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EMERGENCE OF A NEW NEOTROPICAL MALARIA VECTOR FACILITATED BY HUMAN MIGRATION AND CHANGES IN LAND USE

JAN E. CONN, RICHARD C. WILKERSON, M. NAZARÉ O. SEGURA, RAIMUNDO T. L. DE SOUZA, CARL D. SCHLICHTING, ROBERT A. WIRTZ, AND MARINETE M. PÓVOA

Department of Biology, University of Vermont, Burlington, Vermont; Department of Entomology, Walter Reed Army Institute of Research,Washington, District of Columbia; Serviço de Parasitologia, Instituto Evandro Chagas, Belém, Pará, Brazil; Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut; Entomology Branch, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, Georgia

Abstract. In a region of northeastern Amazonia, we find a species previously of minor importance, *Anopheles marajoara,* to be the principal malaria vector. In a total of five collections during 1996–97 in three replicated sites near the city of Macapá, Amapá state, this species occurs in much greater abundance compared with the presumed vector *Anopheles darlingi.* Also, a significantly higher proportion of *An. marajoara* is infected with malaria parasites, determined by the ELISA technique. This appears to be the result of increased abundance of *An. marajoara* due to alterations in land use, invasion of its primary breeding sites by human immigrants, and its anthropophilic behavior. This discovery highlights one of the challenges of Neotropical malaria control, namely that the targeting of specific vectors may be complicated by a changing mosaic of different locally important vectors and their interactions with human populations.

INTRODUCTION

In Brazil, with an estimated 500,000 malaria cases annually,[1] *Anopheles darlingi* is considered to be responsible for most of the malaria transmission because of its preference for feeding on humans[2,3] and its relatively high rates of *Plasmodium* infections.[4,5] Recently several additional Neotropical species have been proposed as potentially important local or regional vectors based on malaria parasite detection using ELISA (Enzyme-Linked ImmunoSorbent Assay) or dissection techniques.[6]

The Neotropical species complex *An. albitarsis s.l.* consists of four species (*An. albitarsis s.s., An. deaneorum, An. marajoara,* and *An. albitarsis* sp. B) which can currently reliably be distinguished only using Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) techniques.[7] *Anopheles deaneorum* has been proposed as an important vector in western Amazonian Brazil on the basis of host preference, abundance and experimental infection[8,9] but the status of the other three is unresolved. Most studies concerning the feeding behavior and potential vector status (evidence of natural or experimental infection by human malaria parasites) of *An. albitarsis s.l.*[10–13] are difficult to interpret because they were performed prior to the recognition of the four species.

A major part of our research program is directed at identifying the mosquito species and the ecological factors involved in *Plasmodium* transmission in the Neotropics in order to facilitate the control of malaria. We wished to investigate whether *An. darlingi* is, as has been suggested,[4,5,14,15] the primary malaria vector throughout the Amazonian region. As more research has been done, additional species have been found to be positive for *Plasmodium.* If such species are convincingly implicated as vectors in different localities, the characteristics of their breeding sites, their behaviors and their migration patterns could be used to predict changes in malaria transmission patterns in other parts of the Amazon Basin.

Previous studies have documented or predicted changes in human-mosquito-malaria parasite interactions based on immigration and habitat alteration in the Amazon Basin,[16–20] other endemic areas in the Neotropics,[14] and worldwide.[21–24] In Amapá state (Figure 1), where this study was carried out, two factors have produced a rapid increase in the human population: the change in status from territory to state, which has led to development of rural areas (Póvoa, pers. obs.) and the designation of the city of Macapá (the state capital) as a duty-free zone in the Amazon Basin, which has resulted in greatly increased migration. Some of the immigrants from nearby states have been identified as infected with malaria parasites.[25] The fraction of the population infected with malaria in Amapá state has increased from 4.3% to about 15% during a nine-year period (1990–1998)[13]. This combination of population influx and higher malarial prevalence has resulted in a marked rise in the number of malaria cases.[25]

