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Diversity of *Anopheles* spp. (Diptera: Culicidae) in an Amazonian Urban Area

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Abstract
The genus *Anopheles* encompasses several species considered as vectors of human infecting *Plasmodium.* Environmental changes are responsible for behavior changes in these vectors and therefore the pattern of malaria transmission. To better understand the dynamics of malaria transmission, this study aimed at identify the species of adult anophelines found in a malaria endemic urban area of the Amazon region, Mâncio Lima, located in the Acre State Brazil. Using Shannon-type light traps installed at 11 collection points near fish ponds, a total of 116 anophelines were collected belonging to nine species. *Anopheles darlingi* Root 1926 and *An. albitarsis s.l.* Lynch-Arribalzaga 1878 were the most abundant and predominant species. Despite the low number of captured adult anophelines, the occurrence of *An. darlingi* throughout all urban area and the presence of secondary vectors reinforce the need of a permanent and continuous entomological surveillance.

Introduction

Malaria is still considered one of the most important parasitic diseases in the tropics. It is caused by parasites of the genus *Plasmodium*, and transmitted by mosquitoes of the genus *Anopheles*. Anophelines have also been implicated as local or secondary vectors in the transmission cycles of microfilariae and arboviruses in Africa and Argentina (Mitchell *et al* 1985, Consoli & Lourenço-de-Oliveira 1994, Diallo *et al* 2014). Anthropogenic environmental changes have altered the behavior of *Anopheles* spp. and consequently, patterns of malaria endemicity in the Amazon region.

Brazilian anophelines are diverse, with 55 described species of which 33 occur in the Amazon region (Tadei *et al* 1998, Rebêlo *et al* 2007). It must be noted that the anopheline fauna may be more diverse than found in this study of Rebêlo *et al* (2007). Several of the observed species are part of cryptical species complex, which share some degree of sympatry and have different levels of susceptibility to *Plasmodium* spp. (Mulamba *et al* 2014, Scarpassa *et al* 2016).

The main vector of malaria in the Amazon region is *Anopheles (Nys.) darlingi* Root 1926, but other species belonging to the subgenus *Nyssorhynchus* have also been incriminated as malaria vectors, including *An. albitarsis s.l.* Lynch-Arribalzaga 1878, *An. deaneorum* Rosa-Freitas 1989, *An. braziliensis* Chagas 1907, *An. nuneztovari* Galbadon 1940, *An. oswaldoi s.l.* Peryassú 1922, *An. triannulatus* Neiva & Pinto 1922, *An. strodei* Root 1926, *An. evansae* Brèthes 1926, *An. galvaoi* Causey, Deane & Deane, 1943, and *An. aquasalis* Curry 1932. The subgenus *Anopheles* has comparatively lower association with malaria transmission, for example, *An. mattogrossensis* Lutz & Neiva 1911, *An. peryassui* Dyar & Knab 1908 and *An. mediopunctatus s.l.* Theobald 1903, which are considered secondary vectors of malaria when compared to *An. darlingi* (Deane 1989, Branquinho *et al* 1996, Hiwat & Bretas 2011).

Anopheles darlingi occurs in about 80% of the Brazilian territory, and its distribution coincides with that of malaria (Hiwat & Bretas 2011, Reis *et al* 2015a). *Anopheles darlingi* prefers large water bodies such as dams, and fish ponds for breeding (Vittor *et al* 2009, Hiwat & Bretas 2011). In general, anophelines seek vertebrate hosts for blood meals throughout the night, with peaks in feeding activity at dusk and dawn (Consoli & Lourenço-de-Oliveira 1994).

One the most malariogenic Amazonian municipalities of Brazil and South America, is Mâncio Lima, located in northwestern Acre State. The municipality registered 7763 autochthonous cases of malaria in 2016 (Annual Parasite Index - API 452), representing 23% of the total cases registered in the state. Approximately half of the cases notified in the municipality were located in the urban area (3791 cases; API 220.8) (Ministério da Saúde 2017).

The maintenance of malaria in Mâncio Lima has been attributed to the flooded landscape and the abundance of improperly managed fish ponds, which provide important productive breeding sites for malaria vectors, especially *An. darlingi* (Reis *et al* 2015a, b). To better understand the dynamics of malaria transmission, this study aims to identify adults of *Anopheles* species in urban area of Mâncio Lima near residences and fish ponds.

