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Diversity of Mosquitoes (Diptera: Culicidae) in the Bom Retiro Private Natural Heritage Reserve, Rio de Janeiro State, Brazil

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Abstract

This study registers the diversity of Culicidae in the Bom Retiro Private Natural Heritage Reserve (RPPNBR), Rio de Janeiro state, Brazil, based on the collection of the immature stages in natural and artificial larval habitats. Larvae and pupae were collected monthly at two sites of the RPPNBR from May 2014 to July 2015 using dippers and aquatic pipettes. The diversity of the mosquito community was described using the Shannon–Wiener Diversity Index (H′), as well as diversity, richness, and dominance of species found in different larval habitats (lake, bamboos, bromeliads, and artificial vessels). The Mann–Whitney test was used to calculate differences between the two natural and artificial habitats. Overall, 15,659 specimens belonging to 25 species, ten genera, and two subfamilies were collected. The most abundant species collected at sites that were reforested recently were *Culex pleuristriatus* Theobald, 1903, *Limatus durhamii* (Theobald, 1901), *Aedes albopictus* (Skuse, 1895), *Culex neglectus* (Lutz, 1904), and *Culex retrosus* (Lane & Whitman, 1951). In a forest preserved site, the most abundant species were *Cx. neglectus*, *Culex iridescens* (Lutz, 1905), *Sabethes identicus* (Dyar & Knab, 1907), *Wyeomyia arthrostigma* (Lutz, 1905), and *Li. durhamii.* With respect to larval habitats, 0.1% of the specimens were collected along the edge of a lake, 5.5% in bamboos, 35.9% in bromeliads, and 58.4% in artificial containers. Only 5.5% of the specimens were collected in the forest preserved site, with the remaining samples from the site with altered vegetation. A greater species richness and diversity were found in forest-altered sites compared to the forest preserved site. Several species were collected in the water accumulated in the nylon lids of plastic water tanks. Such vessels can promote an increase in mosquito population density in the environment surrounding the study area.

Key words: distribution, abundance, vector ecology, immature, Atlantic Forest

Mosquitoes are the most important and well-studied group of blood-sucking insects in Brazil (Consoli and Lourenço-de-Oliveira 1994). Studies on the ecology of mosquitoes provide critical epidemiological insights since they are potential vectors of important pathogens, such as dengue, yellow fever, zika, and chikungunya viruses; *Plasmodium* causing malaria; and worm that causes lymphatic filariasis. Therefore, ecological investigations can facilitate the identification, monitoring, and control of mosquitoes in scenarios of human-driven environmental changes that may result in the emergence of vector-borne diseases epidemics (Guimarães et al. 2000b).

The Atlantic Forest tropical rain forest biome suffered an intense exploitation process with reduction and degradation of its natural forest cover for many years, and in recent decades, some reserve areas have been in the process of reforestation. In the past, the Bom Retiro Private Natural Heritage Reserve (RPPNBR) area was deforested for timber as well as coffee and banana plantation, and its legal conservation has started in 1984 (MMA 2014). Studies

