Species Composition and Distribution of Adult *Anopheles* (Diptera: Culicidae) in Panama

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ABSTRACT *Anopheles* (Diptera: Culicidae) species composition and distribution were studied using human landing catch data over a 35-yr period in Panama. Mosquitoes were collected from 77 sites during 228 field trips carried out by members of the National Malaria Eradication Service. Fourteen *Anopheles* species were identified. The highest average human biting rates were recorded from *Anopheles* (*Nyssorhynchus*) *albimanus* (Wiedemann) (9.8 bites/person/night) and *Anopheles* (*Anopheles*) *punctimacula* (Dyar and Knab) (6.2 bites/person/night). These two species were also the most common, present in 99.1 and 74.9%, respectively, of the sites. *Anopheles* (*Nyssorhynchus*) *aquasalis* (Curry) was encountered mostly in the indigenous Kuna Yala Comarca along the eastern Atlantic coast, where malaria case history and average human biting rate (9.3 bites/person/night) suggest a local role in malaria transmission. *An. albimanus*, *An. punctimacula*, and *Anopheles* (*Anopheles*) *vestitipennis* (Dyar and Knab) were more abundant during the rainy season (May–December), whereas *An. aquasalis* was more abundant in the dry season (January–April). Other vector species collected in this study were *Anopheles* (*Kerteszia*) *neivai* (Howard, Dyar, and Knab) and *Anopheles* (*Anopheles*) *pseudopunctipennis* s.l. (Theobald). High diversity of *Anopheles* species and six confirmed malaria vectors in endemic areas of Panama emphasize the need for more detailed studies to better understand malaria transmission dynamics.

KEY WORDS *Anopheles*, human biting rate, malaria, Panama

Entomologists from Instituto Conmemorativo Gorgas de Estudios de la Salud (ICGES) and the National Malaria Eradication Service (SNEM) have led field studies on *Anopheles* bionomics across Panama since 1921 and 1956, respectively. These studies, focused mostly on species distribution, infection rate, feeding behavior, and pesticide resistance, have resulted in few publications on *Anopheles* bionomics since 1960. Furthermore, before 1956, most studies on *Anopheles* ecology were undertaken nearly exclusively in the Canal Zone, which represents <5% of the country.

Early attempts to describe the distribution of *Anopheles* in Panama were conducted by Baxter and Zetek (1944), Arnett (1947), Blanton et al. (1955) and Blanton and Peyton (1956). These surveys reported 19 species of *Anopheles* collected by light traps, horse bait traps, Shannon traps, and larval collections. Additional reports summarized in Wilkerson and Strickman (1990) elevated the total to 22 species within the *Anopheles* genus and included *Anopheles* (*Anopheles*) *malefactor* (Dyar and Knab), which was recovered from synonymy with *Anopheles* (*Anopheles*) *punctimacula* (Dyar and Knab) in the Canal Zone (Wilkerson 1990).

Seven *Anopheles* species have been previously found infected with *Plasmodium sp.* in Panama (Darling 1910, Simmons 1936a, b, 1937). These studies were based on dissection of individual mosquitoes after being fed on humans with circulating gametocytes, but not all of the parasites reached the sporozoite stage in mosquito salivary glands (see summary in Table 1). In addition, Darling (1910) and Rozeboom (1935) reported naturally infected specimens of *An.* (*Nyssorhynchus*) *argyritarsis* and *An.* (*Nyssorhynchus*) *bachmanni* [Syn. *An.* (*Nyssorhynchus*) *triannulatus*], adding two more species to the earlier list. Although the procedure used in these experiments is now unethical, the results described the known complexity of malaria transmission during this time in Panama.

