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GEOGRAPHICAL DISTRIBUTION OF *ANOPHELES DARLINGI* IN THE AMAZON BASIN REGION OF PERU

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ABSTRACT. Malaria has reemerged as a significant public health disease threat in Peru, especially within the Amazon Basin region. This resurgence of human cases caused by infection with *Plasmodium falciparum* and *Plasmodium vivax* is thought to be associated with the spread of *Anopheles darlingi,* the principal South American malaria vector, into new areas of the Amazon Basin. However, comprehensive studies of the distribution for this species have not been conducted in Peru for several years, nor are historical accounts accurate enough to determine if *An. darlingi* was actually present and not collected or misidentified. Therefore, the objective of this study is to define the distribution of *An. darlingi* as well as obtain data on distribution and abundance of other *Anopheles* species in this region. Mosquitoes were collected during 2001 in the Departments of Loreto and Ucayali, the two largest Amazonian Departments of Peru. A total of 60,585 specimens representing 12 species of the subgenera *Nyssorhynchus* and *Anopheles* were collected at 82 (88.2%) of 93 collecting sites. The majority of mosquitoes obtained were identified as *An. benarrochi,* comprising 70.7% of mosquitoes collected, followed by *An. darlingi* (24.0%), *Anopheles mattogrosensis* (2.4%), and *Anopheles triannulatus* (1.5%). *Anopheles darlingi* was collected from 48.8% of sites, indicating that this species is established throughout central Loreto, including further west in the Amazon Basin than previously reported. These data suggest that this species is now found in areas of the Amazon Basin region where it has not been previously reported.

KEY WORDS *Anopheles darlingi,* Amazon, Peru, malaria

INTRODUCTION

Human malaria remains the single most important arthropod-borne disease of humans worldwide (Kettle 1995, WHO 1998). While effective chemoprophylactic measures and drug treatment regimes are available to prevent and treat malaria infections, widespread resistance to chemoprophylactic agents and resistance of vectors to insecticides are major factors contributing to the resurgence of malaria in many regions of the world (Lane and Crosskey 1993, Kettle 1995).

Since the early 1990s, the incidence of malaria, caused by *Plasmodium vivax* (Grassi & Feletti) and *Plasmodium falciparum* (Welch), has increased greatly in Peru (OGE 1997, Roberts et al. 1997). The Peruvian Ministry of Health reported a 7-fold increase in the incidence of malaria from 13 per 10,000 persons in 1990 to a high of 88 per 10,000 persons in 1996 (Roper et al. 2000). Malaria caused by *P. falciparum* infection previously occurred only sporadically along the northeastern borders of Peru, but now accounts for approximately one third of all malaria cases in the country (OGE 1997, Roberts et al. 1997, Roper et al. 2000).

The Amazon Basin Region of Peru, with an estimated population of 474,000 has been the major focus of the malaria epidemic in Peru. For example, only 641 cases of malaria were reported to the Loreto Health Department in 1964; 123 of these were caused by *P. falciparum* (Roper et al. 2000). By 1997, the number of *P. falciparum* and *P. vivax* cases reached 121,268, accounting for over 62% of reported malaria cases in Peru (Aramburú et al. 1999). Confirmed *P. falciparum* cases exceeded 54,000, with 85 reported deaths in that year (Roper et al. 2000). From 1992 to 1997, malaria cases increased 50-fold in Loreto, compared with an increase of only 4-fold for the rest of Peru (Aramburú et al. 1999). By 1999, Peru reported the second highest number of malaria cases in South America next to Brazil, most from Loreto (Aramburú et al. 1999).

This resurgence in malaria incidence, both *P. falciparum* and *P. vivax,* is thought to be related to the spread of *Anopheles darlingi* Root, the predominant malaria vector in the Amazon Region (Lounibos and Conn 2000). Prior to 1991, *An. darlingi* was not reported from the city of Iquitos (Faran and Linthicum 1981, Need et al. 1993a); however, by 1996, this species was found in several communities surrounding Iquitos and even within the city itself (Fernández et al. 1996). *Anopheles darlingi* has now been reported from other areas of the Peruvian Amazon Basin, and evidence suggests that this species is spreading into previously uninfested areas of the Amazon Basin, particularly within the Department of Loreto (Fernández et al. 1996, Aramburú et al. 1999). Comprehensive studies of the geographical distribution of this species in the Peruvian Amazon Basin have not been conducted for many years (Calderón et al. 1974, Calderón et al. 1995).