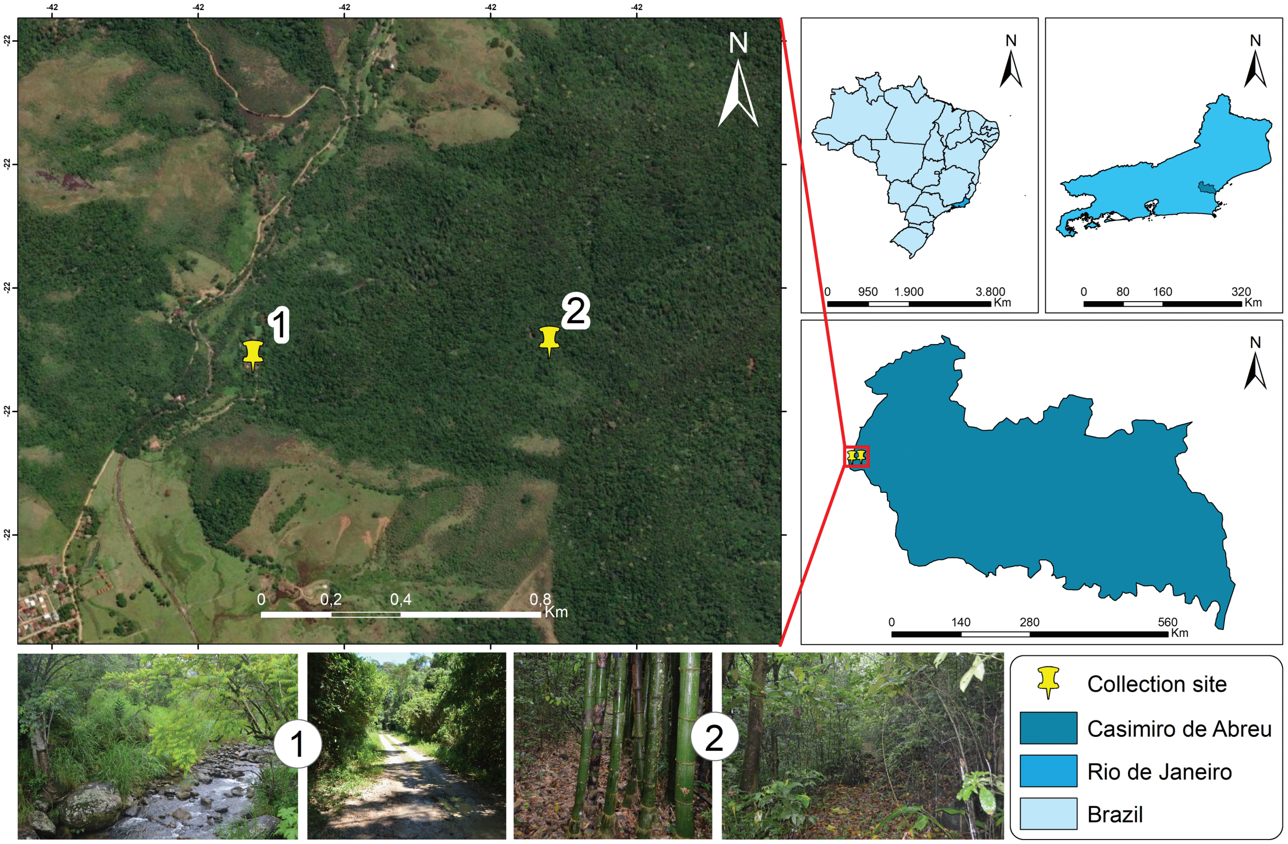

Fig 1. Sampling locations in the Bom Retiro Private Natural Heritage Reserve (RPPNBR), Casimiro de Abreu, Rio de Janeiro state, Brazil. 1) Altered vegetation 2) Preserved vegetation.

on the distribution and abundance of mosquitoes in remnants of the Atlantic Forest in Brazil can help the understanding of the eco-epidemiology of mosquito-borne diseases. The Atlantic Forest biome has a highly diverse flora and fauna, which represents many available niches for mosquitoes. In turn, these mosquitoes can serve as bridge vectors of parasites and pathogens (Alencar et al. 2011).

Demographic changes in Brazil, especially those related to rural–urban migration, can alter the dynamics of some vector-borne diseases, with a reduction of rural endemic diseases and an aggravation of those affecting urban communities, challenging the public health programs (Medeiros-Sousa et al. 2013). Mosquito communities are affected by changes in their environment and microclimate caused by deforestation and changes in land use (Almeida et al. 2020). The capacity of some Culicidae species to adapt to the anthropic environment is one of the reasons for the evolutionary success of the group, which has allowed these insect vectors to live in a great diversity of environments. The mosquito-borne pathogens can undergo a variety of adaptations associated with changes in the communities caused by changes in land use (Guedes 2012).

Mosquitoes can use a wide variety of larval habitats with different dimensions and water volumes. Certain species show a considerable capacity for adaptation to a variety of larval sites and can live in different habitats. Meanwhile, other species are more restricted in their choice of habitats (Lozovei 2001). Anthropogenic changes in remaining forest areas can create conditions for the maintenance and even introduction of new mosquito species in these locations. The use of artificial breeding sites by some Culicidae species may indicate a change of habit or opportunism and shows the capacity of the species to colonize anthropogenic environments (Lopes 1997). Thus, assessing the diversity of mosquito species found in artificial habitats provides a picture of which species are adapted to that type of environment or are merely opportunistic (Almeida et al. 2020).

Recent studies on Culicidae immature communities in areas of Atlantic Forest remnants in Rio de Janeiro have significantly contributed to the knowledge of the immature fauna in these areas. Correa et al. (2014), Alencar et al. (2016), and Aguiar et al. (2020) have reported important data on vectors and potential vectors of pathogens, as well as information on the local fauna, biology, and behavior of mosquitoes in these areas. Therefore, continuing our investigations, we carried out studies focusing on aspects of mosquito ecology and the potential of some species as vectors of pathogens to humans and/or other animals in areas of the Atlantic Forest (Alencar et al. 2015, 2021).

Bioecological information about mosquito vectors helps to detect changes in the disease transmission dynamics (Jesus 2015). The present study aims to identify and analyze the composition of the immature mosquito population in the RPPNBR, a natural forest reserve located in Rio de Janeiro state, Brazil, using the abundance and diversity metrics.

Material and Methods

Study Area and Mosquito Sampling

The RPPNBR is in the municipality of Casemiro de Abreu, in the São João River basin, 140 km from the city of Rio de Janeiro, Rio de Janeiro state, eastern Brazil. The reserve has an area of approximately 556 ha, with most of the vegetation classified as Submontane Dense Ombrophilous Forest in an Advanced Stage of Succession

(MMA 2014). This region has intense solar radiation and is strongly influenced by the Atlantic Ocean, with a predominantly humid tropical/intertropical climate zone (Schobbenhaus 1995).

Two locations were sampled in the area of RPPNBR. One location (22° 27′ 15.4″ S 42° 18′ 02.4″ W) is approximately 500 m from the reserve headquarters. It has altered vegetation, with recent reforestation, small seedlings, a few larger trees, and an artificial lake. Nearby this location, there is a stream and areas with bromeliads. The preserved location (22° 27′ 14.1″ S 42° 17′ 34.9″ W) is inside the forest, where the vegetation is better preserved, dense, and composed of tall trees (Fig. 1). There is a stream crossing part of the area, with a more humid microclimate than the area with altered vegetation.

The field collections were performed from May 2014 to July 2015, except for January 2015, when the heavy rainfall hampered sampling. Fourteen samples were carried out. Collections were carried out for two days monthly, and the mosquitoes' larvae and pupae were collected in the areas where vegetation was altered and in a second area where the forest is preserved in the RPPNBR. The presence of the immatures of mosquitoes was investigated in all potential larval habitats, both natural (lake, bamboos, and bromeliads) and artificial (plastic containers) in each location.

The lake has an area of ~208.27 m^2, the total perimeter is 57.7 m, and it was possible to collect around the whole margin. Nine sampling collections were carried out. The collectors made three samplings to the right, three ahead, and three to the left, respecting a radius of 1 m. The total number of immature mosquitoes was registered, and they were separated by stages.

About 80% of all plants (bamboos and bromeliads) found during the sampling were investigated. The radius of sampling sites was according to the diversity of larval habitats found: in the altered vegetation site, the radius of investigation was 340 m, whereas, in the preserved site, it was approximately 23.50 m.

