

SCIENTIFIC NOTE

OCCURRENCE OF *LIMATUS DURHAMII* IN ARTIFICIAL CONTAINERS IN ATLANTIC FOREST, SÃO PAULO, BRAZIL

KAROLINA MORALES BARRIO-NUOVO, ANTONIO RALPH MEDEIROS-SOUSA, EDUARDO EVANGELISTA, WALTER CERETTI-JUNIOR, ARISTIDES FERNANDES, MARCIA BICUDO DE PAULA, MAURO TOLEDO MARRELLI AND RAFAEL OLIVEIRA-CHRISTE

Epidemiology Department, School of Public Health, University of São Paulo, São Paulo, Brazil

ABSTRACT. *Limatus durhamii* has wild habits with ecological plasticity in anthropized environments. We report the occurrence of this species in several types of artificial containers in 2 fragments of the Atlantic Forest in the city of São Paulo, Brazil. A total of 1,428 larvae were collected, in which plastic containers had the highest abundance of immature forms of *Li. durhamii*, with 579 (40.5%) specimens collected, followed by glass bottles with 464 (32.5%) and metal cans with 223 (15.6%). The high abundance of *Li. durhamii* in artificial containers points to an adaptation process to impacted environments. Therefore, studies that investigate the feeding habits and vectorial capacity of this species are essential to understanding its epidemiological role in the environment.

KEY WORDS Atlantic Forest, artificial containers, breeding sites, *Limatus durhamii*, mosquito, São Paulo

The genus *Limatus* Theobald belongs to the Sabethini tribe and comprises 9 species distributed in the Neotropical region with 5 species in Brazilian territory (Forattini 2002, Harbach 2007). Adults of *Li. durhamii* (Theobald, 1907) are active during the day and are found in the forests of South and Central America (Hervé et al. 1986). This species has been found naturally infected with several arboviruses, such as the Guama virus, Maguari virus, Tucunduba virus, and Caraparu virus (Segura and Castro 2007, Dias et al. 2022), all belonging to *Orthobunyavirus* genus (*Peribunyaviridae* family; Adams et al. 2017). Those viruses cause nonspecific symptoms, such as fever, headache, arthralgia, myalgia asthenia, and chills (Groseth et al. 2017, Matos et al. 2019).

Immature forms of *Li. durhamii* develop in canopy and soil breeding sites, such as broken bamboo, tree holes, fruit peels, escargots, stone holes (Lopes 1999, Hernández-Rodríguez et al. 2018), snail shells (Manguito et al. 2017), and artificial containers (Lopes 1999, Zequi et al. 2005). Researchers observed *Li. durhamii* in the same artificial breeding sites with *Aedes aegypti* (L.), *Aedes albopictus* (Skuse), and *Culex quinquefasciatus* Say (Honório et al. 2006). The species were collected in artificial breeding sites in ecotopes with urban characteristics (Lopes 1999).

Immature forms (larvae and pupae) of the mosquito were collected monthly between March 2015 and April 2017 from artificial containers in 2 remnants of the Atlantic Forest: the Capivari-Monos Protection Area (CMPA) and the Cantareira State Park (CSP). Both sites are located in the metropolitan region of São Paulo state and serve to shelter, protect, and ensure conservation of biodiversity in eastern Brazil. Suction samplers were used for collection

procedures. Three collection sites were selected at CMPA and CSP. Each site shows a different level of anthropization. The collected immature forms were placed in plastic containers with individual information and sent to the Laboratório de Entomologia em Saúde Pública, Faculdade de Saúde Pública, where they were monitored until the adults emerged and were euthanized with ethyl acetate. The dichotomous key by Lane (1953) was used for identification. Figure 1 shows an adult specimen and larvae of *Li. durhamii*.