Anopheles darlingi is considered to be the most

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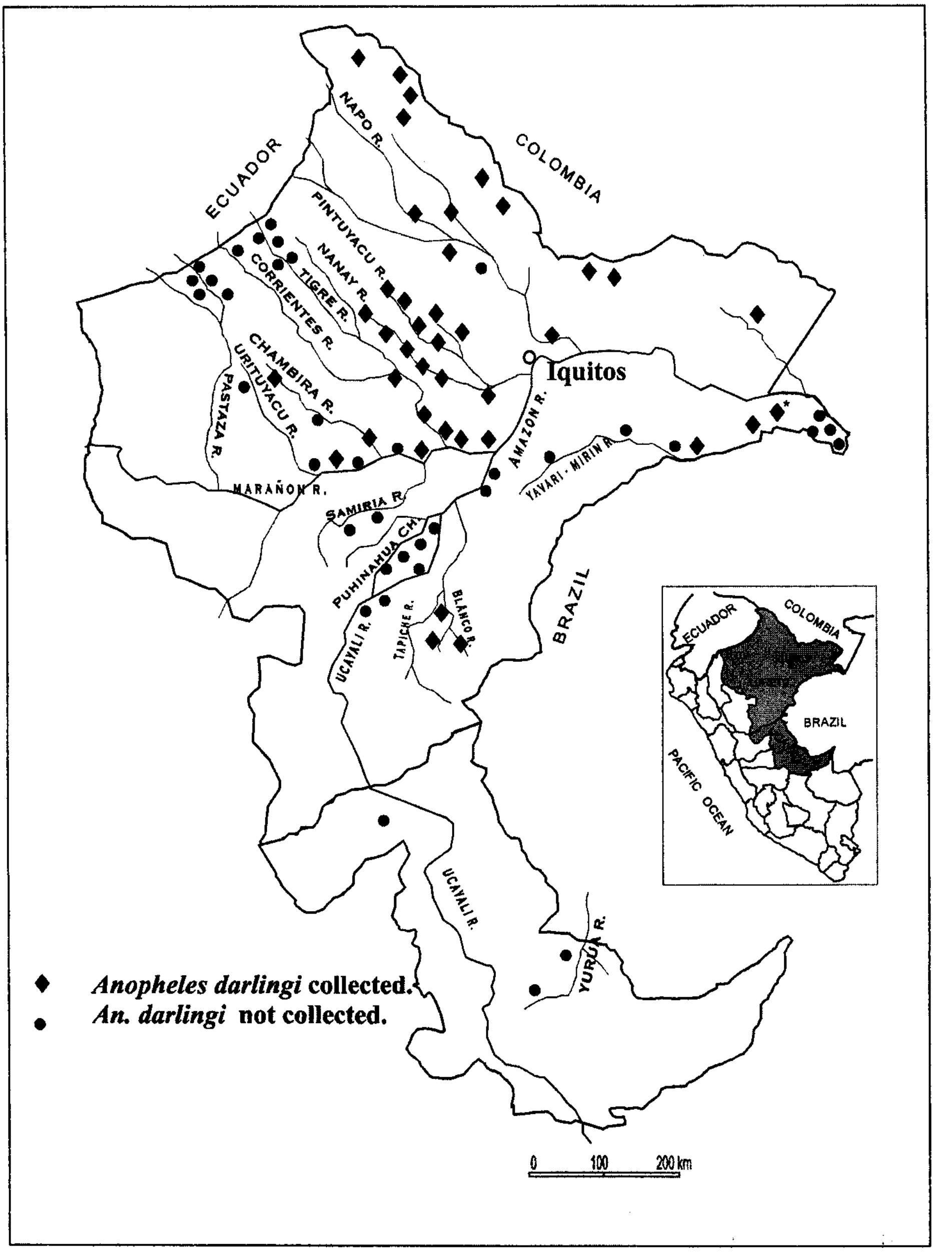