Sampling was performed with dippers, suction samplers, and an entomological dipper, with a 100 cm handle and the collection pan with 11 cm diameter, and an approximate volume of 850 ml. Water was poured into polyethylene trays; larvae and pupae found were quantified and removed with a pipette and placed in 250-ml plastic bags (Whirl-Pak Bags®). For transport, each bag was labeled with the location, date, and habitat type. In the laboratory, immature Culicidae species were screened and transferred to small bowls, with approximately 50 larvae each. Immatures were kept alive in water from the collection sites, and each bowl was periodically supplemented with distilled water to allow the larvae and pupae to complete their development to adulthood. Larval exuviae, pupal skins, and whole fourth-instar larvae that did not complete their development were fixed in 70% ethanol, mounted on a microscope slide with Canada balsam, and covered with coverslip for species identification.

Species identification was performed by direct observation of the morphological characters in a stereomicroscope (ZEISS Stemi SV6), using the morphological identification keys of Lane (1953), Consoli and Lourenço-de-Oliveira (1994), and Forattini (2002). Specimens were deposited in the Entomological Collection of the Oswaldo Cruz Institute, Rio de Janeiro, Brazil, under the title 'Atlantic Forest Collection'.

Data Analyses

Abundance and diversity of species were analyzed separately for each sampling site. The Shannon–Wiener Diversity Index (H′) was used to calculate the species diversity in different types of habitats (lake, bamboo culms, bromeliads, and artificial vessels). This index shows the importance of each species with respect to how frequently they were collected or detected. For species richness (S), we used the number of species identified. We calculated the Simpson Index (D) to assess the distribution of individuals across species. Rarefaction curves were also obtained as a function of the frequency of individuals captured. The standardized index of species abundance (SISA) was used to determine the most abundant species (Roberts and Hsi 1979). We also applied the Kruskal–Wallis test to assess whether the differences between mosquito populations in the different habitats were significant. The Mann–Whitney test was conducted to verify significant differences between the two sampling sites. All statistical analyses were carried out using IBM® SPSS® Statistics 23 (IBM 2015), BioEstat 5.3 (Ayres 2007), and Past Version 3.16 (Hammer et al. 2001).

Results

During the 14 collections, 15,659 specimens were found, 25 species were identified, included in ten genera and two subfamilies (Table 1). Thirteen bamboo plants and 23 bromeliads were investigated. The artificial containers contributed with 58.45% of specimens, bromeliads with 35.90%, bamboos with 5.55%, and the lake with 0.10% (Table 1). The collections in the locations with altered vegetation found the highest number of species (23 species) compared to the forest preserved location (10 species) (Table 3).

The most abundant species at altered vegetation site were *Culex pleuristriatus* Theobald, 1903 (SISA = 0.857); *Aedes albopictus* (Skuse, 1895) (SISA = 0.647); *Limatus durhamii* Theobald, 1901 (SISA = 0.476); *Culex retrosus* Lane & Whitman, 1951 (SISA = 0.262), and *Culex neglectus* (SISA = 0.250). At preserved site, the most abundant were *Culex neglectus* Theobald, 1907 (SISA = 0.786); *Sabethes identicus* Dyar & Knab, 1907 (SISA = 0.448); *Wyeomyia arthrostigma* (Lutz, 1905) (SISA = 0.435); *Culex iridescens* (Lutz 1905) (SISA = 0.305), and *Li. durhamii* Theobald, 1901 (SISA = 0.208).

The natural bamboo habitats had the greatest diversity index (H′ = 1.64; D = 0.24) and evenness (J = 0.71) values, which were significantly larger ($P < 0.05$) than the values found in the lake (Table 2). Ten species, or 40.0% of the total number of species collected at RPPNBR, were found in bamboos (Table 2). However, this larval habitat was the second-lowest in abundance (869).

Artificial containers had the second-highest values of diversity (H′ = 1.62; D = 0.25) and evenness (J = 0.59). Species richness was also high in this type of larval habitat, with 16 taxa or 64.0% of all species collected and the highest density of individuals (9,073). These results were significantly higher ($P < 0.0001$) than those found for the lake. The diversity index was the lowest in the bromeliads (H′ = 0.63; D = 0.77), where species evenness was also low (J = 0.23). However, species richness was high in the bromeliads, with 15 species or 60.0% of all species found. Bromeliads also had a high number of individuals collected (5,521); the difference between these values and those of the lake site was significant ($P < 0.0001$) (Table 2). The lake had a low diversity index (H′ = 0.74; D = 0.59) and the smallest number of species ($n = 3$), representing only 12.0% of the collected species. This habitat had the lowest number of specimens, with only 16 individuals or 0.1% of all mosquitoes collected (Fig. 2; Table 2).

Only 5.55% of the collected specimens were found in areas where the forest is preserved, whereas 94.45% were collected in areas with altered vegetation. This difference may be due to the landscape characteristics of each sampling site (Table 3). While the preserved location is a remnant of native forest, with a diverse set

Table 1. Mosquito species found in each type of breeding site, absolute values, and percentages, in the Bom Retiro Private Natural Heritage Reserve (RPPNBR), Casimiro de Abreu, Rio de Janeiro state, Brazil, from May 2014 to July 2015

