



## Ecology

# Diversity and abundance of mosquitoes (Diptera, Culicidae) in a fragment of Amazon Cerrado in Macapá, State of Amapá, Brazil

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**Abstract.** The Neotropical region has the highest diversity of species, but despite this diversity, this region presents many sampling gaps. The objective of this study was to study the diversity of the mosquitoes of family Culicidae (Diptera: Culicomorpha) as well as to identify the mosquito vectors, in a fragment of Amazon Cerrado in Macapá, Amapá. Three collection methods (Shannon trap, CDC and active collection) were used, and the samples were collected between January to December 2006. 21 species were identified; 11 of them being vectors of malaria, dengue fever, yellow fever, Zika, chikungunya and filariasis; and three of them, *Toxorhynchites h. haemorrhoidalis* (Fabricius), *Wyeomyia melanocephala* Dyar & Knab and *Wyeomyia aporonoma* Dyar & Knab were recorded for the first time from Amapá. The present study contributes to the diversity of mosquitoes (Culicidae) that can be used in additional mapping studies to mitigate epidemic outbreaks in the state of Amapá.

**Keywords:** Arbovirus; malaria; mosquitoes; richness of species; *Toxorhynchites haemorrhoidalis*.

**M**osquitoes of family Culicidae (Diptera: Culicomorpha) are monophyletic and classified into two subfamilies: Anophelinae and Culicinae (HARBACH & KITCHING 2005). Culicidae comprises 3,578 recognized species worldwide, distributed among 42 genera and 140 subgenera (HARBACH 2013) with a higher species diversity in the Neotropical region 1,069 species (31%), distributed in 24 genera (RUEDA 2008). Brazil possesses more than half of this diversity, with 516 described species (WRBU 2018). Although there is a large diversity in the Neotropical region, many sampling gaps still exist. Moreover, recent molecular studies have revealed the existence of several cryptic species complexes (ROSA-FREITAS *et al.* 1998; RUIZ-LOPEZ *et al.* 2012).

Mosquitoes are the most notorious pathogenic transmitters for humans, such as malaria, dengue, Zika, viral encephalitis and lymphatic filariasis (LANE & CROSSKEY 1993). In coastal areas or places with proximal breeding grounds, these insects cause great nuisance, hampering the development of real estates as well as tourism (LANE & CROSSKEY 1993; RUEDA 2008). However, despite their high species richness, only a small part of the mosquitoes actually transmits diseases (RUEDA 2008), for example, human malaria is transmitted only by species of *Anopheles* (DE ARRUDA *et al.* 1986; LANE & CROSSKEY 1993). In Brazil, only *Anopheles darlingi* Root and *Anopheles aquasalis* Curry are considered important vectors of malaria (DE ARRUDA *et al.* 1986; CONN *et al.* 2002; GALARDO *et al.* 2007), other species are considered secondary vectors, and among them, *Anopheles marajoara* Galvão & Damasceno is considered as an important vector in Macapá (CONN *et al.* 2002; GALARDO *et al.* 2007; BARBOSA & SOUTO 2011). Among other

vectors of important re-emerging arboviruses in Brazil, *Aedes aegypti* (Linnaeus) is considered as an important arboviral vector (FIGUEIREDO 2007; WEAVER & REISEN 2010) for dengue fever virus (DENV) (SIMMONS *et al.* 2012; POWELL 2018), yellow fever virus (YFV) (JENTES *et al.* 2011), Chikungunya virus (CHIKV) (LEPARC-GOFFART *et al.* 2014), Zika virus (ZIKV) (WHO 2017) and Mayaro virus (MAYV) (WHO 2017; WIGGINS *et al.* 2018).

In addition to their medical importance, mosquitoes also have ecological relevance as they participate in the trophic chain of other insects as well as of aquatic and terrestrial vertebrates (RUEDA 2008). Various species adopt diverse strategies for their proliferation and dispersion. Even though there are specific breeding sites for certain species of mosquitoes (CONSOLI & OLIVEIRA 1994), they may use permanent or semi-permanent soil breeding sites, man-made or natural containers (palm bracts, leaves, tree holes, bromeliads and *Heliconia* spp.) with accumulated rainwater or accumulated water from other sources (LANE & CROSSKEY 1993; CONSOLI & OLIVEIRA 1994). The purpose of the present study was to record the species diversity of Culicidae in a forest fragment of the Amazonian Cerrado, near the urban perimeter of Macapá, as well as to identify those of medical importance.