The human landing catch (HLC) technique is recommended as the most useful for collecting host-seeking anthropophilic mosquitoes (Service 1993). Its efficiency and low cost far surpass results obtained by any other mosquito collecting technique (WHO 1975), and it remains the only reliable method to assess the degree of human–vector contact, a crucial component of the entomological inoculation rate (EIR)

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Table 1. Summary of *Anopheles* species previously reported as vectors of *Plasmodium vivax* and *P. falciparum* in Panama based on studies conducted by Darling (1910), Simmons (1936a, b, 1937), and Rozeboom (1935)

Mosquito species	*P. vivax*	*P. falciparum*
An. albimanus[a]	1–2	1–2
An. pseudopunctipennis	1	1–2
An. tarsimaculatus (Syn. An. aquasalis)	1–2	—
An. punctimacula (Syn. An. malefactor)[a]	1–2	1–2
An. apicimacula	—	2
An. eiseni	1	—
An. neomaculipalpus	1–2	—
An. bachmanni (Syn. An. triannulatus)[a]	1	—
An. argyritarsis[a]	1	—

[a] Found naturally infected with *Plasmodium sp.* in Panama.
1, oocysts; 2, sporozoites; —, no infection.

(Macdonald 1957). SNEM has used HLC to survey *Anopheles* mosquitoes in Panama since 1970.

To date, the distributions of sibling species within the *Anopheles* (*Nyssorhynchus*) *albitarsis* s.l. (Lynch-Arribálzaga) (Wilkerson et al. 1995, Lehr et al. 2005), *Anopheles triannulatus* s.l. (Neiva and Pinto) (Silva-Do-Nascimento et al. 2006), and *Anopheles* (*Nyssorhynchus*) *nuneztovari* s.l. (Gabaldón) (Kitzmiller et al. 1973, Conn et al. 1998) complexes, as well as other local malaria vectors such as *Anopheles* (*Kerteszia*) *neivai* (Howard, Dyar, and Knab), are poorly known in Panama. Moreover, no systematic mosquito surveys have ever been carried out in Darien Province, eastern Panama, near the Chocó region in Colombia, an area where other *Anopheles* species are likely to be present (Wilkerson and Strickman 1990).

According to the Epidemiological National System (SIVIGILA), northwestern Colombia had the highest levels of malaria morbidity in the Department of Antioquia in 2007 (Estadísticas de la vigilancia en Salud Pública 2007). Both *Plasmodium vivax* (Grassi and Feletti) and *P. falciparum* (Welch) are registered throughout the year near the Panamanian border in Turbo, Colombia (Echeverri et al. 2003), where frequent migration of Kuna (Tule) people into Panama has been reported (Carmona-Fonseca et al. 2005). In this region, two primary malaria vectors, *Anopheles* (*Nyssorhynchus*) *albimanus* and *Anopheles* (*Nyssorhynchus*) *darlingi* (Root) coexist (Lounibos and Conn 2000) and are likely to contribute to the high malaria endemicity. *An. darlingi* occurs from southern Mexico to South America, but it has never been officially reported from Nicaragua, Costa Rica, or Panama (Linthicum 1988). Because no obvious physical or ecological barriers seem to prevent *An. darlingi* from migrating into Panama, the possibility that this important malaria vector may occur in eastern Panama warrants further attention.

Panama reported 5,095 cases of malaria during 2004, a six-fold increase in incidence since 2001. Previously, Panama had never reported >2,000 cases per yr (since 1970, Boletín Epidemiológico 2005). The highest prevalence of malaria occurs in rural areas where indigenous people reside; however, recently, there has been an increase in case numbers in periurban and urban areas as well (Ministerio Nacional de Salud de Panamá MINSA 2005). Some of these urban epidemics may result from circular migration from both rural (endemic) and urban (nonendemic) areas in Panama in recent decades (World Bank 2005). In addition, an increase in resistance to prophylaxis by *Plasmodium* sp. (Samudio et al. 2005) and to insecticides by vectors (Caceres 1999) is altering the epidemiological setting of malaria in unknown ways.

Information on changes in vectorial systems, vector abundance, and species succession is vital for planning effective transmission interventions and for monitoring the efficacy of vector control measures (Ndenga et al. 2006). The objective of this report is to provide up-to-date information on anopheline species collected in close association with human habitations over a 35-yr period in malaria endemic areas in Panama.