Species	Lake		Bamboo		Bromeliad		Artificial breeding[a]		Total	
	N	%	N	%	N	%	N	%	N	%
Ae. (Och.) rhyacophilus Costa Lima, 1933	0	0.00%	0	0.00%	13	0.20%	44	0.50%	57	0.40%
Ae. (Stg.) albopictus (Skuse,1895)	2	12.50%	17	2.00%	169	3.00%	1,148	12.50%	1,336	8.50%
Ae. (Grg.) fluviatilis (Lutz,1904)	0	0.00%	0	0.00%	6	0.10%	0	0.00%	6	0.00%
An. (Ano.) tibiamaculatus (Neiva, 1906)	0	0.00%	0	0.00%	0	0.00%	133	1.50%	133	0.80%
Cq. (Rhy.) venezuelensis Theobald, 1912	0	0.00%	0	0.00%	0	0.00%	2	0.00%	2	0.00%
Cx. (Car.) iridescens (Lutz, 1905)	0	0.00%	126	14.50%	4	0.10%	68	0.70%	198	1.30%
Cx. (Cux.) mollis Dyar & Knab, 1906	0	0.00%	0	0.00%	0	0.00%	12	0.10%	12	0.10%
Cx. (Cux.) quinquefasciatus Say, 1823	0	0.00%	0	0.00%	36	0.60%	16	0.20%	52	0.30%
Cx. (Cux.) sp.	0	0.00%	0	0.00%	63	1.10%	6	0.10%	69	0.40%
Cx. (Mel.) bastagarius Dyar & Knab, 1906	12	75.00%	0	0.00%	0	0.00%	0	0.00%	12	0.10%
Cx. (Mcx.) retrosus Lane & Whitman, 1951	0	0.00%	0	0.00%	208	3.70%	0	0.00%	208	1.30%
Cx. (Mcx.) neglectus Theobald, 1907	0	0.00%	303	34.90%	39	0.70%	327	3.60%	669	4.30%
Cx. (Mcx.) pleuristriatus Theobald, 1903	2	12.50%	4	0.50%	4,835	86.00%	3,429	37.50%	8,270	52.80%
Cx. (Mcx.) sp.	0	0.00%	0	0.00%	10	0.20%	0	0.00%	10	0.10%
Cx. (Pho.) corniger Theobald, 1903	0	0.00%	0	0.00%	13	0.20%	0	0.00%	13	0.10%
Cx. grupo *coronator* Dyar & Knab, 1906	0	0.00%	0	0.00%	19	0.30%	0	0.00%	19	0.10%
Cx. (Lut.) bigoti (Bellardi, 1862)	0	0.00%	0	0.00%	0	0.00%	3	0.00%	3	0.00%
Li. durhamii Theobald, 1901	0	0.00%	20	2.30%	61	1.10%	2,114	23.10%	2,195	14.00%
Li. flavisetosus Oliveira Castro, 1935	0	0.00%	0	0.00%	0	0.00%	155	1.70%	155	1.00%
Li. paraensis (Theobald, 1903)	0	0.00%	0	0.00%	0	0.00%	3	0.00%	3	0.00%
Ps. (Gra.) cingulata (Frabricius, 1805)	0	0.00%	0	0.00%	0	0.00%	1,609	17.60%	1,609	10.30%
Sa. (Pey.) identicus Dyar & Knab, 1907	0	0.00%	201	23.10%	0	0.00%	0	0.00%	201	1.30%
Tx. (Lyn.) bambusicola (Lutz & Neiva, 1913)	0	0.00%	3	0.30%	19	0.30%	0	0.00%	22	0.10%
Tx. purpureus (Theobald, 1901)	0	0.00%	11	1.30%	13	0.20%	3	0.00%	27	0.20%
Tx. sp.	0	0.00%	12	1.40%	21	0.40%	19	0.20%	52	0.30%
Tr. cf. *digitatum* (Rondani, 1848)	0	0.00%	16	1.80%	0	0.00%	0	0.00%	16	0.10%
Wy. (Pho.) edwardsi (Lane & Cerqueira, 1942)	0	0.00%	0	0.00%	51	0.90%	0	0.00%	51	0.30%

Table 1. Continued

Species	Lake		Bamboo		Bromeliad		Artificial breeding[a]		Total	
	N	%	N	%	N	%	N	%	N	%
Wy. (Wyo.) arthrostigma (Lutz, 1905)	0	0.00%	141	16.20%	35	0.60%	7	0.10%	183	1.20%
Wyeomyia (Wyo.) sp.	0	0.00%	15	1.70%	7	0.10%	54	0.60%	76	0.50%
TOTAL	16	100.00% 0.10%	869	100.00% 5.55%	5,622	100.00% 35.90%	9,152	100.00% 58.45%	1,5659	100.00% 100.00%

[a]Artificial container: nylon lid of plastic water tank.

Table 2. Species diversity index per breeding site, Pielou's evenness, and the number of Culicidae species found per breeding site in Bom Retiro Private Natural Heritage Reserve (RPPNBR), Casimiro de Abreu, Rio de Janeiro state, Brazil, from May 2014 to July 2015

Breeding	N	S	H′	D	J
Lake	16	3	0.74	0.59	0.67
Bamboo	842	10	1.64	0.24	0.71
Bromeliad	5,521	15	0.63	0.77	0.23
Artificial breeding*a*	9,073	16	1.62	0.25	0.59

[a]Artificial container: nylon lid of plastic water tank.

N = number of individuals; S = Species; H′ = Shannon–Wiener Diversity Index (H'); D = Simpson's Dominance Index; J = Pielou's evenness.

of forest formations, altered vegetation location has been subjected to recent reforestation, with few natural larval habitats available. On the other hand, it has greater availability of artificial vessels and therefore showed a higher abundance of specimens collected.

Rarefaction curves are graphs that show the number of species as a function of the number of samples. When the curve flattens to the right, it means that a reasonable number of sample specimens have been collected. The rarefaction curves obtained for altered vegetation and preserved sites indicated stability of the number of species in each locality. At altered vegetation site, the curve tends to stabilize around 22 species, while at the preserved site, this stability was reached with ten species (Fig. 3).

The Mann–Whitney test indicated a significant difference between the mosquito populations found in locations with altered vegetation and preserved ($P < 0.05$). The former location had a greater species richness (S = 23) but lower diversity index (H′ = 1.48) than the latter location (S = 10; H′ = 1.64) (Table 3). The *t*-test showed that the H′ index significantly differed between the sampling locations (t = 5.135 and $P = 0.000$).

