The importance of *Anopheles albitarsis* E and *An. darlingi* in human malaria transmission in Boa Vista, state of Roraima, Brazil

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In several districts of Boa Vista, state of Roraima, Brazil we found Anopheles (Nyssorhynchus) albitarsis *E to be the primary vector of human malaria parasites, and during 2001-2002 it was significantly more abundant than* An. darlingi *($p < 0.001$). Other species sampled were* An. (Nys.) braziliensis, An. (Ano.) peryassui, An. (Nys.) nuneztovari, An. (Nys.) oswaldoi *s.l., and* An. (Nys.) triannulatus. *As determined by the ELISA technique* An. darlingi *had a higher overall infection rate (2.1%) compared with* An. albitarsis *E (1.2%). However, a marginally higher proportion of* An. albitarsis *E was infected with* Plasmodium vivax *compared with* An. darlingi, *and the* An. albitarsis *E biting index was also much higher. These results suggest the importance of* An. albitarsis *E in malaria transmission in a savannah ecoregion of northern Amazonian Brazil, and reconfirm the importance of* An. darlingi *even if at lower abundance.*

Key words: *Anopheles albitarsis* E - *Anopheles darlingi* - malaria - Roraima - Brazil

Although the importance of *Anopheles darlingi* in malaria transmission in Amazonia is undiminished (Tadei et al. 1998, Rosa-Freitas et al. 1998, Lounibos & Conn 2000), the biological and epidemiological diversity of other *Anopheles* species involved in regional or local transmission suggests that a variety of control strategies will be needed to effectively reduce the incidence of malaria. Among those species also responsible for malaria transmission are members of the *An. (Nys.) albitarsis* Lynch-Arribalzaga species complex (Conn et al. 2002). This complex consists of four species, three formally named, another identified by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) (*An. albitarsis* s.s., *An. deaneorum*, *An. marajoara*, and *An. albitarsis* B respectively; Wilkerson et al. 1995a,b), and an additional putative species closely related to *An. marajoara*, *An. albitarsis* E (Lehr et al. 2005, but see Wilkerson et al. 2005). Two species are considered to be malaria vectors, *An. deaneorum* (based on experimental infection, Klein et al. 1991a,b) and *An. marajoara* (based on field data including ELISA to test for malaria parasites, Conn et al. 2002). *An. marajoara* appears to be the primary malaria vector in Serra do Navio, state of Amapá (Póvoa et al. 2000b); as well as in Boa Vista, state of Roraima (Silva-Vasconcelos et al. 2002). *An. marajoara* has been identified as the main malaria vector in the port city of Santana and in periurban sections of the city of Macapá in the state of Amapá by Conn et al. (2002), and confirmed as *An. marajoara* by sequences of the mtDNA *COI* gene (Lehr 2003). In Serra do Navio, Amapá, *An. albitarsis* s.l. has also been identified as *An. marajoara* using the mtDNA *COI* gene (Lehr 2003). These data indicate that *An. marajoara* is a significant regional vector in northeastern Amazonian Brazil, and it is important to know more about its local distribution and role in malaria transmission throughout Amazonian Brazil.

The taxonomic status of *An. albitarsis* E is not yet clearly resolved. It is readily identified as monophyletic using appropriate outgroups and phylogenetic approaches such as maximum parsimony (MP), maximum likelihood (ML) and Bayesian analysis when analyzed using complete sequences of the mitochondrial DNA (mtDNA) *COI* gene (Lehr et al. 2005). On the other hand, partial sequences of nuclear genes (ribosomal DNA ITS2 and D2) and mtDNA genes (*COI* and *ND4*) using some of the same sample localities, and some of the same analyses, could not detect this taxon (Wilkerson et al. 2005). The new molecular key to distinguish among members of the Albitarsis complex, based on ribosomal DNA internal transcribed spacer 2 (ITS2) differences (Li & Wilkerson 2005), cannot distinguish *An. albitarsis* E from *An. marajoara* because their ITS2 sequences are identical. It is evident that additional analyses are needed, using independent data sources (Rubinoff & Holland 2005), and extensive geographical samples, to resolve the taxonomic status of *An. albitarsis* E.

Approximately 99% of the malaria cases in Brazil occur in the Amazon region (PAHO 2002). The state of

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Roraima, which persists in having one of the highest annual parasitic indices (API; number of cases/1000) for malaria in Brazil (Silva-Vasconcelos et al. 2002) is the most northern Brazilian state, sharing international borders with Guyana and Venezuela. The capital of Roraima, Boa Vista, is in the savannah ecoregion of Rubio-Palis and Zimmerman (1997) and is characterized by: a long rainy season (April-November), a short dry season (December-March), a mean annual temperature of 27.8°C, and a mean rainfall of 429 mm (Silva-Vasconcelos et al. 2002).

