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ANOPHELINE SPECIES, SOME OF THEIR HABITS AND RELATION TO MALARIA IN ENDEMIC AREAS OF RONDÔNIA STATE, AMAZON REGION OF BRAZIL

RICARDO LOURENÇO-DE-OLIVEIRA, ANTHONY ERICO DA GAMA GUIMARÃES, MONIQUE ARLÉ, TEREZA FERNANDES DA SILVA, MÁRCIA GONÇALVES CASTRO, MONIQUE ALBUQUERQUE MOTTA & LEONIDAS M. DEANE*

Instituto Oswaldo Cruz, Departamento de Entomologia, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brasil

In view of recent studies incriminating several species of anophelines, besides Anopheles darlingi, *as malaria vectors in the Brazilian Amazon, we performed an anopheline survey in four localities – Ariquemes, Cujubim, Machadinho and Itapoã do Oeste – in Rondônia, the most malarious State in the Country.*

Twenty species were found. An. darlingi *was, by far, the dominant species and the only one whose density coincided with that of malaria. On human baits it was more numerous in the immediate vicinity of houses than indoors where, however, it was almost the only species encountered. On both situations it fed mostly at sunset and during the first half of the night. It was less numerous far from houses and scarce inside the forest. Other species* (An. triannulatus, An. evansae, An. albitarsis, An. strodei) *appeared in appreciable numbers only in Ariquemes, both in areas with and without malaria. The remaining species were scanty.* An. darlingi *was confirmed as the primary local vector.*

Key words: Anopheline species – habits – relation to malaria – Brazilian Amazon

In 1985, the year we started the present study, 399,462 confirmed cases of malaria were recorded in Brazil, more than 99% acquired in the Amazon Region (Map), which accounts for 60% of the Country's area of 8.5 million km^2 but holds only 16.5 million inhabitans, or about 12% of the Country's population of 135 million. In the Amazon, the most malarious State is Rondônia, where in that year 168,690 cases were reported, or 42% of the Country's total. The more recent figures, corresponding to year 1987 were the following: 508,864 malaria cases in Brazil, with 228,866 from Rondônia, or 45% (Marques, 1988).

Rondônia State is on the Southwestern part of the Brazilian Amazon, occupying an area of 242,844 km^2 which, in the past, was almost all covered by primitive forest. It was part of the States of Amazonas and Mato Grosso until 1943, when it became a separate Federal Territory under the name of Guaporé which, in 1967, was changed to Rondônia in honour of the half-indian military engineer Cândido Rondon, a protector of the indians who led a group of dedicated men in the outstanding task of taking the telegraph lines to that part of the Amazon through 1,500 km, most of which in the dense jungle.

In Rondônia the climate is equatorial, warm and humid. Temperature ranges from monthly averages of 18 °C to 34 °C, but there are a few days when cold spells of as low as 10 °C are recorded – the so-called "friagens", due to polar winds. Average monthly humidity is above 85% during the year round. Annual rainfall is about 2,300 mm and there is a long rainy season from September to May, and a short dry season, when only a few rainy days occur each month.

Until the middle of last century the Rondônia area was inhabited almost exclusively by

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* To whom correspondence should be sent.

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indian tribes, most of which with no contact with civilization. Later, a small number of immigrants from neighbouring areas came to collect native cocoa which grew near the river margins. In the 1870s attention was called to rubber, which was to become one of the chief sources of income in Brazil for nearly one-half a century, until 1912. Immigrants, most of which fleeing from the severe drought that struck the Northeastern State of Ceará, spread through the forest in Rondônia to work on the dispersed rubber trees. Most of them caught malaria and many died of it.

A rich rubber area existed on the upper Mamoré River, but navigation between the village of Guajará-Mirim, on this river and Santo Antônio, on the Madeira River, was prevented by nineteen rapids. So, a railway was to be built between the two villages, 366 km apart. The first attempt, started in 1878, was a herculean enterprise that failed due to the difficult conditions of the terrain, the poor knowledge of the region and the high prevalence of malaria, as vividly described by Neville B. Craig (1947). Another not less dramatic attempt did succeed: the Madeira-Mamoré Railroad was finally built between 1907 and 1912, by British and American concerns, linking Porto Velho and Guajará-Mirim, after great sacrifices and at the cost of thousands of deaths due to malaria and beriberi. The anti-malaria measures were compulsory quinine administration and screening of houses and lodgements. Oswaldo Cruz (1910) was called for advice during the construction of the railroad and produced a report in which the malaria situation is presented in detail and suggestions for the control of this and other local diseases are given. The role of the railway was far less usefull than expected: when it became ready, rubber production in Brazil had sharply declined, because the trees were irregularly spread in the forest and their yield could not compete with that of the scientifically organized plantations in the Orient.

During the second World War (1939-1945), when the rubber areas in Asia were taken over by the Japanese, there was an intent of re-exploiting rubber in the Amazon, and of about 50 thousand "nordestinos" then brought to the region many came to Rondônia in 1942 and 1943. The permanent population at that time was very small: in the capital and largest village, Porto Velho, there were only 3,800 people. Malaria was the principal parasitic disease, but prophylaxis was only done through medication (with Atabrine) and sporadic engineering work. In 1946 DDT was already being used as indoor sprayings in Rondônia, but only in the villages of Porto Velho and Guajará-Mirim. In 1947 it was extended to other villages, like Abunã and Jaci-paraná and in 1948 to all settlements along the Madeira-Mamoré Railroad.