Discussion

The Atlantic Forest is the second-largest tropical rainforest in the Americas and is considered a global biodiversity hotspot (Marris 2010, INPE 2020). Culicidae species, in general, can use a variety of habitats with different sizes and water volumes for their development during the aquatic stage. Some species can be found in a wide range of habitats, while others are restricted (Service 1976). Environmental changes can mediate physiological adaptations, with a selection of eutrophic environments occurring during the developmental stages of a species (Alencar et al. 2013). Although research on ecological aspects of Culicidae fauna has been carried out throughout Brazil, mainly in the Rio de Janeiro state, studies have focused on detecting vectors of pathogens of human diseases in the urban environment (Alencar et al. 2011). These studies facilitate the identification, monitoring, and control of mosquitoes to environmental changes caused by direct human action, which may result in major epidemics (Guimarães et al. 2000a).

Culex pleuristriatus was the most abundant species in the artificial and natural containers in both sampling locations. Specimens were collected in nylon lids with accumulated water, used to protect plastic water tanks, and in the bromeliad phytotelmata that was the main natural habitat of the species in the RPPNBR region. Lourenço-de-Oliveira et al. (1986) and Consoli and Lourenço-de-Oliveira (1994) reported that *Culex (Microculex)* spp. were restricted to natural habitats, mainly bromeliads, and Müller and Marcondes (2006) indicated the predominance of *Culex (Microculex)* spp. in all the bromeliad species investigated in forest preserved area in Santa Catarina. Furthermore, Alencar et al. (2016) recorded the presence of *Cx. pleuristriatus* in greater numbers in bromeliads, ground depressions, *Heliconia* plants, bamboos, and fallen leaves, contrasting with 41.5% of *Cx. pleuristriatus* specimens were found in artificial containers in this study. Despite *Cx. pleuristriatus* being commonly found in natural containers in undisturbed environments, the species can occur in bromeliads in urban and peri-urban environments (Marques and Forattini 2008).

In the region studied, *Cx. iridescens* was collected in bamboos. Similarly, Valencia (1973) registered *Cx. iridescens* in cut or broken bamboo and tree holes. Forattini (1965) reported the occurrence of *Cx. iridescens* in bromeliads, flower bracts, fallen leaves, artificial containers, and puddles. The species was found in artificial containers (Lopes and Lozovei 1995) and in urban areas (Lopes et al. 1995). Distinctly, *Culex* (*Culex*) *mollis* Dyar & Knab, 1906 was found only in artificial containers, such as nylon lids covering plastic water tanks.

The maintenance and preservation of biodiversity in natural environments are of paramount epidemiological importance, given that zoonosis spillovers have increased over the last 50 years, mainly due to the environmental impact of agriculture and excessive land exploitation, with deforestation and habitat alteration (Berger 2020). Mosquito populations can be affected by the changes in the natural environment, thus participating in the zoonosis spillovers of mosquito-borne diseases. It is well known that changes in the distribution of environmental factors can decrease mosquito populations or benefit them by increasing the abundance of some species in the absence of limiting factors (Medeiros-Sousa et al. 2013).

Sabethes spp. develop in phytotelmata, such as bracts, leaf axils, bamboos, and tree hollows. Among the species of *Sabethes* recorded in the RPPNBR, *Sa. identicus* was found exclusively in pierced bamboos. Meanwhile, other Sabethini mosquitoes like *Li.*

Table 3. Mosquito species found per capture site, absolute values and percentages, in the Bom Retiro Private Natural Heritage Reserve (RPPNBR), Casimiro de Abreu, Rio de Janeiro state, Brazil, from May 2014 to July 2015

Species	Altered vegetation site		Preserved site		Total	
	N	%	N	%	N	%
Ae. (Och.) rhyacophilus Costa Lima, 1933	57	0.39%	0	0.00%	57	0.36%
Ae. (Stg.) albopictus (Skuse,1895)	1,319	8.92%	17	1.96%	1,336	8.53%
Ae. (Grg.) fluviatilis (Lutz,1904)	6	0.04%	0	0.00%	6	0.04%
An. (Ano.) tibiamaculatus (Neiva, 1906)	133	0.90%	0	0.00%	133	0.85%
Cq. (Rhy.) venezuelensis Theobald, 1912	2	0.01%	0	0.00%	2	0.01%
Cx. (Car.) iridescens (Lutz, 1905)	72	0.49%	126	14.50%	198	1.26%
Cx. (Cux.) mollis Dyar & Knab, 1906	12	0.08%	0	0.00%	12	0.08%
Cx. (Cux.) quinquefasciatus Say, 1823	52	0.35%	0	0.00%	52	0.33%
Cx. (Cux.) spp.	69	0.47%	0	0.00%	69	0.44%
Cx. (Mel.) bastagarius Dyar & Knab, 1906	12	0.08%	0	0.00%	12	0.08%
Cx. (Mcx.) retrosus Lane & Whitman, 1951	208	1.41%	0	0.00%	208	1.33%
Cx. (Mcx.) neglectus Theobald, 1907	366	2.47%	303	34.87%	669	4.27%
Cx. (Mcx.) pleuristriatus Theobald. 1903	8,266	55.89%	4	0.46%	8,270	52.81%
Cx. (Mcx.) sp.	10	0.07%	0	0.00%	10	0.06%
Cx. (Pho.) corniger Theobald, 1903	13	0.09%	0	0.00%	13	0.08%
Cx. grupo *coronator* Dyar & Knab, 1906	19	0.13%	0	0.00%	19	0.12%
Cx. (Lut.) bigoti (Bellardi, 1862)	3	0.02%	0	0.00%	3	0.02%
Li. durhamii Theobald, 1901	2,175	14.71%	20	2.30%	2,195	14.02%
Li. flavisetosus Oliveira Castro, 1935	155	1.05%	0	0.00%	155	0.99%
Li. paraensis (Theobald, 1903)	3	0.02%	0	0.00%	3	0.02%
Ps. (Gra.) cingulata (Frabricius. 1805)	1,609	10.88%	0	0.00%	1,609	10.28%
Sa. (Pey.) identicus Dyar & Knab, 1907	0	0.00%	201	23.13%	201	1.28%
Tx. (Lyn.) bambusicola (Lutz & Neiva, 1913)	19	0.13%	3	0.35%	22	0.14%
Tx. purpureus (Theobald, 1901)	16	0.11%	11	1.27%	27	0.17%
Tx. spp.	40	0.27%	12	1.38%	52	0.33%
Tr. cf. *digitatum* (Rondani, 1848)	0	0.00%	16	1.84%	16	0.10%
Wy. (Pho.) edwardsi (Lane & Cerqueira, 1942)	51	0.34%	0	0.00%	51	0.33%
Wy. (Wyo.) arthrostigma (Lutz, 1905)	42	0.28%	141	16.23%	183	1.17%
Wy. (Wyo.) spp.	61	0.41%	15	1.73%	76	0.49%
TOTALc	14,790	100.00%	869	100.00%	15,659	100.00%
		94.45%		5.55%		100.00%
Diversidade (H′)	1.48		1.64			
Riqueza (S)	23		10			