## MATERIAL AND METHODS

**Study sites.** The study was carried out in the campus of Federal University of Amapá, in Macapá, State of Amapá, Brazil. The area studied was between the geographical coordinates 0° 0'7.82" N 51° 4'54.63" W to 0° 0'8.38" N 51° 5'15.02" W and 0° 0'36.70" S 51° 4'59.62" W to 0° 0'36.32" S 51° 5'20.82" W, with an elevation of 8–19 m. The campus belongs to

the morphoclimatic domain of Amazonian Cerrado, covering an area of 906,722.45 m<sup>2</sup> (Figure 1). The predominant climate in Amapá is of equatorial feature with temperature ranging from 25°C to 30°C (AB'SABER 1977). The annual rainfall is high (2,500 mm average), with the maximum rainfall between March - May (2,112.9 mm) and the minimum rainfall between September - November (177.8 mm). The relative annual humidity is 85% with the annual mean insolation of 2,200 hours (SUDAM 1984).

The area of study has a marked human impact due to local urbanization. The phytophysionomy of this area comprises four non-interconnected forest fragments, three of them being gallery forests, and a grassland in the northern portion (Figure 1). There is predominance of native plant species of Cerrado (Amazonian biome) such as Murucis (*Byrsonima* H.B.K.; Malpighiaceae, Malpighiales), Caimbe (*Curatella americana* Linnaeus; Dileniaceae, Dileniales), Umiri (*Humiria* A.St-Hil; Humiriaceae, Malpighiales), Miriti (*Mauritia flexuosa* Linnaeus and *Mauritia carana* Wallace; Arecaceae, Arecales) (AZEVEDO 1969), as well as bromeliads and *Heliconia* spp., which are important for the propagation of some species of Culicidae (ALBICÓCCO et al. 2011). This area does not have permanent breeding sites, such as river backwaters or depressions of sluggish streams or rivers which favors the creation of mosquito species typical to such breeding sites, like *Anopheles* spp. However, the surrounding low-lying areas, locally know as hangover areas, mostly remain flooded due to riverine tides and rainfall (MACIEL 2001). The Igarapé Fortaleza is the closest, at a distance of 1.6 km from the campus. Moreover, there are 41 fish hatcheries and four treatment sewers (probable artificial breeding grounds) at 1.4 km and 1.2 km (straight line distance) respectively, from the campus. Figure 1 shows the distribution of these artificial breeding sites marked with white triangle signs.

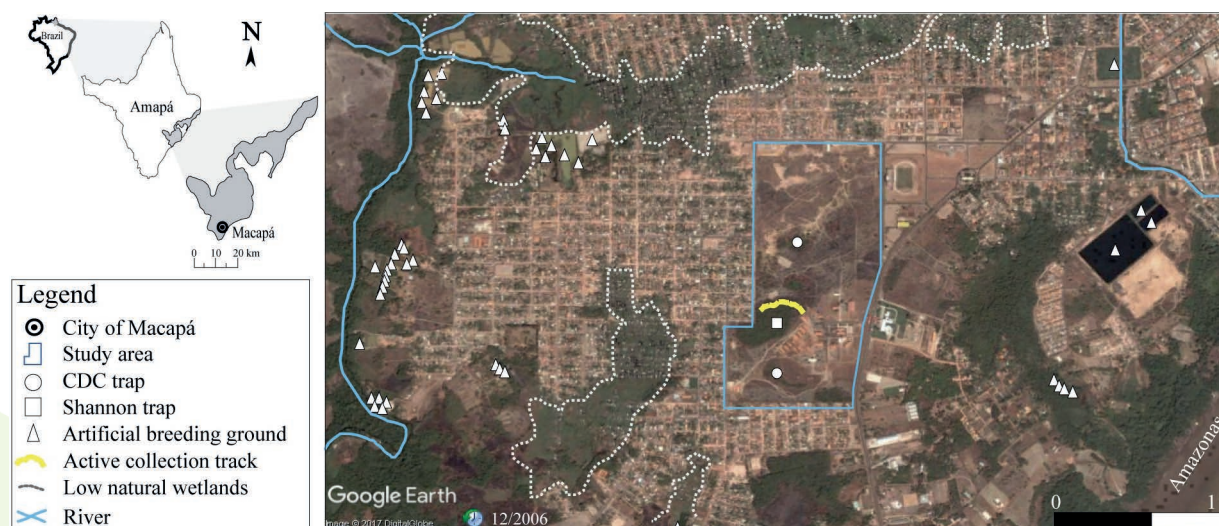
**Data collection.** The monthly samples were collected for one year, between January to December 2006. Three sampling methods were used for three consecutive days: active collection, CDC (LANE & CROSSKEY 1993) and Shannon trap (SHANNON 1939). The active collection was carried out through the capture of mosquitoes with entomological net and glass aspirator "pooter" (LANE & CROSSKEY 1993). This collection was performed along a transect of Cerrado and gallery forest of length 100 m (Figure 1), between 07:00-10:00 h. Periodic stops of five minutes were made during the course (100 m) of collection, facilitating the attraction and capture of mosquitoes. Light traps, such as two CDC traps, were installed at a distance of 200 m from each other, in the

