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; Servicio de Parasitología, 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 humans2,3 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 dissec-
tion 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 infec-
tion,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 hu-
man malaria parasites) of An. albitarsis s.l.10–13 are difficult
to interpret because they were performed prior to the rec-
ognition of the four species.

A major part of our research program is directed at iden-
tifying the mosquito species and the ecological factors in-
volved in Plasmodium transmission in the Neotropics in or-
der to facilitate the control of malaria. We wished to in-
vestigate whether An. darlingi is, as has been suggested,4,5,14,15
the primary malaria vector throughout the Amazonian re-

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 Ma-
capá. Five trips were made to each site (July and September
1996; March, May and July 1997) and mosquitoes were col-
lected from approximately 19:00–21:00 on each of four con-
secutive nights (previous all-night collections in this area in-
dicated this early evening period as the peak biting time).
Informed consent was obtained from all collectors, and the

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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. albitoris s.l. or An. darlingi. Of the 5,223 specimens identified as An. albitoris s.l., 326 were chosen randomly from the three sites and five collection dates, and species determinations were made using RAPD primers: 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).

Malaria parasite identification. All mosquitoes collected were analyzed for Plasmodium (falciparum, malariae, vivax VK210, and vivax VK247) using ELISA following standard protocols. Additionally, for each site and date, both dissection and ELISA were used to assess malarial parasite presence for 10 An. albitoris 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. albitoris s.l. that were positive for any malaria parasite were examined using RAPD-PCR and all were again identified as An. marajoara.

RESULTS

An initial analysis categorizing collection dates as during peak malarial transmission (June–August; November–December) in Amazonian Brazil or not 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 2
Kruskal-Wallis results (F-values) of analysis on ranked data from five collections of An. marajoara and An. darlingi at three replicated sites

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>Site</th>
<th>Species X</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Traits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total collected</td>
<td>41.71***</td>
<td>3.67*</td>
<td>6.15**</td>
<td>2.09</td>
</tr>
<tr>
<td>Total parasitized</td>
<td>24.21***</td>
<td>1.56</td>
<td>5.87*</td>
<td>1.21</td>
</tr>
<tr>
<td># of falciparum</td>
<td>14.74**</td>
<td>2.82</td>
<td>3.76*</td>
<td>2.27</td>
</tr>
<tr>
<td># of vivax</td>
<td>11.31**</td>
<td>3.87*</td>
<td>8.06**</td>
<td>0.67</td>
</tr>
<tr>
<td>Total % parasitized 1</td>
<td>10.87**</td>
<td>0.05</td>
<td>2.34</td>
<td>1.06</td>
</tr>
<tr>
<td>% falciparum</td>
<td>9.69**</td>
<td>1.49</td>
<td>1.85</td>
<td>2.08</td>
</tr>
<tr>
<td>% vivax</td>
<td>7.18*</td>
<td>0.89</td>
<td>3.35*</td>
<td>0.50</td>
</tr>
</tbody>
</table>

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.

pattern) of this region of the Amazon Basin were altered during the periods of our collections (due to El Niño),
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 Fundação Nacional de Saúde 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.

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 Índios 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, 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 in-

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.
cluded 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.\(^\text{32}\) Our data, collected in 1996–97 around the city of Macapá, show a dramatic reduction (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, silted 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.\(^\text{31}\) In Manaus, where growth has been spurred more by urbanization than by agriculture, *An. albittarsis* s.l. has remained at low abundance, and it has not been documented as important in malaria transmission.\(^\text{33}\)

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.\(^\text{4,16–18}\) 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.\(^\text{20}\) 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,\(^\text{21}\) 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.\(^\text{5,15}\) 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. Nazare´ O. Segura, Raimundo T. L. de Souza, and Marinete M. Póvoa, Instituto Evandro Chagas/Fundação Nacional da Saúde, Servic o 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. Richard 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.

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