

BRAZILIAN MOSQUITO (DIPTERA: CULICIDAE) FAUNA. I. *Anopheles* SPECIES FROM PORTO VELHO, RONDÔNIA STATE, WESTERN AMAZON, BRAZIL

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SUMMARY

This study contributes to knowledge of *Anopheles* species, including vectors of *Plasmodium* from the western Brazilian Amazon in Porto Velho, Rondônia State. The sampling area has undergone substantial environmental changes as a consequence of agricultural and hydroelectric projects, which have caused intensive deforestation and favored habitats for some mosquito species. The purpose of this study was to diagnose the occurrence of anopheline species from collections in three locations along an electric-power transmission line. Each locality was sampled three times from 2010 to 2011. The principal adult mosquitoes captured in Shannon trap were *Anopheles darlingi*, *An. triannulatus*, *An. nuneztovari* l.s., *An. gilesi* and *An. costai*. In addition, larvae were collected in ground breeding sites for *Anopheles braziliensis*, *An. triannulatus*, *An. darlingi*, *An. deaneorum*, *An. marajoara*, *An. peryassui*, *An. nuneztovari* l.s. and *An. oswaldoi-konderi*. *Anopheles darlingi* was the most common mosquito in the region. We discuss Culicidae systematics, fauna distribution, and aspects of malaria in altered habitats of the western Amazon.

KEYWORDS: *Anopheles*; Amazon Basin; Culicidae; Fauna records; Malaria epidemiology.

INTRODUCTION

Anophelinae mosquitoes are important vectors of *Plasmodium* that can cause malaria in humans^{2,6}. Additionally, *Anopheles* species may be involved in the dynamics of transmission of microfilariae¹ and arboviruses²². In Brazil, *Anopheles* (*Nys.*) *darlingi* Root, *An.* (*Nys.*) *aquasalis* Curry, *An.* (*Nys.*) *triannulatus* (Neiva and Pinto), *An.* (*Nys.*) *braziliensis* (Chagas), *An.* (*Ker.*) *cruzii* Dyar and Knab, and *An.* (*Ker.*) *bellator* Dyar and Knab are primary vectors of human *Plasmodium*, whereas other *Anopheles* species may be either secondary or local vectors^{10,34}.

Mechanisms of vectorial competence of *Anopheles* species to infection by a *Plasmodium* species are only partially understood. Moreover, they are likely related to the physiological, genetic and immunological characteristics of the species to acquire and transmit the pathogen^{26,27}. The vectorial capacity of competent mosquito populations is determined by ecological factors, e.g., human-blood-feeding frequency, mean longevity, mosquito density relative to humans, and number of infective bites^{3,37}. Consequently, a particular species of *Anopheles* may be an important vector in one region, but of minor or no importance in another.

Relationships between ecological zones and frequency of *Anopheles* species in a small geographical area are unknown. In addition to climate

factors and regional flora and fauna, factors affecting oviposition, feeding and survival of a species include environmental determinants that may be associated to both human activities and alterations in land use. Thus, road construction, mining, human settlements, hydroelectric damming, agriculture and cattle farms have indirectly propitiated increasing in the incidence of human malaria^{9,17,18,33,35}.

The state of Rondônia has been largely impacted by changes in natural ecosystem, caused by intensive deforestation and changes in land use. Moreover, the state possesses a great hydroelectric potential because of the abundance of water resources, and this potential is being exploited to produce energy. Environmental changes of natural ecosystem include intense deforestation, partial obstruction of rivers and creeks, damming of water and formation of large lakes, which are favoring habitats for mosquito vector species, including Anophelinae species.

Currently, there is a concern about the impact of these activities on human and environmental health because they may favor some mosquito vector species, and promote the migration of susceptible humans into the region³⁶. Among the vector species that can be favored by ecological changes it is included *Anopheles* species that are vectors of human *Plasmodium*. Although this scenario is broadly accepted by the entomological surveillance program, the effect of power transmission lines on the epidemiology of human malaria has not been assessed.

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The objective of this paper is to add further records of *Anopheles* species to a regional fauna checklist, providing a compendium of the species recorded in the western Brazilian Amazon. We also comment about malaria prevention in the study area related with the local anopheline fauna.