Materials and Methods

Study Site. The Isthmus of Panama (9°00′ N, 80°00′ W) is the geographical link between Central and South America. Panama borders both the Caribbean Sea and the North Pacific Ocean, between Colombia and Costa Rica, and it encompasses a population of 3,242,173 people (Contraloría General de la República 2004). The annual population growth rate is 5.6%, and its gross domestic product (GDP) is one of the fastest growing in Central America, with a per-capita GDP on par with a middle class nation. Despite this, >60% of the rural population lives in poverty (Ficher and Vasseru 2000) and is more vulnerable to malaria. Malaria, dengue fever, Chagas disease, and leishmaniasis are among the most important vector-borne infections (Boletín Epidemiológico 2002), found predominantly along the Atlantic coast, in Bocas Del Toro, the Ngöbe Buglé Comarca, the Kuna Yala Comarca, and Darien, where up to 45% percent of the intact tropical rain forest exists (ANAM 2003). Some of these locales are currently undergoing extensive changes in landscape because of an increase in tourism; therefore, a potential risk of malaria infection is expected for the influx of susceptible travelers. The annual climate in Panama varies according to two seasons: the dry season, from January to late April (average monthly rainfall, 34.2 mm), and a prolonged rainy season, from early May to December (average monthly rainfall, 507 mm) (Condit et al. 2001). The rainy season triggers changes in relative humidity and human activities throughout the country, and it is when overall mosquito density reaches its maximum in Panama (Wolda and Galindo 1981).

Mosquito Sampling. Mosquito collections were carried out on request from MINSA shortly after notification of malaria outbreaks. Therefore, entomologists from SNEM and ICGES visited virtually all localities in endemic malaria regions of Panama since 1970. Collection teams usually stayed 5–10 d at each specific locality. Mosquitoes were collected outdoors at ≤6 m away from the main entrance of human dwellings. A 6-h collection period (1800–2400 hours) was con-

Table 2. Average HBR of *An. albimanus*, *An. punctimacula*, and *An. aquasalis* in 31 malaria endemic sites of Panama

Province	Localities	*An. albimanus* (mean[a] ± SE)[b]	*An. punctimacula* (mean[a] ± SE)[b]	*An. aquasalis* (mean[a] ± SE)[b]
BC	Barranco Montaña	11.9 ± 0.2a	2.8 ± 0.3a	—
	Barranco Adentro	9.9 ± 0.3b	2.7 ± 0.7a	—
	Guabito Centro	7.5 ± 2.1b	2.9 ± 1.1a	—
	Chiriqui Grande	15.4 ± 1.7a	4.1 ± 0.8a	—
	Finca Debora	5.0 ± 0.8b	1.2 ± 0.3b	—
	Las Delicias	8.3 ± 3.1b	1.8 ± 0.5b	—
	Barra Sixaola	5.4 ± 1.4b	1.3 ± 0.7b	5.2 ± 1.7a
NBC	Bisira	6.8 ± 2.7b	—	—
	Bahia Azul	7.3 ± 4.7b	4.5 ± 1.5b	6.4 ± 0.9a
	Tobobe	2.1 ± 0.8a	3.9 ± 0.7b	—
	Kusapin	4.1 ± 2.8a	3.7 ± 0.8b	—
	Kankintu	5.4 ± 1.3a	4.0 ± 0.9b	—
	Rio Chiriqui	18.5 ± 2.1b	—	—
	Playa Roja	3.7 ± 0.8a	—	—
	Rio Caña	3.2 ± 1.0a	1.1 ± 0.4a	—
	San Pedro	6.1 ± 0.6b	4.4 ± 0.6b	—
DR	Los Monos	4.8 ± 0.9a	9.8 ± 1.1b	—
	El Canglón	5.6 ± 2.8a	6.9 ± 1.4a	—
	Santa Fe	3.7 ± 0.6a	10.1 ± 0.9b	—
	Las Peñitas Jaque	9.2 ± 2.3b	5.3 ± 1.9a	—
	Cañazas-Bayano	7.1 ± 1.1b	8.3 ± 0.5a	—
	El Coco	6.9 ± 1.3b	3.6 ± 0.1b	—
	Biroquera	5.1 ± 0.6b	2.6 ± 1.7b	—
	Zapallal	4.1 ± 1.7b	3.1 ± 1.9b	—
	Punuloso	5.4 ± 0.8b	2.1 ± 1.1b	—
KYC	Navagandi	4.4 ± 1.4b	—	9.1 ± 1.0b
	Playón Grande	2.1 ± 2.8b	3.8 ± 0.7b	5.7 ± 0.4a
	Mansucun	2.6 ± 0.6b	—	6.2 ± 0.7a
	Nva. Anachucuna	2.8 ± 2.9b	3.1 ± 0.5b	6.3 ± 0.5a
	Isla Pino	1.8 ± 1.7b	—	8.2 ± 0.2b
	Carreto	2.1 ± 0.7b	2.8 ± 0.9b	11.1 ± 1.0a

