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BIONOMY OF MOSQUITOES IN BAMBOO INTERNODES IN AN ATLANTIC FOREST REMNANT OF THE STATE OF RIO DE JANEIRO, BRAZIL

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ABSTRACT. Living bamboo stalks are one of the most specialized habitats for mosquito oviposition and immature development. Most of the mosquito species that breed in these habitats are sylvatic, and some are of importance for public health as possible vectors of pathogens. Perforated internodes are a very specialized environment due to the difficulty of access. Furthermore, due to their relatively simple fauna, they represent a valuable model for ecological studies that may be applicable to more complex environments. This study aims to assess the mosquito bionomics of species raised in bamboo internodes. Therefore, the diversity of mosquito species and the influence of abiotic variables (pH and temperature) on the distribution of mosquitoes that breed in this habitat were analyzed. The study area is a fragment of Atlantic Forest within the Association of da Armada (ATA) in Nova Iguaçu, state of Rio de Janeiro, Brazil. Immature mosquitoes were sampled with suction tubes (mouth aspirators) between August 2017 and July 2018. A total of 3,170 larvae were collected in 5 bamboo plants, each with 8 stalks perforated. Of these, 688 larvae reached the adult stage, representing 10 genera and 19 species. The most common species were *Culex neglectus* (43%), *Trichoprosopon digitatum* (22%), *Culex iridescens* (8%), *Sabethes identicus* (7%), and *Orthopodomyia albicosta* (7%). The richness of the immatures collected in the ATA was 19 species, with a diversity of 1.10 and Shannon evenness of 0.57. A diverse composition of Culicidae in bamboo stalks was found, although dominance was low.

KEY WORDS Bamboo internodes, Culicidae, ecology, larvae, natural breeding

INTRODUCTION

Culicid species breed in a wide variety of sites, including bromeliad pools, bamboo internodes, and tree holes, which can significantly range in size and water volume (Lopes 1997, Lozovei 2001). While several species can adapt to considerably different habitats, others are more restricted in their choice of breeding sites (Service 1976). The use of sylvan tree cavities (dendrotelmata) by mosquitoes is restricted to specific natural breeding sites formed by small habitats such as bamboo internodes, which are common in the tropics and subtropics (Lozovei 2001). Several species of native or introduced bamboo, mainly those of the genus *Bambusa*, can be found in fragments of the Atlantic Forest in southern Brazil (Filgueiras and Santos-Gonçalves 2004). When perforated, these bamboos can serve as microhabitats if invaded by sylvatic fauna; they are considered one of the most specialized habitats for the development of some immature forms of mosquitoes (MacDonald and Traub 1960).

In Japan, Moriya (1974) investigated the distribution of dendritic species according to the seasons of the year, using ovitraps made with bamboo internodes. In the USA, Walker et al. (1991) studied the physicochemical factors of water in tree hollows and observed their interaction with biotic processes. In Brazil, Lozovei and Luz (1976), Gomes et al. (1992), Lopes (1997), Lozovei (1998, 2001), Silva and Lozovei (1999), Zequi and Lopes (2001), Silva et al. (2004), Zequi et al. (2005), Marcondes et al. (2009), Muller et al. (2009), Ceretti-Junior et al. (2014, 2020), Maia et al. (2020), and Medeiros-Sousa et al. (2020) during the study in 4 urban parks in the city of São Paulo, Brazil, of collected water from the side holes made in the bamboo culm.

In a study performed in northeast Argentina, Campos (2013) reported that bamboo internodes are more stable than tree holes or bamboo stumps due to the opening being located at a lateral hole in the internodes where rainwater enters. These breeding sites do not depend on the length of the internode but rather on the height of the lateral opening.

