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Mosquitoes of the Caatinga: 2. Species from periodic sampling of bromeliads and tree holes in a dry Brazilian forest

Letícia Silva Marteis[a,*], Delsio Natal[b], Maria Anice Mureb Sallum[b], Antônio Ralph Medeiros-Sousa[b], Roseli La Corte[c]

[a] Colegiado de Medicina, Universidade Federal do Vale do São Francisco, Av. José de Sá Maniçoba, S/N, Centro, 56304-917, Petrolina, PE, Brazil
[b] Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo, Avenida Dr. Arnaldo, 715, 01246-904, São Paulo, SP, Brazil
[c] Departamento de Morfologia, Universidade Federal de Sergipe, Avenida Marechal Rondon, S/N, 49100-000, São Cristóvão, SE, Brazil

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ABSTRACT

The Caatinga is a dry tropical forest, located in the Brazilian semiarid region and rich in phytotelmata. This study investigated the culicid fauna of phytotelmata of the caatinga by sampling for 19 consecutive months aquatic immatures from tree holes and bromeliads. A total of 127 L of water was taken from the plants, containing 6764 immature culicids of 16 species, of which 11 (69%) are undescribed and respond to 90% of the total abundance of the specimens collected. Epiphytic bromeliads harbor a large number of immature Culicidae, although terrestrial bromeliads are the most abundant and widely distributed in the region. The richness of culicid species was similar between terrestrial and epiphytic bromeliads and lower in habitats represented by tree hole phytotelmata. There was no similarity in the composition of culicid species that developed in bromeliads or tree holes. Temperature and humidity were the environmental parameters most strongly associated with the proportion of positive plants. The Caatinga has a great number of endemic species that remain unknown to science and many additional culicid species may await discovery from there.

1. Introduction

Mosquitoes are insects widely distributed around the world. There are approximately 490 species in Brazil (WRBU, 2015), mainly found in tropical rainforest biomes such as the Atlantic Forest and the Amazon Rainforest. Mosquitoes have the ability to develop in a variety of habitats, from wild environments to polluted water from urban areas and affinity for ovipositing in a wide range of sites, including those represented by phytotelmata.

Phytotelmata are aquatic habitats naturally formed by water retention in plants or parts of terrestrial plants, living or dead, detached or not, e.g., tree holes, bromeliad ponds, bamboo internodes, open fruit and fallen leaves (Kitching, 2000). In these environments, which constitute complex micro-ecosystems despite the space and energy limitations, a high diversity of organisms, especially arthropods, coexists. These organisms belonging to different taxonomic groups interact ecologically and contribute to the diversity of patterns observed in nature (Fish, 1983; Kitching, 2000, 2001).

Bromeliad axils play an important role in the development of aquatic insects, including culicids. They store water between the overlaps of their lateral leaves and central well, enabling the establishment of various species in an environment rich in organic matter. In bromeliad phytotelmata, debris not only supports the community of insects but also serves as a source of nutrients for the plant (Ngai and Srivastava, 2006).

Like bromeliads, tree holes constitute important habitats for the development of rich aquatic fauna and were once considered the oldest natural larval habitat of culicids (Jenkins and Carpenter, 1946). The main attraction of these phytotelmata stems is their water storage capacity, maintained mainly by the stemflow that brings nutrients into the holes (Walker et al., 1991) along with the deposition of organic matter in sufficient quantities to form habitats that ensure the development of culicids, even after the decline of rainfall (Jenkins and Carpenter, 1946; Gomes et al., 1992). Tree holes, formed from hard plant parts, are potentially more durable than other phytotelmata, lasting ten or more years (Mogi, 2004).

The Caatinga is a tropical dry forest located in the Brazilian semiarid region, in which the culicid fauna associated with phytotelmata is virtually unknown. Specific information of culicid fauna in the Caatinga is scarce and restricted to transition areas with other biomes (Rebêlo et al., 2007; Santos et al., 2015). There is a recent record of immature fauna in an exclusive conservation area of the Caatinga

* Corresponding author.
E-mail address: leticia.marteis@univasf.edu.br (L.S. Marteis).

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biome, but associated to ponds (Marteis et al., 2015). Furthermore, the biological diversity of the Caatinga has been underestimated for decades, and the fauna and flora of the biome had been alleged to be poor, resulting in few areas of the Caatinga being preserved as fully protected units (Silva and Dinnout, 1999; Zanella and Martins, 2003). This ecosystem assessment was probably due to the Caatingás peculiar environmental conditions, with a seasonality marked by long periods of drought, scarce and erratic rainfall, high temperatures and low relative air humidity (Andrade-Lima, 1981; Trovão et al., 2007). Although its richness and uniqueness are recognized nowadays, the Caatinga is still one of the least studied biomes and, consequently, one of the most neglected in the literature, along with other tropical dry forests of the world (Tabarelli and Vicente, 2004; Santos et al., 2011; Sunderland et al., 2015).

The presence of phytotelmata in this biome suggests that these habitats play a role in the survival dynamics of culicid species adapted to this semi-arid environment. However, there are no systematic studies investigating the diversity and dynamics of culicid that colonize phytotelmata in the Caatinga, nor are there any ecological studies to help understand the behavioral and evolutionary adaptations made by the species based on environmental selective pressures. As such, this study was conducted to investigate both the biodiversity and the population dynamics of the culicid fauna that colonize tank bromeliads and tree holes in the Caatinga biome.

