

## Simian Malaria at Two Sites in the Brazilian Amazon - II. Vertical Distribution and Frequency of Anopheline Species Inside and Outside the Forest

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*An anopheline survey was carried out in two simian malaria areas in the Brazilian Amazon, Balbina and Samuel, to determine the potential vectors of Plasmodium brasilianum. The most abundant and/or acrodendrophilic anophelines in the forest and the most likely vector were Anopheles mediopunctatus, An. nuneztovari, An. oswaldoi, An. triannulatus and An. shannoni. An. darlingi and An. marajoara were captured essentially in anthropic habitats outside the forest and are unlikely to be involved in the transmission of P. brasilianum among monkeys within the forests and from monkeys to man in their surroundings in the Amazon.*

Key words: simian malaria vectors - *Anopheles* - Culicidae - *Plasmodium brasilianum*

The simian malaria caused by *Plasmodium brasilianum* is widespread in the humid forests of Central and South America. Its vector has been discovered only in the Atlantic forest, of southern and southeastern Brazil, where the acrodendrophilic mosquito *Anopheles (Kerteszia) cruzii* was found naturally infected with the parasite (Deane et al. 1970). *An. cruzii* does not occur in the Amazon Region where *P. brasilianum* is suspected to be the cause of a zoonosis (Lal et al. 1988, Arruda et al. 1989). Such a zoonotic malaria vector would need to feed frequently on infected monkeys and humans, mainly indians, rubber gatherers, wood cutters and fishermen, inside the forest or in the surrounding areas.

The current work forms part of a study carried out in two localities in the Brazilian Amazon to describe the zoonotic potential of simian malaria by *P. brasilianum*. The infection rates of this parasite in monkeys from those areas have been published elsewhere (Lourenço-de-Oliveira & Deane 1995). This paper presents the results of a mosquito survey conducted in those sites to describe the anopheline fauna within the forest and in surrounding cleared areas, as well as anopheline species frequencies near the ground and in the tree canopy.

### METHODOLOGY

The anopheline survey was performed near two hydroelectric plants in the Amazon Basin: Balbina, on the Uatumã River, State of Amazonas (1°55'S 59°28'W) and Samuel, on the Jamari River, State of Rondônia (8° 10'S 62°29'W), Brazil, where *P. brasilianum* was detected in, respectively, 15.8% and 9.9% of the primates. Details about these two sites, such as climate, vegetation and fauna are given elsewhere (Lourenço-de-Oliveira & Deane 1995).

Mosquitoes were caught while biting human or animal baits or by light-trap and identified according to Deane et al. (1947) and Faran and Linthicum (1981). The anopheline captures in Balbina were performed in two periods: 6-30 September 1988 and 18 September to 20 October 1989. Within the forest, anopheline catches were carried out on human bait, from 6 to 9 p.m., at two sites: Base I, on both the west and east margins of the Uatumã River, and Base II, an island in the reservoir about 70 km from the dam. At Base I anophelines were collected simultaneously from a human bait at ground level and another on a platform in the canopy, at 14 m above the ground.

For comparison, mosquito captures were also conducted outside the forest, from 27-29 September 1988, in three settlements of the Waimiri-Atroari Indian tribe: Cacau, Curiaú and Maré. In 1989, a total of 424 Waimiri-Atroari lived in 11 settlements near the Balbina forest. The settlements of Cacau and Maré are on the east and west margins of the Camanaú River, with 33 and 37 inhabitants, respectively, while the Curiaú is on the

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north margin of the Curuiú River, with 36 residents. The huts are constructed completely of palm leaves or have wooden walls and a roof of palm leaves. Anophelines were captured simultaneously on four human baits, one inside the hut, one immediately outside, one near the forest, and another in the forest, from 6 to 9 p.m., in Cacau and Curuiú and from 5 to 7 a.m. in Maré. Additionally, a CDC light-trap (Sudia & Chamberlain 1962) was operated near the rivers and the forest.

In Samuel, anophelines were surveyed from 17 February to 28 March 1989 and from August 1990 to July 1991. Collections were performed at three sites within the Samuel Ecological Station, where platforms were built at 15-17 m above the ground in the canopy of selected trees. The Samuel Ecological Station is an uninhabited portion of primary forest between 8°50' and 9°04'S and 63°08' and 63°19'W, on the southeast border of the reservoir, 50 km from the dam and contiguous with the Jamari National Forest. There, anophelines were caught daily, except on rainy nights, from 6 to 9 p.m., on human baits placed simultaneously on the ground and in the canopy.