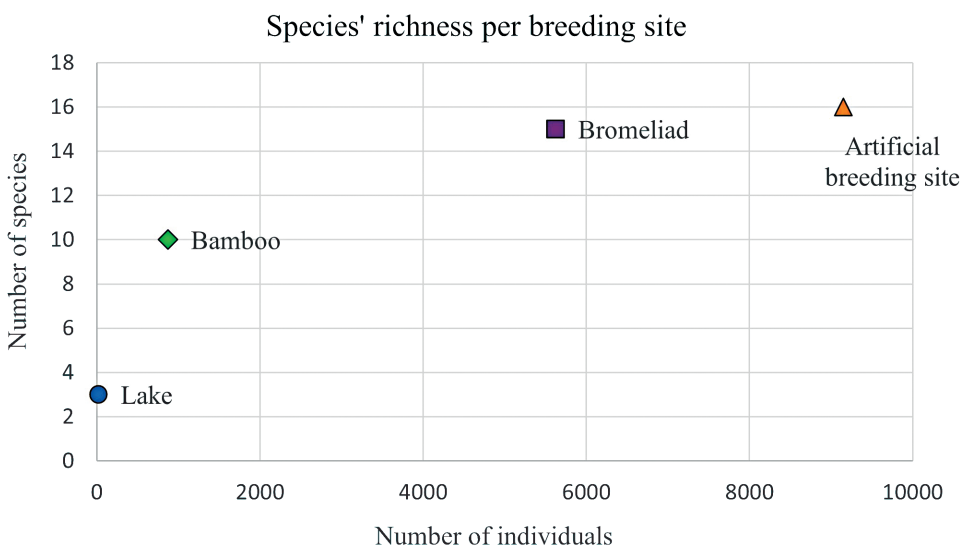

Fig 2. Species richness per sampling site (bamboos, bromeliads, and artificial vessels) in the Bom Retiro Private Natural Heritage Reserve (RPPNBR), Casimiro de Abreu, Rio de Janeiro state, Brazil.

durhamii and *Li. flavisetosus* Oliveira Castro, 1935 were abundant in artificial vessels, i.e., the nylon lid of plastic covering water tanks. Guimarães et al. (1985) observed that *Li. durhamii* is likely capable of establishing and dispersing in the urban environment, with a high potential for breeding in artificial habitats found in domestic and peridomestic environments. Even though the sampling of the present

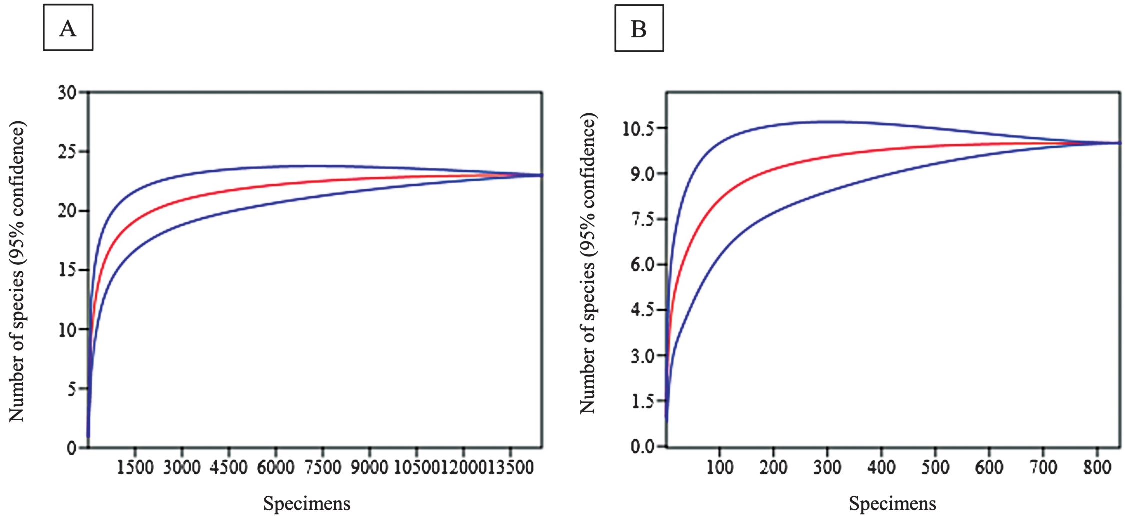

Fig 3. Rarefaction curves representing the accumulation of immature mosquito species in the two sampling sites: (A) Altered vegetation; and (B) Preserved vegetation in the Bom Retiro Private Natural Heritage Reserve (RPPNBR), Casimiro de Abreu, Rio de Janeiro state, Brazil.

study was carried out in a remnant of the Atlantic Forest, considered an area of environmental preservation, the species seems to be capable of colonizing artificial habitats in the region studied. Rezende et al. (2011) reported a high density of *Li. durhamii* in artificial containers in the Atlantic Forest in the city of Linhares, Espírito Santo. These results are consistent with Almeida et al. (2020), who reported that plastic containers were colonized by a great diversity of mosquitoes, suggesting that the species were eclectic in their choice for oviposition habitat, successfully establishing in artificial breeders.