Materials and Methods

Mâncio Lima is one of the most malariogenic Amazonian municipalities in Brazil (upper limit: 7°06′46.60″S, 73°59′19.54″W; inferior limit: 7°46′51.41″S, 72°15′46.59″W), located in northwestern Acre State. Acre is bordered by Bolivia in the southeast and Peru in the south and west, as well as the Brazilian states of Amazonas to the north and Rondônia to the east. Mâncio Lima has an approximate population of 15,206 inhabitants (2.79/km^2), of which 57.6% is distributed in urban areas and 42.39% is distributed among rural or riverine areas (IBGE 2010).

The urban area is divided into seven census tracts and consists of an urban center with a fraction of the streets paved, with heavy commerce and a mostly residential peripheral area where the streets are not paved. The landscape is very heterogeneous, forming a mosaic of forest fragments, pastures, and developed areas, with abundant water bodies consisting mainly of the Japiim River, dams, streams, narrow channels, perennial and temporary pools, and fish ponds.

The climate in the state of Acre is warm and humid equatorial (Köppen-Geiger Af), with rainfall concentrated from November to April (annual values range from 1600 to 2750 mm). The average annual temperature varies from 24.5 to 32°C, with high relative humidity (~ 90%) (SEMA 2012). There are two distinct seasons: dry summers (May to October) and rainy winters (November to April) (Acre State Government).

Adult anophelines were captured using a Shannon light trap and mouth aspirators, by two trained technicians per trap at all times. The traps were installed in peridomiciliary areas at 11 collection points near fish ponds, distributed among 5 census tracts (Fig 1). The selection criterion for the households that received of the traps was to be close to fish ponds where the presence of immature *Anopheles* spp. was previously verified, as described in Reis *et al* (2015a, b). Traps remained in the field from 7:00 to 10:00 pm.

Species identification was carried out using the direct observation of morphological characters with a stereoscopic microscope, following the dichotomous keys by Consoli & Lourenço-de Oliveira (1994) and by Forattini (2002). Demographic data of the municipality of Mâncio Lima were extracted from the Brazilian Institute of Geography and Statistics website (IBGE 2010) and the area calculation for census tracts was performed using the "Calculate Geometry" function in ArcGis 10.1 (ESRI® 2013). The environmental indicator "forested area per km^2" was calculated per census tract by subtracting the total percentage of deforested area from the total land area. Land cover data for 2010 were provided by the Acre Technology Foundation. All fish ponds were georeferenced using GPS, and the number of fish ponds was extracted for each census sector. From this variable, we constructed the indicator "number of fish ponds per population of each sector."

To analyze temporal differences in anopheline captures, we used a chi-square test and a significance level of < 0.05 using the R program (R Core Team) (R Development Core Team 2014). Ethical clearance was obtained from the Ethical Committee in Research (CEP 402/039) from the Oswaldo Cruz Foundation, Ministry of Health, Brazil.

Results and Discussion

During the months of July and October 2012 and February, July and December 2013, a total of 116 *Anopheles* spp. females were collected, 23 of which were only identifiable to genus due to damage to important morphological structures during collection and transportation to Rio de Janeiro, Brazil. The remaining 93 specimens belonged to 2 subgenera and 9 species, as follows: subgenus *Nyssorhynchus* (*An. darlingi*, *An. albitarsis s.l.*, *An. deaneorum*, *An. brasiliensis*, *An. lanei* Galvão & Amaral 1938, *An. oswaldoi s.l.*, *An. triannulatus s.l.*, *An. mattogrossensis*) and subgenus *Lophopodomyia* (*An. gilesi*, Neiva 1908) (Table 1). The most abundant species were *An. albitarsis s.l.* (27%) and *An. darlingi* (26%).

Anopheles darlingi was captured at all collection hours, peaking between 7:00 and 8:00 pm. There was a significant