According to the decennial national census, in 1950 the population of Rondônia was only 37,173 people. In the following decade thousands of immigrants were attracted by the Federal Government's implementation of agricultural settlements and by mining activities, so that by 1960 the population was recorded as of 69,792, an increase due, principally, to the immigration from the other Amazonian States and from the Northeast. In the 1960s, the building of two extensive roads – one from Cuiabá to Porto Velho and the other from Porto Velho to Guajará-Mirim – led to another mass immigration, so that in the 1970 census 113,659 inhabitants were recorded. In the 1970s Rondônia was already linked with Central and Southern Brazil through the Cuiabá-Porto Velho Road (opened to traffic in 1968) and a programme of land distribution by the Government attracted hundreds of thousands of farmers and peasants from the Southern States to Rondônia, where the population, in the 1980 census was 492,810. When we started our studies at the end of 1985 the estimated population of Rondônia was 940,298. It had increased 30-fold since the 1940s, and even the physical type of the inhabitants had become different: previously, the bulk of the population was brown, of mixed indians and whites (the "caboclos"), while now the whites predominate, because the migrants came from the Southern States, chiefly Paraná, colonized by Central-Europeans. The present estimated population is 1.2 million.

Malaria and anopheline surveys performed in Rondônia State during the first half of the present century pointed to *An. darlingi* as the only local important vector of that disease (Shannon, 1933; Deane et al., 1948). Since then, great environmental changes and a huge uncontrolled immigration occurred in the area, and as recent investigations in another Amazonian State, Pará, have led to the incrimination of other anopheline species as carriers (Arruda et al., 1986), studies on the present situation of malaria transmission in Rondônia became imperative.

Our studies here described were made in selected very malarious localities in that State, with the following objectives: a) to determine the anopheline fauna based on adults and larvae; b) to evaluate the prevalence of each species and where, when and on which hosts the females prefer to feed; c) to determine, for each species, the frequency of their contact with man and their coincidence with the presence of malaria.

MATERIAL AND METHODS

Localities surveyed – In Rondônia, the most malarious municipalities were Ariquemes and Porto Velho and for this reason they were chosen for the implementation of our project. Almost 53% of all malaria cases in Rondônia during 1987 were acquired in these two municipalities, out of the fifteen that form the State. In Ariquemes, three areas were included: the town of Ariquemes, capital of the municipality, and two settlements, Machadinho and Cujubim. In Porto Velho, the town was not included, but only a small settlement, Itapoã do Oeste (Map).

Data on the population and malaria prevalence in each locality, in two years of our survey, 1986 and 1987, are seen in Table I. All infections were due to *Plasmodium falciparum*, *P. vivax* or both. Figures were provided by the Superintendency of Health Campaigns (SUCAM), Ministry of Health.

The town of Ariquemes is named after the indian tribe – Arikemes – inhabiting the area when Rondon established a telegraph post there, in 1916. By then, besides the indians and the staff of the post a few rubber gatherers joined them, but in the 1970s miners came to exploit cassiterite and within the last few years intensive immigration mostly from the Southern States, led to a rapid increase so that the population grew, in 1986 and 1987, to about 57,600 and 72,000 respectively.

Machadinho has been recently regarded as the most malarious locality in Brazil. Forty years ago it was pure jungle inhabited by indians, who were displaced by rubber tappers and miners, and in 1984 roads started being built to bring settlers from the Southern States. In 1986 and 1987 it was already holding 9,060 and 12,140 people.

Cujubim is a recent settlement, started in 1985, its population being recorded as of 1,941 and 2,051 respectively in the last two years, most people living in the rural areas.

In Itapoã do Oeste, which started as a bus stop closer to a mining site, the records indicate a population of 3,156 and 4,713 in 1986 and 1987.

Malaria is highly endemic in all four localities. In Ariquemes, thick blood smears examined by the SUCAM staff in 1986 and 1987 revealed 15,779 and 25,597 with plasmodia, or 27.4% and 35.6% of the number of inhabitants, respectively. In Machadinho there were almost twice positive blood slides in relation to the number of dwellers: 17,711 and 23,972, or 195.5% and 185.3% of the population. In Cujubim too, the number of blood smears with plasmodia exceeded that of inhabitants: 2,259 and 2,571 in the two years, or 116.4% and 128.4%. In Itapoã there were 2,537 and 4,284 positive blood exams, or 81.0% and 90.9% of the persons in the area. It can be seen that while in Ariquemes and Itapoã there was yearly less than one case of malaria *per* inhabitant, in Machadinho and Cujubim there were more malaria cases than people, indicating that many dwellers had more than one attack during each year.

In all four localities *P. falciparum* predominated among the positive blood slides, in 1986 and 1987, except within the town of Ariquemes where *P. vivax* came first in 1987. In Ariquemes the proportion of *P. falciparum* was 52.2% and 38.3%; in Machadinho, 73.4% and 61.4%; in Cujubim, 59.1% and 61.7% and in Itapoã, 63.2% and 57.1%.

The Anopheline survey – The Anopheline studies consisted of a survey to determine the local species, their prevalence in the different localities, some of their habits such as endophily, anthropophily, nycthemeral cycle of hematophagy, and relation to malaria.

The field work was performed by a team formed by one scientist, two assistants and one driver, who stayed in Rondônia with headquarters in Ariquemes for periods of four weeks each, in November 1985, February, July and November-December 1986, March-May, July-August and November-December 1987 and May-June 1988. The laboratory work was carried out by one scientist and two technicians, at the Department of Entomology, Instituto Oswaldo Cruz, in Rio de Janeiro.

TABLE I

Population and malaria prevalence in four localities – Ariquemes, Machadinho, Cujubim and Itapoã do Oeste – Rondônia State, Brazil, in 1986 and 1987*

Population and malaria prevalence	Ariquemes		Machadinho		Cujubim		Itapoã	
	1986	1987	1986	1987	1986	1987	1986	1987
Population	57,600	72,000	9,060	12,940	1,941	2,051	3,156	4,713
with positive blood smears:								
number	15,779	25,597	17,711	23,972	2,259	2,571	2,537	4,284
%	27.4	35.6	195.5	185.3	116.4	125.4	81.0	90.9
with *P. falciparum:*								
number	8,239	9,795	12,994	14,721	1,336	2,571	1,604	2,448
% among total positives	52.2	38.3	73.4	61.4	59.1	61.7	63.2	57.1
Average monthly number of malaria cases	1,315	2,133	1,475.9	1,997.7	188.3	347.3	211.4	357

* Data obtained by SUCAM, Ministry of Health.