Fig. 1. Localities where *Anopheles darlingi* were collected on human bait within the Departments of Loreto and Ucayali, Peru, 2001.

efficient malaria vector in the New World (Foote and Cook 1959, Manguin et al. 1999), including areas of the Peruvian Amazon (Faran and Linthicum 1981, Fernández et al. 1996, Aramburú et al. 1999, Manguin et al. 1999). *Anopheles darlingi* is highly anthropophilic and susceptible to both *P. falciparum* and *P. vivax* infections (Manguin et al. 1999). Because of its importance as a vector of malaria in the Amazonian region, spread of *An. darlingi* into new areas of the Peruvian Amazon Basin could lead to an intensification of malaria transmission in endemic areas and the spread of malaria into new areas of Peru. The objective of this study was to define the geographic distribution of *An. darlingi* in the Amazon Basin Region of Peru for use in follow-up studies to determine the overall pattern of spread of this species in Peru as well as to obtain information on the abundance and distribution of other *Anopheles* species from this region.

Table 1. Total number of anophelines collected on human bait, percentage of the total collected, and the number of localities where each species was collected in the Peruvian Amazon Basin, 2001.

Species of anophelines (Subgenus)	Total collected	Percentage of total collected	No. of localities collected from (%)
Nyssorhynchus			
darlingi	14,521	24.0	40 (48.8)
benarrochi	42,855	70.7	32 (39.0)
rangeli	152	0.3	8 (9.7)
triannulatus	942	1.5	18 (22)
oswaldoi s.l.[1]	249	0.4	23 (28.0)
nuneztovari	3	<0.1	1 (1.2)
Anopheles			
mattogrossensis	1,478	2.4	37 (45.1)
Sp. near *forattinii*	140	0.3	18 (21.9)
intermedius	69	0.1	10 (12.2)
Sp. near *fluminensis*	21	<0.1	5 (6.1)
peryassui	52	<0.1	4 (4.9)
nimbus	103	0.2	3 (3.6)
Total	60,585	100.0	—

[1] *An. oswaldoi s.l.* includes *An. konderi* Galvao & Damasceno and *An. oswaldoi s.s.* (Flores-Mendoza 1999).

MATERIALS AND METHODS

Mosquitoes were collected throughout 2001 in the Departments of Loreto and Ucayali, the 2 largest Amazonian Departments of northeastern Peru (Fig. 1). Collections were typically conducted for 1 night in each locality unless *An. darlingi* was not collected, in which case collections often continued for 1–2 additional nights to confirm that this species was not collected at that site.

The Department of Loreto comprises almost one fourth of the landmass of Peru and has an ecosystem characteristic of the Amazon lowlands (Aramburú et al. 1999). The city of Iquitos is the only large urban center in Loreto, with a population of approximately 345,000. The rural population of Loreto, estimated at approximately 474,000 inhabitants, is clustered in towns and villages throughout the Amazon tributary system. The climate in this area is typical of the Amazon Basin, with a rainy season from November to May and a dry season from June through October. The Amazon River level at Iquitos varies up to 9 m annually; the highest levels usually occur from April to May. The mean annual temperature is approximately 25°C, with in excess of 250 cm of precipitation annually (Peñaherrera-del-Aguila 1989, Need et al. 1993a). The peak transmission rates for both *P. falciparum* and *P. vivax* occur during the rainy season, from November through June (Aramburú et al. 1999).

Mosquitoes were collected in areas where the Peruvian Ministry of Health had reported malaria cases as well as areas where *An. darlingi* had, or had not, been reported. Additional collection sites were chosen throughout the region to obtain information from as many distinct geographical areas as possible.