In the present study, we incriminate one of the cryptic species of the *An. albitarsis* complex, *An. marajoara,* as a primary malaria vector in Amapá state by combined evidence from behavioral studies, population density measures, and both ELISA and dissection estimates of infectivity by malaria parasites. We demonstrate that in each of three study sites, malaria parasites are significantly more likely to be acquired by humans from *An. marajoara* than from *An. darlingi.*

MATERIALS AND METHODS

Mosquito Collection and Identification. Adult mosquito collections were made at three endemic malaria sites in the Brazilian state of Amapá near the city of Macapá: Lagoa dos Indios, 0° 00′ N × 51° 06′ W; Granja Alves, 0° 02′ N × 51° 05′ W; and Santana, 0° 01′ S × 51° 09′ W (Figure 1). Lagoa dos Indios and Granja Alves, separated by 4 km, are on the periphery of the city, and Santana is 9 km. southwest of Macapá. Five trips were made to each site (July and September 1996; March, May and July 1997) and mosquitoes were collected from approximately 19:00–21:00 on each of four consecutive nights (previous all-night collections in this area indicated this early evening period as the peak biting time). Informed consent was obtained from all collectors, and the

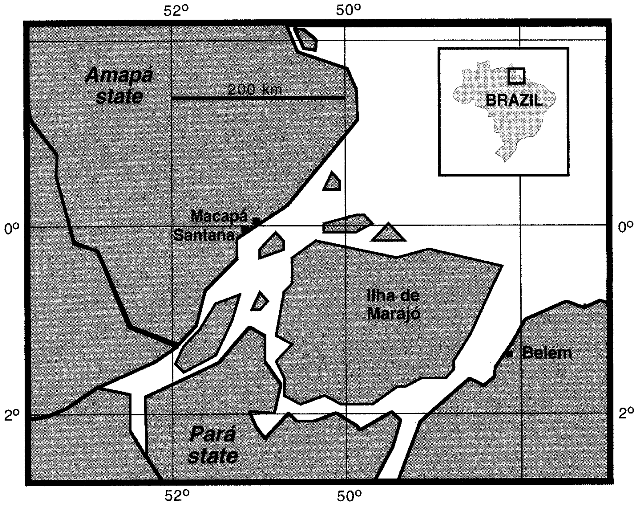

FIGURE 1. Map of Northeastern Amazonia, showing study sites.

Vermont Institutional Review Board approved the project. Each mosquito was dissected into two parts: the abdomen was stored in 95% ethanol and used subsequently for molecular species identification, and the head/thorax was dried and stored for parasite detection using the ELISA technique.

5,493 mosquitoes were identified morphologically as either *An. albitarsis s.l.* or *An. darlingi.*[2] Of the 5,223 specimens identified as *An. albitarsis s.l.*, 326 were chosen randomly from the three sites and five collection dates, and species determinations were made using RAPD primers:[7] all were *An. marajoara.* Given this result, the probability that any of the other three cryptic species were present at appreciable frequencies is minuscule ($P < 0.0001$, binomial test).[26]

Malaria parasite identification. All mosquitoes collected were analyzed for *Plasmodium* (*falciparum, malariae, vivax* VK210, and *vivax* VK247) using ELISA following standard protocols.[27–29] Additionally, for each site and date, both dissection and ELISA were used to assess malarial parasite presence for 10 *An. albitarsis s.l.* and 10 *An. darlingi* (or, if less than 10, all available specimens): head plus thorax and stomachs were dissected for the presence of sporozoites and oocysts, respectively, and then all material was scraped off each slide into individual vials of buffer and tested following ELISA protocols. The two methods showed perfect congruence (data not shown). All the *An. albitarsis s.l.* that were positive for any malaria parasite were examined using RAPD-PCR[7] and all were again identified as *An. marajoara.*