Additionally, some anophelines were collected from 6 to 8 p.m., between 23-31 July 1991, outside the forest, close to dwellings, at three sites near Samuel: Boca do Rio Novo, Caboclo and Japiim Road. At the first two sites, collections were only on human baits, while at Japiim Road simultaneous catches were performed on man and cow. Only a

fisherman's family lives at Boca do Rio Novo, in a wooden house surrounded by the forest, 5 km from the Samuel dam. During the dry season, Caboclo is a temporary settlement of rubber-gatherers, located 4.5 km from the dam, with open, wooden huts, covered with palm leaves, surrounded by the forest. Both Boca do Rio Novo and Caboclo are downstream from the dam on the west bank of Jamari River. Anophelines were captured on a human bait close to the dwellings. At Japiim Road anophelines were collected from a human bait and a cow near two wooden houses close to the forest. This road links the village of Itapoã do Oeste to the Japiim stream, a tributary of the Jamari River, south of the Samuel Ecological Station. All collection sites in Balbina and Samuel are shown on a map in Lourenço-de-Oliveira and Deane (1995:333). G test against a null hypothesis was used to compare the occurrence of anopheline species at ground and in the canopy.

## RESULTS

Ten anopheline species were recognized in the surveyed areas of both Balbina and Samuel (Tables I, IV). *An. nuneztovari* and *An. oswaldoi* were the most abundant species in Balbina, accounting for more than 70% of the total. Nine anopheline species were collected within the forest and only two, *An. darlingi* and *An. oswaldoi*, at the Waimiri-Atroari settlements near Balbina (Table I, Fig. 1). In these anthropic environments only 19

TABLE I

Anopheline mosquitoes caught inside and outside the forest at Balbina, i.e. at three Waimiri-Atroari settlements, State of Amazonas, Brazil, from 6 to 30 September 1988 and 18 September to 20 October 1989

Species	Forest		Total
	Inside	Outside	
<i>Anopheles nuneztovari</i> Gabaldon	840	-	840
<i>Anopheles oswaldoi</i> (Peryassu)	742	3	745
<i>Anopheles</i> ( <i>Nys.</i> ) spp. <sup>a</sup>	300	-	300
<i>Anopheles triannulatus</i> (Neiva & Pinto)	102	-	102
<i>Anopheles evansae</i> (Brethes)	74	-	74
<i>Anopheles mediopunctatus</i> <sup>b</sup> (Theobald)	64	-	64
<i>Anopheles mattogrossensis</i> Lutz & Neiva	6	-	6
<i>Anopheles darlingi</i> Root	-	16	16
<i>Anopheles peryassui</i> Dyar & Knab	3	-	3
<i>Anopheles shannoni</i> Davis	2	-	2
<i>Anopheles galvaoi</i> Causey, Deane & Deane	1	-	1
Total	2133	19	2152
Man-hours	148.5	30	178.5

a: damaged specimens; b: the specimens identified by us as *An. mediopunctatus* probably consists of two related species, neither of which seems to be *An. mediopunctatus* s.s. (Wilkerson pers. comm., Wilkerson 1988).

TABLE II

Anopheline mosquitoes caught on human baits and with a CDC light trap at three Waimiri-Atoari settlements - Cacau, Maré and Curiauí - near Balbina's forest, State of Amazonas, from 27 to 29 September 1989

Species	Human baits <sup>a</sup>				Light trap		Total
	Inside the huts	Near the huts	Near the forest	Into the forest	Near the river	Near the forest	
<i>Anopheles darlingi</i>	8	7	1	-	-	-	16
<i>Anopheles oswaldoi</i>	-	-	-	-	-	3	3
Total	8	7	1	-	-	3	19
Man-hours	8	8	4	2	6	2	30

a: one person per site.

anopheline mosquitoes were captured during 22 hr of human baiting and 8 hr of light-trapping (Table II). *An. darlingi* was collected only outside the forest (84.2% of the anophelines from the settlements) while *An. oswaldoi* was collected both in and outside, although more commonly within the forest. *An. darlingi* was also the only anopheline caught in and close to huts (Fig. 1).