Zequi and Lopes (2001) reported that water accumulates in the internodes due to rain running down the bamboo stem, penetrating the holes made by the insects. Bamboo groves and native taquara

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bamboo (Merostachys sp.) are also frequently cut down by humans, creating openings near the base of their stalks and leading to rainwater accumulation (Silva et al. 2004). The water carries organic particles that feed the larvae and promote the development of microorganisms that also become a source of nutrients for filtering larvae. According to Marques and Forattini (2008), in a study conducted at the municipality of Ilhabela, north coast of the state of São Paulo, the larvae are subject to macrohabitat variations, mainly due to rainfall, as they live in an aquatic environment. The immature populations will occur in internodes of green thatches if they have water in their interior and one or more lateral holes to allow female oviposition (Lozovei 1998).

The development and survival of immature stages are influenced by environmental characteristics and physicochemical factors of the habitat. These include the availability of food resources, microorganisms, pH, temperature, salinity, and composition and content of organic matter, as well as intra- and interspecific interactions such as competition, predation, and mutualism (Juliano 2009, Yee et al. 2010).

This study aims to assess the composition of the mosquito fauna that breeds in bamboo internodes in an Atlantic Forest remnant in Nova Iguaçu, state of Rio de Janeiro, Brazil. We thus identified and compared the diversity of immature mosquitoes and evaluated the influence of abiotic factors on their population distribution throughout the sampling period.

MATERIALS AND METHODS

Study area

Nova Iguaçu is a city in the metropolitan region of Rio de Janeiro State, Brazil, with an area of 524.04 km^2 and located northwest and approximately 28 km from the city of Rio de Janeiro, with an area covered mainly by the typical vegetation of the Atlantic Forest. Around 67% of its territory is composed of environmental reserves, sheltering a large hydrographic basin having as main rivers the Iguaçu and Guandu (Prefeitura Municipal de Nova Iguaçu 2017). The average annual temperature of the region is 30.2°C.

The Atlantic Forest has been intensively fragmented and destroyed due to the exploitation of its resources since the early 16th century. Nonetheless, it remains one of the most biodiverse forests worldwide, with record levels of species diversity per hectare (Lino and Simões 2004).

Sampling points

Sampling was carried out for 1 year, from August 2017 to July 2018, at the Associação dos Taifeiros da Armada (ATA) in the city of Nova Iguaçu, state of Rio de Janeiro, Brazil (22°62′52.05″S, 43°45′49.14″W) (Fig. 1). According to the Köppen–Geiger classification, the local climate is tropical (Aw), characterized by a rainy summer and dry winter (PMRebio Tinguá 2006). The collection point is surrounded by bamboo, trees measuring approximately 30 m in height, lianas, and low-growing epiphytes; there is a waterfall about 10 m away (PMRebio Tinguá 2006).

Immature mosquitoes were collected in bamboo plants using suction tubes. Five bamboo plants were drilled with 8 holes in the ¾ portion of the thatch of each plant in order to obtain the greatest amount of water from the breeding site. A total of 40 samples were collected monthly.

Sampling started 15 days after the drilling and was repeated monthly for 12 months. The water collected was poured into a polyethylene winnower, and the temperature and pH were measured. Any larvae and pupae found were pipetted and placed in 250-ml plastic bags (Whirl-Pak Bags®, Madison, WI) and labeled according to their location, date, and type of breeding. They were then transported to the laboratory for screening of larvae. For this purpose, the content of each sample was transferred to small individual vats. Larvae were kept alive with water from the breeding site, which was periodically supplemented with dechlorinated water in a controlled experimental environment (greenhouse) regulated at a temperature of 28° ± 1°C, RH from 75% to 90%, and a photoperiod of 12 h light and 12 h dark.

Adults reared from the larvae were observed under a stereoscopic microscope for the observation of the morphological characters. Identification at the species level was based on the dichotomous keys and redescriptions made by Lane (1953a, 1953b), Consoli and Lourenço-de-Oliveira (1994), and Forattini (2002). Abbreviations of generic and subgeneric names follow Reinert (2009).

Statistical analysis

To evaluate and compare differences in the composition of mosquito populations, the Shannon–Wiener diversity index (H), richness (S), dominance (D), and Shannon equitability index (J) were used (Shannon 1948). H and S were chosen to measure the occurrence of random species in a community or subcommunity. J refers to the distribution of individuals belonging to each species, proportional to diversity, and inversely proportional to dominance (D). In addition, the significance of these interrelationships was tested using the correlation coefficient (0.5 confidence limit) to analyze the possible influence of climatic factors (pH and temperature of the water in the breeding site) on the population density of the culicid fauna for each sampling month.

