Night and crepuscular mosquitoes and risk of vector-borne diseases in areas of piassaba extraction in the middle Negro River basin, state of Amazonas, Brazil

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A study of crepuscular and night-biting mosquitoes was conducted at remote settlements along the Padauiri River, middle Negro River, state of Amazonas, Brazil. Collections were performed with human bait and a CDC-light trap on three consecutive days per month from June 2003-May 2004. In total, 1,203 h of collection were performed, of which 384 were outside and 819 were inside houses. At total of 11,612 specimens were captured, and Anophelinae (6.01%) were much less frequent than Culicinae (93.94%). Anopheles darlingi *was the most frequent Anophelinae collected. Among the culicines, 2,666* Culex (Ae.) clastrieri *Casal & Garcia, 2,*394 Culex. (Mel.) vomerifer *Komp, and 1,252* Culex (Mel.) eastor *Dyar were the most frequent species collected. The diversity of insects found reveals the receptivity of the area towards a variety of diseases facilitated by the presence of vectors involved in the transmission of* Plasmodium, *arboviruses and other infectious agents.*

Key words: mosquitoes - vector-borne disease - piassava extraction areas - Negro River basin - state of Amazonas - Brazil

Currently, the presence of emerging and re-emerging diseases is demonstrated by the increasing number of notifications from different localities within the Amazon region. Vasconcelos et al. (2001) reported the circulation of 187 different arboviruses in the Amazon region. Because of the constant appearance of infectious diseases, it is important to obtain knowledge regarding the receptivity in areas where poverty-stricken populations are in contact with possible agents. Furthermore, it is essential to determine the medically relevant vectors of infectious diseases, especially the various mosquito species that carry pathogens (Vieira et al. 1998, Hutchings et al. 2005).

One susceptible population includes people living along the Padauiri River, which is located in the middle of the Negro River basin (municipality of Barcelos, state of Amazonas) (Suárez-Mutis et al. 2007). A major source of this population's income comes from harvesting piassaba, a plant fibre used for making brooms and craftwork. Because the piassaba harvesters enter the forest to search for this plant fibre, they invade environments in which infectious agents present in vectors and reservoirs frequently circulate. Currently, the entomological fauna of this area remains unknown. The present study reports the results from an initial survey of mosquitoes collected inside and outside of the homes in communities along the Padauiri River. The results suggest the need to establish both entomological and epidemiological surveillance of vector-borne diseases in this region of Brazil.

MATERIALS AND METHODS

Collection, identification and storage of adult mosquitoes - Collections were performed on three consecutive days per month between June 2003-May 2004. Mosquitoes were captured using human bait and a manual aspirator. The individuals who operated the aspirators were trained members of the research team or associated with the municipality entomological surveillance team. Collections were carried out from 18-22 h over two days and from 18-6 h on the following day. Between 18-22 h, collections using human bait were performed both inside and around the outside of houses and after 22 h, only inside houses.

Mosquitoes were collected in CDC light traps equipped with an incandescent light bulb (Centers for Disease Control, CDC miniature light trap, model #2836, BioQuip Products, Inc, Rancho Dominguez, CA, USA, http://www.bioquip.com). CDC traps were positioned inside domiciles one metre above floor level for 12 h (from 18-6 h).

All specimens were stored in Vacutainer® tubes (Becton-Dickinson) containing silica gel until identification according to genus or species. Tubes were kept separated according to date, time, community, location (inside or outside of houses) and collection technique. Specimen identifications were performed in the Entomological Division of the Institute of Tropical Medicine of Amazonas using the identification keys of Lane (1953), Forattini (1962) and Consoli and Lourenço-de-Oliveira (1994). It was not possible to identify all specimens due to the absence of males. The samples obtained in this study are deposited in the entomological collection of the Malaria Division at the Institute of Tropical Medicine of Ama-

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zonas, except for mosquitoes of genus *Anopheles*, which are located at the Parasitic Diseases Laboratory of the Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, Brazil.

Study area - Adult mosquitoes were collected over the course of one year in localities along the Padauiri River from Cachoeira da Aliança to its confluence with the Negro River, comprising a total of 124 km (Fig. 1). There are five small settlements in the survey area; all of them are located very close to the forest (on average, 5-50 m from houses): Tapera (area 1: 00°11'41.3"S 64°04'42"W), Acú-Acú (area 2: 00°06'24"S 64°01'45"W), Acuquaia (area 3: 00°13'15"N 63°59'24"W), Ararinha/Ararão (area 4: 00°29'63"N 64°03'30"W) and Nova Jerusalém (area 4: 00°40'37"N 64º10'34"W).