*An. mediopunctatus* was far the most common mosquito in Samuel (66.3% of the total, Table IV). Two other anopheline species were also frequent in the area: *An. nuneztovari* (13.7%) and *An. shannoni* (9.9%). *An. mediopunctatus* was the most common species in the forest (almost 70% of the total), where it occurred unlikely; by contrary *An. darlingi* was caught almost exclusively in anthropic environments, accounting for 80.6% of the anophelines captured at Boca do Rio Novo, Caboclo and Japiim Road. *An. marajoara* was found only outside the forest, primarily attracted by the cow (Table V, Fig. 2).

Most of the anophelines in the forest at Balbina were collected at both levels of the vegetation (Table III, Fig. 3). These included *An. nuneztovari*, *An. oswaldoi* and *An. triannulatus*, which were not significantly more abundant in the canopy (62.5%, 58.5% and 64.9% caught in the canopy, respectively) (Eq = 0.6998). *An. mediopunctatus* was more common in the canopy (71.9%), while *An. mattogrossensis* was captured only at ground level.

Almost 80% of the anophelines caught within the forest in Samuel were collected in the canopy (Table III). *An. oswaldoi* was slightly more frequent on the ground while *An. mediopunctatus* and *An. shannoni* were the most common canopy feeders (Fig. 3). *An. mediopunctatus* exhibited the strongest acrodendrophilic behavior in Samuel, with 85.5% of the females captured in the tree canopy and accounted for 74% of the total mosquitoes collected at that level.

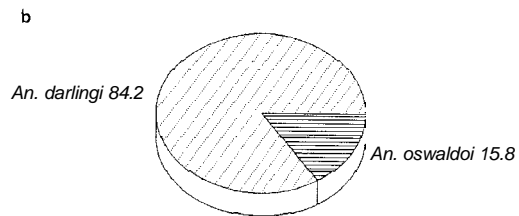
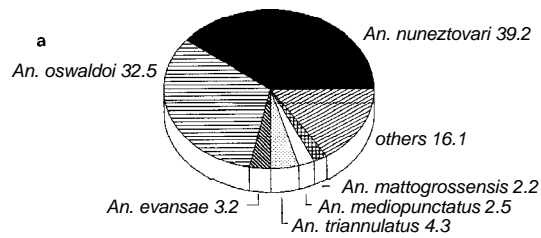


Fig. 1: percentage of each anopheline species collected inside (a) and outside the forest in Balbina (b) i.e. in three Waimiri-Atoari settlements, State of Amazonas, Brazil, from 6 to 30 September 1988 and 18 September to 20 October 1989.

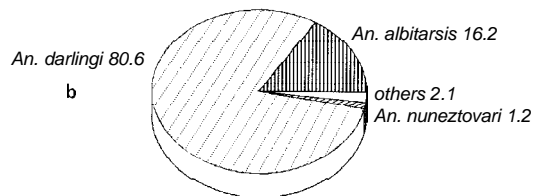
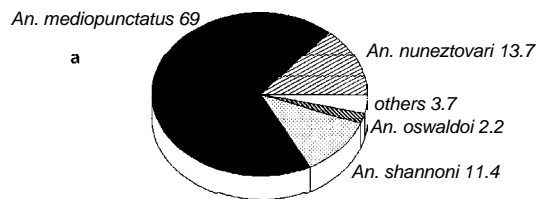


Fig. 2: percentage of each anopheline species collected inside (a) and outside the forest (b) in Samuel, State of Rondônia, Brazil, from 17 February to 28 March 1989 and from August 1990 through July 1991.

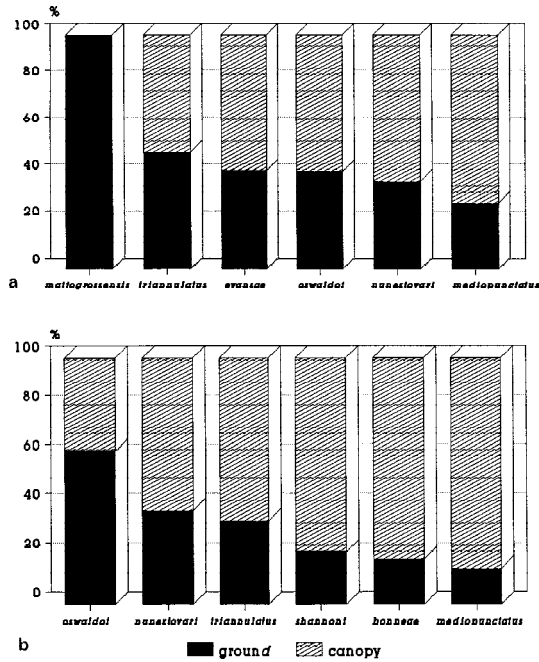


Fig. 3: percentage of each anopheline species caught on humans on the ground and in the forest canopy in Balbina (a) and Samuel (b), respectively in the States of Amazonas and Rondônia, Brazil.