In order to assess whether there was dominance of any species among the mosquitoes found in the ATA, the Kruskal–Wallis test was performed to compare the means of the number of individuals of each species collected per month. The influence of abiotic factors (temperature and pH) on mosquito abundance

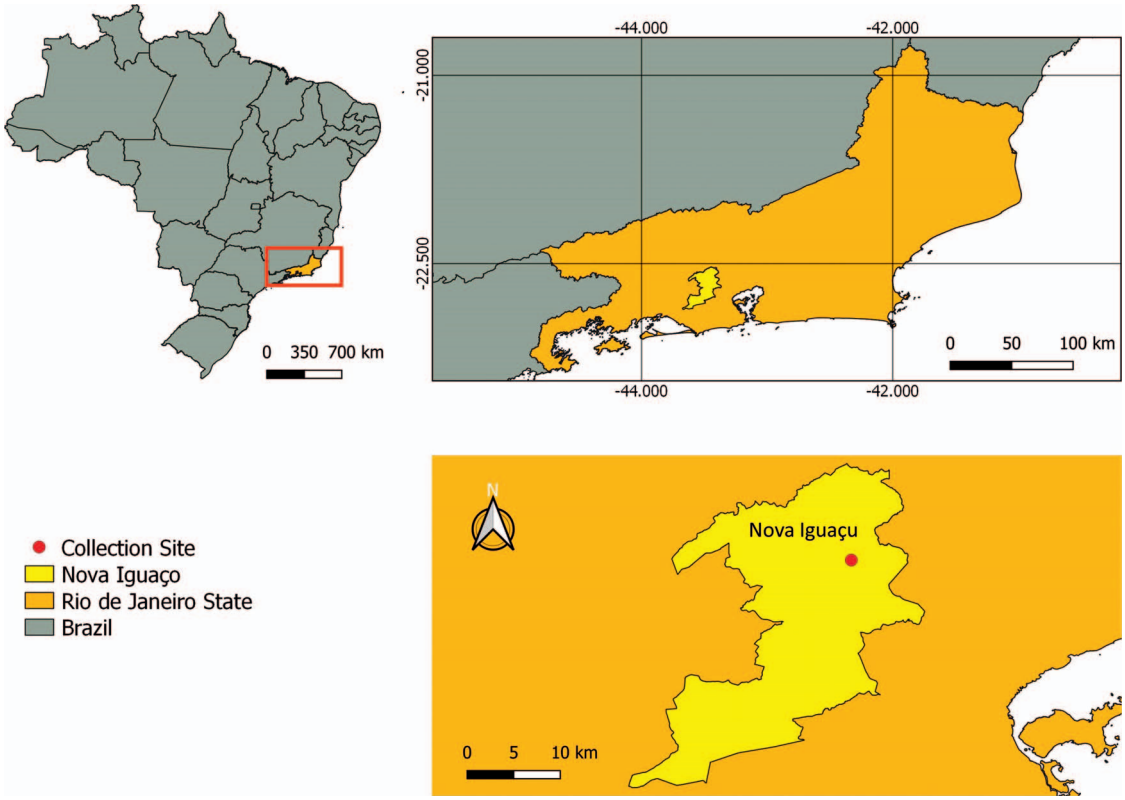

Fig. 1. Sampling sites in the Associação dos Taifeiros da Armada (ATA) in the city of Nova Iguaçu, state of Rio de Janeiro, Brazil.

was observed through a canonical correspondence analysis and a simple linear regression test using Past software version 4.05 (Galway, Ireland).