Adults and larvae were collected.

The adults were obtained in distinct types of systematic captures: (a) inside houses; (b) outdoors, in the immediate vicinity of the houses, on human baits; (c) outdoors, far from the human dwellings, on human baits; (d) outdoors, in the forest, on human baits; (e) outdoors, on animal baits and (f) in hourly captures performed on human baits, simultaneously indoors and in the immediate vicinity of houses, during 24 hour periods.

Captures *a*, *b*, *c* and *e* were performed at sunset, from 6 to 9 p. m., while captures *d* were carried out in the morning (8 to 10 a. m.), in the afternoon (1 to 3 p. m.) and at sunset. The human baits were members of the team or local inhabitants during their normal daily activities. The animal baits were a horse or a cow. Local houses are routinely sprayed indoors with DDT by personnel of the anti-malaria campaign (SUCAM).

The majority of the anophelines caught were keep in a dessicator to be later submitted to Zavala's (1982) immuno-radiometric assay with anti-sporozoite species-specific monoclonal antibodies for the search of malaria infections, in the Department of Immunology.

For the larval studies water collections were recorded and the immature stages were preserved in 70% alcohol and later identified to species.

RESULTS

As a result of the work in the four areas the following twenty anopheline species were collected, belonging to genus *Anopheles (An.)*, subgenera *Nyssorhynchus (Nys.)*, *Anopheles (Ano.)* and *Stethomyia (Ste.)* and to genus *Chagasia (Ch.)*:

An. (Nys.) darlingi Root, 1926
An. (Nys.) argyritarsis Robineau-Desvoidy, 1827
An. (Nys.) albitarsis Lynch-Arribalzaga, 1878
An. (Nys.) braziliensis (Chagas, 1907)
An. (Nys.) evansae (Brethes, 1926)
An. (Nys.) galvãoi Causey, Deane & Deane, 1948
An. (Nys.) nuñeztovari Gabaldon, 1940
An. (Nys.) oswaldoi (Peryassú, 1922)
An. (Nys.) rangeli Gabaldon, Cova Garcia & Lopez, 1941
An. (Nys.) benarrochi Gabaldon, Cova Garcia & Lopez, 1941
An. (Nys.) strodei Root, 1926
An. (Nys.) triannulatus (Neiva & Pinto, 1922)
An. (Ano.) mattogrossensis Lutz & Neiva, 1911
An. (Ano.) peryassui (Dyar & Knab, 1908)
An. (Ano.) minor Costa Lima, 1929
An. (Ano.) intermedius (Chagas, 1908)
An. (Ano.) fluminensis Root, 1927
An. (Ano.) mediopunctatus (Theobald, 1903)
An. (Ste.) nimbus (Theobald, 1902)
Ch. bonneae Root, 1927

TABLE II

Number of adult anophelines of each species, average per ten hours of captures and percent, in the areas studied in Rondônia State, Brazil, November 1985 to June 1988: the town of Ariquemes and the settlements of Machadinho and Cujubim, in the Municipality of Ariquemes, and the settlement of Itapoã do Oeste, in the Municipality of Porto Velho

Species of Anophelines	Numbers					Per 10 hours of captures					%				
	Ariquemes	Machadinho	Cujubim	Itapoã	Total	Ariquemes	Machadinho	Cujubim	Itapoã	Total	Ariquemes	Machadinho	Cujubim	Itapoã	Total
An. darlingi	5,614	2,547	1,156	591	9,908	48.8	138.4	54.8	103.7	61.8	67.0	98.3	97.2	99.7	77.7
An. argyritarsis	17	–	–	–	17	0.1	–	–	–	0.1	0.2	0.0	–	–	0.1
An. albitarsis	353	1	–	–	354	3.1	0.1	–	–	2.2	4.2	–	–	–	2.8
An. braziliensis	152	–	–	–	152	1.3	–	–	–	0.9	1.8	–	–	–	1.2
An. evansae	386	–	–	–	386	3.4	–	–	–	2.4	4.6	–	–	–	3.0
An. galvãoi	50	–	–	–	50	0.4	–	–	–	0.3	0.6	–	–	–	0.4
An. nuñeztovari	35	–	5	1	41	0.3	–	0.2	0.2	0.3	0.4	–	0.4	0.2	0.3
An. oswaldoi	291	–	2	–	293	2.5	–	0.1	–	1.8	3.5	–	0.2	–	2.3
An. rangeli	49	–	–	–	49	0.4	–	–	–	0.3	0.6	–	–	–	0.4
An. benarrochi	7	–	–	–	7	0.1	–	–	–	0.0	0.1	–	–	–	0.1
An. strodei	208	1	3	–	212	1.8	0.1	0.1	–	1.3	2.5	0.0	0.3	–	1.7
An. triannulatus	944	7	8	–	959	8.2	0.4	0.4	–	6	11.3	0.3	0.7	–	7.5
An. (Nys.) sp.	255	35	13	–	303	2.2	1.9	0.6	–	1.9	3.0	1.4	1.1	–	2.4
An. peryassui	–	–	–	1	1	–	–	–	0.2	0.0	–	–	–	0.2	0.0
An. mattogrossensis	1	–	–	–	1	0.0	–	–	–	0.0	0.0	–	–	–	0.0
An. minor	3	–	–	–	3	0.0	–	–	–	0.0	0.0	–	–	–	0.0
An. fluminensis	1	–	–	–	1	0.0	–	–	–	0.0	0.0	–	–	–	0.0
An. mediopunctatus	9	–	–	–	9	0.1	–	–	–	0.1	0.1	–	–	–	0.1
An. (Ano.) sp.	1	–	–	–	1	0.0	–	–	–	0.0	0.0	–	–	–	0.0
Ch. bonneae	2	–	2	–	4	0.0	–	0.1	–	0.0	0.0	–	0.2	–	0.0
Total	8,378	2,591	1,189	593	12,751	72.8	141.3	56.4	104.0	79.5	100	100	100	100	100
Hours spent	1,151	184	211	57	1,603	–	–	–	–	–	–	–	–	–	–