Upon arrival at each study site, the technician in charge of collections solicited volunteers from the area and trained them in procedures for collecting mosquitoes that landed on humans. Therefore, different volunteers participated in mosquito collections at each of the study sites. Individuals trained were health technicians, health promoters, and residents from each study site where collections were performed. Individual collecting team members were randomly assigned as pairs to each collecting area at each study site. Mosquitoes were collected at hourly intervals from 1800 to 0600 h in each site during the study. Anophelines were collected following standard procedures using humans to attract mosquitoes (WHO 1975). Briefly, this involved a human volunteer using a flashlight and mouth aspirator to aspirate all mosquitoes landing on both exposed legs, from the knee to the ankle. Human-landing collections were made for 50 min, with a 10-min rest/break period, every hour during each 12-h collecting period. Collected mosquitoes were placed in appropriately labeled paper cartons and transported to laboratory facilities in Iquitos for identification and further processing. All *Anopheles* mosquitoes collected were identified to species using morphological characters and taxonomic keys available for the anopheline species of this region (Gabaldon et al. 1941, Galvão and Damasceno 1942, Faran 1980, Faran and Linthicum 1981).

The study protocol was approved by institutional review boards at the Naval Medical Research Center (protocol DoD 31557) in compliance with all Federal regulations governing the protection of human subjects. The use of humans was essential for collecting anthrophilic *Anopheles,* which are not often collected using other methods. For example, in an arbovirus surveillance study conducted in a forested area near Iquitos in 1999, in 84 trap-nights where CO_2-baited light traps and human bait were used for mosquito collections, 97% of *An. darlingi*

Table 2. Species of anophelines collected with human landing collections in the Amazon Region of Peru, 2001.

Department, province, grid coordinate, and collection sites (River)	Number of days collected	Species of *Anopheles*[1]											
		1	2	3	4	5	6	7	8	9	10	11	12
Loreto Department													
Requena Province													
Requena District													
5°03′S and 73°51′W													
Caro Corahuayte (Ucayali R.)	1				31			57					
Cajamarca (Ucayali R.)	1				9			3					
Contamanillo (Ucayali R.)	1				8								
Emilio San Martin District													
5°47′S and 74°17′W													
Zapatilla I (Ucayali R.)	1				22			8					
Zapatilla II (Ucayali R.)	1				21	1		13					
Tapiche District													
5°40′S and 74°00′W													
Iberia	3	1											
San Pedro (Tapiche R.)	2	60											76
Soplín Vargas District													
5°56′S and 74°W													
Nva. Esperanza (Blanco R.)	1	42											
Maquia District													
5°46′S and 74°33′W													
Obreros (Puhinahua R.)	2							2					
Victoria (Puhinahua R.)	5				629			476					
Oceanía (Puhinahua R.)	2						3						
Liberal (Puhinahua R.)	2							3					
Bolivar (Puhinahua R.)	2							4					
Nvo. Encanto (Puhinahua R.)	3							22					
Independencia (Puhinahua R.)	2							1					
San Antonio (Puhinahua R.)	2							2					
Loreto Province													
Nauta District													
4°30′27″S and 73°34′36″W													
Nauta (Marañon R.)	4	38		1								13	
Urarinas District													
4°32′S and 74°46′W													
Nvo. San Juan (Chambira R.)	5	3	20	9	6			290	7			7	
Concordia (Marañón R.)	3			148				131					

Table 2. Continued.

Department, province, grid coordinate, and collection sites (River)	Number of days collected	Species of *Anopheles*[1]											
		1	2	3	4	5	6	7	8	9	10	11	12
S.J. Lagunillas (Marañón R.)	1	9					12						
Pampa Caño (Marañón R.)	1	14					57						
San Pedro Z-I (Marañón R.)	1	23	1				45						
4°52′28″S and 75°7′17″W													
Maypuco (Marañón R.)	1		14					10					
4°51′7″S and 75°26′5″W													
Urarinas (Marañón R.)	1	2	15		27	1		2					
4°57′S and 75°34′W													
Nva. Alianza (Chambira R.)	1		93			5							
Tucunaré (Chambira R.)	4	11	16,179			6		24	2			3	22
4°17′S and 76°18′W													
Reforma (Urituyacu R.)	3		7,571							3			
Nva. Alianza (Urituyacu R.)	2		1,245			2							
Tigre District													
4°32′S and 74°46′W													
Nueva York (Tigre R.)	6	625	1					18					
Pto. Orlando (Tigre R.)	1	22						38					
4°29′S and 74°45′W													
Lamas Tapishca (Tigre R.)	2		2			8			32	17	4		
Nuevo Remanente (Tigre R.)	1					5		2	16	8	3		
San Juan de Bartra (Tigre R.)	1					4			1	2			
12 de Octubre (Tigre R.)	3							1					
Parinari District													
4°34′00″S and 74°28′30″W													
Nuevo Arica (Samiria R.)	1							8					
Trompeteros District													
3°46′02″ and 74°54′02″													
José Olaya (Corrientes R.)	2		27	57	14	13		125	6	13			
Alto Amazonas Province													
Napo District													
2°54′S and 76°24′W													
Tambo (Pastaza R.)	1				4								
A. Capahuari (Pastaza R.)	1		71		1	1		32	1				
Villa los Jardines (Pastaza R.)	1		69					3		5			