two largest forest fragments of the campus (Figure 1). The collections were carried out during the new moon phase, as the nights are darker, making the luminous attractions more effective (LANE & CROSSKEY 1993). The CDC traps were installed at a height of 1.5 m from the ground at 18:00h and removed the next day at 07:00 h. Shannon's luminous trap was set inside the largest forest fragment, about 50 m from the edge (Figure 1). This trap was installed at 1800h and withdrawn at 21:00 h. The mosquitoes collected by active collection method, and by Shannon trap were separated as per their time of collection and subsequently analyzed for registering the hourly activity of the species collected.

The captured mosquitoes were stored inside plastic cups and labeled as per their time of collection (active collection and Shannon). The material was transported in a Styrofoam box to the Arthropod laboratory of the Federal University of Amapá (UNIFAP) and morphologically identified using the dichotomous keys of CONSOLI AND OLIVEIRA (1994), FORATTINI (2002), and LANE (1953). The terminologies, such as abbreviations of genera and subgenera, follow REINERT (2009).

All collected mosquitoes were deposited at the Zoological Collection of the Universidade Federal do Amapá - UNIFAP as voucher specimens and are available for future studies.

**Data analysis.** Sample rarefaction / extrapolation curves based on the sample size and sampling methods used were analyzed to compare the species richness and the diversity of nocturnal and diurnal species. Three Hill numbers (HILL 1973) were used in this study, which are associated with species richness estimators and their respective dominances. Hill numbers are diversity indices that quantify diversity in units of equivalent numbers of equally abundant species (HILL 1973; COLWELL et al. 2004; LOU 2006; GOTELLI & CHAO 2013; CHAO et al. 2014). The first number ( $q = 0$ ) analyzes the observed species richness, the second ( $q = 1$ ) is the exponential value of the Shannon entropy index and, the third ( $q = 2$ ) is the inverse Simpson index of dominance and measures the dominance of species (most abundant and prevalent species) in each sampling method (COLWELL et al. 2004). This analysis was performed with iNEXT package (CHAO et al. 2014), using 1000 randomizations with 95% confidence intervals. Subsequently, the abundance of the collected species was arranged per month and monthly precipitation to determine the temporal fluctuation of the species. All analyzes were performed in program R 3.5.0 (<http://www.r-project.org/>) (R CORE TEAM 2018).



**Figure 1.** Study area on the campus of the Federal University of Amapá. The collection points are indicated (Shannon trap, CDC trap and Active Collection). The map also shows the artificial permanent breeding places near the study area, the transect used for active collection, low natural wetlands areas and rivers that allow creation of mosquitoes (Diptera: Culicidae). Adapted from Google Earth PRO (2015).

## RESULTS

**Species composition.** 1,918 mosquitoes were collected, corresponding to 21 species and 11 genera. Out of which, 642 were collected by Shannon trap, 460 by active collection and 816 by CDC trap (Table 1). The Shannon trap and CDC trap sampled 15 species each, where four species were collected exclusively with Shannon-trap and only one species was collected exclusively with CDC-trap. Active collection sampled 11 species, including the only specimen of *Toxorhynchites haemorrhoidalis* (Fabricius) (Table 1).