The Padauiri River forms the boundary between the municipalities of Barcelos (East) and Santa Isabel do Rio Negro (West) in the north of the state of Amazonas. The drainage basin encompasses a humid tropical forest situated close to sea level, with a mean annual rainfall greater than 2,600 mL (Maia-Neto 1998). The climate is characterised by two seasons: a wet season (April-September) and a dry season (October-March). Maximum flooding occurs in July and August. The lowest water levels are observed between January-March, when the heat is intense. The Padauiri River flows from north to south and has yellowish, sediment-rich, alkaline water. It is a tributary of the Negro River and, at the site of confluence, its water mixes with black, acidic water of other black rivers. The changes in water characteristics contribute to the different habitats observed in this region, producing wide biological diversity. The Padauiri River is one of the locations of greatest piassaba extraction activity in the Amazon region.

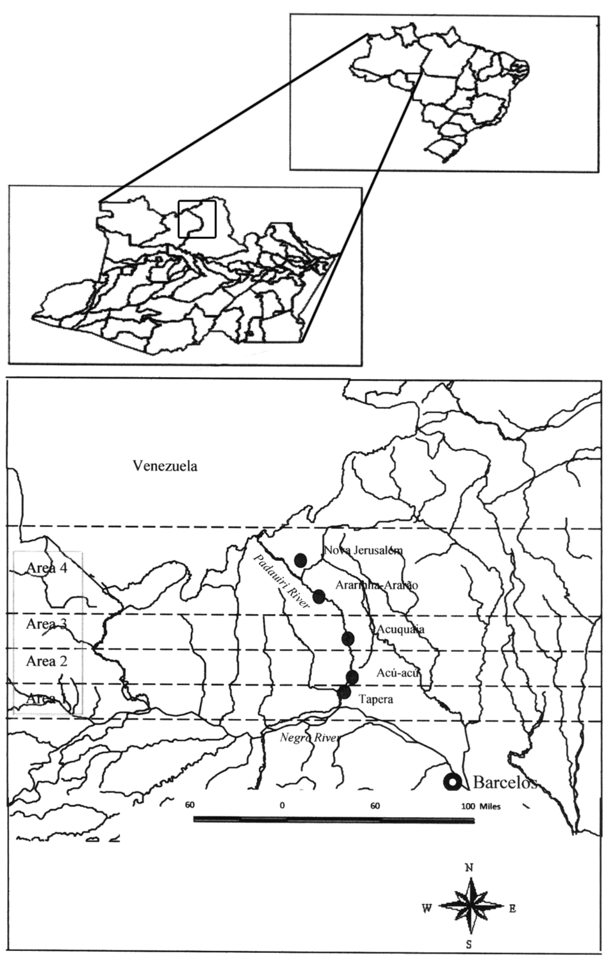

Fig. 1: study area in localities of Padauiri River, affluent of Negro River, state of Amazonas, Brazil. The black dots correspond to five small communities along the river: Tapera, Acú-acú, Acuquaia, Ararinha-Ararão and Nova Jerusalém.

Ethics committee - The Ethical Committee of the Oswaldo Cruz Foundation-Fiocruz approved this study as part of the research project entitled "Study on malaria morbidity in areas of the middle Negro River basin, Amazonas" (protocol n. 157-02).

RESULTS

A total of 1,203 h of collection were performed, of which 384 were outside houses and 819 were inside houses. Overall, 11,612 specimens were captured, including 698 (6.01%) Anophelinae and 10,914 (93.99%) Culicinae. Among the Culicinae were 10,192 mosquitoes of the genus *Culex* (87.77%), 554 of the genus *Psorophora* (4.77%), 164 of the genus *Aedes* (1.41%) and four of *Haemagogus janthinomys* (0.03%) (Table I). Only mosquitoes caught using human bait will be discussed. The mosquitoes collected are listed in Table I.

Six hundred ninety-eight anophelines were collected, of which 631 (5.43%) belonged to *Anopheles darlingi* Root and 67 to other species. Considering that 83.83% (529/631) *An. darlingi* were captured indoors and 16.16% (102/631) outdoors, the bite rate per man-h was 0.52, but for each separate environment, it was 0.63 indoors and 0.23 outdoors. Differences between indoors and outdoors were statistically significant ($p < 0.001$). Among other species of anophelines, 28 individuals (4.4%) were captured indoors, whereas 39 (6.2%) were captured outdoors (Table II). Regarding *An. darlingi*, 409 (64.8%) were obtained inside houses during the rainy season and 120 (19.0%) were obtained during the dry season. Fifty-four specimens (8.6%) were collected outdoors during the rainy season and 48 (7.6%) were obtained during the dry season. The chance of being bitten by *An. darlin*gi inside houses during the rainy season was 3.03 times greater (95% CI: 1.91-4.81) than that during the dry season. This difference was statistically significant ($p = 0.001$) (Table II).