MATERIAL AND METHODS

Study area. Collections were undertaken near Porto Velho, Rondônia State (8°45' S 63°53' W) at approximately 84 m altitude. The investigated area is inserted in the Amazon biome and the vegetation is composed of humid tropical forest of the Madeira River Basin. The annual temperature varies from 20 to 30 °C, and rainfall is higher than 2000 mm¹⁶. Landscape in the study area was severely modified decades ago by excessive land use. The local environmental levels predominant open fields used for pasture and restricted spot of residual forest. The human settlements are restricted to small villages and scattered rural habitations. All the area is permeated by streams and pools for cattle water supplies; on the other hand it characterizes favorable conditions for Culicidae development.

A new hydroelectric dam is currently under construction in Porto Velho, which already hosts a large hydroelectric complex. The mosquito collection sites include three rural localities situated along the 22 km planned trajectory of a new power transmission line. This extension line will interconnect the city of Porto Velho to the main line which is being prepared to transfer electric force, from the generation site to the consumption area, for about more than 2000 km eastern. Around each site, five ponds were searched for larvae and one point was chosen for adult collection using a Shannon trap. Mosquitoes were collected three times between February 2010 and January 2011, totaling 54 collections. Collection sites are shown in Figure 1.

Adult collections. Adult mosquitoes were captured using a Shannon trap with a light power-supply³². The traps were installed adjacent to rural

areas considered to represent anthropic environments³¹. Shannon traps allow the capture of adult females attracted by light and humans near the collection area. According to MISSAWA *et al.*²³, Shannon traps can be used as alternatives to human baiting for capture of anthropophilic mosquitoes in studies that have the objective of detecting the presence of vectors. Due to the light effects, this technical alternative is useful to sort a lot of mosquito's species. Since anophelinae mosquitoes are known by different adaptations for zoophily and endophily³.

After capture, mosquitoes were quickly anesthetized with ethyl acetate and separated into mini plastic vials in silica gel. Species were identified by morphological characters using adult identification keys^{19,13}. Approximately 20% of adults were pinned and deposited in the Entomological Reference Collection of the *Faculdade de Saúde Pública (FSP-USP), Universidade de São Paulo*, Brazil. Other samples were labeled and frozen at -70 °C for future reference.

Immature collections. Immature stages were collected along the future electrical extension line, in small or large, permanently or temporarily ponds. Samples of immature mosquitoes were collected with 80 mL plastic dippers along the pond's border. A volume proportional to the flooded area of each pond was estimated: about one dipper-volume per square meter¹². Larvae were fixed *in situ* in 70% ethanol and pupae were stored in plastic containers and kept alive in the laboratory until adult emergence. Larval and pupal exuviae and genitalia associated with adult forms were mounted on microscope slides and deposited in the FSP-USP collection.

RESULTS

Distribution. The numbers of anopheline adults collected in the study area is shown in Table 1. A total of 862 adults were captured using a Shannon trap. *Anopheles darlingi* represented 98% of all mosquitoes