DISCUSSION

The preference of an anopheline species for feeding in the forest and particularly in the canopy is essential for considering it a potential vector of simian malaria because hosts are primarily arboreal. Attention must be given to the difference between the composition of the anopheline fauna within and outside the forests of Balbina and Samuel. Five species were the most frequent sylvatic anophelines: *An. mediopunctatus*, *An. nuneztovari*, *An. oswaldoi* and *An. triannulatus* were relatively common at both sites but *An. shannoni* was abundant only at Samuel. By contrary, *An. darlingi* predominated outside the jungle. This and other mosquito species found in Balbina and Samuel were rare or absent in the tree canopy, and are unlikely to be involved in the transmission of quartan simian malaria in the Amazon.

*An. mediopunctatus* has always been associated with the forested environments and has been found in all simian malaria areas in the Amazon (Almeida & Deane 1970, Ferreira Neto et al. 1970, Deane et al. 1971, Deane 1976, 1992). It was captured only within the forests at both Balbina and Samuel, and was the most common anopheline species at the latter site.

TABLE III

Capture frequency of anopheline at human baits on the ground and in the forest canopy at Samuel<sup>a</sup> and Balbina<sup>b</sup>, respectively, in the States of Rondônia and Amazonas, Brazil

Species	Mosquitoes/man-hour (x10)			
	Samuel		Balbina	
	ground	canopy	ground	canopy
<i>Anopheles darlingi</i>	-	0.0	-	-
<i>Anopheles evansae</i>	0.3	0.3	4.2	5.8
<i>Anopheles galvaoi</i>	-	-	0.1	-
<i>Anopheles nuneztovari</i>	13.1	21.3	42.7	71.3
<i>Anopheles oswaldoi</i>	3.8	2.3	41.7	58.8
<i>Anopheles triannulatus</i>	1.3	2.4	6.8	6.9
<i>Anopheles (Nys) spp.<sup>c</sup></i>	1.6	3.3	21.0	19.3
<i>Anopheles mattogrossensis</i>	-	-	0.7	-
<i>Anopheles mediopunctatus</i>	24.2	142.9	2.4	6.4
<i>Anopheles peryassui</i>	-	-	0.1	0.3
<i>Anopheles shannoni</i>	5.3	19.7	0.1	0.1
<i>Anopheles (Ano.) spp.<sup>c</sup></i>	-	0.0	-	-
<i>Anopheles nimbus</i>	0.1	-	-	-
<i>Chagasia bonneae</i>	0.1	0.5	-	-
Total	49.7	192.8	119.9	168.9
Man-hours	356	356	76.5	72

a: from 17 February to 28 March 1989 and August 1990 to July 1991; b: from 6-30 September 1988 and 18 September to 20 October 1989; c: damaged specimens.

TABLE IV  
Anopheline mosquitoes caught inside and outside the forest at Samuel, State of Rondônia, Brazil, from 17 February to 28 March and from August 1990 to July 1991

Species of anophelines	Forest		Total
	Inside	Outside	
<i>Anopheles mediopunctatus</i> <sup>a</sup>	5946	-	5946
<i>Anopheles nuneztovari</i>	1227	4	1231
<i>Anopheles shannoni</i>	888	1	889
<i>Anopheles darlingi</i>	1	274	275
<i>Anopheles oswaldoi</i>	216	-	216
<i>Anopheles (Nys.) spp.</i> <sup>b</sup>	174	6	180
<i>Anopheles triannulatus</i>	132	-	132
<i>Anopheles marajoara</i> Galvão & Damasceno	-	55	55
<i>Chagasia bonneae</i> Root	22	-	22
<i>Anopheles evansae</i>	22	-	22
<i>Anopheles nimbus</i> (Theobald)	3	-	3
<i>Anopheles (Ano.) spp.</i>	1	-	1
Total	8632	340	8972
Man-hours	712	16	728

a: the specimens identified by us as *An. mediopunctatus* probably consists of two related species, neither of which seems to be *An. mediopunctatus* s.s. (Wilkerson pers. comm., Wilkerson 1988); b: damaged specimens.