2. Material and methods

2.1. Study area

The study was conducted at the Ecological Station (ESEC) Raso da Catarina (38°44′00" W to 39°29′20" W and 9°33′13" S to 9°54′30" S), Conservation Unit (CU) of the Caatinga biome located in Bahia, northeastern Brazil (Fig. 1). The CU is classified as a strictly protected area (IUCN, Category 1a), with approximately 99,772 ha and a 135,279 m perimeter (Paes and Dias, 2008). Raso da Catarina is the oldest CU in Bahia, the state with the largest area − 30,092,536 ha − of the Caatinga biome in Brazil (Hauff, 2008).

The ESEC Raso da Catarina is located at low tropical latitudes and has a hot semi-arid climate, characterized by the predominance of high temperatures combined with strong insolation and low cloudiness. The temperature range undergoes pronounced daily fluctuations (around 12 °C) in the warmer months of the year. Rainfall is sparse, irregular, heavy and poorly distributed in space and time throughout the year. There is a long dry season punctuated by short rainy periods, reaching annual rainfall averages ranging between 300 mm and 500 mm (Almeida and Figueroa, 1983). The water found on the surface is scarce and certain parts of streams and creeks are intermittent, resulting in a high water deficit. The landscape is marked by vegetation on sandy soil, predominantly shrubby, very dense and less thorny than that observed in the Caatinga overlying crystalline soils (Velloso et al., 2002).

There are two areas with different vegetation types within the CU. The first type covers almost all the CU, with branched and thorny shrub-tree vegetation consisting mainly of Euphorbiaceae, Bromeliaceae and Cactaceae. This area is marked by deciduity in the dry season, with tree species that reach an average of 5 m in height, and the predominance of shrubs of less than 3 m in height. The second environment is a forest area called Mata da Pororoca, represented by a natural area of isolated forest that occupies 29 ha of the Station, or 0.03% of its total area and has a width between 200 and 400 m.

The forest area is not associated with any drainage or watercourse, has trees that sometimes reach as much as 15 m in height, has a closed understorey, dry shrubs and the ground is supposedly more fertile than that of its surroundings. Epiphytic species of the Bromeliaceae and Orchidaceae families are found in the forest area. There is also the presence of litter and a large number of lianas. This environment differs both in physiognomy and flora from other areas of the ESEC (Paes and Dias, 2008).

2.2. Collection, maintenance and identification of culicids

Immature samples were collected over 19 consecutive months, from March 2013 to September 2014. In each collection, seven tree holes and 40 bromeliads – 30 terrestrial and 10 epiphytes – were investigated. The bromeliads surveyed were close to established trails and roads within the CU and were divided into three areas located in different transects: terrestrial bromeliads present along a trail, in an area with shrub-tree vegetation and sandy soil, from which 20 bromeliads were haphazardly sampled; a cluster of epiphytic bromeliads, also in the same shrub-tree vegetation area and at a distance of approximately 800 m from the previous cluster, from which 10 bromeliads were haphazardly sampled; forest area, at a distance of about 25 km from the previous locations, from which 10 epiphytic bromeliads were haphazardly sampled. Epiphytic bromeliads sampled ranged from 50 cm to 6 m height from the soil.

Of the seven tree holes investigated, six were in the forest area and one in the shrub-tree Caatinga area where large trees are scarce. Other holes were monitored but since they were permanently dry, they were excluded from the study. In each survey, 40 sampled bromeliads were randomly selected, with a total of 760 samples at the end of the study. The seven sampled tree holes were the same in all larval surveys, totaling 133 investigations.

All examined bromeliads, both terrestrial and epiphytic, belonged to *Aechmea aquilega*, abundant throughout the CU region. Trees that had phytotelmata habitats belonged to the species commonly known as Angico-manjola (*Parapiptadenia zehntneri*), Guava-bravo (*Psidium myrsinites*) and Jaqueira-brava (*Richeria grandis*).

The water in the central well and the additional cavities formed by the outer axial leaves of each bromeliad and in tree holes was drawn using a manual suction pump (700 mL). The aspirated content was measured and carefully transferred into plastic basins to facilitate the capture of immature culicids. The larvae and pupae found were quantified and transferred into lidded plastic containers, along with water from the larval habitat, before being stored in a Styrofoam box for transportation to Laboratório de Entomologia e Parasitologia Tropical (LEPaT) of the Universidade Federal de Sergipe, where the immature forms were maintained. The fluid contents and imamatures from each sampled plant were maintained separately. The aspirated and unused water was immediately returned to the plants and rainwater was added to replace the volume removed.

Specimens collected as pupae were transferred into disposable cups and placed in cages until the emergence of adults. Larvae were kept in the same container with water from their larval habitat and fed with fish food (TetraMarine Saltwater Granules®; Tetra GmbH Herrenteich, 78–49324 Melle − Germany). Upon reaching the fourth stage of larval development, specimens were individualized for the emergence of adults and the collection of larval and pupal exuvia. The adults were killed with water at 60 °C and preserved in 70% ethanol until mounted on a slide with coverslip, and were then identified at a species level or as morphologically similar species.

To ensure reliable taxonomic identification of culicids, the associated sets of larval and pupal exuvia and male and female genitalia were examined. Samples of the species collected were sent to the Laboratório de Entomologia em Saúde Pública – LESP of the Faculdade de Saúde Pública da Universidade de São Paulo, to confirm their species identification and to provide samples of the species to the Entomology Reference Collection.

2.3. Data analysis

The species were characterized by means of dominance and constancy indices. Dominance was defined as the total number of culicids collected in all samples using the formula D% = (i/t)*100