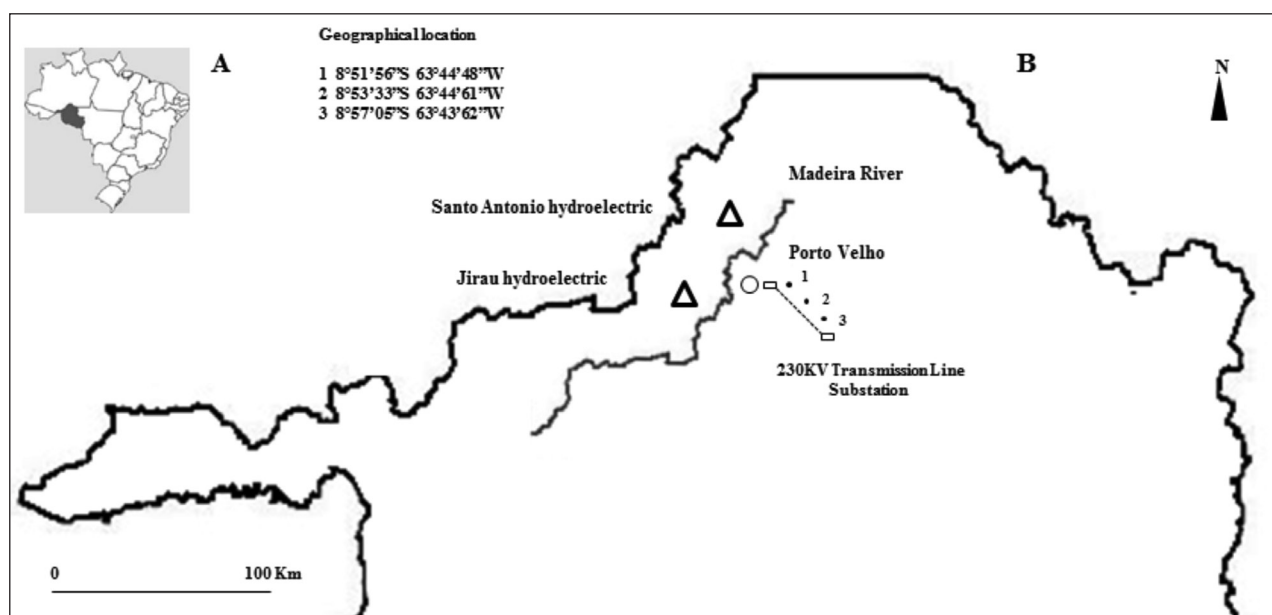


Fig. 1 - Map of anopheline mosquito collection sites. A Rondônia State in Brazil. B northern Rondônia. Numbers indicate sampling sites 1, 2 and 3.

collected. Collection site number 2 had a higher number of adults. In few numbers, the other species are: *An. triannulatus*, *An. nuneztovari*, *An. gilesi* and *An. costai*.

The results of anopheline larvae are shown in Table 2. A total of 201 larvae were collected. *Anopheles braziliensis* was the most present species, representing 40.2% of the total, followed by *An. triannulatus* (26.8%), *An. darlingi* (13.4%) and *An. deaneorum* Rosa-Freitas (5.4%). Less collected species were: *Anopheles marajoara* Galvão and Damasceno, *An. peryassui* Dyar and Knab, *An. nuneztovari* l.s., and *An. oswaldoi* l.s., which together accounted for 14.2% of the total. Larvae were collected more frequently in site 1.

Approximately 20% of adult mosquitoes captured in the field were deposited in the FSP-USP Collection under accession numbers E13342-E13433 and E13507-E13517. Larval and pupal exuviae and genitalia slides associated with adult forms were deposited under accession numbers E13434-E13506 and E13518-E13611. Other specimens were labeled, stored in tubes with alcohol, and preserved at -70 °C.

DISCUSSION

Shannon trap collections resulted in a greater number of captures, but low species variation, with *An. darlingi* being the most collected

Table 1
Species of the genus *Anopheles* collected as adults in Shannon trap, in Porto Velho, Rondônia State, Brazil. Samples were collected in three distinct occasions, from February 2010 to January 2011

Collection Dates	Adult species	Site 1	Site 2	Site 3	Total	%
February 2010	<i>An. (Nys.) darlingi</i>	22	30	212	264	100
					264	100
September 2010	<i>An. (Nys.) darlingi</i>	2	184	45	231	96.25
	<i>An. (Nys.) triannulatus</i>	-	2	1	3	1.25
	<i>An. (Nys.) nuneztovari</i> l.s.	4	-	-	4	1.7
	<i>An. (Lph.) gilesi</i>	1	-	-	1	0.4
	<i>An. (Ano.) costai</i>	-	-	1	1	0.4
					240	100
January 2011	<i>An. (Nys.) darlingi</i>	4	299	49	352	98.4
	<i>An. (Nys.) triannulatus</i>	1	-	-	1	0.2
	<i>An. (Nys.) nuneztovari</i> l.s.	3	1	1	5	1.4
					358	100
Total		37	516	309	862	

Table 2
Anopheles larvae collected in Porto Velho, Rondônia, Brazil. Samples were collected three times from February 2010 to January 2011

Collection Dates	Larvae species	Site 1	Site 2	Site 3	Total	%
February 2010	<i>An. (Nys.) darlingi</i>	8	1	-	9	11.6
	<i>An. (Nys.) triannulatus</i>	13	8	-	21	27.3
	<i>An. (Nys.) braziliensis</i>	1	4	35	40	52.0
	<i>An. (Nys.) marajoara</i>	-	4	-	4	5.2
	<i>An. (Nys.) oswaldoi-konderi</i>	1	-	-	1	1.3
	<i>An. (Ano.) peryassui</i>	1	-	-	1	1.3
	<i>An. (Nys.) nuneztovari</i> l.s.	1	-	-	1	1.3
					77	100
September 2010	<i>An. (Nys.) darlingi</i>	18	-	-	18	21.4
	<i>An. (Nys.) triannulatus</i>	12	-	-	12	14.3
	<i>An. (Nys.) braziliensis</i>	34	3	-	37	44.1
	<i>An. (Nys.) marajoara</i>	-	3	-	3	3.6
	<i>An. (Ano.) peryassui</i>	5	-	1	6	7.1
	<i>An. (Nys.) nuneztovari</i> l.s.	7	1	-	8	9.5
					84	100
January 2011	<i>An. (Nys.) braziliensis</i>	4	-	-	4	10.0
	<i>An. (Nys.) triannulatus</i>	8	10	3	21	52.5
	<i>An. (Nys.) nuneztovari</i> l.s.	2	1	1	4	10.0
	<i>An. (Nys.) deaneorum</i>	-	10	1	11	27.5
					40	100
Total		115	45	41	201	