The studied areas were distinct with regard to frequency of each mosquito species in the collections. Five hundred thirty-one specimens of *An. darlingi* (84.2%) were collected in area 4, in which the sampling localities were near large piassaba trees. For both indoor and outdoor houses in area 1, the biting rate per man-h was 0.03; in area 2 it was 0.09; in area 3 it was 0.16; and in area 4 it was 5.6. Inside houses, the biting rate was 0.03 in area 1, 0.05 in area 2, 0.15 in area 3 and 6.7 in area 4. The chance of collecting one specimen of *An. darlingi*

TABLE I

Mosquitoes collected in four areas of the Padauiri River, middle Negro River, state of Amazonas, Brazil

	Intradomiciliar (ID)						Peridomiciliar (PD)			
	CDC Trap		Human bait		Total ID		Human bait		Total	
Species	n	%	n	%	n	%	n	%	n	%
Anophelinae										
Anopheles (Nyssorhynchus) darling Root, 1926	0	0.00	529	4.56	529	4.56	102	0.88	631	5.43
Anopheles (Nyssorhynchus) nuneztovari Gabaldón, 1940	3	0.03	1	0.01	4	0.03	7	0.06	11	0.09
Anopheles (Nyssorhynchus) oswaldoi (Peryassú, 1922)	0	0.00	0	0.00	0	0.00	14	0.12	14	0.12
Anopheles (Anopheles) mediopunctatus (Lutz, 1903)	11	0.09	6	0.05	17	0.15	20	0.17	37	0.32
Anopheles (Anopheles) peryassui Dyar & Knab, 1908	2	0.02	1	0.01	3	0.03	0	0.00	3	0.03
Anopheles (Stethomyia) nimbus (Theolbad, 1902)	1	0.01	1	0.01	2	0.02	0	0.00	2	0.02
Subtotal Anophelinae	17	0.15	538	4.63	555	4.78	143	1.23	698	6.01
Culicinae										
Culex (Culex) coronator Dyar & Knab, 1906	5	0.04	11	0.09	16	0.14	24	0.21	40	0.34
Culex (Culex) sp.	36	0.31	46	0.40	82	0.71	38	0.33	120	1.03
Culex (Culex) declarator Dyar & Knab, 1906	0	0.00	2	0.02	2	0.02	4	0.03	6	0.05
Culex (Aedinus) amazonensis (Lutz, 1905)	0	0.00	2	0.02	2	0.02	4	0.03	6	0.05
Culex (Aedinus) clastrieri Casal & Garcia, 1968	125	1.08	1,273	10.96	1,398	12.04	1,268	10.92	2,666	22.96
Culex (Anoedioporpa) originator Gordon & Evans, 1922	5	0.04	11	0.09	16	0.14	6	0.05	22	0.19
Culex (Carrollia) anduzei Cerqueira & Lane, 1944	1	0.01	5	0.04	6	0.05	16	0.14	22	0.19
Culex (Melanoconion) atratus Theobald, 1901	175	1.51	133	1.15	308	2.65	332	2.86	640	5.51
Culex (Melanoconion) bastagarius Dyar & Knab, 1906	190	1.64	48	0.41	238	2.05	272	2.34	510	4.39
Culex (Melanoconion) crybda Dyar, 1924	5	0.04	15	0.13	20	0.17	38	0.33	58	0.50
Culex (Melanoconium) sp.	233	2.01	249	2.14	482	4.15	594	5.12	1,076	9.27
Culex (Melanoconion) eastor Dyar, 1920	167	1.44	509	4.38	676	5.82	576	4.96	1,252	10.78
Culex (Melanoconion) pedroi Sirivanakarn & Belkin, 1980	0	0.00	14	0.12	14	0.12	42	0.36	56	0.48
Culex (Melanoconion) spissipes (Theolbald, 1903)	14	0.12	14	0.12	28	0.24	16	0.14	44	0.38
Culex (Melanoconion) taeniopus Dyar & Knab, 1907	25	0.22	101	0.87	126	1.09	234	2.02	360	3.10
Culex (Melanoconion) theobaldi (Lutz, 1904)	1	0.01	53	0.46	54	0.47	74	0.64	128	1.10
Culex (Melanoconion) vaxus Dyar, 1920	0	0.00	82	0.71	82	0.71	100	0.86	182	1.57
Culex (Melanoconion) vomerifer Komp, 1932	28	0.24	1,362	11.73	1,390	11.97	1,004	8.65	2,394	20.62
Culex (Melanoconion) ybarmis Dyar, 1920	286	2.46	16	0.14	302	2.60	232	2.00	534	4.60
Culex (Melanoconion) zeteki Dyar, 1918	0	0.00	0	0.00	0	0.00	2	0.02	2	0.02
Culex (Microculex) chryselatus Dyar & Knab, 1919	11	0.09	21	0.18	32	0.28	30	0.26	62	0.53
Culex (Microculex) stonei Lane & Whitman, 1943	0	0.00	2	0.02	2	0.02	10	0.09	12	0.10
Subtotal Culicini	1,307	11.26	3,969	34.18	5,276	45.44	4,916	42.34	10,192	87.77
Psorophora (Janthinosoma) albigenu (Peryassu, 1908)	0	0.00	26	0.22	26	0.22	0	0.00	26	0.22
Psorophora (Janthinosoma) albipes (Theobald, 1907)	3	0.03	9	0.08	12	0.10	2	0.02	14	0.12
Psorophora (Janthinosoma) amazônica Cerqueira, 1960	64	0.55	208	1.79	272	2.34	142	1.22	414	3.57
Psorophora (Janthinosoma) ferox (von Humboldt, 1819)	16	0.14	36	0.31	52	0.45	22	0.19	74	0.64
Psorophora (Janthinosoma) sp.	18	0.16	2	0.02	20	0.17	0	0.00	20	0.17
Psorophora (Psorophora) cilipes (Fabricius, 1805)	3	0.03	1	0.01	4	0.03	2	0.02	6	0.05
Haemagogus (Haemagogus) janthinomys Dyar, 1921	1	0.01	3	0.03	4	0.03	0	0.00	4	0.03
Aedes (Ochlerotatus) hortator Dyar & Knab, 1907	0	0.00	0	0.00	0	0.00	2	0.02	2	0.02
Aedes (Ochlerotatus) scapularis Rondani, 1848	1	0.01	13	0.11	14	0.12	4	0.03	18	0.16
Aedes (Ochlerotatus) fluviatilis (Lutz, 1904)	0	0.00	6	0.05	6	0.05	0	0.00	6	0.05
Aedes (Ochlerotatus) fulvus (Wiedemann, 1828)	1	0.01	3	0.03	4	0.03	4	0.03	8	0.07
Aedes (Ochlerotatus) serratus Theobald, 1901	5	0.04	77	0.66	82	0.71	48	0.41	130	1.12
Subtotal Aedini	112	0.96	384	3.31	496	4.27	226	1.95	722	6.22
Subtotal Culicinae	1,419	12.22	4,353	37.49	5,772	49.71	5,142	44.28	10,914	93.99
Total	1,436	12.37	4,891	42.12	6,327	54.49	5,285	45.51	11,612	100.00