As seen in Table II, among the 12,751 adult females of eighteen species in the area, *An. darlingi* was by far the most abundant (77.7% of the total anophelines), followed at some distance by *An. triannulatus* (7.5%), *An. evansae* (3.0%), *An. albitarsis* (2.8%), *An. oswaldoi* (2.3%), *An. strodei* (1.7%) and *An. braziliensis* (1.2%), all remaining species being scarce.

By splitting the results by each of the four areas we find that in the town of Ariquemes, where captures were more numerous and diversified seventeen species were collected, with an average of 72.8 specimens per 10 hours of captures. *An. darlingi* was the most numerous, accounting for 67.0% of the fauna, with an average of 48.8 specimens per 10 hours, but other species were well represented, like *An. triannulatus*, *An. evansae*, *An. albitarsis*, *An. oswaldoi*, *An. strodei* and *An. braziliensis*. In Machadinho the anopheline density was much higher – 141.3 per 10 hours – and *An. darlingi* was almost the only species encountered, being much more numerous than in Ariquemes: 138.4 specimens per 10 hours, and corresponding to 98.3% of the total anophelines. In Cujubim, the anophelines were decidedly fewer than in the other two settlements of the municipality – 56.4 specimens per 10 hours – but *An. darlingi* was, again, almost the only species detected, representing 97.2% of all species. In Itapoã the anopheline density was high – 104.0 per 10 hours – and *An. darlingi* was almost the only anopheline found – 99.7% of all species and with 103.7 specimens per 10 hours of captures.

As in the town of Ariquemes some districts had become free or almost free of malaria while in others the disease persisted, it seemed appropriate to divide the results of the entomological survey according to those two situations. This can be seen in Table III. In the malarious districts thirteen species of anophelines were present, with an average of 96.6 per 10 hours; in the non-malarious there were eighteen species, with 36.9 per 10 hours. In the former districts *An. darlingi* was much more numerous than in the latter: 75.2 specimens against 9.1 per 10 hours, and 77.8% *versus* 24.7% of the total anophelines.

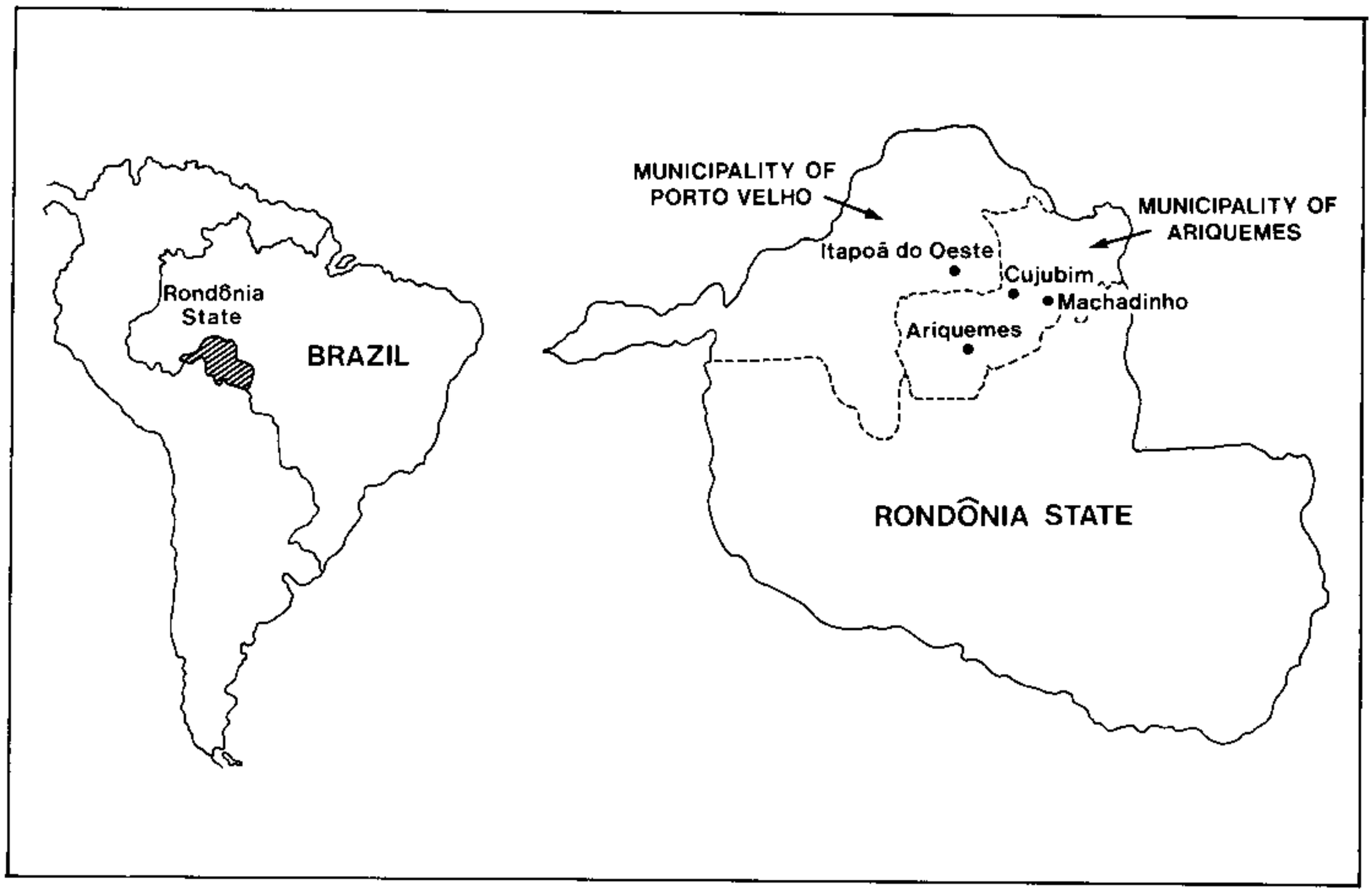
As referred above, our mosquito captures were performed so as to give some indication of the endophily and anthropophily of the local species of anophelines. Results are summarized in Tables IV, V and VI. They revealed that inside houses, most of which, as afore mentioned, were routinely sprayed with DDT, there were 30.1 anophelines per 10 hours of captures. *An. darlingi* was found in smaller numbers than outdoors close to houses – an average of 29.2 specimens per 10 hours –, but represented 96.9% of the total anophelines. In the immediate vicinity of the houses, biting humans, anophelines were much more numerous – 125.8 specimens per 10 hours, and this was the situation where *An. darlingi* was most abundant – 113.6 specimens per 10 hours –, accounting for 90.3% of all anophelines; the next species in numbers was *An. triannulatus*, with 3.6 specimens per 10 hours and 2.6% of the total species.