Rarefaction and extrapolation estimators for biodiversity (species richness  $q = 0$ , Shannon entropy exponential  $q = 1$ , and Simpson inverse concentration  $q = 2$ ) based on sample coverage, showed that our sampling was large enough to record the majority of Culicidae species in the study area. Diversity curves showed little gain in species richness,

dominance, and equally common species in relation to increased sampling effort > 250 sampled units (Figure 2a).

The species richness recorded by each type of trap (Active = 11, Shannon and CDC = 15), was close to the estimation observed in the rarefaction / extrapolation analysis, which is expected up to 12 species for active, 16 species for Shannon and 15 species for CDC. Therefore, a probable increase of ~ 2 to 3 species is expected if more collections are done in the study area (Figure 2b). The species richness among the three collection methods was statistically close to each other when compared to > 250 individual units (Figure 2b). The exponential Shannon entropy estimates ( $q = 1$ ) also showed that the number of observed and predicted species was remarkably similar, indicating that the sampling included almost all equally common species. However, the number of common species was significantly lower in active

**Table 1.** List of mosquito species in the campus of the Federal University of Amapá, Macapá, Brazil. The species were recorded according to their method of collection, absolute number, and relative number of individuals during the period from January to December 2006.

SPECIES	Shan	A.C.	CDC	N	%
<i>Aedeomyia (Aedeomyia) squamipennis</i> (Lynch Arribáizaga)	90	0	122	212	10.4
<i>Aedes (Stegomyia) aegypti</i> (Linnaeus)	6	6	0	12	0.63
<i>Aedes (Ochlerotatus) scapularis</i> (Rondani)	80	61	99	240	1.5
<i>Aedes (Ochlerotatus) serratus</i> (Theobald)	43	38	109	190	9.91
<i>Anopheles (Nyssorhynchus) braziliensis</i> (Chagas)	18	0	3	21	1.09
<i>Anopheles (Nyssorhynchus) darlingi</i> Root	9	0	0	9	0.47
<i>Anopheles (Nyssorhynchus) marajoara</i> Galvão & Damasceno	20	0	0	20	1.04
<i>Anopheles (Nyssorhynchus) nuneztovari</i> s.l. Galbadon	7	0	0	7	0.36
<i>Anopheles (Anopheles) mattogrossensis</i> Lutz & Neiva	1	0	0	1	0.05
<i>Coquillettidia (Rhynchoaenia) venezuelensis</i> (Theobald)	13	0	20	33	1.72
<i>Culex (Culex) cf. nigripalpus</i> Theobald	190	138	260	588	30.7
<i>Culex (Culex) quinquefasciatus</i> Say	65	18	60	143	7.46
<i>Culex (Melanocolion) portesi</i> Senevet & Abonnenc	27	0	30	57	2.97
<i>Limatus durhamii</i> Theobald	0	62	18	80	4.17
<i>Mansonia (Mansonia) titillans</i> (Walker)	48	0	55	103	5.37
<i>Psorophora (Janthinosoma) ferox</i> (Von Humboldt)	25	76	21	122	6.2
<i>Psorophora (Janthinosoma) amazonica</i> Cerqueira	0	25	7	32	1.67
<i>Toxorhynchites (Lynchiella) haemorrhoidalis</i> (Fabricius)	0	1	0	1	0.05
<i>Uranotaenia (Uranotaenia) hystera</i> Dyar & Knab	0	0	6	6	0.31
<i>Wyeomyia melanocephala</i> Dyar & Knab (Subgenus uncertain)	0	22	2	24	1.25
<i>Wyeomyia (Triamyia) aporonoma</i> Dyar & Knab	0	13	4	17	0.89
<b>TOTAL</b>	<b>642</b>	<b>460</b>	<b>816</b>	<b>1,918</b>	<b>-</b>

