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Short Communication

Culicidae fauna (Diptera: Culicidae) survey in urban, ecotonal and forested areas, from the Moreno municipality - Pernambuco State, Brazil.

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Abstract

Introduction: Mosquitoes are vectors of several pathogens in tropical regions. However, information on Culicidae in the northeast region of Brazil is scarce despite many municipalities having environmental conditions favorable for these insects. **Methods:** We collected mosquitoes monthly for one year from urban, ecotonal, and ecological reserve areas in Moreno, Brazil, by using CDC Miniature light traps, aspiration, ovitraps, and liquid suction. **Results:** We collected 1,401 insects in the Culicidae family. Fifteen species of seven genera of Culicidae were identified and five specimens were identified only at the genus level. **Conclusions:** There is a wide diversity of mosquito species in the sampled areas, some of which are important for human health.

Keywords: Culicidae species. Environmental changes. Mosquitoes, Urbanization. Sylvatic mosquitoes.

Culicidae species are vectors for several pathogens in tropical areas worldwide[1,2]. In Brazil, *Aedes aegypti* (Linnaeus, 1762) and *Culex quinquefasciatus* (Say, 1823) are considered the main vectors of annual epidemics of current and emerging pathogens, such as Zika and Chikungunya viruses and human filarial nematodes, which are found in urban environments[3-6]. Although there is some information on the distribution and biology for some of the sylvatic mosquito species, a large knowledge gap exists on mosquito species diversity and distribution in neotropical regions. Moreover, no information is available regarding the role of sylvatic species for pathogen emergence, propagation, and maintenance of the zoonotic cycle[7]. These species preferentially inhabit forested and ecotonal areas and feed mostly on small mammals and bird species. However, continuing urbanization and the expansion of human populations over those areas increase the contact between mosquitos and humans, which increases the probability of the eventual spillover of new pathogens to humans[7,8]. Data from the World Health Organization (WHO) have shown that three-fourths of all emerging infectious diseases originate or are currently transmitted from animals to humans[9], and 15% of these diseases have a direct association with the forested environment[10].

In the Northeast region of Brazil, land use changes and human expansion across forested areas have promoted the contact between sylvatic mosquito species and human populations[1]. Among the few studies regarding Culicidae fauna across this region, at least 10 mosquito genera were detected: *Anopheles*, *Aedeomyia*, *Psorophora*, *Wyeomyia, Limatus, Mansonia, Coquillettidia, Aedes, Haemagogus*, and *Culex*[11,12]. Moreover, among the detected species, two are particularly important in the transmission of pathogens: *Aedes (Ochlerotatus) scapularis* (Rondani, 1848) and *Aedes (Ochlerotatus) taeniorhynchus* (Wiedemann, 1821). These two species are adapted to breed and feed in flooded areas and mangroves, respectively, which are normally near secondary forests[13]. Individuals of these species were found to be infected with Japanese encephalitis virus (JEV), Rocio virus (ROV), Melão virus, *Dirofilaria immitis*, and *Wuchereria bancrofti* nematodes, which are the main etiological agents for canine and human filariasis, respectively[13]. Moreover, Aragão et al.[11] also detected *Haemagogus janthinomys* (Dyar, 1921), which is a vector of yellow fever[1,2].

The main goal of this study was to characterize the mosquito species that circulate between three areas in the Moreno municipality of the Pernambuco State, which is a region prone to dengue infections. The Moreno municipality is approximately

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30km from Recife, the capital City of the Pernambuco State in Brazil. Moreno has a human population of 5,648, distributed across 1,629 properties in an area that is approximately 206m². Moreno has a hot and humid climate and is at an altitude of 97 m with an average temperature of 28°C. Collection sites were in three areas with different degrees of anthropization: 1) the Moreno urban area (MUA) (João Paulo II Subdivision, latitude -8.1236260, longitude -35.0975200); 2) the Moreno ecotonal area (MEA), (latitude -8.1262880, longitude -35.0940660) is a transition zone between João Paulo II and the Carnijó Ecological Reserve, has a village that is inhabited by 911 people, and has Atlantic forest vegetation; and, 3) Carnijó Ecological Reserve (CER) (latitude -8.1400090, longitude -35.0844500) is a Private Natural Heritage Reserve (PRNP) that spans 135.5ha with 26 ha of native Atlantic forest. The MUA is located 500m from the MEA and 2km from the CER.