in area 4 was 46.27 times greater than that for any other area ($p < 0.001$). Excluding *An. darlingi*, other species of Anophelinae were more frequently found in areas other than area 4. These data correlate with the incidence of malaria, which is higher in area 4 than in any other three locations (Suárez-Mutis & Coura 2007).

TABLE II

Anophelinae species collected indoors and in peri-domestic area of the Padauiri River localities by season

	Area 1		Area 2		Area 3		Area 4				
	Tapera		Acú-acú		Acuquaia		Ararinha-Ararão and Nova Jerusalém		Total		
	ID	PD	ID	PD	ID	PD	ID	PD	ID	PD	Total
1st rainy season											
An. darlingi	5	1	6	5	14	8	188	24	213	38	251
Other anophelines	4	4	7	7	1	14	0	3	12	28	40
1st dry season											
An. darlingi	0	0	4	12	6	0	103	32	113	44	157
Other anophelines	0	0	0	0	0	0	0	0	0	0	0
2nd rainy season	0										
An. darling	4	0	4	3	16	12	172	1	196	16	212
Other anophelines	3	4	8	4	1	3	4	0	16	11	27
2nd dry season											
An. darling	0	0	0	0	0	0	7	4	7	4	11
Other anophelines	0	0	0	0	0	0	0	0	0	0	0
Total											
An. darlingi	9	1	14	20	36	20	470	61	529	102	631
Other anophelines	7	8	15	11	2	17	4	3	28	39	67

ID: indoor; PD: peri-domestic.

Different nycthemeral bite-peak cycles were found. In area 1, the number of specimens collected was very low, thus compromising the analysis, but apparently the anophelines continued to bite throughout the night. In area 2, the number of collected specimens was also low, but *An. darlingi* continued to bite throughout the night, peaking at dusk. In area 3, mosquito activity peaked at dusk, but frequent and great numbers of mosquitoes continued until almost 22 h. In area 4, indoor collections showed that the mosquitoes peaked between 19-20 h and decreased thereafter, with a second peak between 4-6 h. Regarding *An. darlingi*, 31.4% of the specimens were collected between 18-20 h and 68.6% were collected throughout the night. In area 4, mosquitoes were abundant most of the time during the year (Fig. 2).