mosquitoes. It is not surprising that *An. darlingi* is an important malaria vector in endemic areas of the Amazon: it is considered highly anthropophilic and is ubiquitous in the region's considerable freshwater bodies^{14,17}.

Although immature collections resulted in fewer mosquitoes, they yielded higher species variation (Table 2). *Anopheles braziliensis* was the most common, followed by *An. triannulatus*, *An. darlingi*, *An. nuneztovari* l.s., *An. marajoara*, *An. deaneorum*, *An. peryassui* and *An. oswaldoi-konderi*. Surprisingly, *An. darlingi* larvae were rarely found in the larval habitats sampled. The low number of *An. darlingi* immatures suggests that the breeding sites of this species were not located in the study area. *Anopheles triannulatus* and *An. braziliensis* were frequently collected in the region. These species are probably less anthropophilic than *An. darlingi* and are characterized as secondary malaria vectors³⁴. These results justify the use of Shannon traps in combination with immature collections, because the Shannon operates attractively and the latter passively. Both techniques enlarge fauna evaluation quality.

We found one female with morphological characteristics typical of Series Arribalzagia (Table 1). This specimen was identified as *An. costai* based primarily on the geographical distribution previously described for the species^{27,28}.

Anopheles (Ano.) costai Fonseca and Ramos, *An. (Ano.) forattinii* Wilkerson and Sallum and *An. (Ano.) mediopunctatus* (Lutz) belong to the Arribalzagia Series of *Anopheles (Anopheles)*. Because of morphological similarities, *An. costai* and *An. forattinii* have been previously misidentified as *An. mediopunctatus*. More recently, these species have been diagnosed based on features of the male genitalia, larvae and pupae. As a result, *An. costai* was resurrected from synonymy with *An. mediopunctatus* and *An. forattinii* was described and validated as a member species of the Arribalzagia Series^{28,29}.

Although *Anopheles (Nyssorhynchus) nuneztovari* Gabaldón is commonly recorded in the Brazilian Amazon⁸, BERGO *et al.*⁴ suggested that under the name *An. nuneztovari* cytotype A could be more than one species, including *An. goeldii*. *Anopheles nuneztovari* A is morphologically similar to, but chromosomally distinct from *An. nuneztovari* cytotypes B/C which occur in Venezuela and Colombia, respectively⁷. CALADO *et al.*⁵ corroborated the morphological hypothesis of BERGO *et al.*⁴, asserting that *An. goeldii* was a valid species, using morphology, the mitochondrial *cox1* gene and the nuclear *White* gene. CALADO *et al.*⁵ resurrected *An. goeldii* from synonymy with *An. nuneztovari*. However, it is possible that both species occur in Brazil and we consequently adopted the nomenclature *An. nuneztovari* l.s. in the present study until further research is conducted in Rondônia State.

Anopheles (Nys.) konderi Galvão and Damasceno are morphologically similar to *An. (Nys.) oswaldoi* in larval, pupal and adult female characters, but are distinguished by characters of the male genitalia¹¹. Based on morphological comparisons, LANE²⁰ synonymized *An. konderi* with *An. oswaldoi*. Later, FLORES-MENDOZA *et al.*¹¹ observed differences in the apex of the aedeagus in the male genitalia that were consistent in samples from distinct geographic locations, and thus resurrected the species from synonymy. As only one immature of this species was found, which emerged as a female, it was not possible to identify the

species with certainty. The specimen was therefore labeled in Table 2 as *An. oswaldoi-konderi*. Both species have been reported from Rondônia State²¹. Recently, SALLUM *et al.*²⁹ demonstrated to have more than one species under the name *An. konderi*. Furthermore, MOTOKI *et al.*²⁴ using sequences of COI mtDNA, single copy *White* nuclear gene, and the second internal transcribed spacer of the ribosomal DNA corroborate the presence of *An. konderi* in Rondônia State, showing that Rondônia population is closely related to representatives from Paraná State.

