológica de Poço das Antas*; **RPPNBR:** *Reserva Particular do Patrimônio Natural do Bom Retiro;* **D%:** dominance index; **SISA:** standardized index of species abundance.

H. leucocelaenus was the predominant species. Although Alencar et al.[9] reported that egg-laying by this species peaked in April in areas under the influence of the Simplício hydroelectric dam in Minas Gerais State, Brazil, and that egg-laying varied seasonally, in this locality *H. leucocelaenus* was the predominant species in all seasons[9]. *Aedes albopictus* is a potential vector of dengue virus, chikungunya virus, West Nile virus, yellow fever virus, Eastern equine encephalitis virus, and Western equine encephalitis virus, and several other arboviruses[14].

According to the Shannon diversity index, RBioPA had the highest mosquito diversity; however, species richness was highest in PARNA-Itatiaia. The diversity may be reduced by stress in biotic communities, according to Richardson[15].

Although there is no evidence of active sylvatic yellow fever virus transmission in the nature reserves studied here, the abundance of the main mosquito vector for this disease in Brazil necessitates active surveillance for the emergence of the virus in neighboring communities.

Conflict of interest

The authors declare that there is no conflict of interest.

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