Table 2. Continued.

Department, province, grid coordinate, and collection sites (River)	Number of days collected	Species of *Anopheles*[1]											
		1	2	3	4	5	6	7	8	9	10	11	12
Titiyacu (Pastaza R.)	2		3,069		3	44		32	2	7			
Andoas Viejo (Pastaza R.)	1		263	30		1							
Nuevo Andoas (Pastaza R.)	1		19										
Maynas Province													
Napo District													
2°29′S and 73°40′W													
Santa Clotilde (Napo R.)	1	2											
Patria Nueva (Napo R.)	1		2,392					10					
Huiririma (Napo R.)	1	16	8										
Santa Elena (Napo R.)	1	63	18			4			8				
San Felipe (Napo R.)	1	20	80			5		12					
V. de Zapote (Napo R.)	2	10,227	1						5				5
Alto Nanay District													
3°29′S and 73°33′W													
San Antonio (Pintoyacu R.)	1	13											
Atalaya (Pintoyacu R.)	2	35											
M. Calvario (Pintoyacu R.)	1	205											
Miraflores (Pintuyacu R.)	1	40	1										
Saboya (Pintoyacu R.)	1	20											
S. J. Raya (Pintoyacu R.)	1	112							2				
3°39′S and 73°13′W													
Albarenga (Nanay R.)	2	200											
Pucahurco (Nanay R.)	1	7											
S. Pavayacu (Nanay R.)	1	57											
S. J. Hungurahual (Nanay R.)	1	18											
Diamante Azul (Nanay R.)	1	12							1				
Sta. M. Alt. Nanay (Nanay R.)	2	225											
Putumayo District													
00°58′S and 74°55′W													
Bellavista (Jubineto R.)	1	232	42			11			22	1			
San Belin (Yaricaya)	1	1	529			106			3				
00°21′S and 74°9′W													
Soplin Vargas (Putumayo R.)	1	1,138	1										
Santa Teresita (Peneya)	2	430	13						2				
Atalaya (Putumayo R.)	1	3	59			13		8	13	12	6		

Table 2. Continued.

Department, province, grid coordinate, and collection sites (River)	Number of days collected	Species of *Anopheles*[1]											
		1	2	3	4	5	6	7	8	9	10	11	12
01°47′S and 73°24′W													
Sta. Mercedes (Putumayo R.)	1	423											
2°27′S and 72°40′W													
S. Fsco. Ere (Putumayo R.)	1	23	68					5	1		1		
El Estrecho (Putumayo R.)	1	128											
R. Castilla Province R. Castilla District 04°13′S and 69°45′W													
St. Rosa (Amazon R.)	1							2					
S. Rita Mochila (Amazon R.)	1							5					
Leoncio Ramirez (Yavari R.)	1							1					
Yavari District 04°21′S and 72°10′W													
San Pedro (Yavari R.)	1	3				1		2					
Yarina (Yavari R.)	1	11	2			6		9					
S. Jose Parinari (Yavari R.)	1					5							
Carolina (Yavari Mirin R.)	1					1							
04°30′S and 72°40′W													
El Sol (Atacuari R.)	1	7											
Dpto, Ucayali Atalaya District 09°45′S and 72°45′W													
Puesto Breu (Yurua R.)	1		1										
Dorado (Yurua R.)	1		2	13	7					1			
Dulce Gloria (Yurua R.)	1			9	2								
San Pablo (Yurua R.)	1			21	4								
Victoria (Yurua R.)	1			17	1								
C. Portillo Province Campo Verde District 8°29′45″S and 75°48′W													
San José	12		10,979	4	2			1	16			36	
Total		14,521	42,855	152	942	249	3	1,478	140	69	21	52	103