Aedes albopictus showed an eclecticism of oviposition, with specimens present in all larval habitats in the two sampling locations and more abundant in artificial vessels. This species grows in natural and artificial containers and has been present in urban and suburban areas of all Brazilian regions (Carvalho et al. 2014). The presence of *Ae. albopictus* can increase the risk for the transmission of arboviruses such as the Zika virus that has spread rapidly in Brazil (Vorou 2016). Therefore, systematic and continuous monitoring of the mosquito fauna in this area is critical because of their role as pathogen vectors.

Mosquitoes of the genus *Psorophora* are known to lay their eggs primarily in ephemeral pools of water (Forattini 2002). However, we found *Psorophora cingulata* Fabricius, 1609 in artificial containers, representing 17.58% of immatures found in artificial habitats. This result contrasts the findings of Lourenço-de-Oliveira et al. (1986), who reported *Psorophora* in ground pools only. Lozovei (2001) stated that some mosquito species show considerable plasticity for adapting to different breeding habitats. Finally, Alencar et al. (2021) evidenced that studies on the mosquito fauna could help assess the degree of environmental change that may occur in a given region.

Considering the variety of larval habitats present in the RPPNBR, we observed that the type of container (natural or artificial) could influence the composition and abundance of the mosquito fauna. Several species were collected in the water accumulated in nylon lids covering the plastic water tanks found in the area studied with more altered vegetation, where we also found higher species richness. Such nylon lids can promote an increase in mosquito populations in the environment surrounding the study area.

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Author Contributions

A.Q.B. and J.A. contributed with experimental design, data analysis and manuscript writing, C.F.M. and H.R.G.S. contributed with the organism identification and data analysis, J.S.S. and S.O.F.S. contributed with data analysis and manuscript review. All authors revised successive drafts of the manuscript. All authors read and approved the final manuscript.

References Cited

Aguiar, D. M., A. Q. Bastos, P. J. Leite, H. R. Gil-Santana, J. S. Silva, J. Alencar. 2020. Comparative analysis between sampling methods for immature mosquitoes in an Atlantic Forest fragment in Brazil. J. Am. Mosq. Control Assoc. 36: 245–248.

Alencar, J., Z. M. Fereira, C. M. Lopes, N. M. Serra-Freire, R. P. de Mello, J. d. o. s. S. Silva, and A. E. Guimarães. 2011. Biodiversity and times of activity of mosquitoes (Diptera: Culicidae) in the biome of the Atlantic Forest in the State of Rio de Janeiro, Brazil. J. Med. Entomol. 48: 223–231.

Alencar, J., F. Morone, C. F. Mello, H. R. Gil-Santana, and A. É. Guimarães. 2013. Immature mosquitoes (Diptera: Culicidae) in a eutrophic landfill tank from State of Rio de Janeiro, Brazil. Rev. Soc. Bras. Med. Trop. 46: 769–771.

Alencar, J., C. F. de Mello, A. É. Guimarães, H. R. Gil-Santana, J. S. Silva, J. R. Santos-Mallet, and R. M. Gleiser. 2015. Culicidae community composition and temporal dynamics in Guapiaçu Ecological Reserve, Cachoeiras de Macacu, Rio de Janeiro, Brazil. PLoS One. 10: e0122268.

Alencar, J., C. F. de Mello, N. M. Serra-Freire, A. É. Guimarães, H. R. Gil-Santana, and R. M. Gleiser. 2016. Biodiversity and temporal distribution of immature Culicidae in the Atlantic Forest, Rio de Janeiro State, Brazil. PLoS One. 11: e0159240.

Alencar, J., C. F. de Mello, L. Rodríguez-Planes, J. dos Santos Silva, H. R. Gil-Santana, A. Q. Bastos, H. G. Albuquerque, W. C. Rodrigues, and A. É. Guimarães. 2021. Ecosystem diversity of mosquitoes (Diptera: Culicidae) in a remnant of Atlantic Forest, Rio de Janeiro state, Brazil. Austral. Entomol. 60: 244–256.

Almeida, J. F., H. C. M. Belchior, C. M. Ríos-Velásquez, and F. A. C. Pessoa. 2020. Diversity of mosquitoes (Diptera: Culicidae) collected in different types of larvitraps in an Amazon rural settlement. PLoS One. 15: 1–15.

Ayres, M., Jr. M. Ayres, D. L. Ayres, and A. A. Santos. 2007. BioEstat: aplicações estatísticas nas áreas das ciências biológicas e biomédicas. Sociedade Civil Mamirauá: Ministério da Ciência e Tecnologia, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Belém, Brasil, pp.1–364.

Berger, K. 2020. The man who saw the pandemic coming - Marcellus. (https://marcellus.in/story/the-man-who-saw-the-pandemic-coming/).

Carvalho, R. G., R. Lourenço-de-Oliveira, and I. A. Braga. 2014. Updating the geographical distribution and frequency of Aedes albopictus in Brazil with remarks regarding its range in the Americas. Mem. Inst. Oswaldo Cruz. 109: 787–796.

Consoli, R. A. G. B., and R. Lourenço-de-Oliveira. 1994. Principais Mosquitos de Importância Sanitária no Brasil - Fundação Oswaldo Cruz (Fiocruz): Ciência e tecnologia em saúde para a população brasileira. FIOCRUZ, Rio de Janeiro.

Correa, F. F., R. M. Gleiser, P. J. Leite, E. Fagundes, H. R. Gil-Santana, C. F. Mello, R. Gredilha, and J. Alencar. 2014. Mosquito communities in Nova Iguaçu Natural Park, Rio de Janeiro, Brazil. J. Am. Mosq. Control Assoc. 30: 83–90.