RESULTS

The 688 specimens from the ATA that were reared to the adult stage belonged to 16 species and 10 genera: *Culex neglectus* Lutz (43% of individuals collected), *Trichoprosopon digitatum* (Rondani) (22%), *Cx. iridescens* (Lutz) (8%), *Sabethes identicus* (Dyar and Knab) (7%), *Orthopodomyia albicosta* (Lutz) (7%), *Tr. pallidiventer* (Lutz) (3.5%), *Wyeomyia codiocampa* (Dyar and Knab) (2.9%), *Toxorhynchites* sp. (1.3%), *Wy. arthrostigma* (Lutz) (1.2%), *Limatus durhamii* (Theobald) (0.6%), *Wyeomyia* sp. (0.6%), *Haemagogus leucocelaenus* (Dyar and Shannon) (0.4%), *Onirion personatum* (Lutz) (0.4%), *Wy. oblita* (Lutz) (0.4%), *Aedes albopictus* (Skuse) (0.3%), *Culex* sp. (0.3%), *Li. pseudomethysticus* (Bonne-Wepster and Bonne) (0.3%), *Cx. imitator* Theobald (0.1%), and *Wy. aporonoma* (Dyar and Knab) (0.1%) (Table 1).

The months with the highest diversity (H index) were November 2017 (1.75), June 2018 (1.55), October 2017 (1.43), July 2018 (1.22), and September 2017 (1.10). In contrast, the months with the lowest H values were March 2018 (0.47), August 2017 (0.64), and January 2018 (0.74). March 2018 had the highest dominance index (D = 0.83) with a prevalence of *Cx. neglectus* ($n = 59$) species, representing 91% of the Culicidae collected in that month. On the other hand, the greatest richness was found in May 2018 (S = 11), with a high diversity index (H = 1.40) and evenness (J = 0.58) (Table 1).

The Kruskal–Wallis test showed an extremely significant difference between the species ($P < 0.001$). *Culex neglectus* had the highest number of individuals ($n = 297$), representing 49% of all mosquitoes collected in the ATA. This species was significantly more abundant ($P < 0.05$) than all other species collected, except for *Or. albicosta* ($P = 0.177$).

The canonical correspondence graph depicts the distribution of species around the abiotic variables—pH and temperature—of the breeding sites (bamboo plants) in which they were collected (Fig. 2). It was found that the greater the proximity of the culicid species with the vectors (green lines) of the analyzed biotic variables, the stronger their interaction. The species most associated with temperature are located on the left side of the graph, including *Ae. albopictus*, *Li. durhamii*, *Wy. aporonoma*, *Cx. neglectus*, *Cx. iridescens*, *Wy. codiocampa*, *On. personatum*, and

Table 1. Monthly distribution of mosquito species, including absolute values (*N*) of specimens collected, dominance (D%), specific richness (S), Shannon–Weiner diversity index (H), and Shannon equitability (J) in bamboo in the Associação dos Taifeiros da Armada, city of Nova Iguaçu, state of Rio de Janeiro, Brazil, between August 2017 and July 2018.

Species	2017					2018							Total
	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	
Aedes albopictus	0	0	0	0	0	0	0	0	0	1	0	1	2
Culex iridescens	0	3	0	0	0	0	1	1	10	0	24	17	56
Culex sp.	0	0	0	0	0	0	1	0	0	0	0	1	2
Cx. imitator	0	0	0	0	0	1	0	0	0	0	0	0	1
Cx. neglectus	0	0	2	9	0	6	34	59	71	41	26	49	297
Hemagogus leucocelaenus	0	0	2	0	0	0	0	0	0	1	0	0	3
Limatus durhamii	0	0	0	0	0	0	0	0	1	3	0	0	4
Li. pseudomethysticus	0	0	0	0	0	0	0	1	1	0	0	0	2
Onirion personatum	0	0	0	0	0	0	1	0	0	2	0	0	3
Orthopodomyia albicosta	4	2	1	1	0	0	1	0	0	8	17	13	47
Sabethes identicus	12	26	2	5	0	1	1	0	0	2	0	0	49
Toxorhynchites sp.	1	1	1	1	0	0	1	1	0	0	2	1	9
Trichoprosopon digitatum	71	57	22	1	0	0	0	0	2	0	0	0	153
Tr. pallidiventer	0	1	8	5	0	0	0	1	0	4	4	1	24
Wyeomyia aporonoma	0	0	0	0	0	0	0	0	1	0	0	0	1
Wy. arthrostigma	0	5	0	0	0	0	0	0	0	1	2	0	8
Wy. codiocampa	0	0	2	7	0	0	4	1	3	1	2	0	20
Wy. oblita	0	0	0	0	0	0	0	0	1	1	1	0	3
Wyeomyia sp.	0	0	0	1	0	0	0	1	0	0	0	2	4
N	88	95	40	30	0	8	44	65	90	65	78	85	688
S	4	7	8	8	0	3	8	7	8	11	8	8	16
H	0.64	1.10	1.43	1.75	0	0.74	0.93	0.47	0.83	1.40	1.55	1.22	—
D	0.67	0.44	0.35	0.20	0	0.59	0.61	0.83	0.64	0.42	0.26	0.40	—
J	0.46	0.57	0.69	0.84	0	0.67	0.45	0.24	0.40	0.58	0.75	0.59	—