[1] 1, *An. darlingi*; 2, *An. benarrochi*; 3, *An. rangeli*; 4, *An. triannulatus*; 5, *An. oswaldoi s.l.*; 6, *An. nuneztovari*; 7, *An. mattogrossensis*; 8, *An.* sp. near *forattinii*; 9, *An. intermedius*; 10, *An.* sp. near *fluminensis*; 11, *An. peryassui*; 12, *An. nimbus*.

collected were from human bait (M. J. Turell, 1999, unpublished data). In another 10-wk study conducted in Padre Cocha, a village located on the River Nanay near Iquitos, 2,162 *An. darlingi* were collected from human-landing collections, while only 88 were collected in light traps baited with CO_2 and octanol, and 77 from light traps baited only with CO_2 (A.S.T. Chan, unpublished data).

RESULTS

Within the Amazonian Departments of Loreto and Ucayali, adult anopheline mosquitoes were collected at 82 of 93 (88.2%) collection sites. The remaining 11 sites yielded no anophelines. A total of 60,585 *Anopheles* mosquitoes were collected from human-landing collections, representing 12 species from two subgenera (*Nyssorhynchus* and *Anopheles*) within the genus *Anopheles* (Table 1). The majority (70.7%) of *Anopheles* species collected from all sites were *An. benarrochi* Gabaldon, Cova García, & López, followed by *An. darlingi* (24.0%), *An. mattogrossensis* Lutz & Neiva (2.4%), and *An. triannulatus* (Neiva & Pinto) (1.5%). The remaining anopheline species collected, *An. rangeli* Gabaldon, Cova-García & López, *An. oswaldoi* s.l. (Peryassu), *An.* sp. near *forattinii* Wilkerson & Sallum, *An. intermedius* (Peryassu), *An.* sp. near *fluminensis*, *An. peryassui* Dyar & Knab, *An. nuneztovari* Gabaldon, and *An. nimbus* (Theobald) represented less than 2% of the total mosquitoes collected (Table 1).

Among all *Anopheles* species collected, *An. darlingi* was found at the greatest number of sites (48.8%) (Table 1). *An. darlingi* was not collected in the Department of Ucayali. Although only 1,478 *An. mattogrossensis* were collected, it was captured at more sites (37) than any of the other anopheline species, except *An. darlingi*. Further, even though *An. benarrochi* was collected frequently, it was collected from fewer sites (32) than was *An. darlingi* or *An. mattogrossensis*. Figure 1 shows the sites within the Department of Loreto from which *An. darlingi* was collected during the present study. Table 2 provides coordinates of districts within the Department of Loreto in which collection localities were located, and number of anophelines, of each species, collected at each study site. All collecting sites were located along the rivers listed in Table 2, typically within 10 km of the grid coordinates provided. These data demonstrate that *An. darlingi* is established throughout central Loreto, including further west in the Amazon Basin than previously reported, suggesting that this species has spread into previously uninfested areas of the Peruvian Amazon Basin.

DISCUSSION

In Peru, malaria has become a significant public health threat during the last decade, especially within the Amazon Basin region. This increase in malaria occurrence is thought to be due to the spread of *An. darlingi* into the Amazon Basin region, combined with changing river-use patterns, jungle exploitation, and the reduction in residual insecticide treatments that were conducted prior to the 1990s (Roberts et al. 1997, Aramburú et al. 1999, Roper et al. 2000). Because the spread of *An. darlingi* into new areas of the Amazon Basin may be a major factor in the resurgence of human malaria in Loreto, this study was conducted to determine the current distribution of this species within the Amazon region of Peru. Current distribution data are needed to establish the present range of this species and is critical to determine if *An. darlingi* is indeed spreading into previously uninfested regions in the Peruvian Amazon.