Outdoors, on open terrain and far from human dwellings, thirteen species were caught while feeding on human bait, 124.5 specimens per 10 hours; here, other species were also frequent besides *An. darlingi:* of this species, 47.2 specimens were caught per 10 hours, accounting for 37.9% of the anopheline fauna, while the corresponding figures for *An. triannulatus* were 31.0 and 24.9%, and for *An. oswaldoi* 23.1 and 18.6%.

Still feeding on humans outdoors but inside the forest, anophelines were much fewer – only 6.0 in the average per 10 hours, but including eleven species, the most numerous being *An. triannulatus* and *An. oswaldoi:* of the former, 3.4 specimens were obtained per 10 hours, accounting for 56.8% of all anophelines, the corresponding figures being 1.8 and 30.1% for *An. oswaldoi; An. darlingi* came third, with only 0.4 specimens per 10 hours and 6.8% of the total species.

A very diversified fauna with more abundant specimens was obtained outdoors, on animal bait. Either on a cow or a horse placed on open terrain, 338 anophelines of fourteen species were obtained per 10 hours of captures, but here *An. darlingi* accounted for only 21.1% of the total anophelines, and other species were also numerous: *An. triannulatus* 25.9%, *An. evansae* 13.9%, *An. albitarsi* 9.9%, *An. strodei* 9.4%, *An. oswaldoi* 6.6% and *An. braziliensis* 6.0%.

In hourly captures performed simultaneously on human baits inside houses and outdoors, in



Map Localities surveyed in Rondônia State.

TABLE III

Number of anopheline adults of each species, average per 10 hours of captures and percent, in districts of the town of Ariquemes with (+) and without or almost without (–) cases of malaria, November 1985 to June 1988

Species of Anophelines	Number		Per 10 hours of captures		%	
	+	–	+	–	+	–
An. darlingi	5,194	420	75.2	9.1	77.8	24.7
An. argyritarsis	4	13	0.0	0.3	0.1	0.8
An. albitarsis	129	224	1.9	4.9	1.9	13.2
An. braziliensis	86	66	1.2	1.4	1.3	3.9
An. evansae	92	294	1.3	6.4	1.4	17.3
An. galvãoi	47	3	0.7	0.1	0.7	0.2
An. nuñeztovari	13	22	0.2	0.5	0.2	1.3
An. oswaldoi	164	127	2.4	2.8	2.5	7.5
An. rangeli	21	28	0.3	0.6	0.3	1.6
An. benarrochi	–	7	–	0.2	–	0.4
An. strodei	70	138	1.0	3.0	1.0	8.1
An. triannulatus	625	319	9.0	6.9	9.4	18.8
An. (Nys.) sp.	226	29	3.3	0.6	3.4	1.7
An. mattogrossensis	–	1	–	0.0	–	0.1
An. minor	1	2	–	0.0	0.0	0.1
An. fluminensis	–	1	–	0.0	–	0.1
An. mediopunctatus	6	3	0.1	0.1	0.1	0.2
An. (Ano.) sp.	–	1	–	0.0	–	0.1
Ch. bonneae	–	2	–	0.0	–	0.1
Total	6,678	1,700	96.6	36.9	100	100
Hours spent	691	460	–	–	–	–

TABLE IV

Number of adult anophelines of each species, captured in different situations in Rondônia State, Brazil (areas mentioned in Table II), November 1985 to June 1988

Species of Anophelines	Indoors	Outdoors				Total
		On human bait			On animal bait	
		Close to houses	Far from houses	In forest		
An. darlingi	1,580	7,759	137	12	420	9,908
An. argyritarsis	–	4	3	–	10	17
An. albitarsis	21	131	3	1	198	354
An. braziliensis	–	27	5	–	120	152
An. evansae	–	89	19	1	277	386
An. galvāoi	2	13	15	–	20	50
An. nuñeztovari	–	19	4	1	17	41
An. oswaldoi	–	42	67	53	131	293
An. rangeli	–	14	2	1	32	49
An. benarrochi	2	5	–	–	–	7
An. strodei	1	16	8	–	187	212
An. triannulatus	9	244	90	100	516	959
An. (Nys.) sp.	16	220	4	–	63	303
An. peryassui	–	1	–	–	–	1
An. mattogrossensis	–	1	–	–	–	1
An. minor	–	1	–	–	2	3
An. fluminensis	–	–	–	1	–	1
An. mediopunctatus	–	1	4	3	1	9
An. (Ano.) sp.	–	–	–	1	–	1
Ch. bonneae	–	2	–	2	–	4
Total	1,631	8,589	361	176	1,994	12,751
Hours spent	541	683	29	291	59	1,603

the immediate vicinity of houses during 24-hour periods, 2,634 *An. darlingi* females were collected, 685 indoors and 1949 outdoors, or 26.1% against 73.9%. In both situations feeding started at sunset, went through the whole night until the early morning hours, the majority of specimens biting during the first half of the night, with the peak around sunset (Table VII).