Using human bait, 8,885 mosquitoes representing six subgenera of the genus *Culex* were collected. Specimens of the subgenus *Melanoconion* Theobald, 6,112 individuals (52.6%) were most frequently collected. Among these, *Culex vomerifer* Komp (2,366/11612; 20.38%) and *Culex (Mel.) eastor* Dyar (1,085/11612; 9.34%) were most frequently collected. It was not possible to identify 1,076 specimens because they were damaged.

A total of 2,547 specimens (21.93%) belonged to the subgenus *Aedinus* Lutz and 21.88% (2541) were *Cx. (Aed.) clastrieri* Casal & Garcia. Regarding the subgenus *Culex* Linnaeus, 125 specimens were collected (1.07%); *Microculex* Theobald, 0.54% (63/11612) and *Carrollia* Lutz, 0.18% (21/11612) (Table I).

Using human bait, 450 mosquitoes of the genus *Psorophora* Robineau-Desvoidy were collected, 3.85% (447/11612) belonged to the subgenus *Janthinosoma* and *Psorophora amazonica* Cerqueira was the most frequently found. One hundred fifty-seven mosquitoes of the genus *Aedes* Meigen were captured (1.35%), among which *Aedes (Och.) serratus* (Theobald) was the species most frequently captured (125/157). No *Aedes aegypti* or *Aedes albopictus* specimens were found.

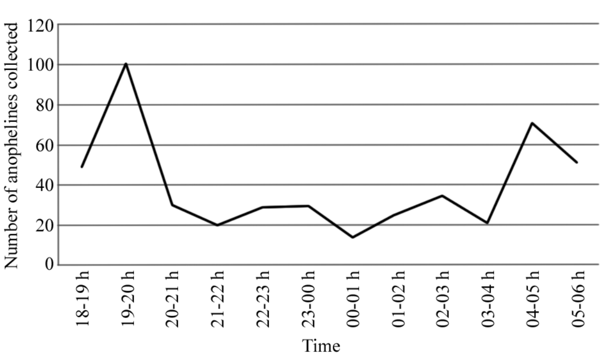

Fig. 2: bimodal indoors biting activity of *An. darlingi* in the Area 4 (Ararinha-Ararão and Nova Jerusalém).

DISCUSSION

Measures to control the spread of infectious diseases include surveys of the dynamics of parasite transmission. For human malaria, the primary vectors are mosquitoes. The first entomological survey of mosquitoes in the area of the middle Negro River basin, an area with a high incidence of malaria, was conducted in the Jaú National Park between 1993-1995 (Hutchings et al. 2005). In the north of the state of Amazonas, another study was conducted in the municipality of São Gabriel da Cachoeira (Hutchings et al. 2002). The present study expands our knowledge of the entomological fauna responsible for the incidence of malaria in the area of the Padauiri

River, which has its source in the Venezuelan Amazon region and is a tributary of the Negro River.

Among 55 anophelines species registered in Brazil by Rebêlo et al. (2007), we collected six species in the Padauiri River basin. The most frequently captured species in our survey was *An. darlingi*. This species exhibits a high survival rate in comparison to other anophelines (Kiszewski et al. 2004) and is highly anthropophilic, endophilic and endophagic. Additionally, *An. darlingi* is the mosquito species most frequently found infected with sporozoites of any of the three human *Plasmodium* species circulating in Brazil (Deane et al. 1948, 1988, Deane 1986, Tadei et al. 1998, Tadei & Thatcher 2000). Several studies show that *An. darlingi* is involved in outdoor malaria transmission at dusk (Deane 1986, Lourenço-de-Oliveira et al. 1989, Consoli & Lourenço-de-Oliveira 1994), although this species also transmits *Plasmodium* spp. to humans indoors (Rosa-Freitas et al. 1998). Among the *An. darlingi* specimens captured in our study, 83.8% were collected on human bait indoors, revealing that, in the Padauiri River area, this species is endophagic. Fig. 2 shows that the activity of this species peaked at dawn and dusk in area 4, but remained active throughout the night. This pattern of feeding was not observed in other areas, maybe because of the small number of specimens collected in other areas. Similar to the results obtained for the area 4, bimodal feeding activity has been observed in other areas of the Amazon region (for example, near the Ituxi River (Roberts et al. 1987) in Ariquemes, (Tadei et al. 1988) and in Costa Marques, both in Rondônia (Klein & Lima 1990). Interestingly, it is known that great variability in biting activity patterns can be observed in *An. darlingi,* in contrast with the more stable patterns observed for other anophelines (Voorham J 2002). Although we did not carry out infectivity studies in the Padauiri River area, the results for both malaria cases together with asymptomatic infected individuals suggest that area 4 poses the greatest risk for human infection with *Plasmodium* (Suárez-Mutis & Coura 2007).