Data Analysis. The variances for the data on abundances of both mosquitoes and parasites were quite high, and could not be transformed to meet the assumptions of normality or homoscedasticity. Thus, all data were transformed to ranks and Kruskal-Wallis analysis was performed on the following variables: total mosquitoes collected, total mosquitoes parasitized, total mosquitoes positive for *P. falciparum,* total mosquitoes positive for *P. vivax,* total percent mosquitoes parasitized, total percent mosquitoes positive for *P. falciparum,* and total percent mosquitoes positive for *P. vivax* (the two forms of *Plasmodium vivax* were combined in all analyses). Class effects in the analysis were: species, site, and date, and their two-way interactions. The interactions of species and date, and site and date were not significant and were dropped from the final model.

RESULTS

An initial analysis categorizing collection dates as during peak malarial transmission (June–August; November–December) in Amazonian Brazil or not[4] revealed no significant effects of the distinction of peak/non-peak for any of the traits. Although this result was somewhat surprising, the rainfall patterns (and consequently the malaria transmission

TABLE 1

Total number of *An. marajoara* and *An. darlingi* individual mosquitoes collected and percentages infected by malaria parasites by collection date, using 3 localities as replicates

	Locality														
	Lagoa dos Indios					Granja Alves					Santana				
Species	T	Pf	Pv1	Pv2	Pm	T	Pf	Pv1	Pv2	Pm	T	Pf	Pv1	Pv2	Pm
07/96															
MJA	**567**	**0.35**	**0.17**	**7.58**	**0**	**214**	**0.47**	**0**	**7.94**	**0**	**1**	**0**	**0**	**0**	**0**
DAR	**8**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
09/96															
MJA	362	0	0	0.83	0.27	37	0	0	0	0	12	0	0	0	0
DAR	5	0	0	0	0	1	0	0	0	0	29	0	3.45	3.45	3.45
03/97															
MJA	1543	0.06	0.06	0.13	0.84	202	0	0	0.50	0	59	0	0	0	3.39
DAR	6	0	0	0	0	25	0	0	0	0	6	0	0	0	0
05/97															
MJA	1144	0.26	0	0.70	0.70	90	0	0	0	0	106	0.94	0	0	0
DAR	36	0	0	0	0	52	0	0	1.92	0	16	0	0	0	0
07/97															
MJA	**728**	**2.75**	**1.65**	**1.37**	**0.41**	**92**	**1.09**	**0**	**0**	**0**	**66**	**0**	**1.52**	**0**	**1.52**
DAR	**0**	**0**	**0**	**0**	**0**	**54**	**1.85**	**0**	**0**	**0**	**26**	**0**	**0**	**0**	**0**

MJA = *An. marajoara*; DAR = *An. darlingi.*

T = total number of mosquitoes collected. All individuals were assayed with ELISA monoclonals except MJA at Lagoa dos Indios 07/97 where 748 MJA were collected and 379 were analyzed.

Pf = *Plasmodium falciparum*; Pv1 = *P. vivax* VK210; Pv2 = *P. vivax* VK247; Pm = *P. malariae.*

Months in bold are during the presumed peak malaria transmission season in the Amazon Basin (Deane, 1988).

TABLE 2

Kruskal-Wallis results (F-values) of analysis on ranked data from five collections of *An. marajoara* and *An. darlingi* at three replicated sites

	Source			
	Species	Site	Species × Site	Date
df	*1*	*2*	*2*	*4*
Traits				
Total collected	41.71***	3.67*	6.15**	2.09
Total parasitized[1]	24.21***	1.56	5.87*	1.21
# of *falciparum*	14.74**	2.82	3.76*	2.27
# of *vivax*[2]	11.31**	3.87*	8.06**	0.67
Total % parasitized[1]	10.87**	0.05	2.34	1.06
% *falciparum*	9.69**	1.49	1.85	2.08
% vivax[2]	7.18*	0.89	3.35*	0.50

[1] This includes *P. falciparum, P. vivax* VK210, *P. vivax* VK247 and *P. malariae.*

[2] This includes both *P. vivax* VK210 and *P. vivax* VK247.