Our objectives were to identify which species of *An. albitarsis* s.l. is responsible for malaria transmission in Boa Vista and to extend the initial studies by Silva-Vasconcelos et al. (2002) by examining anophelines from several additional endemic malaria localities there.

MATERIALS AND METHODS

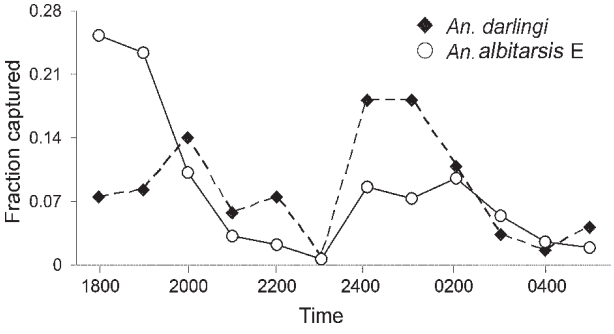
Study sites - For continuity with and an extension of the study by Silva-Vasconcelos et al. (2002) we collected in two localities, 13 de Setembro (along the Rio Branco) and Caranã (along the Rio Cauamé). We chose four additional localities (Cauamé, Jardim da Copaiba, Caçari, and Distrito Industrial) where malaria cases had recently (2000-2001) been reported to the Secretaria Municipal de Saúde in Boa Vista.

Adult collection and identification - Mosquitoes were collected between 18:30-21:00 h, coincident with the peak biting time of the majority of anophelines found in Boa Vista as reported by Silva-Vasconcelos et al. (2002). There were seven collection periods: March, May, and September of 2001, and March, June, August, and November of 2002. To detect infected mosquitoes that might be biting at other than crepuscular and post-crepuscular times (Voorham 2002) we added two 12-h collections at Jardim da Copaiba, one in August 2002 and one in April 2003. We collected primarily during the rainy season because there is a significant positive correlation between rainfall and malaria cases in Boa Vista (Silva-Vasconcelos et al. 2002). Informed consent was obtained from all collectors, and the Biosafety Committee at the Instituto Evandro Chagas in Belém, state of Pará, the Vermont Institutional Review Board, and New York State Department of Health Institutional Review Board approved the project. Mosquitoes were identified within 24 h using the taxonomic key of Deane et al. (1946). The samples of *An. albitarsis* s.l. were initially identified as *An. marajoara* based on RAPD banding patterns in Wilkerson et al. (1995a), but subsequently as *An. albitarsis* E based on the mtDNA *COI* gene sequence (Lehr et al. 2005). Biting indices (BI) were calculated by dividing the number of mosquitoes captured by the number of collectors and the number of hours they collected.

Malaria parasite identification - Each mosquito specimen was divided into two parts: the head and thorax were maintained dry for ELISA analysis and then processed using standard protocols (Wirtz et al. 1987, 1991, 1992) to detect *Plasmodium* (*falciparum, malariae, vivax* VK210, and *vivax* VK247); and the abdomens were preserved for molecular identification and DNA analysis. *Anopheles* species other than *An. darlingi* or *An. marajoara* were analyzed by ELISA in pools of a maximum of five individuals (except in one case, Table I).

Data analysis - The variances for the data on abundances of *An. darlingi* and *An. albitarsis* E and the malaria parasites were too high to be transformed to meet the assumptions of normality or homoscedasticity. Therefore, all data were transformed to ranks and a Kruskal-Wallis analysis was performed with the following variables: total mosquitoes collected, total mosquitoes positive for *P. falciparum* and total mosquitoes positive for *P. vivax* (both variants were combined in all analyses). A separate analysis for *P. malariae* was not undertaken because the sample size was too small (see Table I). Class effects in the analysis were species, site, month, and their two-way interactions. Data from only four of the six localities (Jardim da Copaiba, Caçari, Caraña, and 13 de Setembro) were analyzed using Kruskal-Wallis because in the Distrito Industrial we did not detect *An. darlingi*, and in Cauamé after the first very dismal showing (March 2001, Table I) we failed to find either of our key target species.

The data from the 12-h collections at Jardim da Copaiba (Results and Figure) were not included in this analysis because of small sample size.