Forattini, O. P. 1965. Entomologia médica. Culicini: Culex, *Aedes e Psorophora*. Mosquito Taxonomic Inventory, São Paulo.

Forattini, O. P. 2002. Culicidologia Médica: Identificação, Biologia, Epidemiologia. Edusp - Editora da Universidade de São Paulo, São Paulo.

Guedes, M. P. 2012. Culicidae (Diptera) in Brazil: relationships between diversity, distribution and diseases. Oecol. Aust. 16: 283–296.

Guimarães, A. E., M. Arlé, and R. N. M. Machado. 1985. Mosquitoes in Serra dos Órgãos National Park, State of Rio de Janeiro, Brazil. II. vertical distribution. Mem. Inst. Oswaldo Cruz. 80: 1–2.

Guimarães, A. É., R. P. De Mello, C. M. Lopes, and C. Gentile. 2000a. Ecology of mosquitoes (Diptera: Culicidae) in areas of Serra do Mar State Park, State of São Paulo, Brazil. I - monthly frequency and climatic factors. Mem. Inst. Oswaldo Cruz. 95: 1–16.

Guimarães, A. É., C. Gentile, C. M. Lopes, A. Sant'Anna, and A. M. Jovita. 2000b. Ecology of mosquitoes (Diptera: Culicidae) in areas of Serra da Bocaina National Park, Brazil. I - Habitat distribution.Rev. Saude Publica. 34: 243–250.

Hammer, Ø., Harper D., and Ryan P. 2001. PAST: paleontological statistics software package for education and data analysis. Palaentol. Electronica. 4: 1–9.

IBM, S. P. S. S. 2015. Ibm Spss statistics version 23. International Business Machines Corp., Boston.

INPE. 2020. INPE - National Institute for Space Research. (http://www.inpe.br/).

Jesus, G. M. De. 2015. Ecological aspects of mosquitoes (Diptera: Culicidae) in an urban forest fragment and its vector implications in the municipality of Taubaté, State of São Paulo, Brazil. p. 115.

Lane, J. 1953. Neotropical Culicidae, vol. 1. ed. University of São Paulo, São Paulo.

Lopes, J. 1997. Ecology of mosquitoes (Diptera: Culicidae) in natural and artificial breeding sites in a rural area of Northern Paraná State, Brazil. V. Larva collection in artificial containers installed in riparian forest. Rev. Saúde Públ. 31: 370–377.

Lopes, J., and A. L. Lozovei. 1995. Ecology of mosquitoes (Diptera: Culicidae) in natural and artificial breeding sites in a rural area of Northern Paraná State, Brazil: l - collections along the ribeirão stream bed. Rev. Saude Publica. 29: 183–191.

Lopes, J., M. A. N. da Silva, V. D. R. B. de Oliveira, Â. M. Borsato, and M. C. P. Braga. 1995. Ecology of mosquitoes (Diptera: Culicidae) in natural and artificial breeding sites in a rural area of northern Paraná State, Brazil, III. Viability of containers as breeding sites. Semin. Ciências Biológicas e da Saúde. 16: 244–253.

Lourenço-de-Oliveira, R., R. Heyden, and T. F. da Silva. 1986. Some aspects of the ecology of mosquitoes (Diptera, Culicidae) of an area of plains (granjas Calábria), in Jacarepaguá, Rio de Janeiro: V. Breeding places. Mem. Inst. Oswaldo Cruz. 81: 265–271.

Lozovei, A. L. 2001. Microhabitats de Mosquitos (Diptera, culicidae) em Internódios de Taquara na Mata Atlântica, Paraná Brasil. Iheringia, Série Zool. 90: 3–13.

Marques G. R. A. M., and O. P. Forattini. 2008. Culicidae in bromeliads: diversity of species by anthropic environments, coastal area of Southeastern Brazil. Rev. Saúde Pública. 42: 979–1985.

Marris, E. 2010. Conservation: Biodiversity as a bonus prize. Nature. 468: 895.

Medeiros-sousa, A. R., W. Ceretti-junior, and P. R. Urbinatti. 2013. Biodiversity of mosquitoes (Diptera: Culicidae) in parks in the city of São Paulo I introduction material and methods results. 13: 317–321.

Ministério do Meio Ambiente. 2014. Plano de Manejo da Reserva Particular do Patrimônio Natural Bom Retiro, capitulo 2. Ed. Laboratório de Ecologia Aplicada, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil. p. 159.

Müller G. A., and C. B. Marcondes. 2006. Bromeliad-associated mosquitoes from Atlantic forest in Santa Catarina Island, southern Brazil (Diptera, Culicidae), with new records for the State of Santa Catarina. Iheringia Sér. Zool. 96: 315–319.

Rezende, A. R., T. M. das Virgens, M. A. Liberato, F. I. Valente, A. Fernandes, and P. R. Urbinatti. 2011. Ecological aspects of immature mosquito in larvitraps in forest and adjacent anthropic environment in the municipality of Linhares, State of Espírito Santo, Brazil. Epidemiol. Serv. Saúde. 20: 385–391.

Roberts, D. R., and B. P. Hsi. 1979. An index of species abundance for use with mosquito surveillance data. Environ. Entomol. 8: 1007–1013.

Schobbenhaus, C. 1995. Mapa Geológico do Brasil e da Área Oceânica Adjacente Incluindo Depósitos Minerais, escala 1:2.500.000. MME/DNPM, Brasília.

Service, M. 1976. Mosquito Ecology. Field Sampling Methods, 3rd ed. Springer, Dordrecht.

Valencia, J. D. 1973. Mosquito studies (Diptera, Culicidae) XXXI. A revision of the subgenus *Carrollia of Culex*. Contrib. Amer. Ent. Inst. 9: ii + 1–134.

Vorou, R. 2016. Zika virus, vectors, reservoirs, amplifying hosts, and their potential to spread worldwide: what we know and what we should investigate urgently. Int. J. Infect. Dis. 48: 85–90.