During 2001, collections conducted at 93 localities within the Departments of Loreto and Ucayali yielded anophelines from 82 (88.2%) of the collection sites. *anopheles darlingi* was not collected at any of the sites within the Department of Ucayali. However, *An. darlingi* has previously been reported from Madre de Dios, another Amazonian Department of Peru (Calderón et al. 1995). These data, combined with previous reports, suggest that at present this species is found within the Amazon Basin region of Peru only in the Departments of Loreto and Madre de Dios.

Anopheles darlingi previously was reported only from the eastern areas of the Peruvian Amazon Basin near the borders of Brazil and Colombia, extending westward to the Iquitos area (Calderón et al. 1974, 1995). Additionally, during mosquito collections, including human-landing collections, conducted between 1988 and 1991 at 3 forested sites near Iquitos, over 35,000 mosquitoes of 13 genera and 25 species were obtained, and these collections yielded no *An. darlingi* (Need et al. 1993). Fernández et al. (1996) reported the presence of *An. darlingi* in the vicinity of Iquitos for the first time during collections conducted in 1995; this species is now well established in the Iquitos area (Aramburú et al. 1999, Roper et al. 2000). In addition, *An. darlingi* had not been collected previously from the western regions of the Amazon Basin extending to the foothills of the Andes. For example, in Andoas, a region located about 350 km west-northwest of Iquitos near the Ecuadorian border, six 2-wk mosquito collections conducted in 1990–1991 yielded over 4,000 anopheline mosquitoes; no *An. darlingi* were collected (Need et al. 1993b). However, during the present study, *An. darlingi* was found much farther west in the Department of Loreto than had been previously reported. Specifically, we collected *An. darlingi* along the Chambira River, at a site located approximately 265 km west of Iquitos, and approximately 130 km southeast of Andoas (Fig. 1). Our collection data show that *An. darlingi* is now established throughout the central region of Loreto, including far to the west of Iquitos, where it was first reported in 1995 (Fernández et al. 1996). Therefore, these data suggest that *An. darlingi* is indeed spreading into previously uninfested areas of the Amazon Basin of Peru. Alternatively, this species may always have been present in these areas but not previously collected due to very low population densities. For example, *An. darlingi* may have always been present in these areas, but kept below detectable levels during the intensive malaria control programs conducted during the 1960s through the early 1980s, when such programs were abandoned. Therefore, a combination of increasing abundance after extensive vector-control programs were discontinued, followed by population expansion into new areas, may help explain the appearance of *An. darlingi* in areas of the Peruvian Amazon Basin where it was not previously reported.

Our collections conducted during 2001 show that *An. darlingi* is found in the Amazonian Department of Loreto along the borders of Colombia and Brazil from 70°W longitude to approximately 76°W longitude, and south from approximately 0°2′ to 6°40′S latitude. These data indicate that *An. darlingi* is found in a much larger area of the Peruvian Amazon than previously reported and may help to explain the resurgence of human malaria cases in this area in recent years. The data obtained from this study should be used as baseline data; surveillance for *An. darlingi* should continue in this area to determine if *An. darlingi* is spreading further into Loreto and other areas within the Amazon Basin region of Peru, which could have important consequences in the epidemiology of malaria in this area.

The distribution of malaria in Loreto is uneven, with several zones of high transmission and larger areas where malaria transmission and disease incidence occur at lower levels (OGE 1997, MINSA, Loreto 1998, Aramburú et al. 1999, Roper et al. 2000). Epidemiological evidence suggests that other *Anopheles* species might be responsible for maintaining and transmitting human malaria in endemic areas of Peru where *An. darlingi* has not been reported. For example, in Pucallpa, the largest urban center in the Department of Ucayali, 2,852 cases of malaria were reported in 1997, 557 cases were reported in 1998, and 363 malaria cases occurred in 1999, all in an area where *An. darlingi* has not been found (MINSA, Ucayali 2001). In Yurimaguas, a city in the extreme western area of Loreto, 82 cases of malaria were reported in 2001, and in Andoas, 176 cases were reported in 1999, both areas where *An. darlingi* has not been reported (MINSA, Loreto 2001). Therefore, in malaria-endemic areas of the Peruvian Amazon, other *Anopheles* species apparently are responsible for transmitting malaria in areas where *An. darlingi* does not occur. For that reason, the objectives of this study also included investigating the abundance and distribution of other *Anopheles* species in this region.