Or. albicosta. On the other hand, the species most associated with pH are on the right side of the graph; these include *Hg. leucocelaenus*, *Tr. pallidiventer*, *Tr. digitatum*, *Sa. identicus*, and *Wy. arthrostigma* (Fig. 2).

The simple linear regression test showed that *Tr. pallidiventer* ($P = 0.009$) was significantly and positively correlated with pH ($P \leq 0.001$). *Sabethes identicus* also had a significant positive correlation ($P \leq 0.05$) with this variable ($P = 0.01$). *Onirion*

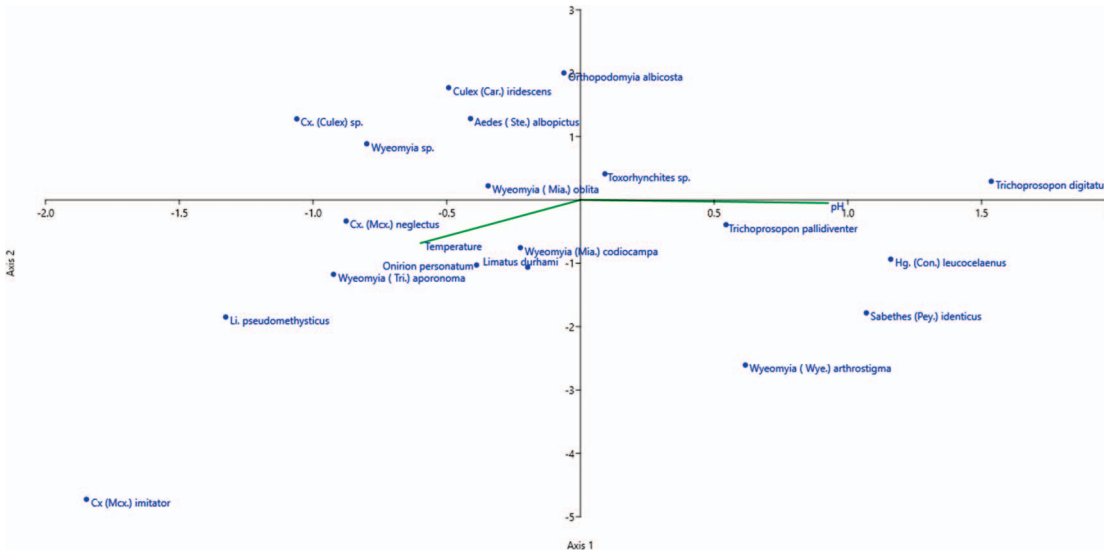

Fig. 2. Canonical correspondence. The green vectors indicate the direction and proximity of climatic variables in relation to each studied species. The closer these vectors are to the species, the stronger the interaction between the two.