Fraction of captured *Anopheles albitarsis* E and *An. darlingi* by hour. August 2002, Jardim da Copaiba, Boa Vista, Roraima, Brazil.

RESULTS

We collected and identified 4673 mosquitoes using human landing catches in six districts in Boa Vista. The samples of *An. albitarsis* s.l. were identified as *An. albitarsis* E using *COI* sequences (Lehr et al. 2005). *An. albitarsis* E was most abundant (80.9%) followed by *An. darlingi* (9.8%), *An. braziliensis* (6.3%), and *An. peryassui* (1.6%). Each of *An. nuneztovari*, *An. oswaldoi* s.l., and *An. triannulatus* was detected at less than 1% (Table I). Significantly more ($P < 0.001$; Table II) *An. albitarsis* E were collected compared with *An. darlingi* and it is obvious from the abundance of *An. albitarsis* E attracted to humans (mean = 140.17 versus mean of 15.9 for *An. darlingi*) that in Boa Vista this species is anthropophilic. The abundance of *An. albitarsis* E is also evident from the BI that range from 0.25-30.5, whereas for *An. darlingi* the range is 0.03-6.5 (Table I). Despite these differences in mosquito abundance and biting rate, only marginally significantly more ($p = 0.058$) *An. albitarsis* E were infected with *P. vivax* compared with *An. darlingi*

and there was no significant difference for *P. falciparum* (Table II). *P. vivax* (70.5%, both variants) was the most common parasite detected in mosquitoes, followed by *P. falciparum* (27.3%) and *P. malariae* (2.3%).

There was a significant monthly difference (Table II) in total mosquito abundance between May 2001 (mean = 37.0) and June 2002 (mean = 168.3), although both are during the rainy season. Months also differed significantly for number of mosquitoes infected with *P. falciparum*, probably because there were no mosquitoes

TABLE I

Total number of *Anopheles* mosquitoes collected, biting index (BI), and number infected by collection date from several localities in Boa Vista, state of Roraima, Brazil, 2001-2002

	Species											
	An. darlingi						*An. albitarsis* E					
Locality	T	BI	Pf	VK 210	VK 247	Pm	T	BI	Pf	VK 210	VK 247	Pm
Mar 01												
13 Setembro [a]	1	0.03	0	0	0	0	22	0.56	2	1	0	0
Cauamé [b]	0						1	0.25	0	0	0	0
May 01												
13 Setembro	0						55	4.4	1	2	0	0
D. Industrial [b]	0						44	2.93	0	0	0	0
Jar. Copaiba	3	0.2	0	0	0	0	49	3.27	0	1	1	0
Caçari [c]	12	0.44	0	0	0	0	105	3.89	0	4	1	0
Caraña [b]	4	0.25	0	1	0	0	68	4.25	0	1	0	0
Sept. 01												
13 Setembro [d]	2	0.11	0	0	0	0	239	13.28	2	3	0	0
D. Industrial [e]	0						48	3.0	0	0	0	0
Jar. Copaiba [f]	1	0.03	0	0	0	0	184	5.75	2	3	0	0
Caçari [g]	18	0.41	0	1	0	0	61	1.39	1	1	0	0
Caraña [h]	23	0.58	1	1	0	0	24	0.6	0	0	0	0
Mar 02												
13 Setembro	0						6	1.5	0	0	0	0
Jar. Copaiba	3	0.75	0	0	0	0	15	3.75	0	0	0	0
Caçari	0						16	0.76	0	0	0	0
Caranã	0						4	0.22	0	0	0	0
D. Industrial [j]	0						25	2.08	0	0	0	0
June 02												
13 Setembro [l]	2	0.08	0	0	0	0	295 [k]	12.29	1	8	0	0
Jar. Copaiba [m]	12	0.24	0	0	0	0	558	11.16	0	0	0	0
Caçari [n]	4	0.5	0	0	0	0	244	30.5	0	0	0	0
Caranã [h]	7	0.7	0	0	0	0	224	22.4	1	0	0	1
Aug 02												
13 Setembro [o]	12	0.4	0	2	0	0	162	5.4	0	1	0	0
Jar. Copaiba [p]	85	1.7	0	0	1	0	601	12.02	0	3 [q]	0	0
Caçari [s]	11	0.92	0	0	0	0	29	2.42	0	0	0	0
Caranã [t]	0						34	2.83	0	1 [u]	0	0
Nov 02												
13 Setembro [v]	0						7	0.88	0	0	0	0
Jar. Copaiba	0						150	25.0	0	0	0	0
Caçari [w]	106		0	0	0	0	129	8.06	0	0	0	0
Caranã [y]	76						105	26.25	0	0	0	0