Significance levels. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

df = degree of freedom.

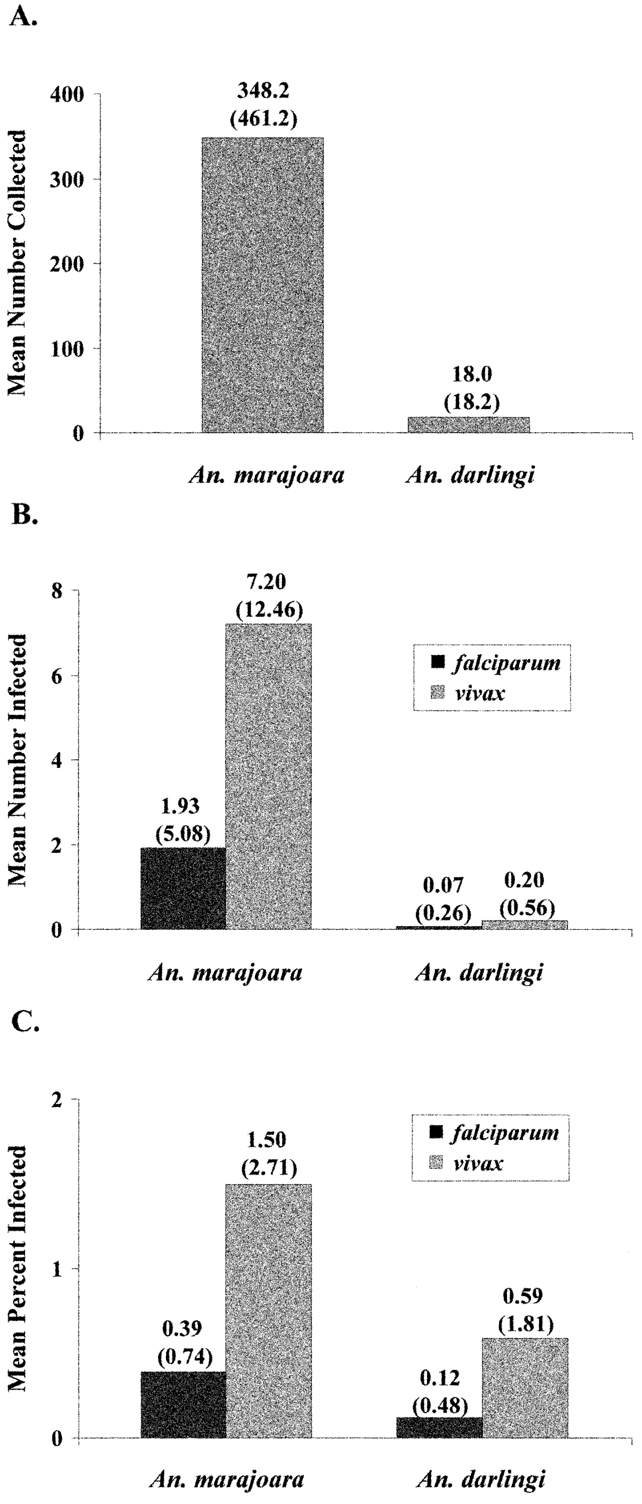

FIGURE 2. (A) Mean numbers of *An. marajoara* and *An. darlingi* collected during the duration of the present study. (B) Mean numbers of mosquitoes infected with *P. vivax* and *P. falciparum.* (C) Mean percent of mosquitoes infected with *P. vivax* and *P. falciparum.* Standard deviations are in parentheses.

pattern) of this region of the Amazon Basin were altered during the periods of our collections (due to El Niño),[30,31] perhaps obscuring the expected differences between seasons.