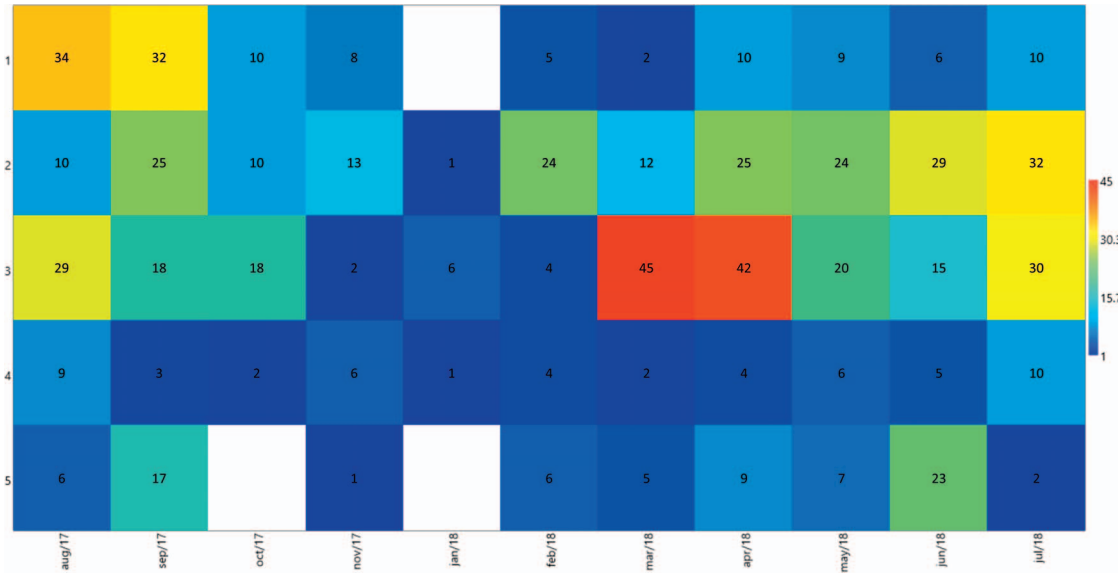

Fig. 3. Matrix plot of Culicidae collected in each bamboo 1 to 5, from August to November of 2017 and January to July of 2018.

personatum also showed a positive correlation with temperature. It should be highlighted that there were no positive correlations or statistically significant associations with the variables analyzed among the other species (Fig. 2).

Figure 3 shows the relationship of each mosquito species collected per month in each bamboo plant. In this matrix plot, the green, yellow, orange, and red squares indicate a greater number of mosquitoes collected in that monthly period, while the light and dark blue squares indicate a lower number of species, and white squares indicate the absence of species collected in that month (Fig. 3). It is noteworthy that, of the 5 bamboo plants from which collections were made each month, bamboo 1 had a high number of immature mosquitoes collected in August and September 2017, while the abundance of collected immatures decreased from October 2017 to July 2018 (Table 2).

Bamboo 2 had a low abundance of specimens from August 2017 to March 2018, apart from September 2017 and February 2018. However, from April 2018 onward, the number of specimens increased at this breeding site, with the highest numbers observed in June and July 2018. On the other hand, at bamboo 3, a high number of immatures was collected in March and April 2018, with a decrease from November 2017 to February 2018. Other sampled bamboos showed low numbers of individuals, except for bamboo 2, which also had a slight increase in the number of immatures during June and July 2018. Bamboos 4 and 5 showed a low abundance throughout the collection period (Fig. 3).

According to the data from the dendrogram performed on all bamboo plants analyzed, bamboos 4 and 5 showed the highest degree of similarity in species composition relative to the other bamboo samples. Likewise, bamboos 2 and 3 were also very similar to each other. Bamboo 1 was closer, or more similar, to bamboos 4 and 5 and further, or more different, from bamboos 2 and 3 (Fig. 4).

DISCUSSION

Studying the diversity of mosquitoes in the Atlantic Forest is crucial for evaluating changes in the pattern of activities of their populations. However, few studies have been conducted on the immature forms of mosquitoes colonizing bamboo internodes and tree hollows, which are similar biotopes in terms of their aquatic substrates and microhabitats. Considering that the environmental characteristics of these microhabitats are essential for species development, studies on the culicid fauna that use them provide insights into the survival and persistence of these insects during climatically unfavorable periods, especially during the low-rain period.