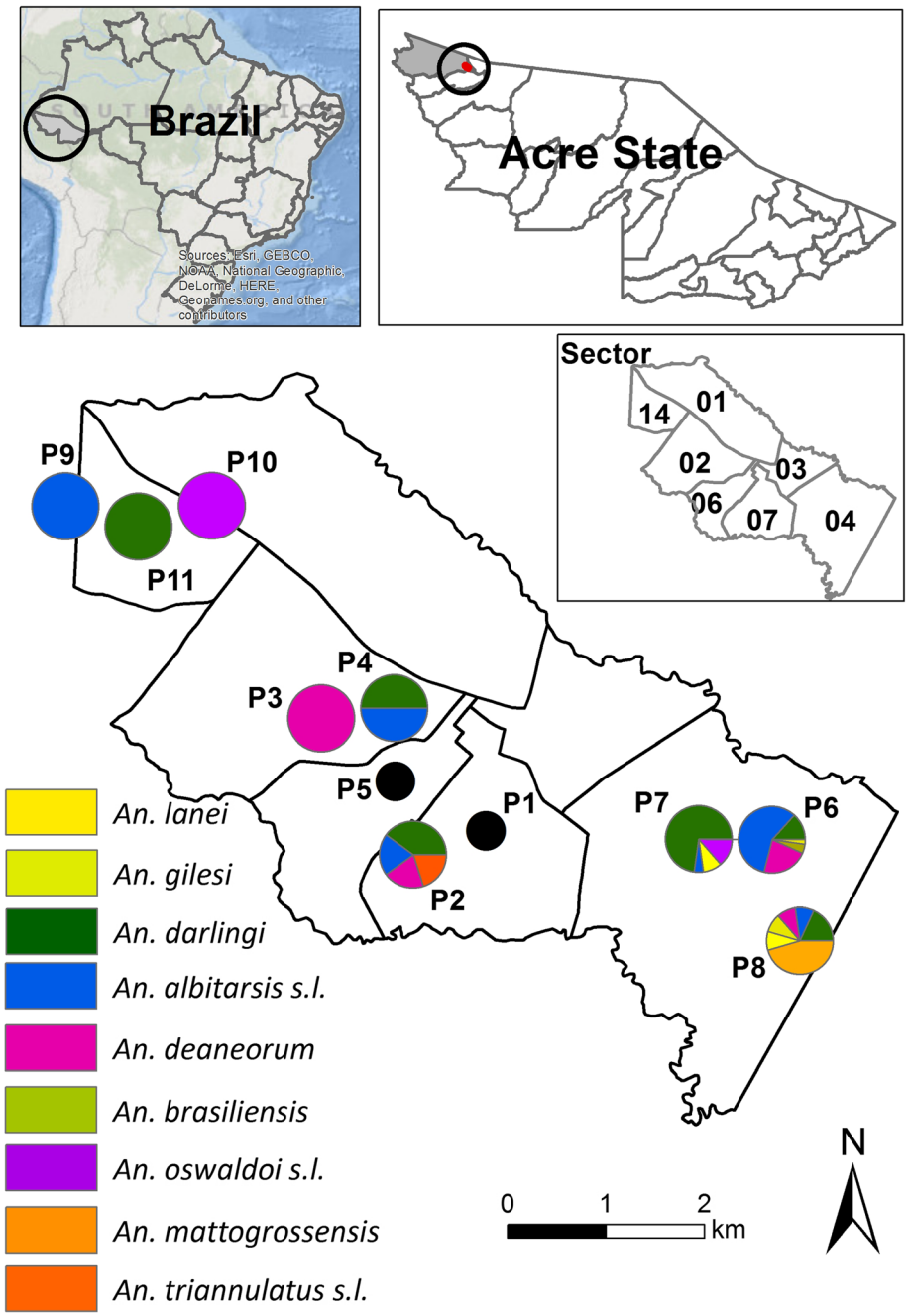

Fig 1 Distribution map for *Anopheles* spp. adults in the urban zone of Mâncio Lima, Acre from July 2012 to December 2013.

difference between the number of *Anopheles* captured and time of capture ($X^2 = 89.2$; $p < 0.0001$). Ninety-two specimens were captured between 7:00 and 8:00 pm, including *An. albitarsis s.l.* (34%), *An. darlingi* (25%), and *An. deaneorum* (11%). From 8:00 to 9:00 pm, 19 specimens were captured, in which *An. darlingi* was predominant with 6 specimens captured (32%). From 9:00 to 10:00 pm, only 5 specimens were captured, 2 of which were *An. darlingi*. Our results agree with Silva-Vasconcelos *et al* (2002) which indicate the early hours of the night as the peak of feeding activity of *Anopheles* species. *Anopheles darlingi* and *An. albitarsis s.l.* were the most frequently captured species, occurring in more than 50% of the collection points. *Anopheles mattogrossensis*, *An. triannulatus*, and *An. brasiliensis* were the least common, each being captured at only one collection point (Table 1).

In the present study, the total number of captured *Anopheles* adults was low ($N = 116$), probably due to method used, compared to surveys conducted in other Brazilian areas. For example, Xavier & Rebêlo (1999) used human bait for 12 collections throughout the year at São Luís Island in Raposa (Maranhão State), and captured 1407 adult

Table 1 Total numbers of *Anopheles* spp. captured using Shannon traps in Mâncio Lima, Acre, from July 2012 to December 2013.