[a] Average obtained from three collectors over 5–10 d/6 h per day replicates.
[b] Values followed by different letters are significantly different from each other, $P < 0.05$.
—, species absence.

ducted daily using manual aspirators (WHO 1975). Mosquitoes were morphologically identified to species based on available keys (anonymous document from ICGES; Wilkerson and Strickman 1990). Information was recorded by locality and collection time, and human biting rate (HBR) per species was calculated. Voucher specimens were deposited in entomological collections at ICGES in Panama City. Two neighboring localities in Colombia (Capurgana and Acandi) were surveyed by SNEM in collaboration with its Colombian counterpart Servicio de Erradicación de la Malaria (SEM) in 1977. The collecting procedure for these field trips was as described above.

Data Analyses. Overall average HBRs were log transformed (x + 1) before testing for differences among species using one-way analysis of variance (ANOVA). Means for significant main effects were separated using Scheffé test, which makes unplanned comparisons among the means with unequal sample sizes. Least significant difference (LSD) was used to compare the average HBR between the dry and rainy season for each *Anopheles* species using PROC GLM function and mean comparison. To control for differences in the average HBR of prevalent species (*An. albimanus, An. punctimacula,* and *An. aquasalis*) among only 31 of 77 collecting sites, a one-way ANOVA was performed with collecting site or locality as a fixed treatment (Table 2). The localities for the latter statistical procedure were visited most frequently and have the best statistical representation and the highest degree of malaria endemicity in Panama. They were distributed as follows: seven sites in Bocas Del Toro (BC), nine in the Ngöbe Buglé Comarca (NBC), nine in Darien (DR), and six in the Kuna Yala Comarca (KYC; Table 2). The first two regions (BC and NBC) are located in western Panama, and the latter two (DR and KYC) are in eastern Panama (Fig. 1). We used SAS 9.0 version software package (SAS Institute, Cary, NC) for statistical analysis and $P < 0.05$ as a cut-off for statistical significance. We visited all sampling sites, obtained the geographic coordinates by using handheld global positioning system (GPS) units (Garmin International, Olathe, KS), and imported them into ArcView GIS software (Environmental Systems Research Institute, Redlands, CA) to create maps of *Anopheles* species distribution in relation to hydrology, vegetation type, and altitude. Updated GIS data sets were obtained from Naos Molecular Biology and Evolution Laboratories of Smithsonian Tropical Research Institute (STRI), Panama City.

Results

Species Composition and Distribution. Fourteen *Anopheles* species in four subgenera and *Chagasia bathana* (Dyar) were caught using human landing