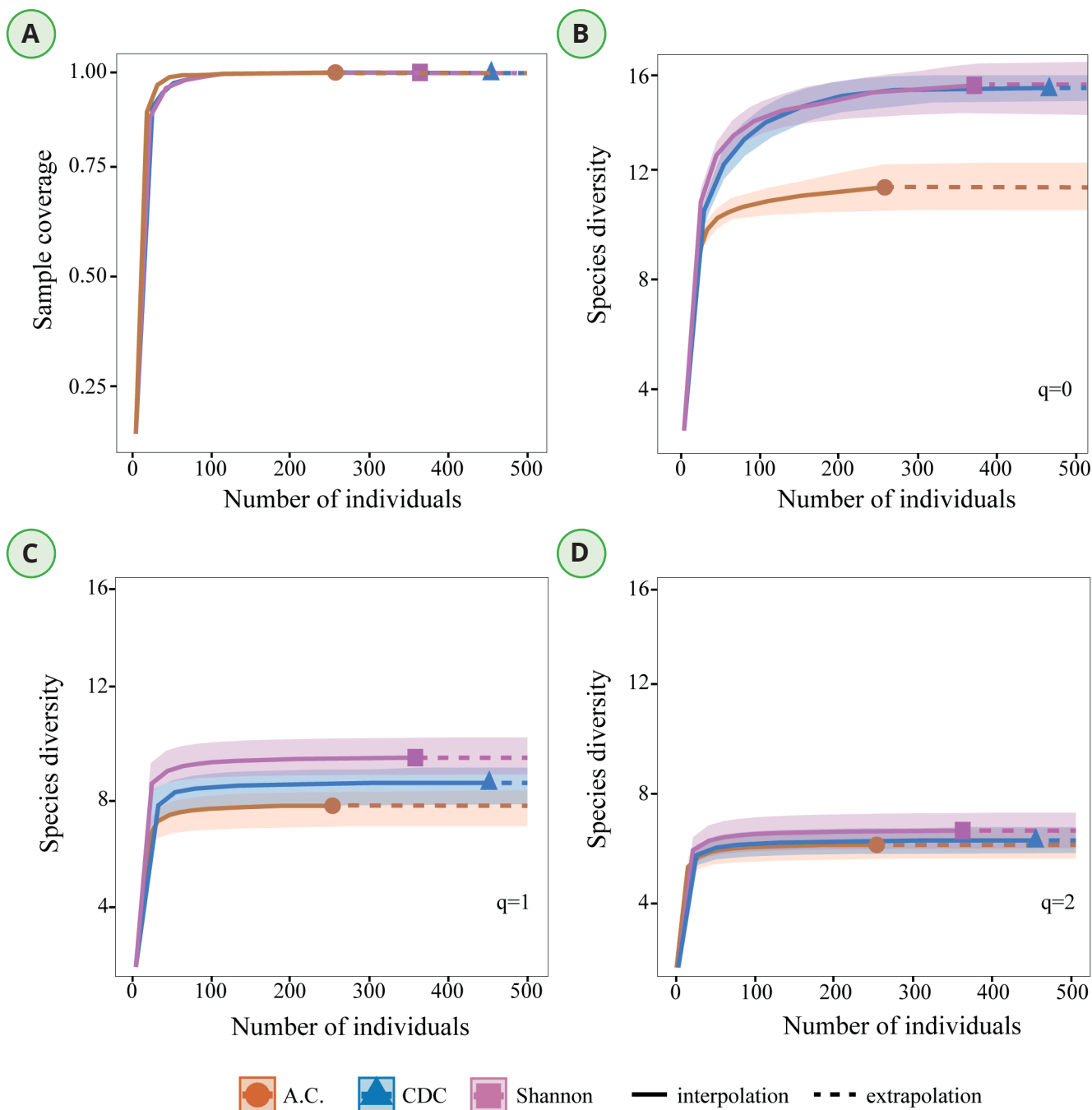
\*Shan: Shannon's trap. A.C.: Active Collection. CDC: automatic CDC light trap. N°: absolute number of specimens collected and (%): Relative frequency of the species in the sample. \*\*Abbreviations of the genera and subgenera followed REINERT (2009).

collection than with Shannon and CDC (Figure 2c). Also, the species dominance index estimated using Simpson's inverse concentration ( $q = 2$ ) was slightly higher with Shannon. Although the difference between the 3 sampling methods was of the order of 0.5 'species', this difference was significant (Figure 2d).

**Temporal variation of species.** The three most abundant species observed were: *Culex cf. nigripalpus* Theobald (30.7%), *Aedes scapularis* (Rondani) (12.5%) and *Aedeomyia squamipennis* (Lynch-Arribáizaga) (10.4%) (Table 1). The monthly collection of Culicidae, ranged from five species in the first month (January) to 17 species in June, the highest record in a single month's collection (Figure 3). When the richness of Culicidae was compared with the meteorological data obtained in this study, no significant correlation was found. However, the second semester of the year showed a greater variety of species than the first semester, except for

June, in which 17 species were collected (Figure 3).

