

Research Article

Mosquitoes (Diptera: Culicidae) in oviposition traps set in forest fragments of a semideciduous seasonal forest, Atlantic Forest domain, in the state of Rio Grande do Sul, Brazil

Mosquitos (Diptera: Culicidae) en trampas de oviposición puestas en fragmentos de selva estacional semidecidua de dominancia Atlántica, Estado de Rio Grande do Sul, Brasil

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Abstract. This study aimed to compare the mosquito fauna found in three remnant forest fragments of a semideciduous seasonal forest by using oviposition traps in the northwestern region of the state of Rio Grande do Sul, Brazil. Between 2018 and 2019, larvae were collected quarterly in three forest fragments, one situated in an urban area, another in a rural area close to the city and a third formed by an extensive area of native forest far from the urban environment. Four species were caught: *Aedes terrens* (Walker, 1856) (282), *Limatus durhamii* Theobald, 1901 (30), *Aedes aegypti* (Linnaeus, 1762) (23) and *Toxorhynchites theobaldi* (Dyar and Knab, 1906) (6). The Morisita-Horn index indicated a high similarity between the fragments studied (>80%). The mean number of mosquitoes collected in the rural area was significantly higher than in the other areas ($F = 6.073$; d.f. = 17; $p < 0.05$). The calculation of the ϕ coefficient to assess the co-occurrence of two species in the same trap did not indicate significant values ($p > 0.05$). *Aedes terrens* demonstrated a good ability to colonize and inhabit the different types of forest fragments studied. In addition, the forest fragment located in a rural area close to the urban area offered conditions for the survival of species of wild and urban mosquitoes, such as *Ae. aegypti*.

Key words: *Aedes*; larvae; *Limatus*; *Toxorhynchites*.

Resumen. Este estudio tuvo como objetivo comparar la fauna de mosquitos encontrada en tres fragmentos remanentes de un bosque estacional semideciduo a partir del uso de trampas de oviposición en la región noroeste del Estado de Rio Grande do Sul, Brasil. Entre los años 2018 y 2019 se recogieron larvas trimestralmente en tres fragmentos de bosque, uno insertado en una zona urbana, otro en una zona rural cercana a la ciudad y un tercero formado por una extensa zona de bosque nativo alejado del entorno urbano. Se capturaron cuatro especies: *Aedes terrens* (Walker, 1856) (282), *Limatus durhamii* Theobald, 1901 (30), *Aedes aegypti* (Linnaeus, 1762) (23) y *Toxorhynchites theobaldi* (Dyar y Knab, 1906) (6). El índice Morisita-Horn indicó una alta similitud entre los fragmentos estudiados (> 80%). El número medio de mosquitos recogidos en la zona rural fue significativamente mayor que en las otras zonas ($F = 6,073$; d.f. = 17; $p < 0,05$). Por otro lado, el cálculo del coeficiente phi para evaluar la co-ocurrencia de dos especies en la misma trampa no indicó valores significativos ($p > 0,05$). *Aedes terrens* mostró una buena capacidad para colonizar y habitar los diferentes tipos de fragmentos de bosque estudiados. Además, el fragmento de bosque insertado en una zona rural

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cercana al área urbana ofrecía condiciones para la supervivencia de especies de mosquitos silvestres y urbanos, como *Ae. aegypti*.

Palabras clave: *Aedes*; larva; *Limatus*; *Toxorhynchites*.

Introduction

The Atlantic Forest Domain, which involves different vegetation types, has a high diversity of mosquito species. Its topographic complexity and spatial variability, with the formation of different microclimates, allows mosquitoes to find habitats capable of sustaining them, promoting their proliferation (Pereira *et al.* 2006; Cardoso *et al.* 2011; Santos *et al.* 2020).

The process of hyperfragmentation of the Atlantic Domain landscapes has promoted the phenomenon of taxonomic homogenization of the biota, promoting the proliferation and dominance of groups of animal species with generalist characteristics (Lôbo *et al.* 2011). This forest fragmentation also affects mosquito communities, changing their composition, genetic diversity and behavior (Multini *et al.* 2020; Wilk-da-Silva *et al.* 2020).