The species of anopheline larvae and their prevalence are found in Table VIII. Comparing the figures in this Table with those of the adults in Table II, no correlation is to be seen as to the frequency of the species. Among the adults, *An. darlingi* was predominant (77.7% of all anophelines), followed by *An. triannulatus* (7.5%), *An. evansae* (3.0%), *An. oswaldoi* (2.3%) and *An. strodei* (1.7%), while for the larvae the sequence was *An. strodei* (30.9%), *An. triannulatus* (27.9%), *An. albitarsis* (23.5%), *An. darlingi* (only 8.0%) and *An. oswaldoi* (2.3%).

The larvae of mosquitoes identified in the adult stage as *An. triannulatus* are of two types, one diagnosed as *An. triannulatus triannulatus* (Neiva & Pinto, 1922) and the other as *An. triannulatus davisi* Paterson & Shannon, 1927, which we believe should be regarded as a distinct species; in a sample of *An. triannulatus* larvae obtained in our survey, more than 99% belonged to the latter type. The larve of *An. albitarsis* were also of two types: in one the outer anterior clypeal hairs are aciculate, in the other they are definitely branched, as previously observed by Deane et al. (1948) and Rosa-Freitas et al. (1987).

DISCUSSION

Studies on anophelines and their relation to malaria in Rondônia were performed, in the first half of the present century by Cruz (1910), Chagas (1913), Shannon (1933), Causey et al. (1946) and Deane et al. (1948).

TABLE V

Number of adult anophelines of each species, per 10 hours of captures in different situations, in Rondônia State, Brazil (areas mentioned in Table II), November 1985 to June 1988

Species of Anophelines	Indoors	Outdoors				
		On human bait			On animal bait	Total
		Close to houses	Far from houses	In forest		
An. darlingi	29.2	113.6	47.2	0.4	71.2	61.8
An. argyritarsis	–	0.1	1.0	–	1.7	0.1
An. albitarsis	0.4	1.9	1.0	0.0	33.6	2.2
An. braziliensis	–	0.4	1.7	–	20.3	0.9
An. evansae	–	1.3	6.6	0.0	46.9	2.4
An. galvãoi	0.0	0.2	5.2	–	3.4	0.3
An. nuñeztovari	–	0.3	1.4	0.0	2.9	0.3
An. oswaldoi	–	0.6	23.1	1.8	22.2	1.8
An. rangeli	–	0.2	4.8	0.0	5.4	0.3
An. benarrochi	0.0	0.1	–	–	–	0.0
An. strodei	0.0	0.2	2.8	–	31.7	1.3
An. triannulatus	0.2	3.6	31.0	3.4	87.5	6.0
An. (Nys.) sp.	0.3	3.2	1.4	–	10.7	1.9
An. peryassui	–	0.0	–	–	–	0.0
An. mattogrossensis	–	0.0	–	–	–	0.0
An. minor	–	0.0	–	–	0.3	0.0
An. fluminensis	–	–	–	0.0	–	0.0
An. mediopunctatus	–	0.0	1.4	0.1	0.2	0.1
An. (Ano.) sp.	–	–	–	0.0	–	0.0
Ch. bonneae	–	0.0	–	0.1	–	0.0
Total	30.1	125.8	124.5	6.0	338.0	79.5

Cruz and Chagas found in Rondônia what they thought to be three species of anophelines: *"Cellia albimana"* (which could have been any *Nyssorhynchus* of the *albimanus* series), *"Ce. argyrotarsis"* (any *Nyssorhynchus* of the *argyritarsis* series) and *An. nimbus*, but they regarded the first two as the carriers of malaria. Shannon (1933) visited five localities along the Madeira-Mamoré Railroad, finding seven species of anophelines, the only present indoors being *An. darlingi*, which outdoors, on animal baits, accounted for only 14% of all anopheline specimens; it was the most ubiquitous species and was found naturally infected with sporozoites, being regarded as the main local malaria vector, while *"An. tarsimaculatus"* (a name given to various species of the *albimanus* series), in spite of being more numerous, would play but a minor role in the region, due to its exophily and zoophily. Causey et al. (1946) in their keys for the identification of anophelines of the Amazon and Northeastern Regions of Brazil, included the species found in the present Rondônia area, which at that time was part of the States of Amazonas and Mato Grosso. Deane (1947), in an article summarizing the knowledge on the prevalence, transmission and control of malaria in the Brazilian Amazon, mentions the presence and distribution of anophelines in Rondônia, mostly along the Madeira-Mamoré Railroad and confirmed the role of *An. darlingi* as the principal vector by the finding of specimens infected with sporozoites in Porto Velho and Jaci-Paraná. In the extensive report of Deane et al. (1948) on the distribution and biology of the anophelines of the Northeastern and Amazon Regions of Brazil, the informations of the previous paper are largely amplified. From Rondônia, they identified 12,095 imagoes, 6,878 larvae and 5,066 ovipositions of individual females belonging to twenty species, referring to breeding-places, endophily, anthropophily, biting hours, parasites and relation to malaria, regarding *An. darlingi* as the only important local vector.