The temporal fluctuations in the abundance of mosquito species are shown in Figure 3. There was a marked variation in the composition of species through the periods of high and low precipitation. For example: *Ae. scapularis*, *Aedes serratus* (Theobald), *Psorophora ferox* (Von Humboldt) and *Psorophora amazonica* Cerqueira, were more abundant at the beginning of the rainy season and decreased during the subsequent rainy months until they were absent in the driest month (September); *Anopheles mattogrossensis* Lutz & Neiva and *Uranotaenia hystera* Dyar & Knab, rare species, were also recorded during the rainy season. *Anopheles nuneztovari* s.l. was the only species which was observed to be highly abundant at the peak of driest period, while *An. darlingi* and *An. marajoara* (rare species) were more abundant in the collections from rainy months than dry months. On the other hand, *Ad. squamipennis* and *Mansonia titillans* (Walker)



**Figure 2.** Rarefaction and extrapolation curves based on individual and coverage, based on Hill numbers ( $q = 0, 1, 2$ ). **A)** Sample coverage; **B)** Species diversity, species richness ( $q = 0$ ); **C)** Exponential of Shannon's entropy (Shannon diversity,  $q = 1$ ) and **D)** Simpson's inverse concentration (Simpson diversity,  $q = 2$ ). The 95% confidence intervals (shaded) were obtained by bootstrapping (1,000 replications). Reference samples are indicated by solid shapes. The proposed estimators are accurate for both the rarefaction (solid line) and the short-range extrapolation.

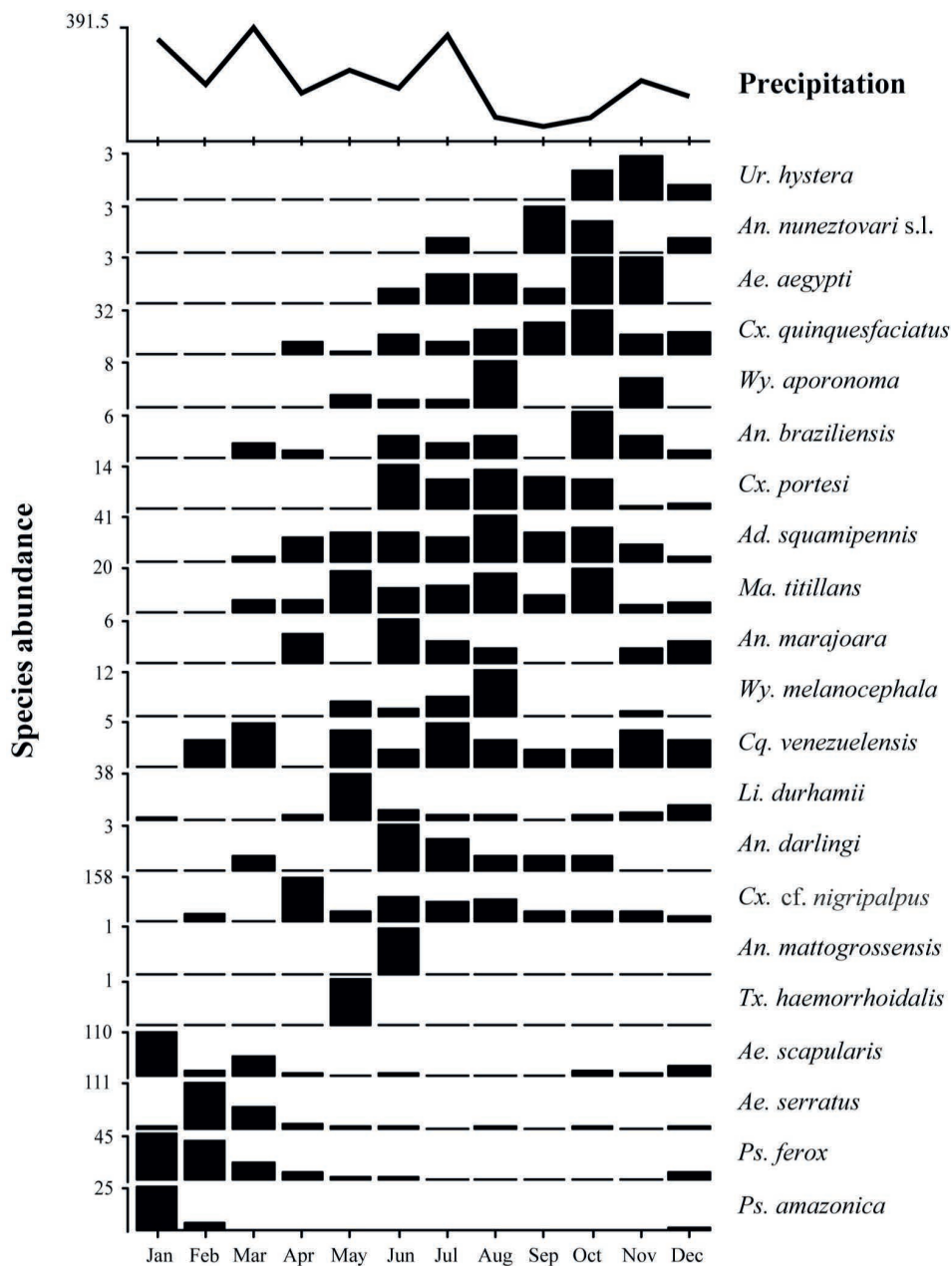
showed higher abundance at the end of rainy season and were reduced during the interval of dry and early rainy season. *Coquillettidia venezuelensis* (Theobald) did not show any seasonal pattern, except for being absent from the samples of January and March.