The semideciduous seasonal forest, a type of vegetation belonging to the Atlantic Domain, and other Brazilian ecosystems as well, has undergone an intense process of degradation and fragmentation associated with the transformation of native vegetation into extensive areas for livestock or extensive farming (Lopes *et al.* 2012). The semideciduous seasonal forest, even in the face of this process of environmental degradation, still harbors a diverse fauna of mosquitoes that find breeding sites in water accumulated in the soil, in phytohelms and in artificial breeding sites, especially those formed by the disposal of irregular garbage in nearby forest areas or cities (Rezende *et al.* 2011; Silva *et al.* 2019).

Thus, understanding how anthropogenic changes in the semideciduous seasonal forest affect mosquito fauna, especially with regard to forest fragmentation, is essential for the development of more effective strategies to control potential vectors of arboviruses. This study aimed to compare the mosquito fauna found in three remnant forest fragments of semideciduous seasonal forest by using oviposition traps in the northwestern region of the state of Rio Grande do Sul, Brazil.

Materials and Methods

Study area. The study was conducted in three forest fragments located in the northwestern region of Rio Grande do Sul, Brazil (Fig. 1). These fragments are situated in an area of semideciduous seasonal forest in the Atlantic domain. All the selected areas have been cut down in the past, and currently, it is still possible to observe logging activity with selective cuttings of trees of economic interest.

The three collection points were in an altitude range between 450 and 500 m above sea level and ranged in size from 15.5 to 103 ha. The region has a climate classified as temperate, with the absence of a dry season and characterized by hot summers, making it subtype Cfa in the world climate classification of Köppen-Geiger (Peel *et al.* 2007).

The first fragment, located in the urban area (URB) (28°16'36.95"S; 53°31'10.34"W) of the city of Panambi-RS, was surrounded by a residential area of low population density with recent occupation. Also, in the vicinity of this fragment, other anthropized forest fragments were observed, crossed by roads, in addition to crop fields. This fragment had an area of 15.5 ha, consisting of a low canopy (up to 8 m) and a poorly developed understory with clearing areas. In addition, it was crossed by a stream with a width that varied between two and three meters, which received domestic effluents from nearby residences. The second fragment was

located in the rural area (RUR) (28°15'27.29" S; 53°28'41.20" W) of the same city, and it had an area of 17.5 ha, being surrounded by a large area of extensive farming and by a road. This fragment was 0.8 km from the urban area of the city of Panambi-RS. Its canopy was low (up to 8 m) and had many clearings, while its understory was poorly developed. The third sampled fragment was located in the city of Pejuçara-RS, situated in the rural area, but had a 103-ha area of native forest with different levels of preservation (NAT) (28°22'17.24" S; 53°41'45.42" W). In the most preserved areas within this fragment (secondary forest), where mosquito collections were carried out, the canopy reached 12 m in height. In addition, the understory showed marked stratification, with the presence of plant species at risk of extinction, for example, *Dicksonia sellowiana* Hook. (Cyatheales: Dicksoniaceae). This fragment was 4.8 km away from the urban area of the city of Pejuçara-RS.



Figure 1. Location of three fragments of a semideciduous seasonal forest in the northwestern region of the state of Rio Grande do Sul, Brazil. URB: forest fragment located in the urban area of the city of Panambi-RS. RUR: forest fragment located in the rural area close to the urban area of Panambi-RS. NAT: forest fragment located in the rural area far from urban area of the city of Pejuçara-RS. / Ubicación de tres fragmentos de un bosque estacional semideciduo en la región noroeste del estado de Rio Grande do Sul, Brasil. URB: fragmento de bosque ubicado en el casco urbano de la ciudad de Panambi-RS. RUR: fragmento de bosque ubicado en el área rural cercana al área urbana de Panambi-RS. NAT: fragmento de bosque ubicado en el área rural alejada del área urbana de la ciudad de Pejuçara-RS.

Procedures for collection and identification of specimens:

Mosquitoes were sampled once every three months in each of the three forest fragments (URB, RUR and NAT) between February 2018 and April 2019, totaling six collections in each area. In each fragment, six oviposition traps were placed at random, more than 10 m apart and positioned in trees at a height of 1.5 m from the ground. All traps were installed at a minimum distance of 100 m from the edge of the fragment.

The traps used were made with 1-L matte black pots without a lid. In each pot four vertical plates of 2.5 x 14 cm plywood (Eucatex® plates) were affixed with metal clips according to Alencar *et al.* (2016). In each trap, 0.5 L of distilled water was added with the addition of 10% grass (hay water) (Chadee *et al.* 1995), which was collected and replenished every three months.