A total of 1,428 *Li. durhamii* larvae were collected in artificial breeding sites (Table 1). The highest abundance of immature forms of this species was found in plastic containers, with 579 specimens collected, followed by glass bottles (464 specimens) and metal cans (223). Specimens of *Li. durhamii* were found in more types of artificial breeding sites in areas of CMPA than in the CSP. Some artificial containers e.g., water reservoirs, tarpaulins, tires, and asbestos roofs, were not found in CSP. Glass bottles were present only in the CSP environment. Plastic, metal, and ceramic containers were present in both sites. Even with less artificial container variability, CSP showed 28% more presence of *Li. durhamii* larvae than CMPA. It is worth noting that abundance may have been affected by the number of different kinds of containers scattered throughout the collection sites. The most abundant artificial containers were plastics (49 units), glass (21 units), and metal (20 units) containers.

The occurrence of *Li. durhamii* in artificial containers (tires, plastic, and metal containers) was previously reported (Lopes 1999, Zequi et al. 2005,

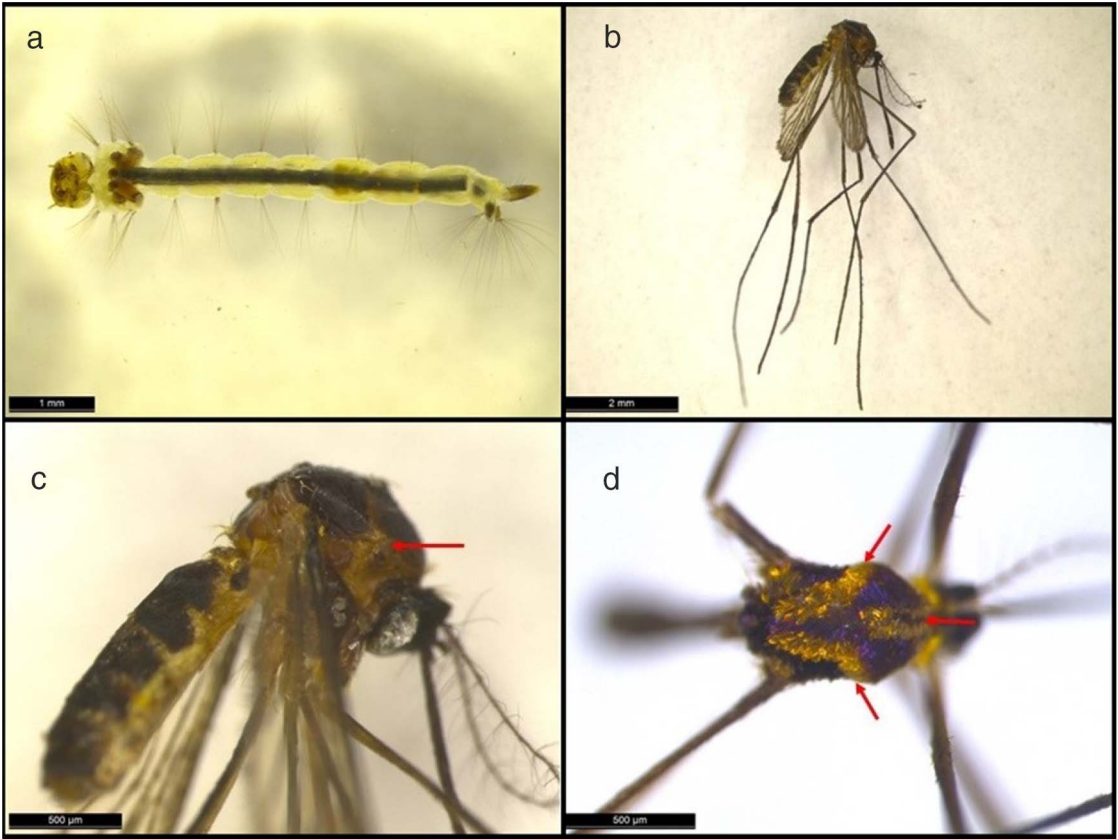


Fig. 1. Images of sampled specimens. (a) *Limatus durhamii* larvae photographed alive. (b) *Li. durhamii* adult specimen. (c, d) The arrows indicate the yellow metallic aspect of mesonotum scales: lateral and dorsal views (characteristic of this species).