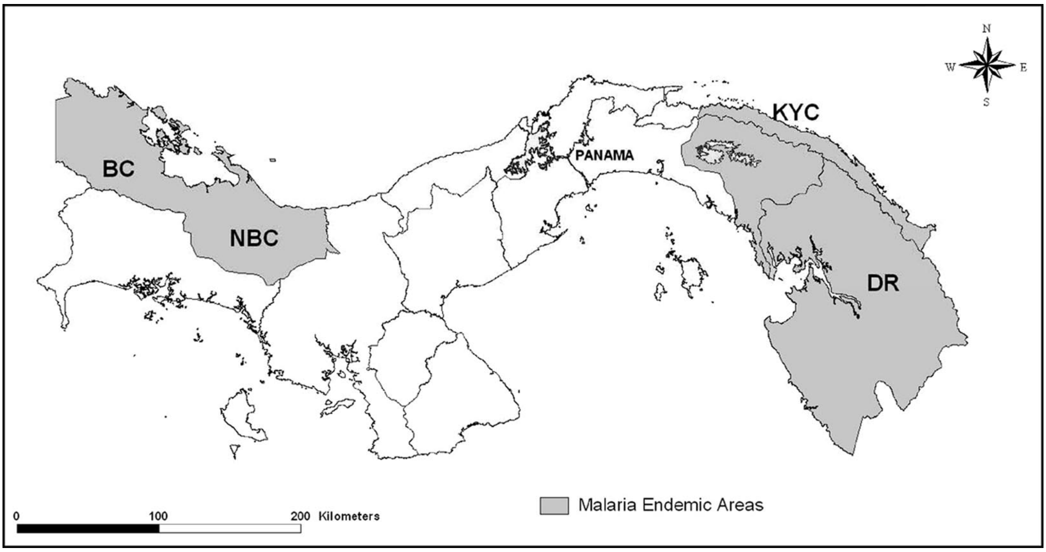

Fig. 1. Malaria endemic areas of Panama: BC, NBC, KYC, and DR.

catch from 1970 to 2005, in malaria endemic areas of Panama (Table 3). A total of 33,917 specimens was gathered from 77 localities during 228 field trips and roughly 13,680 h of sampling. Forty percent of collecting sites were visited >10 times during the dry and the rainy seasons (Figs. 2 and 3). *An. albimanus* was the most abundant, common, and widespread species, accounting for 24,490 (72.2%) of the total, and it was collected in 76 localities (99.1%) and 216 (94.7%) of 228 field trips. *An. punctimacula* was the second most common species, accounting for 6,081 (17.9%) of the total, collected in 57 localities (74.9%) and in 129 (56.8%) field trips. *An.* (*Nyssorhynchus*) *aquasalis* (Curry) represented 3.6% (1,240) of the total mosquitoes collected, found in 13 localities (16.8%), 11 of which were located in KYC along the eastern Atlantic coast (Fig. 2).

The remaining 11 species accounted together for <10% (2,334) of the total (Table 3). *An. neivai* and *An.* (*An.*) *vestitipennis* (Dyar and Knab) were mostly recorded from western Panama. The former species was collected from seven (9.1%) localities of NBC near the Atlantic coast, whereas *An. vestitipennis* was recorded more inland in seven (9.1%) localities of BC, near the Costa Rican border (Fig. 3). In July, 1977, in Colombia, *An. darlingi* was identified from Capurgana ($n = 29$) and Acandi ($n = 6$), ≤15 km from the Panamanian border (data not shown). However, *An. darlingi* was not found on the Panamanian side of the border.

Anopheles (*Anopheles*) *pseudopunctipennis* s.l. (Theobald) was collected in higher numbers from Uala, Nurra, and Punuloso, all situated in eastern Panama (Fig. 2), whereas *An. albitarsis* s.l. was caught in Zapallal, Meteti, and Biroquera. Furthermore, *An. tri-*

Table 3. ***Anopheles*** **species collected using HLC from 1970 to 2005 in Panama**

Anopheles species by subgenera	Total no.–% mosquitoes by species	Number–% localities where species was found	Number –% collecting trips where species was found
Nyssorhynchus			
An. albimanus	24,490–72.2	76–99.1	216–94.7
An. aquasalis	1,240–3.6	13–16.8	37–16.2
An. triannulatus s.l.	496–1.5	<5–<7	<10–<5
An. albitarsis s.l.	112–0.3	<5–<7	<10–<5
An. oswaldoi s.l.	<50–0.1	<5–<7	<10–<5
An. strodei s.l.	<50–0.1	<5–<7	<10–<5
Anopheles			
An. punctimacula	6,081–17.9	57–74.9	129–56.8
An. vestitipennis	868–2.6	7–9.1	21–10.5
An. pseudopunctipennis	155–0.5	7–9.1	15–6.6
An. apicimacula	124–0.4	<5–<7	<10–<5
An. neomaculipalpus	<50–0.1	<5–<7	<10–<5
An. malefactor	<50–0.1	<5–<7	<10–<5
Kerteszia			
An. neivai	279–0.9	7–9.1	19–8.1
Lophopodomyia			
An. squamifemur	<50–0.1	<5–<7	<10–<5
Chagasia bathana	<50–0.1	<5–<7	<10–<5
Total	33,917 individuals	77 localities	228 field trips