The aquatic content of the traps and the Eucatex® plates were collected and taken to the laboratory to breed the immature forms of mosquitoes until adulthood in transparent plastic bottles using the water from the traps, rich in organic matter.

Specimen identification was based on morphological characters under a stereomicroscope. The dichotomous keys presented by Consoli and Oliveira (1994) and Forattini (2002) were used to identify the species. *Toxorhynchites* Theobald specimens were identified according with Darsie (1985), Stein *et al.* (2018) and from the original description (Dyar and Knab 1906) and by comparison with specimens from the laboratory's entomological collection. After identification, all specimens were deposited in the didactic zoological collection of the Federal Farroupilha Institute. The abbreviations for mosquito genera are those proposed by Reinert (2009).

Data analysis. The data obtained in this study were organized in spreadsheets using Microsoft Excel. To analyze the composition between forest fragments using relative abundances, a cluster analysis was performed with the Morisita-Horn index, which measures the probability that two individuals selected at random, each from a different location or group, are of the same species (Magurran 2004). This analysis and the construction of the cluster were performed by the statistical software PAST 4.03. One-way analysis of variance (ANOVA) and Tukey's comparison test were used to compare the means of culicids obtained between the three forest fragments using the statistical software GraphPad Prism 6. The *phi* coefficient was calculated to test whether there was a significant association (positive/negative) between species as described by Warrens (2008), using the statistical software BioEstat 5.0.

Results

A total of 341 specimens, belonging to four species, were caught; in decreasing order of abundance, they were: *Aedes terreus* (Walker, 1856), *Limatus durhamii* Theobald, 1901, *Aedes aegypti* (Linnaeus, 1762) and *Toxorhynchites theobaldi* (Dyar and Knab, 1906). *Aedes terreus* and *Li. durhamii* were recorded in the three forest fragments, the first being the most abundant in all of them. *Aedes aegypti* was found only in traps installed in the forest fragments URB and RUR, and *Tx. theobaldi* was found only in the fragments RUR and NAT (Tab. 1).

Table 1. Mosquito species collected (total specimens) in oviposition traps in three fragments of a semideciduous seasonal forest in the northwestern region of the state of Rio Grande do Sul, Brazil. / Especies de mosquitos recolectadas (total de especímenes) en trampas de oviposición en tres fragmentos de un bosque estacional semideciduo en la región noroeste del estado de Río Grande do Sul, Brasil.

Species	URB (%)	RUR (%)	NAT (%)	Total number (%)	Mean per trap (range)	Positive samples (%) ¹
<i>Ae. aegypti</i>	22 (31.0)	1 (0.5)	0 (0.0)	23 (6.7)	0.21 (0 - 15)	4 (3.7)
<i>Ae. terreus</i>	42 (59.1)	182 (91.9)	58 (80.5)	282 (82.7)	2.61 (0 - 24)	49 (45.4)
<i>Li. durhamii</i>	7 (9.9)	11 (5.6)	12 (16.7)	30 (8.8)	0.28 (0 - 3)	24 (22.2)
<i>Tx. theobaldi</i>	0 (0.0)	4 (2.0)	2 (2.8)	6 (1.8)	0.06 (0 - 2)	6 (5.6)
Total number	71 (100.0)	198 (100.0)	72 (100.0)	341 (100.0)		

¹ Samples indicate total number of traps and sampling dates positive for Culicidae

In RUR, all four species observed in this study were recorded. In URB and NAT, three species were found in each (Tab. 1). The Morisita-Horn index indicated a high similarity between the study fragments (>80 %), with RUR and NAT forming a cluster in relation to URB (Fig. 2). When comparing the mean abundances of mosquitoes collected in the three forest fragments, RUR showed significantly higher values compared to URB and NAT ($F = 6.073$; $d.f. = 17$; $p < 0.05$) (Fig. 3).

Up to two species were recorded co-occurring in the same breeding site/ trap (*Ae. terreus* and *Ae. aegypti*; *Ae. terreus* and *Li. duhamii*; *Li. duhamii* and *Tx. theobaldi*). The determination of the *phi* coefficient to assess the co-occurrence of two species in the same trap did not indicate significant values ($p > 0.05$), demonstrating a pattern of occurrence that does not depend on the presence or absence of other mosquito species at the breeding site (Tab. 2).