During operation period, apparently, power transmission line does not represent a major environmental impact in Rondônia State because they do not interfere directly with water resources or flooding. By the construction period, moreover, the potential risk for new malaria transmission cycles in the region is low because workers are primarily active during daylight, whereas vectors generally blood feed at night in the peri/intradomicile^{3,15}. Nevertheless, it is important that construction companies should maintain malaria prevention and vector control programs, because the main vector was detected along the line. Immigration of susceptible humans to malaria is worrisome because the primary regional vector occurs in areas around the power transmission line.

RESUMO

Fauna brasileira de mosquitos (Diptera: Culicidae). I. Espécies de *Anopheles* de Porto Velho, estado de Rondônia, oeste da Amazônia, Brasil

Este estudo contribui para o conhecimento de espécies de *Anopheles*, incluindo vetores de *Plasmodium* do oeste da Amazônia brasileira, em Porto Velho, no estado de Rondônia. Esta região vem passando por mudanças ambientais, como consequência de agricultura extensiva e projetos hidroelétricos que causam desmatamento, favorecendo o desenvolvimento de algumas espécies de mosquitos. Assim, a proposta deste estudo é registrar a presença de espécies de anofelinos na área, sendo conduzidas coletas de mosquitos em três locais, ao longo de uma linha de transmissão de energia elétrica. Cada uma das localidades foi amostrada três vezes, no período de 2010 a 2011. Os principais mosquitos adultos capturados em armadilhas de Shannon foram *Anopheles darlingi*, *An. triannulatus*, *An. nuneztovari* l.s., *An. gilesi* e *An. costai*. Assim como as formas larvárias *Anopheles braziliensis*, *An. triannulatus*, *An. darlingi*, *An. deaneorum*, *An. marajoara*, *An. peryassui*, *An. nuneztovari* l.s. e *An. oswaldoi-konderi*, coletadas em criadouros. *Anopheles darlingi* foi a espécie mais coletada na região. Em adição, discutiu-se sistemática de Culicidae, distribuição de fauna e aspectos da malária em ambientes modificados do oeste da Amazônia brasileira.

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The screenshot shows the website for the Biblioteca do Instituto de Medicina Tropical de São Paulo. At the top left is the logo of the Instituto de Medicina Tropical de São Paulo (IMT USP) and the Universidade de São Paulo. To the right is an 'Intranet' button and social media icons. A navigation bar includes 'Início', 'Sobre o IMTSP', 'Pesquisa', 'Ensino', 'Extensão', and 'Buscar'. Below this is a secondary menu with 'Organização', 'Notícias', 'Administração', 'Biblioteca', 'Revista IMTSP', 'Apoio', 'Concursos e Editais', and 'Contato e Localização'. The main content area is titled 'Biblioteca do Instituto de Medicina Tropical de São Paulo' and includes a mission statement, objectives, and vision. It also features a 'Biblioteca Menu' with links to 'Guia do Usuário', 'Links', 'Livros com texto integral', 'Congressos da Sociedade Brasileira de Medicina Tropical', 'Espaço do Pós-Graduando', 'Pesquisa na Internet', 'Publicações da Biblioteca', 'Revistas Eletrônicas', 'Revistas Gratuitas', 'Sites (Bases de Dados)', and '+informações'. Logos for 'Lattes' and 'QUALIS' are displayed, along with contact information: 'Contato: bibimt@usp.br' and 'Atualizada em 01/12/2010'. A 'Voltar ao Topo' link is at the bottom left.

The Library of the São Paulo Institute of Tropical Medicine (IMTSP Library) was created on January 15, 1959 in order to serve all those who are interested in tropical diseases. To reach this objective, we select and acquire by donation and / or exchange appropriate material to be used by researchers and we maintain interchange between Institutions thorough the Journal of the São Paulo Institute of Tropical Medicine, since the Library has no funds to build its own patrimony.

The IMTSP Library has a patrimony consisting of books, theses, annals of congresses, journals, and reference works.

The collection fo journals existing in the Library can be verified through the USP – Bibliographic Database – OPAC – DEDALUS <http://dedalus.usp.br:4500/ALEPH/eng/USP/USP/DEDALUS/start> of the USP network.