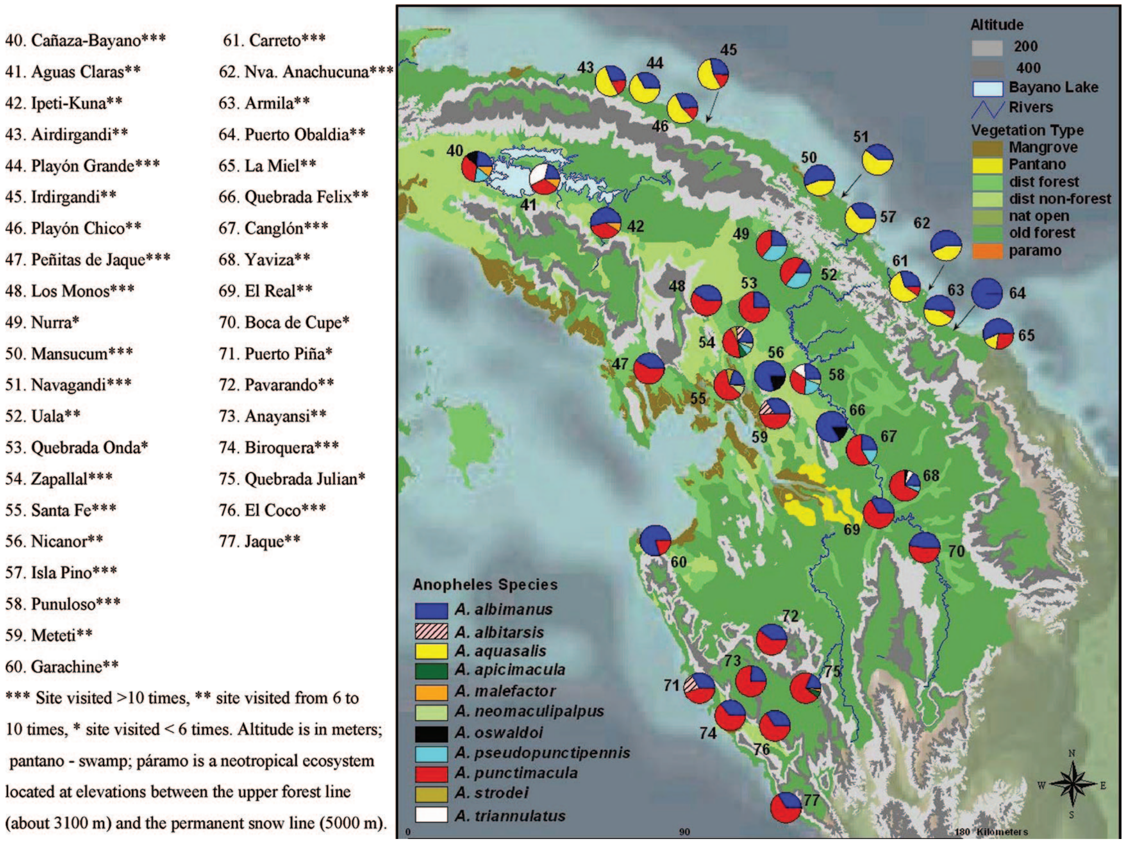

Fig. 2. *Anopheles* collecting sites, eastern Panama.

annulatus s.l., *An.* (*Nyssorhynchus*) *oswaldoi* s.l. (Peryassú), *An.* (*Nyssorhynchus*) *strodei* s.l. (Root), *An.* (*An.*) *neomaculipalpus* (Curry), *An.* (*An.*) *apicimacula* (Dyar and Knab), *An. malefactor,* and *Chagasia bathana* were caught in only a subset of localities (Figs. 2 and 3). *An.* (*Lophopodomyia*) *squamifemur* Antunes was collected only in Las Cumbres near Panama City (locality data not shown on Fig. 2). Ninety-eight percent of mosquitoes were collected in localities <100 m above sea level, and 37 (48%) localities had more than two *Anopheles* species (Figs. 2 and 3). In general, species within the subgenus *Anopheles* (*An. punctimacula*, *An. vestitipennis*, and *An. pseudopunctipennis* s.l.) were encountered in woody areas, whereas *An. triannulatus* s.l. (*Nyssorhynchus*) was collected in areas with permanent water bodies.