Mosquito sampling was performed using ovitraps in natural breeding sites, and we actively searched for individuals in the larvae/pupae stages. The CDC Miniature Light Trap Model 512 developed by the US Centers for Disease Control for the collection of mosquitoes was used for the collection of species with nocturnal activity, and we used aspiration in resting places to capture adult mosquitoes during the day. Collections were conducted once a month at five points per location. Sampling was carried out between May 2007 and February 2008. Light traps were installed 1.2m aboveground for a period of 14h (18:00 to 08:00). Sampling for mosquitoes during the day was carried out by aspiration in a radius of 3m² for all collection points. Each collection event occurred for 1h, immediately before noon. Pasteur pipettes were used to obtain samples of mosquitoes in the young stage (larvae and pupae); the collections were made in natural breeding sites such as tree holes and leaf plants, such as Bromeliaceae and Heliconiaceae, that accumulate rain water. Oviposition traps that contained grass infusions of 30% were placed at five capture points in the shaded areas around houses for seven days. Eggs, larvae, and pupae were allowed to develop and emerge at room temperature. Emerged larvae were fed Friskies® cat food so the entire developmental cycle could be completed, and adult insects were stored in plastic containers with lids in a mixture of paraffin and naphthalene. Adult mosquito species were identified in the Entomology Laboratory of the Department of Epidemiology, School of Public Health, University of São Paulo (USP). The rainfall data, average monthly temperature, and relative humidity during the study period were obtained from the meteorological substation at Vitória de Santo Antão, which is located 20-27km from the Moreno municipality.

All of the collection point data, the metadata for all of the species collected during this study, and the data from Aragão et al.[11] are available on the Microreact website (https://microreact.org/project/By7-vf7il)[14]. A user can navigate and color-code each sample by species. Microreact does not allow the same species name to be entered twice, and so we added a three letter code that represented each of the sampling locations [MUA, MEA, CER, and Dois Irmãos ecological reserve (DIER) sampled by Aragão et al.[11]] followed by a number that represented the different sampling times, sampling location, the pathogen transmitted, and, if known, the feeding behaviors [e.g., Mammals broad sense (MBS), Mammals including Homo (MIH), *Homo sapiens* specific (HSS), Birds-Mammals broad sense (BMBS), Birds specific (BS), and Birds-Mammals including Homo (BMIH)]. These details are mostly unknown for many species, but future studies should incorporate this valuable information.

A total of 3,183 insect specimens were captured in the three areas: 1,111 from the MUA, 968 from MEA, and 1,104 from the CER. Ninety-five percent (95.8%-3,048 species) belonged to the Diptera order, and 1,401 (44%) belonged to the Culicidae family. Around 60% of all collected Culicidae specimens were caught in the MUA, 24.1% in the CER, and 15.2% in the MEA. We detected twenty Culicidae species that represented nine genera: *Anopheles*, *Aedes, Culex*, *Coquillettidia*, *Aedeomyia*, *Psorophora*, *Mansonia*, *Limatus*, and *Wyeomyia* (**Table 1**). Fifteen individuals could be identified to the species level, and the remaining five individuals could not be identified with the current literature and may represent new species.

Both the MUA and MEA areas only had species from three Culicidae genera: *Aedes, Culex*, and *Coquillettidia.* Among the identified species, only *Aedes albopictus* and *Culex quinquefasciatus* were found to be representative of their corresponding genus, and the other three *Coquillettidia* species were collected at a lower frequency, with only one collected from the MUA and two from the MEA (**Table 1**). A higher diversity of Culicidae species, within nine genera, were identified in the CER (**Table 1**). Overall, the CER had a higher species diversity when compared to that of the other two sites. It is important to note that *A. albopictus* and *C. quinquefasciatus* were the only species present at all of the sampled sites. *Coquillettidia venezuelensis* and *Coquillettidia hermanoi* were found only in the MEA. There were several species only present in the CER (**Table 1**), which indicates those species can be considered a target species for the evaluation of environmental impacts on preserved areas.