After those studies, during about forty years, anopheline adults and larvae from several

TABLE VI

Percent of adult anophelines of each species captured in different situations in Rondônia State, Brazil (areas mentioned in Table II), November 1985 to June 1988

Species of Anophelines	Indoors	Outdoors				Total
		On human bait			On animal bait	
		Close to houses	Far from houses	In forest		
An. darlingi	96.9	90.3	37.9	6.8	21.1	77.7
An. argyritarsis	–	0.1	0.8	–	0.5	0.1
An. albitarsis	1.3	1.5	0.8	0.6	9.9	2.8
An. braziliensis	–	0.3	1.4	–	6.0	1.2
An. evansae	–	1.0	5.3	0.6	13.9	3.0
An. galvãoi	0.1	0.2	4.2	–	1.0	0.4
An. nuñeztovari	–	0.2	1.1	0.6	0.9	0.3
An. oswaldoi	–	0.5	18.6	30.1	6.6	2.3
An. rangeli	–	0.2	0.6	0.6	1.6	0.4
An. benarrochi	0.1	0.1	–	–	–	0.1
An. strodei	0.1	0.2	2.2	–	9.4	1.7
An. triannulatus	0.6	2.6	24.9	56.8	25.9	7.5
An. (Nys.) sp.	1.0	2.6	1.1	–	3.2	2.4
An. peryassui	–	0.0	–	–	–	0.0
An. mattogrossensis	–	0.0	–	–	–	0.0
An. minor	–	0.0	–	–	0.1	0.0
An. fluminensis	–	–	–	0.6	–	0.0
An. mediopunctatus	–	0.0	1.1	1.7	0.1	0.1
An. (Ano.) sp.	–	–	–	0.6	–	0.0
Ch. bonneae	–	0.0	–	1.1	–	0.0
Total	100	100	100	100	100	100

localities in Rondônia continued to be routinely collected and identified in relation to malaria control measures by the SUCAM personnel, but the results were not presented as research activities. In the present decade, especially due to the recrudescence of malaria in the State, interest in the ecology of anophelines and their relation to malaria is rising, so that several groups of workers are becoming devoted to those subjects and publishing their findings.

An extensive work has been recently carried out on anophelines and malaria in Rondônia in the town of Ariquemes by Tadei et al. (1988). The authors found fourteen anopheline species. *An. darlingi* was dominant, biting chiefly at sunset and during the first half of the night, with a small peak at sunrise, being regarded as the principal vector (it was the only species dissected and the only one found with sporozoites). The spacial and seasonal transmission in the town is discussed in connection with the larval breeding-places, and control measures are suggested.

Of our present survey three progress reports have been recently presented. One, by Lourenço-de-Oliveira et al. (1987) includes the data obtained until May 1987, when *An. darlingi* had been the only anopheline found infected with sporozoites. Others, by Deane et al. (1988) and Oliveira-Ferreira et al. (1988), summarize the data on anopheline behaviour and relation to malaria and the degree of anti-sporozoite immunity in the local population obtained until August 1987; by then, two other species were detected with sporozoites by the IRMA, althought in much smaller numbers than *An. darlingi (An. triannulatus* with *P. vivax* and *An. braziliensis* with *P. falciparum).*

In the present report of our anopheline studies in Rondônia the results obtained lead to the following comments.

According to the data obtained by SUCAM the four areas studied are very malarious, two of them with more cases each year than the number of inhabitants. *P. falciparum* is more

TABLE VII

Nycthemeral cycle of *Anopheles darlingi* females feeding on human baits inside houses and outdoors in the immediate vicinity of houses in Ariquemes, Rondônia State, Brazil, 1986-1988

Hour	Number			%		
	Indoors	Outdoors	Total	Indoors	Outdoors	Total
6- 7	21	44	65	3.1	2.3	2.5
7- 8	8	10	18	1.2	0.5	0.7
8- 9	1	1	2	0.1	0.1	0.1
9-10	2	–	2	0.3	–	0.1
10-11	–	–	–	–	–	–
11-12	–	–	–	–	–	–
12-13	–	–	–	–	–	–
13-14	1	–	1	0.1	–	0.0
14-15	–	–	–	–	–	–
15-16	1	–	1	0.1	–	0.0
16-17	–	3	3	–	0.2	0.1
17-18	5	5	10	0.7	0.3	0.4
18-19	141	306	447	20.6	15.7	17
19-20	104	296	400	15.2	15.2	15.2
20-21	118	281	399	17.2	14.4	15.1
21-22	104	207	311	15.2	10.6	11.8
22-23	80	224	304	11.7	11.5	11.5
23-24	33	183	216	4.8	9.4	8.2
0- 1	14	100	114	2.0	5.1	4.3
1- 2	8	96	104	1.2	4.9	3.9
2- 3	9	55	64	1.3	2.8	2.4
3- 4	18	53	71	2.6	2.7	2.7
4- 5	9	34	43	1.3	1.7	1.6
5- 6	8	51	59	1.2	2.6	2.2
Total	685	1,949	2,634	100	100	100

common than *P. vivax* and *P. malariae* was not detected (Table I).

Of the twenty species of anophelines we encountered, *An. darlingi* was by far the dominant in all localities. In Ariquemes it accounted for 67% of all anophelines, and in the other three localities to more than 97% (Table II).

In Ariquemes *An. darlingi* was much more numerous in districts with active malaria transmission than in those without or almost without malaria, and there was no correlation with the density of the other species and the prevalence of the disease (Table III).

Inside houses *An. darlingi* was almost the only species present, the six others being very scarce, but it was much more abundant biting man outdoors, in the immediate vicinity of the houses. It was less numerous far from houses, where other species (like *An. triannulatus* and *An. oswaldoi*) were also caught in large numbers and it was scarce in the forest, where those two other species predominated. In captures on animal bait *An. darlingi* came second to *An. triannulatus* and was followed by also numerous *An. evansae*, *An. albitarsis*, *An. strodei*, *An. oswaldoi* and *An. braziliensis* (Tables IV, V and VI).