Over the study period, the annual parasitic index (API) reported was 602.3/1,000 inhabitants, thus classifying the area as high epidemiological risk (Suárez-Mutis 2007). However, there were differences among communities that were statistically significant. For area 1 the API was 303.6/1,000; for area 2 it was 268.0/1,000; for area 3 it was 879.4/1,000; and for area 4 it was 946.7/1,000. Nearly 20% of the individuals living in the Padauiri River are asymptomatic for *Plasmodium* infection (Suárez-Mutis 2007, Suárez-Mutis & Coura 2007). We think that the high number of human reservoirs that are asymptomatic for *Plasmodium* infection may be the cause of the high incidence of malaria. Considering all of these characteristics, we hypothesise that *An. darlingi* is the primary human malaria vector in the Padauiri River area.

Although it displayed low density, the anopheline *Anopheles mediopunctatus s.l.* was also collected in this study. Because we collected only adult females, it was not possible to identify the specimens correctly. According to Sallum et al. (1999) and Wilkerson and Sallum (1999), it is necessary to examine characteristics of the immature stages and male terminalia to discriminate among *Anopheles costai*, *Anopheles forattini* and *An. mediopunctatus*. *Anopheles oswaldoi* was incriminated as a vector of *Plasmodium* in deforested areas of Acre (Branquinho et al. 1996). Although different groups have shown that *An. oswaldoi* possesses low susceptibility to *Plasmodium* infection, it seems that this mosquito is an important vector in the absence of *An. darlingi* (Branquinho et al. 1996). *Anopheles nuneztovari* is an important vector for malaria in Colombia, Venezuela and Peru. In Brazil, the species was incriminated as a vector of *Plasmodium vivax* VK210 and VK247 and *Plasmodium malariae* in localities of Pará and Amapá, where *An. nuneztovari* is present at high densities (Galardo et al. 2007). However, the importance of *Plasmodium falciparum* as a vector is incompletely understood (Tadei et al. 1988, Galardo et al. 2007). *An. nuneztovari* was also incriminated as vector of Guaroa (Bunyaviridae) virus in Pará (Dégallier et al. 1992). Out of the eight subgenera of *Culex* occurring in Brazil (Consoli & Lourenço-de-Oliveira 1994), six were found in the Padauiri River area: *Culex, Aedinus, Carrollia, Melanoconion, Anoedioporpa* and *Microculex*. Mosquitoes from subgenus *Melanoconion* were the most frequently found in the studied area, and *Cx. vomerifer* was the most common species detected (20.38%). In contrast, Hutchings et al. (2005) obtained only 51 specimens in Parque Nacional do Jaú. *Cx. vomerifer* was found to be infected with certain arboviruses such as Caraparu (Brazil, Panama), Guama (Colombia, Peru), Itaqui (Brazil, Venezuela), Moju (Venezuela), Murutucu (Belém), Ossa (Panama), Ananindeua, Vinces, Madrid and other types of Bunyaviridae (Shope et al. 1988, Walter Reed Report 1998, Carrara et al. 2005). *Cx. vomerifer* is recognised as one of the vectors of both the enzootic and the epizootic cycle of Venezuelan equine encephalitis (Turrell et al. 2000).

The subgenus *Aedinus* contributed to 2,546/11,612 (21.93%) of the mosquitoes found, among which *Culex clastrieri* was the most frequent species captured. No specimens of *Cx. clastrieri* were obtained in Jaú National Park, but specimens were caught in CDC traps in Querari, in the municipality of São Gabriel da Cachoeira (Hutchings et al. 2002). *Cx. clastrieri* was previously found infected with Iridovirus (Jancovich et al. 2005), although the public health importance of this finding is unknown. Only 1.07% of the culicines collected belonged to the subgenus *Culex*.

Ps. amazonica accounted for less than 4% of all culicines captured in this area, but it was among the 10 species most frequently caught. This was similar to the findings for Jaú National Park. It is not known whether *Ps. amazonica* is implicated in the transmission of infectious agents to humans. *Psorophora ferox* are mosquitoes with diurnal and zoophilic habits, potentially providing an explanation for the small number of individuals collected in our study.

The small number of *Ha. janthinomys* specimens caught in the Padauiri River area may be related to the capture method used in this study, which prioritised nocturnal collection inside and around the outside of houses. This mosquito species has also been found in the Jaú National Park (Hutchings et al. 2005).

Among the four subgenera of *Aedes* present in Brazil, only some specimens of *Ochlerotatus* mosquitoes were collected. One important point to emphasise is that no specimens of *Ae. aegypti* and *Ae. albopictus* (the vectors of urban yellow fever and dengue) were collected.

Using the CDC traps, six specimens of *Lutzomya flaviscutelata* were collected. This species is a vector for *Leishmania (L.) amazonensis* in the Amazon Region (Falqueto & Ferreira 2005). In this area, several cases of American cutaneous leishmaniasis have been documented (unpublished data).