No marked tendency of a regular abundance of species during collections was observed. Lozovei (1998) found 17 species of dendritic mosquitoes in 2 sites of the Pico do Marumbi State Park, Serra do Mar, southern Atlantic Forest, a richness value that is very close to our findings (19 species in bamboo stalks in the ATA).

Alencar et al. (2010) reported that bamboo internodes had a higher frequency of immature mosquitoes compared with other breeding sites in the Serra do Mar State Park, in the state of São Paulo. Campos (2016) reported that mosquito species richness was greater in stumps than in bamboo

Table 2. Monthly abundance of immature mosquitoes collected in the Associação dos Taifeiros da Armada, city of Nova Iguaçu, state of Rio de Janeiro, Brazil, between August 2017 and July 2018.

Bamboo	Aug	Sept	Oct	Nov	Jan	Feb	Mar	Apr	May	Jun	Jul
1	34	32	10	8	0	5	2	10	9	6	10
2	10	25	10	13	1	24	12	25	24	29	32
3	29	18	18	2	6	4	45	42	20	15	30
4	9	3	2	6	1	4	2	4	6	5	10
5	6	17	0	1	0	6	5	9	7	23	2

internodes, and the abundance was higher in internodes than in bamboo stumps. Our study showed a greater richness than abundance in the internodes of the 5 bamboo plants collected.

The combination of dendritic fauna and water retention in bamboo internodes results in mosquito breeding sites in bamboo culms that are isolated from the environment. The success of these species depends on the physical space and adequate volumes of water so that the larvae can escape from predators (Lozovei 1998).

Culex neglectus was the dominant species, representing 43% of the specimens collected. Marques and Forattini (2008) state that the species must exert control over other species in order to become dominant and thus be more abundant, indicating its ecological success in the community. In our study, *Cx. neglectus* was the most abundant species, with 297 specimens collected, thus differing from the results of Silva et al. (2004), who found a greater abundance of *Li. durhamii* in bamboo internodes, in the Atlantic Forest biome, Paraná State, Brazil. Marques and Forattini (2008) collected mosquitoes from bromeliads and found 2 dominant species: *Cx. pleuristriatus* (Theobald) and *Cx. ocellatus* (Theobald). Representatives of the subgenus *Microculex* are usually found colonizing breeding sites represented by permanent natural containers such as bromeliads, tree holes, and bamboo internodes (Forattini 1965).

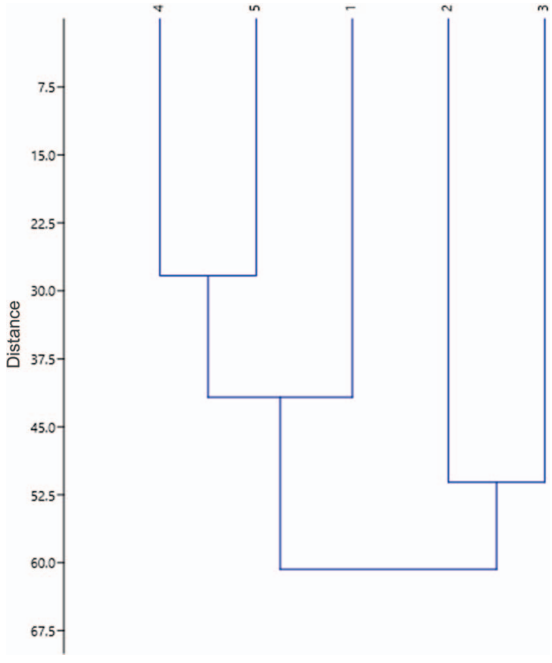

Fig. 4. Dendrogram of the bamboos in which immature samples were collected in the Associação dos Taifeiros da Armada (ATA) Preservation area, city of Nova Iguaçu, state of Rio de Janeiro, Brazil, from August 2017 to July 2018.