In this region, *An. marajoara* clearly poses a substantially greater threat of biting humans than does *An. darlingi* (Table 1). Significantly more ($P < 0.001$; Table 2) *An. marajoara* were collected than *An. darlingi,* and it is evident from the abundance of *An. marajoara* attracted to humans (mean = 348.2; Figure 2) that this species is anthropophilic; *An. darlingi* was typically found only at low frequencies (range 0–54; Table 1).

Significantly more *An. marajoara* were also infected with *Plasmodium spp.* than *An. darlingi* (Table 2; Figure 2). When *Plasmodium* is analyzed by species, this pattern holds true for both *P. vivax* and *P. falciparum.* When analyzed as percent of individual mosquitoes infected, *An. marajoara* also shows greater infection rates by both *P. vivax* and *P. falciparum* (Table 1; Figure 2). Of greatest importance to malaria transmission are the significantly higher total abundances and infection fractions of *An. marajoara* compared to *An. darlingi.* Data from Fundacão Nacional de Saude indicate the occurrence of new malaria cases (both *P. falciparum* and *P. vivax*) for our three collection sites during the months of our collections even when no *An. darlingi* were present.[25]

The significance of the species by site interaction term (Table 2) indicates that the patterns of mosquito abundance and numbers of mosquitoes parasitized differed among sites for the two species. *Anopheles marajoara* shows its highest abundance at Lagoa dos Indios whereas *An. darlingi* is at its highest frequencies at Granja Alves (Table 1).

DISCUSSION

When compared with the only other major study of Brazilian anophelines in Amapá, which occurred in the 1940's,[32] our data suggest a striking change in the relative abundances of *An. marajoara* (as *An. albitarsis s.l.*) and *An. darlingi.* During the earlier study (1939–44), researchers collected a total of 1,779 adult *An. darlingi* compared with a total of 247 adult *An. albitarsis* from 3 collecting sites in Amapá state (including the city of Macapá). Their methodology included 12-hr captures from humans, using aspirators both indoors and outdoors. We collected only outdoors because our preliminary data demonstrated that both *An. marajoara* and *An. darlingi* are exclusively exophilic in our three study sites, an interesting change from the behavior of both species during the earlier collections.[32] Our data, collected in 1996–97 around the city of Macapá, show a dramatic reversal (5,223 *An. marajoara* compared with 270 *An. darlingi*; Table 1). When combined with the reduction in forest habitat (one of the major breeding sites of *An. darlingi*) by burning and clear cutting, and the concurrent increase in agricultural sites around Macapá which create habitat such as marshy, sunlit pools (ideal for *An. marajoara*; Segura, M.N.O., unpublished data) this evidence is indicative of an increased abundance of *An. marajoara.* As a comparison, in Manaus in Amazonas state, *An. darlingi* temporarily disappeared from the city in 1975; this has been suggested to be the result of a reduction in breeding sites as the city grew rapidly.[33] In Manaus, where growth has been spurred more by urbanization than by agriculture, *An. albitarsis s.l.* has remained at low abundance, and it has not been documented as important in malaria transmission.[33]

For many regions in the Amazon Basin, populations of *An. darlingi* have increased because road construction in the forest has considerably expanded the breeding sites—large areas of neutral, partially shaded and unpolluted water. These characteristics also attract human inhabitants.[5,16–18] Subsequently, clearing of forests and water pollution reduce the suitability of these for *An. darlingi* breeding. However, these sites, and newly created ponds for agricultural use, attract other mosquito species.[20] In Macapá such changes have led to an increase in breeding sites for *An. marajoara.* In addition, humans have colonized land near extensive marshy areas, another preferred breeding habitat of *An. marajoara* (Segura, M.N.O., unpublished data). We hypothesize that these changes, in combination with an increase in human host abundance, and the immigration of individuals infected with malaria parasites,[25] have led to a population increase in *An. marajoara* and to its current position as the primary malaria vector in this region.