Figure 1 shows the total number of specimens from the three most abundant species that were collected in all three areas. Both *C. quinquefasciatus* and *A. albopictus* had an abundance peak during the rainy season (July and August) in the MUA, and there was a second abundance peak for *C. quinquefasciatus* in January/February. There were two smaller abundance peaks for *C. quinquefasciatus* and *A. albopictus* in the MEA during July/August and November/December. In the CER, there was a high peak of abundance of *Limatus durhamii* during the dry season (September/October) with a relatively constant abundance of *C. quinquefasciatus* and *A. albopictus* from September 2007 until February 2008. Overall, the abundance of *A. albopictus* and *C. quinquefasciatus* mostly aligns with rainfall, which may be due to a higher availability of breeding sites in peri-domiciliary areas. Conversely, the peak abundance of *L. durhamii* occurred during the dry season, which suggests that it is not influenced by rainfall; this may be due to this species being found more frequently in forested areas where oviposition sites are available even in the dry season. Another interesting finding was the absence of *Aedes aegypti* in the samples collected from the MUA and the MEA, even though entomological data collected

TABLE 1: Culicidae species occurrence at the three sampling sites in Moreno municipality, Pernambuco State, Brazil.

Sampling site	Species	Number of Mosquitoes/Period					Total
		2007				2008	
		May/Jun	Jul/Aug	Sep/Oct	Nov/Dec	Jan/Feb	
Urban Area (MUA)	*Aedes (Stegomyia) albopictus* (Skuse, 1894)	-	198	2	20	145	365
	Coquillettidia (Rhynchotaenia) juxtamansonia (Chagas, 1907)	-	-	-	-	3	3
	Culex (Culex) quinquefasciatus Say, 1823	49	186	105	76	67	483
Ecotonal area (MEA)	*Aedes (Stegomyia) albopictus*	-	20	2	31	-	53
	Culex quinquefasciatus	9	34	-	66	48	157
	Coquillettidia (Rhynchotaenia) venezuelensis (Theobald, 1912)	2	-	-	-	-	2
	Coquillettidia (Rhynchotaenia) hermanoi (Lane & Coutinho, 1940)	-	-	-	1	-	1
Carnijó ecological reserve (CER)	*Aedeomyia (Aedeomyia) squamipennis* (Lynch Arribálzaga, 1878)	-	-	-	-	01	01
	Aedes (Stegomyia) albopictus	-	1	25	21	23	70
	Aedes (Stegomyia) aegypti (Linnaeus, 1762)	-	-	-	-	2	1
	Anopheles (Nyssorhynchus) albitarsis Lynch Arribálzaga, 1878	-	-	-	-	1	1
	Culex quinquefasciatus	-	-	28	24	31	83
	Culex (Culex) nigripalpus Theobald, 1901	-	-	-	-	2	2
	Culex (Microculex) pleuristriatus Theobald, 1903	-	-	-	-	2	2
	Culex (*Melanoconion*) sp.1*	-	-	-	-	8	8
	Culex (Melanoconion) spissipes (Theobald, 1903)	-	-	-	-	5	5
	Culex (*Melanoconion*) sp.2*	-	-	-	-	4	4
	Culex (Melanoconion) ocossa Dyar & Knab, 1919	-	-	-	-	1	1
	Coquillettidia (Rhynchotaenia) juxtamansonia	-	-	2	-	-	2
	Coquillettidia (Rhynchotaenia) nigricans (Coquillett, 1904)	1	-	1	-	6	9
	Limatus durhamii Theobald, 1901	-	-	118	-	9	127
	Mansonia (Mansonia) indubitans Dyar & Shannon, 1925	2	-	-	-	-	2
	Mansonia (Mansonia) titillans (Walker, 1848)		-	1	-	-	01
	*Mansonia (Mansonia) sp.1**	-	6	6	-	1	13
	*Psorophora sp.1**	-	-	4	-	-	4
	*Wyeomyia sp.1**	-	-	1	-	-	1
	Total	**63**	**445**	**295**	**239**	**359**	**1,401**

MUA: Moreno urban area; **MEA:** Moreno ecotonal area; **CER**: Carnijó Ecological Reserve. * Individuals that were not identified according to the current literature, and may be new species.