Trichoprosopon digitatum was the second-most abundant species in this study, with 153 specimens collected in bamboo internodes. In the North region of the state of Paraná, Brazil, Lopes (1997) collected *Tr. compressum* (Lutz) and *Tr. pallidiventer* only in bamboo containers, albeit with low frequency and abundance. In the city of Londrina, Paraná State, Brazil, Zequi et al. (2005) reported that *Tr. compressum* was not found colonizing tires but only bamboo internodes. Both previous studies suggested that these species have sylvatic characteristics related to reproductive requirements, although with the potential to colonize urban areas. On the other hand, our results differ from those of Ceretti-Júnior et al. (2014), who reported species of *Wyeomyia* as the most prevalent in internodes of perforated bamboos and that *Tr. pallidiventer* and *Tr. digitatum* prevailed in cross sections of bamboo plants.

Some species of *Haemagogus* develop in hollow trees but can also be found in bromeliads and the punctured internodes of bamboos (Forattini 2002). In this study, the water from the bamboo stalks usually had a cloudy aspect and a slightly unpleasant odor, probably due to the decomposition of organic matter. Moreover, pH values varied widely, with values ranging from 2.64 to 7.8 (average of 5.51). On the other hand, Lozovei (1998) stated that the immature forms that coexist in living green internodes choose the types of holes based on their bionomic requirements, usually opting for internodes with clear water, without sediment, with a pleasant odor, and with a pH varying from acidic to neutral, ranging between 3.50 and 7.0.

Haemagogus leucocelaenus showed low abundance throughout the collection period. When Cerretti-Júnior et al. (2014) evaluated immatures that developed in bamboo internodes in environmental preservation areas in the state of São Paulo, they also recorded a low abundance of this species.

Despite their finding, the authors recommended continuous monitoring.

The results of our canonical correspondence analysis showed that *Ae. albopictus* was associated with temperature. Docile et al. (2017) observed that this species had a strong association with the temperature and pH when collected in bromeliads.

Medeiros-Sousa et al. (2013) stated that some species of mosquitoes collected in areas of city parks show predatory behavior, as is the case of *Toxorhynchites*, which play the role of immature controllers in their breeding sites. Zequi and Lopes (2001) reported that predation is an important factor driving insect population dynamics in breeding sites. Lozovei (1998) observed a low number of *Toxorhynchites* specimens in bamboo internodes, at Taquara da Floresta Atlântica, Serra do Mar and the geomorphological unit of Primeiro Planalto, Paraná State, Brazil. Campos (2013, 2016) found a single *Toxorhynchites* larva per vessel in northeastern Argentina, which may be due to the ecological relationship of intraspecific cannibalism. In the present study, few specimens of *Toxorhynchites* were also collected in 12 sampling months, with no specimens detected in 3.

Limatus durhamii is considered a nonobligatory predator in the absence of food or casual encounter with the prey (Medeiros-Sousa et al. 2013). We detected no *Toxorhynchites* in 3 sampling months in which *Li. durhamii* was found, suggesting that this species may be occupying the ecological niche in the absence of the main predator. Ceretti-Júnior et al. (2014) reported that the larvae of *Tr. pallidiventer* and *Tr. digitatum* found in bamboo are predators and cannibals. Lopes (1997) stated that *Tr. pallidiventer* was collected only in bamboo containers, which is useful for recording the occurrence of this species as it did not accept another container.

This study was conducted in an ecosystem full of habitats for numerous species of macroinvertebrates. Therefore, further studies are necessary to recognize the species that cohabit with mosquitoes in bamboo. A richness value of 19 species of mosquitoes was found inhabiting bamboo microhabitats. Gomes et al. (2007) reported that richness and abundance are related to the adaptive value of culicids and domiciliation processes. The presence of humans and domestic animals can therefore influence the predominance of species.

The species found that are identified as potential vectors of etiological agents, namely *Ae. albopictus*, *Hg. leucocelaenus*, and *Sa. identicus*, showed a low abundance.

The bamboo internodes analyzed in the ATA area constituted a specialized microhabitat for some species of mosquitoes found during the sampling period due to their restricted access. Since they represent a restricted environment with relatively simple fauna, bamboos are a useful model for bioecological studies, possibly applicable to more complex environments.

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