T: total number of mosquitoes identified; Pf: *P. falciparum*; VK210: *P. vivax* VK210; VK247: *P. vivax* VK247; Pm: *P. malariae*. Additional mosquito species collected (below) were all negative for *Plasmodium* except where noted. *a*: 1 *An. nuneztovari*; *b*: 1 *An. braziliensis*; *c*: 24 *An. braziliensis* and 1 *An. peryassui*; *d*: 7 *An.braziliensis*; *e*: 51 *An. peryassui*, 27 *An. braziliensis*, 1 *An. nuneztovari* and 1 *An. triannulatus*; *f*: 53 *An. braziliensis*, 10 *An. peryassui*, 1 *An. triannulatus*; *g*: 27 *An. braziliensis*; *h*: 4 *An. braziliensis*; *j*:1 *An. peryassui*; *k*: all mosquitoes analyzed in pools of n = 3; *l*: 12 *An. braziliensis*; *m*: 38 *An. braziliensis*, 9 *An. peryassui*, 1 *An. nuneztovari*; *n*: 8 *An. braziliensis*, 1 *An. peryassui*; *o*: 7 *An. braziliensis*, 4 *An. nuneztovari*, 2 *An. oswaldoi* s.l., and 1 *An. triannulatus*; *p*: 30 *An. braziliensis* and 6 *An. nuneztovari*; *q*: pool of n = 5; *r*: 5 *An. braziliensis* and 1 *An. nuneztovari*; *s*: 26 *An. braziliensis* (1 pool of n = 7 positive for Pv1); *t*: 15 *An. braziliensis*, 2 *An. peryassui*; *u*: pool of n = 6 mosquitoes; *v*: 1 *An. braziliensis*, 1 *An. triannulatus*; *w*: 8 *An. braziliensis*; *x*: 3 *An. braziliensis*, 1 *An. triannulatus*; *y*: these mosquitoes were not tested. All collections peridomestic from 18:00-21:00 h unless otherwise noted.

collected that were infected with this malaria parasite in March 2002, August 2002, and November 2002.

The significance of the species-by-site interaction term in Table II indicates that mosquito abundance differed among sites for these two species. *An. albitarsis* E is at its highest frequency at Jardim da Copaiba, whereas *An. darlingi* shows its greatest abundance at Caçari. Similarly, the significant species-by-month interaction term demonstrates that among months, the patterns of each species' abundance differed. In this case, *An. albitarsis* E is most abundant in June 2002 and *An. darlingi* is most abundant in November 2002.

The two 12-h collections undertaken in 2002 and 2003 at Jardim da Copaiba demonstrate, at least for 2002 (Figure), that *An. albitarsis* E in this district bites predominately in the early evening, has a smaller peak between 12:00 and 03:00 h, but can bite throughout the night. *An. braziliensis* was collected in 2002 at 18-19 h (n = 2), 19-20 h (n = 1) and at 23-24 h (n = 2). In 2003 *An. albitarsis* E was uncommon (18-19 h, n = 2; 19-20 h, n = 1) and *An. darlingi* was collected in low numbers biting through the night (18-19 h, n = 7; 19-20 h, n = 2; 20-21 h, n = 3; 21-22 h, n = 2; 22-23 h, n = 3; 23-24 h, n = 1; 24-01 h, n = 2; 01-02 h, n = 2; 02-03 h, n = 2; 04-05 h, n = 4). In both temporal collections, *An. darlingi* bit throughout the night, with a peak about the same time as *An. albitarsis* E's smaller peak (after midnight), and there was no discernible peak in 2003.

We detected infected mosquitoes during the 2002 collections: one *An. darlingi* infected with *P. vivax* VK210 and two *An. albitarsis* E, one infected with each of *P. falciparum* and *P. vivax* VK210. For all collections, all mosquito species collected were tested for *Plasmodium* but among the five additional species encountered, only one pool of 7 *An. braziliensis* was positive for *P. vivax* VK210 (Table I).