During the present study, 12 anopheline species representing 2 subgenera (*Nyssorhynchus* and *Anopheles*) were collected from sites within the Departments of Loreto and Ucayali (Table 1). The most abundant anopheline species collected was *An. benarrochi*, representing 70.7% of mosquitoes obtained from these areas. Of the 12 species collected, the following are considered to be primary or secondary malaria vectors in forested areas of eastern Peru, based on salivary gland infections or circumsporozoite enzyme-linked immunosorbent assay (Wirtz et al. 1992) data: *An. darlingi*; *An. nuneztovari*; *An. rangeli*; *An. oswaldoi; An.* sp. near *fluminensis;* and, *An. mattogrossensis* (Hayes et al. 1987, Need et al. 1993b, Calderón et al. 1995, Fernández et al. 1996). Some or all of these species may serve as malaria vectors in forested areas of eastern Peru where *An. darlingi* has not been collected. For example, in the Department of Junin, a high-jungle area located on the eastern slopes of the Andes southwest of Iquitos, hyperendemic malaria occurs sporadically, and *P. vivax* is apparently maintained by anopheline species other than *An. darlingi*. These include *An. oswaldoi, An. nuneztovari, An. triannulatus, An.* sp. nr. *fluminensis, An. trinkae,* and *An. rangeli* (Hayes et al. 1987) species also collected during the present study.

The malaria vector situation in endemic areas of the Peruvian Amazon is not at all clear, especially with regard to *An. benarrochi,* the most abundant mosquito collected during this study. Specifically, Aramburú et al. (1999) states "*An. benarrochi* is the dominant malaria vector in western Loreto, while *An. triannulatus* is the dominant vector in eastern Loreto." However, no data are presented to confirm the vector status of these species (Aramburú et al. 1999). Indeed, *An. benarrochi,* which may be the dominant species in malaria-endemic areas in the absence of *An. darlingi,* and which represented over 70% of the mosquitoes collected during this study, was discounted as a vector in Northern Brazil based on the dissection of 31 females in 1948 (Deane et al. 1948). In another study conducted by Klein et al. (1991), *An. benarrochi,* experimentally infected with *P. vivax,* developed oocysts, but were never observed to have sporozoites in the salivary glands, suggesting that this species may not be an efficient malaria vector. However, *An. benarrochi* has been implicated as the primary vector of malaria in areas of the Peruvian Amazon, where it occurs in high numbers, accounting for up to 98% of the anopheline fauna in the Amazonia lowlands, areas where both *P. falciparum* and *P. vivax* are endemic (Calderón et al. 1974, Aramburú et al. 1999). Additionally, *An. benarrochi* was reported as a principal malaria vector in the Departments of Loreto and Ucayali by Calderón et al. (1995). Fernández et al. (1996) reported the presence of *Plasmodium* circumsporozoite proteins in 9 pools containing 37 female *An. benarrochi* collected near Iquitos (Fernández et al. 1996), demonstrating natural *Plasmodium* infection in this species. Further studies are required to determine the importance of *An. benarrochi* and other *Anopheles* species as malaria vectors in the Amazon Basin region of Peru, especially due to the abundance and wide distribution of this species in the Peruvian Amazon.

The data obtained from this study clearly show that *An. darlingi* is more widely distributed within the Amazon Basin region of Peru than previously reported. However, the question remains as to whether the distribution determined during the course of this study represents an increase in the range of this species, represents collections from sites not previously sampled for this species, or perhaps an increase in abundance of this species in these areas following the decline of organized vector-control programs. To determine if the distribution of *An. darlingi* is indeed spreading into new areas of the Amazon Basin, continued accumulation of data on the distribution of this species, such as that obtained from this study, is required. These data should be used as baseline data on current *An. darlingi* distribution. Surveillance for *An. darlingi* should continue in this area to determine if indeed *An. darlingi* is spreading further into Loreto and other areas within the Amazon Basin region of Peru. This information, coupled with further studies on the bionomics of other suspected and known vector species will aid greatly in understanding the epidemiology of human malaria within the Amazon Basin and may aid in helping to control the resurgence of malaria among human populations in this area.

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