Calderón-Arguedas et al. 2009, Ortega-Morales et al. 2010). Its tolerance to environmental pressures makes it the Sabethini species best adapted to artificial breeding sites (Forattini 2002). Our findings corroborate those of Lopes et al. (1993), who also found *Li. durhamii* in artificial containers such as plastic, tires, and metal, with the greatest abundance being in plastic ones. Calderón-Arguedas et al. (2009) also reported the presence of *Li. durhamii* in plastic and

metal containers; however, it was more abundant in metal ones. Nonetheless, Lopes (1999) and Zequi et al. (2005) reported that *Li. durhamii* was the most abundant of all species in tires.

In addition, *Li. durhamii* larvae feed on decaying matter present in water, but in the absence of resources, they can be predate on larvae on other mosquito species as well (Lopes 1999). Lopes (1999) reported larvae of *Li. durhamii* coexisting in an artificial container with larvae of *Culex eduardoi* Casal and Garcia, *Culex bigoti* Bellardi, *Cx. quinquefasciatus*, and *Toxorhynchites* sp. in a riparian forest in an urban area in southern Brazil, and Marin et al. (2009) observed *Li. durhamii* and *Ae. aegypti* larvae living in the same artificial container. Honório et al. (2006) and Ortega-Morales et al. (2010) reported *Li. durhamii* preying on *Ae. aegypti* larvae, and Olano et al. (2015) registered *Li. durhamii* larvae coexisting with *Ae. aegypti* and *Cx. quinquefasciatus* within schools located in ecotypes with wild and peri-urban characteristics. The presence of *Li. durhamii* and *Ae. aegypti* larvae in the same artificial container (Marin et al. 2009, Olano et al. 2015) is indicative of its adaptation process to impacted and peridomestic environments.

Table 1. Types of artificial containers in CMPA and CSP where *Limatus durhamii* specimens were collected.

| Container | CMPA | CSP | Total | % |
|-------------------|------|-----|-------|------|
| Plastic container | 381 | 198 | 579 | 40.5 |
| Water reservoir | 1 | 0 | 1 | 0.07 |
| Glass bottle | 0 | 464 | 464 | 32.5 |
| Metal container | 104 | 119 | 223 | 15.6 |
| Tarpaulin | 9 | 0 | 9 | 0.6 |
| Tire | 78 | 0 | 78 | 5.5 |
| Asbestos roof | 4 | 0 | 4 | 0.28 |
| Ceramic container | 21 | 49 | 70 | 4.9 |
| | 598 | 830 | 1,428 | 100 |

Our scope does not measure water volume for each container; based on local observation, glass bottles, metal, ceramic, and plastic containers, and tires (more effective breeding sites) can accumulate less water volume than water reservoirs. Asbestos roofs and tarpaulins showed less frequency of *Li. durhamii* larvae due to the minimum potential water accumulation capacity, where leaves can occupy all of the spaces.

Although both localities were Environmental Protection Areas, each one has different conservation stages. This becomes evident when observing the different types of artificial breeding sites found. Plastic containers may be the best indicator of human disturbance. This artificial breeding site was the most common in the 2 localities. Tarpaulins, tires, and asbestos roofs were present only in the CMPA area. Glass bottles were found only in the CSP area. Unlike the CMPA, the CSP is the type of park where people use its trails as a place for leisure and nature appreciation. On these occasions, it is common to consume different types of drinks, and as a result, the bottles often are abandoned, becoming breeding sites for mosquito species.