TABLE V  
Anopheline mosquitoes caught on human and cow at three settlements - Boca do Rio Novo, Caboclo and Japiim Road - near Samuel's forest, State of Rondônia, from 23 to 31 July 1991

Species	Boca do Rio Novo Human	Caboclo Human	Japiim Road		Total
			Human	Cow	
<i>Anopheles marajoara</i>	-	-	1	54	55
<i>Anopheles darlingi</i>	243	25	6	-	274
<i>Anopheles nuneztovari</i>	1	1	-	2	4
<i>Anopheles shannoni</i>	-	-	1	-	1
<i>Anopheles (Nys.) spp.</i> <sup>a</sup>	3	2	-	1	6
Total	247	28	7	57	340
Man-hours	4	4	6	2	16

a: damaged specimens.

The presence and abundance of the other four anopheline species in the forest has already been reported for simian malaria areas in the Amazon. In a forested swamp in Porto Nacional, State of Amazonas, Deane et al. (1968) reported that 30% of the collected anophelines were *An. nuneztovari* and *An. oswaldoi*. The latter species was the predominant mosquito in catches performed by Deane et al. (1971) in an enzootic area of simian malaria, Serra do Navio, State of Amapá, accounting for 75.7% of the anophelines collected, while *An. nuneztovari* accounted for only 3.7%. *An. oswaldoi* was the most common anopheline in forested swamp near Tefé, State of Amazonas within the territory of *Cacajao calvus*, from which *P. brasilianum* was first identified, where it repre-

sented 51.3% of the anophelines collected by Almeida and Deane (1970).

Deane et al. (1971) observed that near the Tracajatuba River, an area located 100 km from Serra do Navio, where 16.6% of monkeys were infected with *P. brasilianum*, both *An. nuneztovari* and *An. oswaldoi* were rare (only 3.2% and 4.7% of the total, respectively), but *An. triannulatus* accounted for 63.7% of the anophelines collected. Similarly, Deane and Ferreira-Neto (1973) reported from Cabeceira Grande, State of Tocantins, that 8.1% of monkeys were infected with *P. brasilianum* and 70% of the collected anophelines were *An. triannulatus*. This mosquito species has been detected in all simian malaria areas surveyed in the Brazilian Amazon, but at most sites its den-

sity was low or moderate, except for the two localities mentioned above (Deane et al. 1968, 1971, Almeida & Deane 1970, Ferreira Neto et al. 1970, Deane & Ferreira Neto 1973).

Brazilian species of monkeys and marmosets live predominantly in the forest canopy where the anopheline vector of simian malaria must also take most of its blood meals. This behavior suggested to Deane et al. (1969) that *An. cruzii* was the vector of both *P. brasilianum* and *P. simium* in south and southeast Brazil.

The field work performed in Balbina was restricted to short periods in September and October of two adjoining years. At that time, the most common anophelines, *An. oswaldoi* and *An. nuneztovari*, were slightly more numerous in the canopy than near the ground. One of the acrodendrophilic anopheline, *An. mediopunctatus*, was not abundant during that period of the year in Balbina. On the other hand, *An. mediopunctatus* was the most common and acrodendrophilic anopheline in Samuel and its preference for the high levels of the vegetation in simian malaria areas has already been demonstrated (Deane et al. 1971, Deane 1992) (Table III, Fig. 3).

Previously, *An. oswaldoi* has been shown to feed preferently in the canopy only in Cabeceira Grande (Deane & Ferreira Neto 1973). This anopheline was more common in ground collections at Samuel (Table III, Fig. 3) as well as in Trinidad and other localities in Brazil (Aitken et al. 1968, Deane et al. 1971). *An. oswaldoi* has shown no preference with regard to vertical distribution or tended to be slightly acrodendrophilic, as it was in Balbina, in three other simian malaria areas in the Brazilian Amazon (Deane et al. 1968, 1971, Ferreira Neto et al. 1970).

In contrast to Balbina and Samuel where *An. nuneztovari* was slightly acrodendrophilic, the species was previously collected in larger numbers near the ground, e.g. by Deane et al. (1971) at Serra do Navio (95.8% on the ground), at Tracajatuca (67.1%) and at Porto Mauá (59.4%). Although feeding height preferences of *An. oswaldoi* and *An. nuneztovari* may vary from site to site, their abundance within the forest (Fig. 1) may suggest their possible involvement in simian malaria at Balbina.