Some researchers have implied that if *An. darlingi* were eliminated from the Neotropics, malaria would become an unimportant disease in South America.[5,15] Our data do not dispute the overall importance of *An. darlingi* in the Neotropics, but they suggest an unforeseen problem for long range plans to combat malarial transmission in the Amazonian Basin: the combination of anopheline species that are somewhat anthropophilic (even facultatively), with an influx of human settlers that carry *Plasmodium* is a potentially volatile mix.

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Authors' Addresses: Jan E. Conn, Department of Biology, 321 Marsh Life Sciences Building, University of Vermont, Burlington, VT 05405-0086. Richard C. Wilkerson, Walter Reed Biosystematics Unit, Department of Entomology, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100. M. Nazaré O. Segura, Raimundo T. L. de Souza, and Marinete M. Póvoa, Instituto Evandro Chagas/Fundação Nacional da Saúde, Servico de Parasitologia, Av. Almirante Barroso 492, Belém, Pará 66090-000, Brazil. Carl D. Schlichting, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269-0043. Robert A. Wirtz, Entomology Branch, Division of Parasitic Diseases, Centers for Disease Control, 4770 Buford Highway NE, Atlanta, GA, 30341-3724.

Reprint Requests: Jan E. Conn, Department of Biology, 321 Marsh Life Sciences Building, University of Vermont, Burlington, VT 05405-0086.

REFERENCES

1. Pan American Health Organization, 1998. Status of malaria programs in the Americas, XLVI Report CSP 25/INF/1.
2. Deane LM, Causey OR, Deane MP, 1946. An illustrated key by adult female characteristics for identification of thirty-five species of Anophelini from the Northeast and Amazon regions of Brazil, with notes on the malaria vectors (Diptera: Culicidae). *Am J Hyg Mono Series 18:* 1–18.
3. Charlwood JD, Alecrim WA, 1989. Capture-recapture studies with the South American malaria vector *Anopheles darlingi,* Root. *Ann Trop Med Parasit 83:* 569–576.
4. Deane LM, 1988. Malaria studies and control in Brazil. *Am J Trop Med Hyg 38:* 223–230.
5. Charlwood JD, 1996. Biological variation in *Anopheles darlingi* Root. *Mem Inst Osw Cruz 91:* 391–398.
6. Lounibos LP, Conn JE, 2000. Malaria Vector Heterogeneity in South America. *Amer Ent 46:* 238–249.
7. Wilkerson RC, Parsons TJ, Klein TA, Gaffigan TV, Bergo E, Consolim J, 1995. Diagnosis by Random Amplified Polymorphic DNA Polymerase Chain Reaction of four cryptic species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* (Diptera: Culicidae) from Paraguay, Argentina, and Brazil. *J Med Ent 32:* 697–704.
8. Klein TA, Lima JBP, Tada MS, 1991. Comparative susceptibility of anopheline mosquitoes to *Plasmodium falciparum* in Rondonia, Brazil. *Am J Trop Med Hyg 44:* 234–239.
9. Klein TA, Lima JBP, Tada MS, Miller R, 1991. Comparative susceptibility of anopheline mosquitoes in Rondonia, Brazil to infection by *Plasmodium vivax. Am J Trop Med Hyg 45:* 463–470.
10. Linthicum KJ, 1988. A revision of the Argyritarsis Section of the subgenus *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). *Mosq Sys 20:* 101–271.
11. Rosa-Freitas MG, Deane LM, Momen H, 1990. A morphological, isoenzymatic and behavioural study of ten populations of *Anopheles (Nyssorhynchus) albitarsis* Lynch-Arribalzaga, 1878 (Diptera: Culicidae) including from the type-locality—Baradero, Argentina. *Mem Inst Osw Cruz 85:* 275–289.
12. Narang SK, Klein TA, Perera OP, Lima JP, Tang AT, 1993. Genetic evidence for the existence of cryptic species in the *Anopheles albitarsis* complex in Brazil: allozymes and mitochondrial DNA restriction fragment length polymorphism. *Biochem Genet 31:* 97–112.
13. Povoa MM, Wirtz RA, Lacerda RNL, Miles MA, Warhurst D, 2000. Malaria vectors in the municipality of Serra do Navio, state of Amapá, Amazon region, Brazil. *Mem Inst Osw Cruz 96:* 179–184.
14. Zimmerman RH, 1992. Ecology of malaria vectors in the Americas and future direction. *Mem Inst Osw Cruz 87:* Suppl. 3, 371–383.
15. Forattini OP, 1962. *Entomologia Medica* Vol. 1. São Paulo, Brazil: Faculdade de Higiene e Saude Publica, 311 p.
16. Smith NJH, 1981. Colonization lessons from a tropical Forest. *Science 214:* 755–761.
17. Cruz Marques A, 1987. Human migration and the spread of malaria in Brazil. *Parasit Today 3:* 166–170.
18. Alecrim WD, 1992. Malaria, prospecting activities and govern-