DISCUSSION

In terms of relative species abundance our data are congruent with those of Silva-Vasconcelos et al. (2002): *An. albitarsis* E (as *An. albitarsis*) > *An. darlingi* > *An. braziliensis* > *An. peryassui* > *An. nuneztovari*. Their study also found a higher infection rate for *An. darlingi* in comparison with *An albitarsis* E, and the biting indices for *An. darlingi* are in the same range. However, the present study found higher biting indices for *An. albitarsis* E in three localities: Caçari, Caranã, and Jardim da Copaiba. Although our 12-h collections were limited, they demonstrate the same pattern for *An. darlingi* and *An. albitarsis* E as found by Silva-Vasconcelos et al. (2002). *An. darlingi* is known for its geographically variable patterns of biting activity (reviewed in Rosa-Freitas et al. 1992, Voorham 2002), resulting from either environmental variables, genetic differentiation, or possibly both. Interestingly, *An. marajoara* in Western Venezuela has a similar peak biting period as *An. albitarsis* E in Boa Vista (predominantly before midnight; Rubio-Palis & Curtis 1992). We found a single pool of *An. braziliensis* infected with *Plasmodium*. Although a greater proportion of *An. braziliensis* was infected in the study of Silva-Vasconcelos et al. (2002), in neither study, because of low abundance combined with low infectivity, does this species contribute significantly to malaria transmission.

Both studies found a much higher proportion of mosquitoes infected with *P. vivax* than *P. falciparum*, a reflection of the relative proportions of these two parasites in humans in Roraima (Chaves & Rodrigues 2000), in other parts of the Amazon (e.g., Amapá, Conn et al. 2002; Pará, Póvoa et al. 2003) and throughout most endemic Neotropical countries (PAHO 2002). Both studies also found a higher proportion of mosquitoes infected with variant VK210 compared with variant VK247. In a recent study in three endemic malaria areas of Brazil, VK210 was associated with the highest parasitemia levels in humans compared with VK247 and *P. vivax*-like, although there was no differential response to chloroquine treatment among the three variants (Machado & Póvoa 2000).

P. malariae was detected in only one mosquito (*An. albitarsis* E from Caranã), and in six individuals of *An. darlingi* in the earlier study in Boa Vista (Silva-Vasconcelos et al. 2002) but ELISA slightly underestimates the overall prevalence of malaria parasites in anophelines because it is not as sensitive as ELISA-PCR (Póvoa et al. 2000a). Thick blood smears also underestimate the prevalence of *P. malariae* in humans (Scopel et al. 2004). These findings suggest that accurate diagnosis of *P. malariae* should be a higher priority because this parasite results in low, but significant levels of morbidity in humans

TABLE II

F-values from Kruskal-Wallis analysis of ranked data from six collections of *Anopheles darlingi* and *An. albitarsis* E at four locations in Boa Vista, Brazil

	Source					
	Species	Month	Site	Species × Site	Species × Month	Month × Site
df	1	5	3	3	5	15
Traits						
Total mosq. collected [a]	35.60[c]	5.13 [d]	3.87 [e]	4.03 [e]	4.99 [d]	1.89
# *falciparum*	3.79	2.95 [e]	0.59	1.21	1.52	0.31
# *vivax* [b]	4.25[e]	0.98	0.73	1.01	0.92	0.81

a: this includes *An. darlingi* and *An. albitarsis* E; *b*: this includes both *P. vivax* VK210 and *P. vivax* VK247; significance levels: *c*: $p < 0.001$; *d*: $p < 0.01$; *e*: $p < 0.05$.

(Collins et al. 2004), there has been a recent resurgence in human cases in Pará (Póvoa et al. 2003) as well as in Suriname (PAHO 2002) and misdiagnosis may mean that the wrong antimalarial treatment is given (Scopel et al. 2004).

Because our collections identified *An. albitarsis* E exclusively of the four species in the *An. albitarsis* complex it is very likely that the mosquitoes referred to in Silva-Vasconcelos et al. (2002) as *An. albitarsis* s.l. are also *An. albitarsis* E. Thus the present study confirms *An. albitarsis* E as an important malaria vector, at a minimum in savannah habitat around Boa Vista. This finding does not diminish the importance of *An. darlingi* as the primary malaria vector in the Neotropics (Lounibos & Conn 2000). In fact, in some localities *An. darlingi* has increased recently (Póvoa et al. 2003, Gil et al. 2003, Schoeler et al. 2003). The finding of the importance of *An. albitarsis* E in Boa Vista serves as a reminder that the heterogeneity of malaria vectors (reviewed in Lounibos & Conn 2000) must be taken into account, whether in relation to integrated malaria control strategies that might be implemented (Alilo et al. 2004) or transgenic mosquitoes that could eventually be considered for release (Touré et al. 2004).

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