Other anophelines in Samuel, the common *An. shannoni* and the rare *Ch. bonneae*, showed a marked preference for biting at the forest canopy. The acrodendrophilic behavior of these species was also observed by Deane et al. (1953, 1968, 1971) and Ferreira Neto et al. (1970) in several Amazonian areas with and without simian malaria.

Few mosquito surveys were conducted previously at Balbina and Samuel and in their surroundings. Chagas et al. (1982) reported the presence of

*An. darlingi*, *An. nuneztovari* and *An. oswaldoi* in Balbina at the beginning of the dam construction. When the Uatumã River was dammed, flooding of the forest provided extensive breeding sites for *An. oswaldoi*, which became very abundant (Eletronorte 1988).

The first anopheline survey at Samuel was carried out by Deane (1947) who found 30 *An. darlingi* and 2 *An. nuneztovari* indoors, trapped in bednets, during the day. During the construction of the Samuel Dam, *An. oswaldoi*, *An. mediopunctatus*, *An. nuneztovari* were collected near the Jamari River, but *An. darlingi* was still the predominant mosquito in cleared areas, accounting for 90-100% of the total captured (Tadei 1985, 1986). Most of the mosquito captures in the present survey were carried out within Samuel's forest in contrast to those performed by Deane (1947) and Tadei (1985, 1986) who worked essentially in man-made or disturbed environments.

The few hours spent with captures conducted in anthropic environments outside the forest in both Balbina and Samuel (Tables I, IV) confirmed the results of hundreds of hours of anopheline collections that we have been performing in the Amazon, with regarding to the anopheline fauna constitution and the synanthropy of *An. darlingi* (Lourenço-de-Oliveira et al. 1989, 1994).

During the last three decades, ambitious colonization projects in the Brazilian Amazon were responsible for progressive changes in the landscape. The forest was cleared and replaced to pastures and plantations. With the modifications in the landscape several anopheline species commonly reported in the past have been rare in the present surveys. Concomitantly, *An. darlingi* became the predominant or practically the only species in the anthropic environments. It became the most synanthropic anopheline in the Amazon, as observed at Balbina, Samuel and all over the region (Tadei et al. 1988, Lourenço-de-Oliveira et al. 1989, 1994, Deane 1989, Klein & Lima 1990, Lourenço-de-Oliveira 1994). Obviously the sylvatic anophelines deprived from their natural blood feeding sources among wild animals may leave the forest and invade the open fields, i.e. the anthropic environment. There, they attack mostly domestic animals, while *An. darlingi* is the predominant species feeding on man (Deane et al. 1948, Lourenço-de-Oliveira et al. 1989, Klein & Lima 1990, Oliveira-Ferreira et al. 1992).

*An. darlingi* may take blood in the forest, even near the canopies. Deane et al. (1953) collected 19.4% and 11.8% of *An. darlingi* females at 10m and 15m, respectively, versus 50.6% near ground in a forest where simian malaria has never been detected, in Belém, State of Pará. This forest sur-

rounds one of the principal breeding sites of the species in the area. Lourenço-de-Oliveira et al. (1989) showed that only 6.8% of the total anophelines collected into a small patch of forest near Ariquemes, State of Rondônia belonged to this species, although there were numerous breeding places in the area.

Species belonging to the *An. albitarsis* complex, as *An. marajoara*, are essentially zoophilic mosquitoes that breed and feed outside the forest, where it was not collected at both Balbina and Samuel, confirming previous studies performed by Deane et al. (1948), Lourenço-de-Oliveira and Heyden (1986), Lourenço-de-Oliveira et al. (1989), Rosa-Freitas et al. (1990), Oliveira-Ferreira et al. (1992), Forattini et al. (1993).

We conclude that neither *An. darlingi* nor *An. marajoara* can be suspected as vector of simian malaria among monkeys in the forest nor from monkeys to man in the Brazilian Amazon. For the same reason, i.e. because *An. darlingi* is better adapted to the anthropic environments such as the open fields in the rural areas, cleared river margins and proximity to the secondary woods and recently cleared forests, it is the primary vector of the human malaria throughout this region (Deane 1986, 1989, Lourenço-de-Oliveira 1989, Lourenço-de-Oliveira et al. 1989, Oliveira-Ferreira et al. 1990). On the other hand the anopheline vectors of *P. brasilianum* in the Amazon are likely to be among the most abundant, sylvatic and canopy feeders at Balbina and Samuel: *An. medio-punctatus*, *An. nunezovari*, *An. oswaldoi*, *An. triannulatus* and *An. shannoni*.

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