The scant information about the biology of *Li. durhamii* contrasts with its medical-epidemiological importance (Lorenz et al. 2019). This species was found in high abundance in some culicid fauna investigations (Ceretti-Junior et al. 2020, Evangelista et al. 2021) and was registered as naturally infected with yellow fever virus (Obara et al. 2012). Barrio-Nuevo et al. (2020) detected Zika virus in *Li. durhamii* during a study in forest fragments of São Paulo municipality. Different from other Sabethini species, the high presence of *Li. durhamii* in artificial breeding sites may point to an adaptation process. The evidence of interaction of *Li. durhamii* and etiological agents shows the necessity of blood meal habit and vectorial capacity investigations of this mosquito species and its importance in public health scenarios.

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REFERENCES CITED

- Adams MJ, Lefkowitz EJ, King AMQ, Harrach B, Harrison RL, Knowles NJ, Kropinski AM, Krupovic M, Kuhn JH, Mushegian AR, Nibert M, Sabanadzovic S, Sanfaçon H, Siddell SG, Simmonds P, Varsani A, Zerbini FM, Gorbalenya AE, Davison AJ. 2017. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses. *Arch Virol* 162:2505–2538.
- Barrio-Nuevo KM, Cunha MS, Luchs A, Fernandes A, Rocco IM, Mucci LF, Souza RP, Medeiros-Sousa AR, Ceretti-Junior W, Marrelli MT. 2020. Detection of Zika and dengue viruses in wild-caught mosquitoes collected during field surveillance in an environmental protection area in São Paulo, Brazil. *PLoS ONE* 15:e0227239.
- Calderón-Arguedas O, Troyo A, Solano ME, Avendaño A. 2009. Culicid fauna associated to artificial containers in the neighborhood “La Caprio,” Costa Rica. *Rev Costarr Salud Pública* 18:30–36.
- Ceretti-Junior W, Oliveira-Christe R, Wilk-da-Silva R, Mucci LF, Duarte AMRC, Fernandes A, Barrio-Nuevo KM, Port-Carvalho M, Marrelli MT, Medeiros-Sousa AR. 2020. Diversity analysis and an updated list of mosquitoes (Diptera: Culicidae) found in Cantareira State Park, São Paulo, Brazil. *Acta Trop* 202:105669.
- Dias HG, Santos FB and Pauvolid-Corrêa A. 2022. An overview of neglected Orthobunyaviruses, Brasil. *Viruses* 14:987.
- Evangelista E, Medeiros-Sousa AR, Ceretti-Junior W, Oliveira-Christe R, Wilk-da-Silva R, Duarte AMRC, Vendrami DP, Carvalho GC, Mucci LF, Marrelli MT. 2021. Relationship between vertical stratification and feeding habits of mosquito (Diptera: Culicidae) assemblages collected in conservation units in the green belt of the city of São Paulo, Brazil. *Acta Trop* 221:106009.
- Forattini OP. 2002. *Culicidologia médica*. Volume 2. São Paulo, Brazil: EDUSP.
- Groseth A, Vine V, Weisend C, Guevara C, Watts D, Russell B, Tesh RB, Ebihara H. 2017. Maguari virus associated with human disease. *Emerg Infect Dis* 23:2025–1331.
- Harbach RE. 2007. The Culicidae (Diptera): a review of taxonomy, classification and phylogeny. *Zootaxa* 638:591–638.
- Hernández-Rodríguez JL, Granados-Echegoyen CA, Ortega-Morales BO, Ibáñez-Bernal S, Pérez-Pacheco R, Chan-Bacab M, Alonso-Hernández N, Pérez-Rentería C, Huerta-Jiménez H. 2018. First record of *Limatus durhamii* Theobald (Diptera: Culicidae) in Campeche, Mexico. *Florida Entomol* 101:712–715.
- Hervé JP, Dégallier N, Travassos-da-Rosa APA, Pinheiro FP, Sá-Filho GC. 1986. Arbovírus isolados a partir de mosquitos. In: Fundação Serviços de Saúde Pública (Brasil) ed. *50 anos de contribuição às Ciências Biológicas e à Medicina Tropical*. Volume 2. Belém, Brazil: Instituto Evandro Chagas.
- Honório NA, Cabello PH, Codeço CT, Lourenço-de-Oliveira R. 2006. Preliminary data on the performance of *Aedes aegypti* and *Aedes albopictus* immature developing in water-filled tires in Rio de Janeiro. *Mem Inst Oswaldo Cruz* 101:225–228.
- Lane J. 1953. *Neotropical Culicidae*. São Paulo, Brazil: Universidade de São Paulo.
- Lopes J. 1999. Ecology of mosquitoes (Diptera, Culicidae) in natural and artificial rural breeding sites northern Paraná State, Brazil: VIII. Influence of predator larvae (*Toxorhynchites* sp., *Limatus durhamii* and *Culex bigoti*) on the populations of *Culex quinquefasciatus* and *Culex eduardoi*. *Rev Bras Zool* 16:821–826.