Species	Collection points	July 2012	October 2012	July 2013	October 2013	December 2013	Total
An. darlingi	P2, P4, P6, P7, P8, P11	12	10	3	4	2	31
An.albitarsis s.l.	P2, P4, P6, P7, P8, P9	5	1	2	22	2	32
An.deaneorum	P2, P3, P6, P8	3		1	7	2	13
An. oswaldoi s.l.	P7, P10		3	1			4
An. lanei	P7, P8	1	2				3
An. gilesi	P6, P8	1			1		2
An. mattogrossensis s.l.	P8	5					5
An. brasiliensis	P6				2		2
An. triannulatus s.l.	P2	1					1

anophelines distributed among 6 species of the subgenus *Nyssorhynchus*. Maciel & Missawa (2012) captured 3160 anophelines using human bait during evening collections (6:00 to 10:00 pm) in Porto Velho, Rondônia. On the other hand, Guimarães *et al* (1997) in Guairá, Paraná, demonstrated increased efficiency of *Anopheles* mosquitoes collection in Shannon traps (56.72%) compared to human bait (43.28%).

Our results showed a high species diversity of anopheline in an urban area and corroborate with previous studies. In the municipality of Anájas, Belém, six species of anophelines were collected with the predominance of *An. darlingi* ($N = 381$; 14.1%) and *An. oswaldoi* ($N = 22$, 0.8%) (Santos *et al* 2005). In the Vila Candelária, Rondônia, an urban river community, nine species of anophelines were captured, with predominance of *An. darlingi* ($N = 2.131$; 94%) and *An. nuneztovari* ($N = 87$; 88.4%) (Gil *et al* 2007). In the urban area of Boa Vista, Roraima, seven species were captured with predominance of *An. darlingi* and *An. albitarsis s.l.* (Silva-Vasconcelos *et al* 2002, Póvoa *et al* 2006). Additionally, Póvoa *et al* (2006) showed that in some districts of Boa Vista, *An. albitarsis* E (a member of the Albitarsis complex) was significantly more abundant than *An. darlingi* and was incriminated as the main vector of malaria. However, these authors highlighted the strong role of *An. darlingi* in malaria transmission even at low densities, as occurrence of this species is typically associated with areas of high malaria prevalence.

In Mâncio Lima, census sector 04 had the highest richness and abundance of captured specimens (Fig 1, Table 2). This sector encompasses more than 50% of the deforested territory within the urban zone while, as well as 1/3 of the fish ponds (~ 4 fish ponds/100 inhabitants). In the others sectors, the fish ponds density was < 4 fish ponds/100 inhabitants. Fish ponds have been incriminated as the main breeding sites

Table 2 Environmental and sociodemographic characteristics of experimental census tracts in the urban center of Mâncio Lima, Acre.

Census sector ID	Collection point	Month/year	Population	(Fish ponds/pop) × 100	Total area (km^2)	Forested area (km^2)
1	NA*		986	2.03	6.49547	4.67222
2	P3	July 2012	1243	1.37	4.73051	0
	P4	July 2012				
3	NA*		1266	0.55	2.11324	1.251506
4	P6	July, October, December 2013	1243	4.24	7.57189	2.632327
	P7	July and October 2012				
	P8	July and October 2012				
5	P9	July 2012	1155	1.73	2.08001	0
	P10	July 2013				
	P11	July 2012				
6	P5	July 2012	1488	0.74	2.43121	0
7	P1	July 2012	1369	1.83	3.19614	0.29018
	P2	July and October 2012				

*NA** not sampled

for immature forms of *Anopheles* species (Reis *et al* 2015a) and are capable of supporting anopheline populations throughout the year if proper management practices are not maintained (Reis *et al* 2015b).

This study reinforces the importance of keeping updated the knowledge of anopheline fauna in areas with malaria transmission, especially in places where urban transmission is known. Many urban areas in the Amazon, such as Mâncio Lima, have been suffering large landscape and ecological transformations, such as the large scale development of aquaculture that changes the temporal and spatial receptivity of the area for anophelines. Despite the low number of adult anophelines captured, our study evidences a diverse population of anophelines, composed not only by the main malaria vector in the Amazonian region (*An. darlingi*) but also by several secondary vectors, such as *An. albitarsis s.l.* and others. These results highlight the necessity of a continuous entomological surveillance in the area to inform control strategies.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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