**Species of medical importance.** 11 species were classified as medically important, according to the CDC arbovirus catalog, available at <https://wwwn.cdc.gov/arbocat/> (CDC 2018) (Table 2). The species of high epidemiological importance collected in the present study were: *Ae. aegypti*, *An. darlingi* and *An. marajoara*. However, these species were categorized as accidental species due to their less abundance in the studied area. *Aedes aegypti* ( $n = 12$ ) was recorded from the collection of June to November, between 08:00 h and 10:00 h, and between 18:00 h and 19:00 h; *An. darlingi* ( $n = 9$ ) was recorded in March and from June to October, between 18:00 h and 19:00 h; and *An. marajoara* ( $n = 20$ ) was collected in April, June, July, August, November and December, from 18:00 h – 20:00 h.

## DISCUSSION

To date, a total of 95 species of mosquitoes had been recorded for the State of Amapá (DEANE *et al.* 1948; CERQUEIRA 1961; TADEI *et al.* 1998; PÓVOA *et al.* 2001; SOUTO & PIMENTEL 2006; BERGO *et al.* 2007; GALARDO *et al.* 2007; SOUTO *et al.* 2011). In the present study, we studied the diversity of mosquitoes in a restricted area of Macapá (our study area) with different collection methods (CDC traps, Shannon traps and active collection). The findings of this study contribute to the knowledge of diversity of mosquitoes in Macapá, which in turn, may help other studies regarding the ecological and evolutionary processes of mosquitoes (RUEDA 2008).

*Toxorhynchites* (Fabricius) is the only non – hematophagous genus of Neotropical Culicidae. Its immature forms are predators of other mosquito larvae and small arthropods, and thus, considered as an option for the biological control of *Ae. aegypti* and *Ae. albopictus* larvae (RUEDA 2008; HUTCHINGS 1994).



**Figure 3.** Abundance of species of Culicidae and relative monthly precipitation. The bars indicate the monthly relative abundance (y-axis is not standardized) and the line indicates the monthly precipitation over the sampled period. Precipitation levels were obtained from Núcleo de Hidrometeorologia e Energias Renováveis, Instituto de Pesquisas Científicas e Tecnológicas do Amapá - NHMET/IEPA.

**Table 2.** List of species of mosquitoes and associated diseases. The virus species are abbreviated according to CDC (2018).