- Lopes J, Silva MAN, Borsato AM, Oliveira VDRB, Oliveira FJA. 1993. An ecological study of mosquito *Aedes (Stegomyia) aegypti* L. and associated culicifauna in an urban of Southern Brazil. *Rev Saude Pública* 27:326–333.
- Lorenz C, Alves JMP, Foster PG, Sallum MAM, Suesdek L. 2019. First record of translocation in Culicidae (Diptera) mitogenomes: evidence from the tribe Sabethini. *BMC Genom* 20:721.
- Mangudo C, Campos RE, Rossi GC, Gleiser RM. 2017. Snail shells as larval habitat of *Limatus durhamii* (Diptera: Culicidae) in the Yungas of Argentina. *Acta Trop* 167:204–207.
- Marin R, Marquetti MDC, Álvarez Y, Gutiérrez JM, González R. 2009. Especies de mosquitos (Diptera: Culicidae) y sus sitios de cría en la Región Huetar Atlántica, Costa Rica. *Rev Biomed* 20:15–23.
- Matos GC, Ferreira MS, Filho AJM, Neto OPA, Campos VM, Lima MLG, Rodrigues JCP, Ribeiro ACS, Freitas MZO, Silva FA, Chiang JO, Casseb LMN, Sousa JR, Quaresma JAS, Martins LC, Vasconcelos PFC, Carvalho VL. 2019. Experimental infection of golden hamsters with Guama virus (*Peribunyaviridae*, *Orthobunyavirus*). *Microbial Pathogenesis* 135:103627.
- Obara MT, Monteiro H, Paula MB, Gomes AC, Yoshizawa MAC, Lira AR, Boffil MIR, Carvalho MSL. 2012. *Haemagogus janthinomys* and *Haemagogus leucocelaenus* naturally infected with yellow fever virus in Federal District, Brazil, 2007–2008. *Epidemiol Serv Saúde* 21:457–463.
- Olano VA, Matiz MI, Lenhart A, Cabezas L, Vargas SL, Jaramillo JF, Sarmiento D, Alexandre N, Stenstrom TA, Overgaard HJ. 2015. Schools as potential risk sites for vector-borne disease transmission: mosquito vectors in rural schools in two municipalities in Colombia. *J Am Mosq Control Assoc* 3:212–222.
- Ortega-Morales AI, Avila PM, Elizondo-Quiroga A, Harbach RE, Siller-Rodríguez QK, Fernández-Salas I. 2010. The mosquitoes of Quintana Roo State, Mexico (Diptera: Culicidae). *Acta Zool Mex* 26:33–46.
- Segura MNO and Castro FC. 2007. Atlas of Culicidae in the Brazilian Amazon: characteristics of hematophagous insects of the family Culicidae. Belém, Brazil: Instituto Evandro Chagas.
- Zequi JAC, Lopes J, Medri IM. 2005. Imaturos de Culicidae (Diptera) encontrados em recipientes instalados em mata residual no município de Londrina, Parana, Brasil. *Rev Bras Zool* 22:656–661.