Blood-feeding, for *An. darlingi*, starting at sunset, remained high during the first half of the night, decreasing gradually until early morning and being much more frequent outdoors, in the immediate vicinity of the houses than indoors (Table VII).

Among the larvae collected *An. darlingi* came in fourth place (only 8% of the total anophelines), after *An. strodei*, *An. triannulatus* and *An. albitarsis* (Table VIII). Nearly all *An. triannulatus* larvae were *An. triannulatus davisi* and two morphologically distinct types of *An. albitarsis* were found, one of which is being described as a new species.

TABLE VIII

Anopheline larvae of each species collected in Rondônia State (areas mentioned in Table II), November 1985 to June 1988

Species of Anophelines	Number	%
An. darlingi	287	8.0
An. argyritarsis	17	0.5
An. albitarsis	843	23.5
An. braziliensis	34	0.9
An. nuñeztovari	22	0.6
An. oswaldoi	81	2.3
An. rangeli	31	0.9
An. strodei	1,106	30.9
An. triannulatus	1,000	27.9
An. (Nys.) spp.	124	3.5
An. peryassui	23	0.6
An. mattogrossensis	2	0.1
An. minor	9	0.3
An. intermedius	1	0.0
An. nimbus	2	0.3
Total	3,582	100

By comparing the recent reports on anophelines and malaria in Rondônia with the survey performed about forty years ago by Deane et al. (1948), considerable differences can be detected.

The comparative proportion of the adults of the various species in the anopheline fauna has changed. The *Nyssorhynchus*, and especially *An. darlingi*, became substancially predominant: in the previous survey *An. darlingi* accounted for only 26.7% of the total adults and the non-*Nyssorhynchus* to 14.7%, while in the study here presented the corresponding figures are 77.7% and 0.1% respectively. Formerly, *An. nuñeztovari* was more abundant than *An. darlingi* and much more than *An. triannulatus*, the opposite occuring at present.

Another difference concerns to *An. darlingi* behaviour. The endophilic and anthropophilic habits of this mosquito were emphasized by every research worker through the years, so that malaria was regarded as being transmitted principally inside houses. This was an important aspect for those that had the task of controlling the disease after World War II: DDT spraying of the houses interior was the answer and the measure was efficient in many areas of *darlingi*-transmitted malaria. Now we see *An. darlingi* feeding mostly outdoors, although in the immediate vicinity of houses, or entering houses just to feed and then flying to rest outdoors. What happened? Should we hypothetize that the species is clever enough to change its habits, having learned a way to escape contact with DDT-sprayed walls? A repellent effect of DDT has been suggested. Also the possibility of several species of *Nyssorhynchus*, including *An. darlingi*, being, indeed, complexes of cryptic or sibling species, as indicated by studies of caryotypes and/or electrophoretic profiles of isozymes. Could we admit that the extensive use of DDT has selected populations that, from the beginning, were responder to a repellent effect of the insecticide?

Another point refers to the several recent additions to the list of proven and potential malaria vectors in the Amazon, Rondônia included. Again, everyone that, in the past, studied malaria transmission in the region considered *An. darlingi* as the chief vector, or even the only species of epidemiological importance in most endemic areas. In Rondônia, *An. darlingi* had been the only species found naturally infected, the presence of the other species did not coincide with malaria and all specimens dissected from thirteen species had been negative for plasmodia (Deane et al., 1948).

Of course, new methods have been developed for the diagnosis of salivary gland infections. These are less time-consumming and perhaps somewhat more sensitive than the old methods. Chiefly, they permit the previously impossible identication of plasmodia species. However, the sole use of these new methods cannot account for the present high rates of infections with human plasmodia sporozoites found among several anopheline species that were previously considered unimportant or, at best, with a local or secondary role as malaria vectors.

The present findings, based on immunological tests with monoclonal antibodies, would suggest that perhaps most species of the local anophelines are highly susceptible to human plasmodia. The old notion that every malarious area had its particular and, usually, only vector among the local anopheline fauna would not be true in the "new" Amazon Region. Or we might suggest that the vector capacity of an anopheline species is not related to its capacity to support the complete development of the

plasmodia, but simply to its ability to approach and feed on a human malaria carrier.

In the case of the Amazon Region, particularly in that of Rondônia, we can, at least for the moment, suggest that human mass immigration, increasing the density of the human population, and, on the other hand, the extensive deforestation and disorderly occupation, are indirectly responsible for the modification on the mosquito behaviour, by substracting their former sources of food and bringing man closer to their shelters and breeding-places.

RESUMO

Espécies de anofelinos, alguns de seus hábitos e relação com a malária, em áreas endêmicas do Estado de Rondônia, Amazônia brasileira – Como recentes estudos vêm incriminando diversas espécies de anofelinos como transmissores da malária na Amazônia brasileira além do *Anopheles darlingi*, realizamos um estudo sobre esse grupo de mosquitos em quatro localidades – Ariquemes, Cujubim, Machadinho e Itapoã do Oeste – no Estado de Rondônia, o mais malarígeno do país.

Vinte espécies foram achadas. *An. darlingi* foi, de longe, a predominante e a única cuja densidade coincidiu com a da malária. Sugando iscas humanas, esse mosquito mostrou-se mais numeroso na imediata vizinhança das casas do que em seu interior, onde, entretanto, foi quase a única espécie encontrada. Em ambas as situações picava principalmente ao crepúsculo vespertino e durante a primeira metade da noite, sendo menos freqüente longe das casas e escasso na floresta. Outras espécies, como *An. triannulatus*, *An. evansae*, *An. albitarsis* e *An. strodei* foram capturadas em números apreciáveis, somente em Ariquemes, em áreas com e sem malária, porém as demais mostraram-se raras. O *An. darlingi* foi confirmado como o transmissor primário na área.

Palavras-chave: espécies de anofelinos – hábitos – relação com a malária – Estado de Rondônia – Amazônia brasileira

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