In conclusion, this is the first study relative to Culicidae fauna of localities situated in the middle Negro River basin, state of Amazonas, Brazil. The presence of *An. darlingi* feeding inside houses was expected, given the high prevalence of malaria in the area. The presence of a variety of mosquitoes raises the hypothesis that this area may be need to establish an epidemiological and entomological surveillance system. In addition to subsequent studies aimed at virus isolation, investigations of vector competence, behaviour and biology are needed to determine the true risk of epidemics and endemic diseases in this area.

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REFERENCES

Branquinho MS, Araújo MS, Natal D, Marelli MT, Rocha RM, Taveira FAL, Kloetzel JK 1996. *Anopheles oswaldoi* an important potential malaria vector in Acre, Brazil. *Trans R Soc Trop Med Hyg 90*: 233.

Carrara AS, Gonzales M, Ferro C, Tamayo M, Aronson J, Paessler S, Anishchenko M, Boshell J, Weaver CS 2005. Venezuelan equine encephalitis virus infection of spiny rats. *Emerg Infect Dis 11*: 663-669.

Consoli RAGB, Lourenço-de-Oliveira R 1994. *Principais mosquitos de importância sanitária no Brasil*, Editora Fiocruz, Rio de Janeiro, 225 pp.

Deane LM 1986. Malaria vectors in Brazil. *Mem Inst Oswaldo Cruz 81* (Suppl. II): 5-14.

Deane LM, Causey OR, Deane MP 1948. Notas sobre a distribuição e a biologia dos anofelinos das regiões nordestina e amazônica do Brasil. *Rev Serv Esp Saude Publica 1*: 967-976.

Deane LM, Ribeiro CD, Lourenço-de-Oliveira R, Oliveira-Ferreira J, Guimarães AE 1988. Study on the natural history of malaria in areas of the Rondônia state, Brazil, and problems related to its control. *Rev Inst Med Trop Sao Paulo 30*: 153-156.

DéGallier N, Travassos-Rosa A, Vasconcelos PFC, Hervé JP, de Sá filho GC, Travassos da Rosa JFS, Travassos da Rosa ES, Rodrigues S 1992. Modifications of arbovirus transmission in relation to construction of dams in Brazilian Amazonia. *Ciênc Cult (Sao Paulo) 44*: 124-135.

Falqueto A, Ferreira AL 2005. Reservatórios extra-humanos do complexo Leishmania. Dinâmica de transmissão da infecção ao homem. In JR Coura, *Dinâmica das doenças infecciosas e parasitárias*, Guanabara Koogan, Rio de Janeiro, p. 739-752.

Forattini OP 1962. *Entomologia Médica*, Vol. 2, Universidade de São Paulo, São Paulo, 506 pp.

Galardo AKR, Arruda M, Couto AARDA, Wirtz R, Lounibos LP, Zimmerman RH 2007. Malaria vector incrimination in three rural riverine villages in the Brazilian Amazon. *Am J Trop Med Hyg 76*: 461-469.

Hutchings RSG, Sallum MAM, Ferreira RLM, Hutchings RW 2002. Culicidae (Diptera: Culicomorpha) da Amazônia Ocidental Brasileira: Querari. *Acta Amazônica 32*: 109-122.

Hutchings RSG, Sallum MAM, Ferreira RLM, Hutchings RW 2005. Mosquitoes of the Jaú National Park and their potential importance in Brazilian Amazonia. *Med Vet Entomol 19*: 428-441.

Jancovich JK, Davidson EW, Parameswaran N, Mao J, Chinchar VG, Collins JP, Jacobs B, Storfer A 2005. Evidence for emergence of an amphibian iridoviral disease because of human-enhanced spread. *Mol Ecol 14*: 213-224.

Kiszewski A, Mellinger A, Spielman A, Malaney P, Sachs SE, Sachs J 2004. A global index representing the stability of malaria transmission. *Am J Trop Med Hyg 70*: 486-498.

Klein TA, Lima JBP 1990. Seasonal distribution and biting patterns of *Anopheles* mosquitos in Costa Marques, Rondônia, Brazil. *J Amer Mosq Control Assoc 6*: 700-707.

Lane J 1953. *Neotropical Culicidae,* Vol. II, Universidade de São Paulo, São Paulo, p. 554-1112.

Lourenço-de-Oliveira R, Guimarães AEG, Arlé M, Silva TF, Castro MG, Motta MA, Deane LM 1989. Anopheline species, some of their habits and relation to malaria in endemic areas of Rondônia state, Amazon region of Brazil. *Mem Inst Oswaldo Cruz 84*: 501-514.

Maia Neto RF 1998. Clima e hidrologia. In LI Rojas, L Toledo, *Espaço e doença: um olhar sobre Amazonas*, Fiocruz, Rio de Janeiro, p. VI.