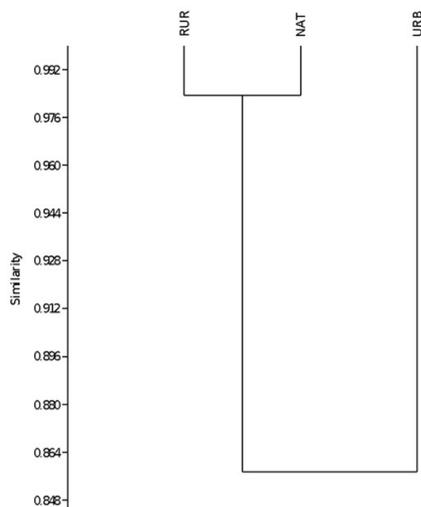


Figure 2. Cluster analysis (Morisita–Horn index) of the mosquitoes collected in oviposition traps in three fragments of a semideciduous seasonal forest in the northwestern region of the state of Rio Grande do Sul, Brazil. / Análisis de conglomerados (índice Morisita-Horn) de los mosquitos recolectados en trampas de oviposición en tres fragmentos de un bosque estacional semideciduo en la región noroeste del estado de Rio Grande do Sul, Brasil.

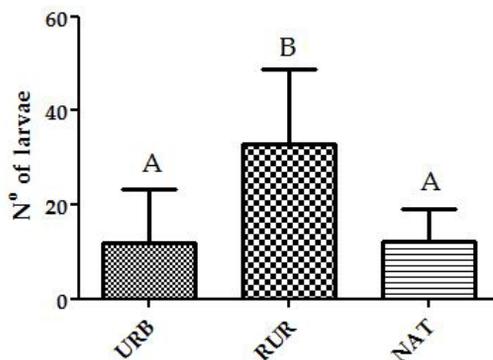


Figure 3. Mean abundance of mosquitoes collected in oviposition traps in three fragments of a semideciduous seasonal forest in the northwestern region of the state of Rio Grande do Sul, Brazil. Means followed by the same letter do not differ by the Tukey test at <0.05 . Vertical bars denote $1 \pm SD$. / Abundancia media de mosquitos recolectados en trampas de oviposición en tres fragmentos de un bosque estacional semideciduo en la región noroeste del estado de Rio Grande do Sul, Brasil. Las medias seguidas por la misma letra no difieren por la prueba de Tukey en $<0,05$. Las barras verticales denotan $1 \pm SD$.

Table 2. Comparison of occurrence of mosquito species in oviposition traps according to *phi* (\emptyset) coefficient. The significance of non-random co-occurrence was determined by the chi-square test ($X^2, \leq 0.05$). / Comparación de ocurrencia de especies de mosquitos en trampas de oviposición según coeficiente *phi* (\emptyset). La significancia de la co-ocurrencia no aleatoria se determinó mediante la prueba de chi-cuadrado ($X^2, \leq 0,05$).

	<i>Ae. aegypti</i>	<i>Ae. terrens</i>	<i>Li. duhamii</i>	<i>Tx. theobaldi</i>
<i>Ae. aegypti</i>	-	$\emptyset = 0.1190$; $X^2 = 0.5417$; $p > 0.05$	No record	No record
<i>Ae. terrens</i>	-	-	$\emptyset = 0.1908$; $X^2 = 3.1303$; $p > 0.05$	No record
<i>Li. duhamii</i>	-	-	-	$\emptyset = 0.0670$; $X^2 = 0.0377$; $p > 0.05$
<i>Tx. theobaldi</i>	-	-	-	-

Discussion

The knowledge of mosquito biodiversity in forest remnants is important for the prediction of possible occurrences of cases of arboviruses in human populations and their circulation in wild animal populations. Among the four recorded species, *Ae. aegypti* stands out for its epidemiological importance, as it is involved in the Dengue, Zika and Chikungunya epidemics that have occurred in Brazil in recent decades, in addition to being involved in the urban transmission cycle of the yellow fever virus (Kotsakiozi *et al.* 2017). *Aedes terrens* and *Li. durhamii*, in turn, seem to play a secondary role in the circulation of some arboviruses, such as the viruses Bunyaviridae and Zika (Forattini 2002; Fernandes *et al.* 2019). *Toxorhynchites theobaldi* is not involved in any transmission cycle of pathogens since their females do not feed on blood (Godoy *et al.* 2015).