ment policies in the Amazon Region. *Rev Inst Med Trop São Paulo 34:* S48.

19. Carmago LMA, Ferreira MU, Kreiger H, de Carmago EP, da Silva LP, 1994. Unstable hypoendemic malaria in Rondonia (western Amazon region, Brazil): epidemic outbreaks and work-associated incidence in an agro-industrial rural settlement. *Am J Trop Med Hyg 51:* 16–25.
20. Tadei WP, Thatcher BD, Santos JMM, Scarpassa VM, Rodrigues IB, Rafael MS, 1998. Ecologic observations on anopheline vectors of malaria in the Brazilian Amazon. *Am J Trop Med Hyg 59:* 325–335.
21. Walsh JF, Molyneaux DH, Birley MH, 1993. Deforestation: effects on vector-borne disease. *Parasitol 106* Suppl: 55–75.
22. Mouchet J, Carnevale P, 1997. Impact of changes in the environment on vector-transmitted diseases. *Santé 4:* 263–269.
23. Molyneux DH, 1998. Vector-borne parasitic diseases-an overview of recent changes. *Int J Parasitol 28:* 927–934.
24. Gratz NG, 1999. Emerging and resurging vector-borne diseases. *Annu Rev Entomol 44:* 51–75.
25. Fundação Nacional de Saude (FUNASA)—Coordenadoria Regional do Estado do Amapá.
26. Hollander M, Wolfe DA, 1973. *Nonparametric Statistical Methods.* New York: Wiley.
27. Wirtz RA, Zavala F, Charoenvit Y, Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, Andre RG, 1987. Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. *Bull WHO 65:* 39–45.
28. Wirtz RA, Charoenvit Y, Burkot TR, Esser KM, Beaudoin RL, Collins WE, Andre RG, 1991. Evaluation of monoclonal antibodies against *Plasmodium vivax* sporozoites for ELISA development. *Med Vet Ent 5:* 17–22.
29. Wirtz RA, Sattabonkgot J, Hall T, Burkot TR, Rosenberg R, 1992. Development and evaluation of an enzyme-linked immunosorbent assay for *Plasmodium vivax*-VK247 sporozoites. *J Med Entomol 29:* 854–857.
30. Webster PJ, Palmer TN, 1997. The past and the future of El Niño. *Nature 390:* 562–564.
31. Rodbell DT, Seltzer GO, Anderson DM, Abbott MB, Enfield DB, Newman JH, 1999. An ~15,000-year record of El Niño-driven alluviation in Southwestern Ecuador. *Science 283:* 516–520.
32. Deane LM, Causey OR, Deane MP, 1948. Notas sôbre a distribuição e a biologia dos anofelinos das regiôes Nordestina e Amazonica do Brasil. *Bras Rev Serv Esp Saude Pub 1:* 827–966.
33. Tadei WP, Thatcher BT, 2000. malaria vectors in the Brazilian Amazon; *Anopheles* of the subgenus *Nyssorhynchus. Rev Inst Med trop S. Paulo 42:* 89–94.