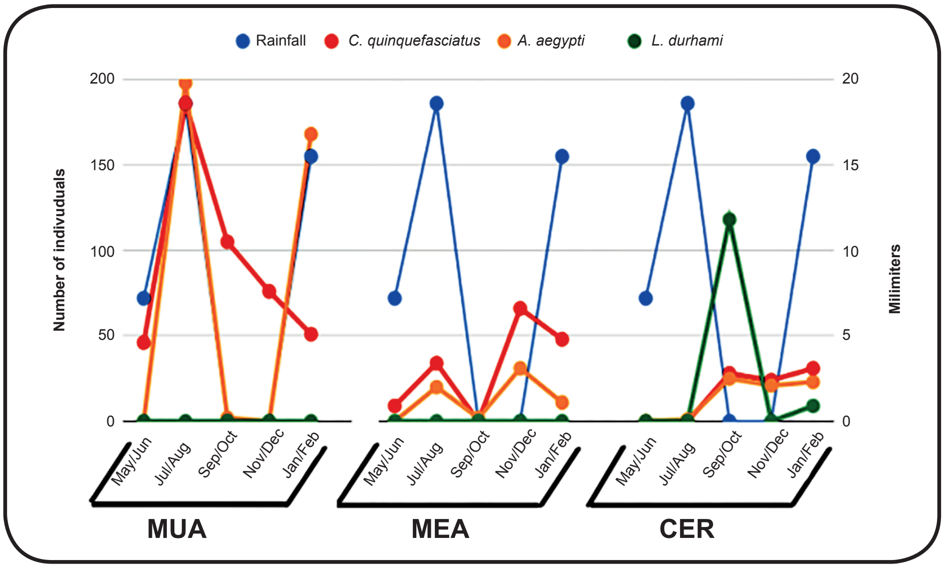

FIGURE 1. Temporal distribution of the three most abundant Culicidae species found in the urban area (MUA), ecotonal area (MEA), and the Carnijó ecological reserve (CER) and the rainfall during the collection period. **MUA**: Moreno urban area; **MEA:** Moreno ecotonal area; **CER**: Carnijó Ecological Reserve.

by the Programa Nacional de Controle da Dengue (PNCD) shows the municipality has been infested with this species since 1990 and has caused multiple dengue fever cases every year (139 confirmed cases in 2007 and 34 confirmed cases in 2008) (Moreno Epidemiological data, personal communication). Conversely, *A. albopictus* was the most frequently collected and most abundant species in all of the areas, which suggests it could be involved in the local dengue transmission cycle. The presence of *A. albopictus* could be from the Moreno municipality being surrounded by several sugar cane crop fields and forested areas, which form a green-belt of suitable environment that may lead to the proliferation of *A. albopictus*[12].

There is only one published work[11] describing the Culicidae species found in the Pernambuco State, and there were 17 new species reported in this study from the *Anopheles*, *Culex*, and *Aedes* genera, including those with a known distribution in this state (*C. quinquefasciatus*, *A. aegypti*, and *A. albopictus)*. In this study, we sampled a different municipality of the Pernambuco State (Moreno) and recovered 11 newly reported species for the state, including *Aedeomyia squamipennis*, *Anopheles albitarsis*, *Coquillettidia juxtamansonia*, *Coquillettidia nigricans*, *C. hermanoi*, *C. venezuelensis*, *Culex nigripalpus, Culex pleuristriatus*, *Mansonia indubitans*, *Mansonia titillans*, and *L. durhamii*.

The occurrence of several Culicidae species, both those previously described and the newly reported species in the Pernambuco State, highlights the need for a deeper characterization of Culicidae fauna in different environments using complementary sampling methods. Our results bring new knowledge regarding mosquito species distribution in Northeast Brazil and, more specifically, in the Pernambuco Sate, which was the epicenter of the Zika virus epidemic[15]. In addition, further studies are needed to evaluate the role of *A. albopictus* in the transmission of arboviruses in municipalities that are surrounded by agricultural and forested areas. We predict that many more Culicidae species will be found if sampling is performed in different sylvatic environments throughout the Pernambuco State. The emergence and annual epidemics of arboviruses that are transmitted by mosquitoes, as well as the transmission of parasitic nematodes that are the agents of lymphatic filariasis in the Pernambuco State, provide evidence that surveillance strategies are necessary to study other mosquitoes species and their vector capacity. The data generated in subsequent studies can be readily uploaded on the Microreact webserver to make all information available for the decision-making on vector control.

Conflict of interest

The authors declare that there is no conflict of interest.

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