Limatus durhamii and especially *Ae. terrens*, which was the most abundant species in the three fragments, showed the ability to inhabit forest fragments with different characteristics and inhabit different landscapes. These species can be found in a variety of natural and artificial breeding sites, having been recorded in both anthropized and preserved wild environments (Lopes 1997; Zequi *et al.* 2005; Alencar *et al.* 2016).

The occurrence of *Tx. theobaldi* in fragments located in wild environments (RUR and NAT), situated in landscapes of rural areas, and its absence in URB, contrasts with the results obtained by Silva and Lozovei (1999), who found this species in a forest fragment situated in an area urban of Curitiba-PR. Calderón-Arguedas *et al.* (2009) reported the occurrence of this species in a park located in an urban area of Puntarenas in Costa Rica, growing in artificial containers, including cans. This indicates that this species can occur both in wild environments and in anthropized environments.

The presence of *Ae. aegypti* in URB and the recording of a larva in RUR indicates that this species can occur in forest fragments, as long as they are situated in an anthropized environment. This species is considered adapted to synanthropism, being essentially urban (Wilke *et al.* 2017). Its occurrence in forest fragments situated in urban and rural areas is widely recorded in the literature, and it is less present in rural areas (*e.g.*, Zequi *et al.* 2005; Cox *et al.* 2007). The fact that the RUR fragment is closer to the urban area (0.8 km) when

compared to the NAT fragment (4.8 km), could explain the occurrence of *Ae. aegypti* in the former and its absence in the latter. This species, in infested urban environments, ends up not dispersing much, but under certain conditions, it can disperse for up to 800 m in search of hosts and places for oviposition (Reiter 1996). In addition, more extensive areas with more preserved forests, as observed in NAT, offer a selective pressure of resource competition and predation that disadvantage *Ae. aegypti* (Forattini 2002; Juliano 2007).

The highest mean numbers of larvae and the highest species richness were observed in the RUR fragment. This denotes the importance of forest remnants in areas of extensive farming for the maintenance of the fauna of Culicidae, since they serve as a refuge for wild vertebrate animals (Chiarello 2000), which serve as a source of food, and offer breeding facilities (e.g., tree hollows). These forest remnants also provide artificial breeding sites from the illegal disposal of garbage containing containers capable of accumulating water.

The high similarity observed between the mosquito communities of the three fragments studied (Morisita-Horn index >80%), even though they have visually distinct characteristics, indicated that the use of oviposition traps possibly resulted in a reduced number of species sampled, even in the NAT fragment, where a greater species richness was expected due to its level of preservation and size. In a study conducted by Silva *et al.* (2018) at the Bom Retiro Private Natural Heritage Reserve, state of Rio de Janeiro, oviposition traps were used at different points in the forest area, with only six species of mosquitoes being obtained. The formation of a cluster between the URB and RUR communities indicated that because they were fragments with a higher level of similarity to each other, especially with regard to size and high level of anthropic disturbance, even with one being situated in an urban area and the other in a rural area, the composition of their mosquito fauna tended to be more similar than when compared to that found in NAT.

There is little information on the co-occurrence of mosquitoes in breeding sites in wild environments. In this study, although more than one mosquito species was observed in the same breeding site, none of the species showed a significant co-occurring behavior with another in the installed traps. Some studies indicate that semiochemicals produced by Culicidae larvae can mediate the oviposition behavior of other species, enabling pregnant females to avoid ovipositing, for example, in breeding sites previously colonized by predators or by interspecific competitors (Navarro-Silva *et al.* 2009). Thus, apparently, eventual semiochemicals produced by the larvae do not seem to mediate the oviposition pattern of the recorded species.

Conclusions

The composition of the Culicidae fauna and its distribution pattern in the oviposition traps installed in the three forest fragments suggest that *Ae. terrens* has a good capacity to colonize and inhabit different types of forest fragments of the semideciduous seasonal forest, in the Atlantic domain. This species, still little studied, has demonstrated its ability to become infected with some arboviruses and, therefore, must be evaluated in surveillance and vector control operations in the study region. In addition, our findings point out that forest fragments situated in rural areas closer to urban areas, that is, located around cities, offer conditions for the survival of species of wild and urban mosquitoes, such as *Ae. aegypti*.

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