Species	Associated human diseases
<i>Aedeomyia squamipennis</i>	GAMV
<i>Aedes aegypti</i>	CHIK, DENV, YFV and ZIKV
<i>Aedes scapularis</i>	ILHV, SLEV, VEEV, WYOV, YFV and <i>W. bancrofti</i>
<i>Aedes serratus</i>	SLEV, VEEV and WYOV
<i>Anopheles darlingi</i>	Malaria
<i>Anopheles marajoara</i>	Malaria
<i>Anopheles nuneztovari</i> s.l.	Malaria
<i>Coquillettidia venezuelensis</i>	BSQV, CATUV, COTV, GMAV, MAYV, MURV and OROV
<i>Culex cf. nigripalpus</i>	EEE, SLE and WNV
<i>Culex quinquefasciatus</i>	SLE, WEE and <i>W. bancrofti</i>
<i>Mansonia titillans</i>	VEE
<i>Psorophora ferox</i>	CVV, EEEV, ORIV, SLEV, WNV and WYOV

Virus names and abbreviations: BSQV – Bussuquara; CATUV – Catu; CHIK – Chikungunya; COTV – Cotia; CVV – Cache Valley; DENV – Dengue; EEEV – Eastern Equine Encephalitis; GAMV – Gamboa; GMAV – Guama; LHV – Ilheus; MAYV – Mayaro; MURV – Murutucu; ORIV – Oriboca; OROV – Oropouche; SLEV – St. Louis Encephalitis; VEEV – Venezuelan Equine Encephalitis; WB – Wuchereria bancrofti; WNV – West Nile; WYOV – Wyeomyia virus; YFV – Yellow Fever; ZIKV – Zika;

Out of the nine species of *Toxorhynchites*, which are present in Brazil, *Tx. h. haemorrhoidalis* was confirmed in Amazonas and Pará, in addition to Cayenne, French Guiana. However, until the present study, *Tx. h. haemorrhoidalis* had not been reported in Amapá. A similar situation has been observed with *Wy. melanocephala* and *Wy. aporonoma*, as both of them have been recorded in the state of Pará and Amazonas (CONFALONIERI & COSTA NETO 2012; HUTCHINGS *et al.* 2013; HUTCHINGS *et al.* 2018), but not in Amapá. The above scenario possibly demonstrates the lack of sufficient mosquito surveys in Amapá.

*Culex* cf. *nigripalpus* and *Ae. scapularis* are highly aggressive human blood feeders (FORATTINI 2002). Currently, *Cx.* cf. *nigripalpus* is incriminated as a vector of equine encephalitis, St Louis Encephalitis and Wyeomyia virus. Adult females of *Ad. squamipennis*, the third most frequent species observed in this study, are ornithophilic but may accidentally bite humans (GABALDON *et al.* 1977; DÉGALLIER *et al.* 1992). *Aedeomyia squamipennis* is a vector of West Nile virus and *Plasmodium* spp., which infect birds (GABALDON *et al.* 1977). The last bird census in 2004 for this study area, registered 142 bird species, of which five were migratory birds (CAMPOS *et al.* 2008). Hence, the large number of *Ad. squamipennis*, collected in our study, may be related to the high diversity of birds in this campus. Moreover, migratory birds can pose a threat for arboviral outbreaks with the presence of *Ad. squamipennis*, because infected birds with West Nile virus bitten by these mosquitoes can consequently transmit the pathogens to the residents as well as the students on the campus (MARCONDES *et al.* 2017).

Although the presence of main vectors of malaria were observed in the study area, the risk of transmission of malaria on campus may be low as a small number of vectors were actually collected. On the other hand, the risk of malaria transmission may be quite high in nearby neighborhoods due to their close proximity to favorable breeding sites and the high population density of these neighborhoods. BARBOSA & SOUTO (2011) studied the ecological aspects of *An. darlingi* and *An. marajoara*, in two districts situated at the outskirts of Macapá (Bairro Zerão and Bairro Marabaixo I); one of which, the neighborhood Zerão, is the campus (our study area). The peak hourly activities observed by the authors is the same as recorded in this study for *An. darlingi* (between 18:30 h and 19:30 h), and *An. marajoara* (between 19:30 h and 20:30 h), while in Marabaixo I, peak activity for both species were between 19:30 h and 20:30 h.

This study reports the presence of three culicid species for the first time in Amapá and also makes a contribution towards the diversity of Culicidae in Macapá, Amapá. The presence of important mosquito vectors as observed in this study enforces the importance of similar surveys and recommends extensive mosquito surveillance activities in different ecosystems, to prevent and decrease the transmission of mosquito-borne diseases.

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