Rebêlo JM, Moraes JL, Alves GA, Leonardo FS, da Rocha RV, Mendes WA, Costa E, Câmara LE, Silva MJ, Pereira YN, Mendonça JA 2007. Distribution of species from genus *Anopheles* (Diptera: Culicidae) in the state of Maranhão, Brazil. *Cad Saude Publica 23*: 2959-2971.

Roberts DR, Alecrim WD, Tavares AM, Radke MG 1987. The house-frequenting, hostseeking and resting behavior of *An. darlingi* in Southeastern Amazonas, Brazil. *J Am Mosq Control Assoc 3*: 433-441.

Rosa-Freitas MG, Lourenço-de-Oliveira R, Carvalho-Pinto CJ, Flores-Mendoza C, Silva do-Nascimento TF 1998. Anopheline species complexes in Brazil. Current knowledge of the related to malaria transmission. *Mem Inst Oswaldo Cruz 93*: 651-655.

Sallum MAM, Wilkerson RC, Forattini OP 1999. Taxonomic study of species formerly identified as *Anopheles mediopunctatus* and resurrection of *An. costai* (Diptera: Culicidae). *J Med Entomol 36*: 282-300.

Shope RE, Woodhall JP, Da Rosa AT 1988. The epidemiology of diseases caused by viruses in Groups C and Guama (Bunyaviridae). In TP Monath, *The arboviruses: epidemiology and ecology*, CRC Press, Boca Raton, p. 37-52.

Suárez-Mutis MC 2007. *Epidemiologia da malária em comunidades do Rio Padauiri, médio Rio Negro, uma área de extrativismo vegetal da piaçaba no estado de Amazonas*, PhD Thesis, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, 169 pp.

Suárez-Mutis MC, Coura JR. 2007. Changes in the epidemiological pattern of malaria in a rural area of the middle Rio Negro, Brazilian Amazon: a retrospective analysis. *Cad Saude Publica 23*: 795-804.

Suárez-Mutis MC, Cuervo P, Leoratti FMS, Morães-Avila SL, Ferreira AW, Fernandes O, Coura Jr. 2007. Cross-sectional study reveals a high percentage of asymptomatic *Plasmodium vivax* infection in the Amazon Rio Negro area, Brazil. *Rev Inst Med Trop São Paulo 49*: 159-164.

Tadei WP, Santos JMM, Costa WLS, Scarpassa VM 1988. Biologia de anofelinos amazônicos. XII - Ocorrência de espécies de *Anopheles*, dinâmica de transmissão e controle da malária na zona urbana de Ariquemes (Rondônia). *Rev Inst Med Trop Sao Paulo 30*: 221-251.

Tadei WP, Thatcher BD 2000. Malaria vectors in the Brazilian Amazon: *Anopheles* of the subgenys *Nyssorhynchus*. *Rev Inst Med Trop Sao Paulo 42*: 87-94.

Tadei WP, Tatcher BD, Santos JMM, Scarpassa VM, Rodrigues IB, Rafael MS 1998. Ecologic observations on Anophelinae vectors of malaria in the Brazilian Amazon. *Am J Trop Med Hyg 59*: 325-335.

Turell MJ, Jones JW, Sardelis MR, Dohm DJ, Coleman RE, Watts DM, Fernandez R, Calampa C, Klein TA 2000. Vector competence of Peruvian mosquitoes (Diptera: Culicidae) for epizootic and enzootic strains of Venezuelan equine encephalomyelitis virus. *J Med Entomol 37*: 835-839.

Vasconcelos PFC, Travassos da Rosa APA, Rodrigues SG, Travassos da Rosa ES, Degallier N, Travassos da Rosa JFS 2001. Inadequate management of natural ecosystem in the Brazilian Amazon region results in the emergence and reemergence of arboviruses. *Cad Saude Publica 17* (Suppl.): 155-164.

Vieira C, Velez ID, Montoya MN, Agudelo S, Alvarez MI, Genchi C, Simon F 1998. *Dirofilaria immitis* in Tikuna Indians and their dogs in the Colombian Amazon. *Ann Trop Med Parasitol 92*: 123-125.

Voorham J 2002. Intra-population plasticity of *Anopheles darlingi's* (Diptera: Culicidae) biting activity patterns in the state of Amapá, Brazil. *Rev Saude Publica 36*: 75-80.

Walter Reed Report 1998. Diseases vector ecology profile. Colombia [monograph on the Internet]. Washington: Defense Pest Management information Analysis Center. Armed Forced Pest Management Board, 1998 [cited 2008 March 13]. Available from http://www.afpmb.org/pubs/dveps/colombia.pdf.

Wilkerson RC, Sallum MAM 1999. *Anopheles (Anopheles) forattini*: a new species in Series Arribalzagia (Diptera: Culicidae). *J Med Entomol 36*: 345-354.