HBR. Overall average HBR varied significantly among mosquito species ($F_{8,2188} = 170.01$; $P < 0.05$), with the greatest rates obtained in *An. albimanus* and *An. punctimacula* (Table 4). The average HBR of *An. albimanus* ($F_{1,438} = 299.9$; $P < 0.05$), *An. punctimacula* ($F_{1,438} = 9.1$; $P < 0.05$), *An. vestitipennis* ($F_{1,438} = 5.7$; $P < 0.05$), and *An. aquasalis* ($F_{1,438} = 4.49$; $P < 0.05$) varied significantly between seasons (Table 4). The greatest HBR for *An. albimanus*, *An. punctimacula*, and *An. vestitipennis* was recorded during the rainy season (May–December), ranging from 1.1–18.5, 1.3–10.1, and 0.8–5.9 bites per person per night (b/p/nt), respectively. In contrast, *An. aquasalis* showed the highest HBR during the dry season (January–April), ranging from 2.2 to 11.1 b/p/nt.

There were significant among-site variations in the average HBRs of *An. albimanus* ($F_{30,184} = 2.6$; $P < 0.05$), *An. punctimacula* ($F_{30,184} = 8.16$; $P < 0.05$), and *An. aquasalis* ($F_{30,184} = 10.4$; $P < 0.05$). The highest HBR for *An. albimanus* was recorded from western Panama, in Barranco Montaña (11.9 b/p/nt), Chiriqui Grande (15.4 b/p/nt), and Rio Chiriqui (18.5 b/p/nt). The first two sites are located in BC; Rio Chiriqui is in NBC (Table 2; Fig. 3). On the contrary, *An. punctimacula*'s highest HBR was recorded from Santa Fe (9.8 b/p/nt), and Los Monos (10.1 b/p/nt), both located in DR (Table 2; Fig. 2). The highest HBR for *An. aquasalis* was recorded from three sites in KYC in the Atlantic coast, near the Colombian border: Navagandi (9.1 b/p/nt), Isla Pino (8.2 b/p/nt), and Carreto (11.1 b/p/nt) (Table 2; Fig. 2).

Discussion

The fact that mosquito collections for this study were carried out on the occurrence of malaria outbreaks in Panama and not based on a more comprehensive seasonal or temporal collection protocol may

1. Las Delicias***
2. Finca Debora***
3. Barra Sixaola***
4. Barranco Adentro***
5. Las Tablas**
6. Barranco Montaña***
7. San-San Pond*
8. Finca California**
9. Finca Once*
10. Guabito Centro***
11. Milla-11**
12. Almirante**
13. Pumuna*
14. Robalo*
15. Chiriqui Grande***
16. Manati*
17. Cricamola**
18. Bahia Azul***
19. Kusapin***
20. Tobobe***
21. Playa Roja***
22. Bisira***
23. Kankintu***
24. Playa Chiriqui**
25. Vegay*
26. Guazaro**
27. Rio Caña***
28. Rio Chirirqui***
29. San Pedro***
30. Palo Blanco*
31. Santa Catalina**
32. Cahuita*
33. Calobebora**
34. Concepcion**
35. Veraguas**
36. Belem**
37. Progreso*
38. Pto. Armuelles*
39. Punta Burica*

*** Site visited >10 times, ** site visited from 6 to 10 times, * site visited < 6 times. Altitude is in meters; pantano - swamp; páramo is a neotropical ecosystem located at elevations between the upper forest line (about 3100 m) and the permanent snow line (5000 m).

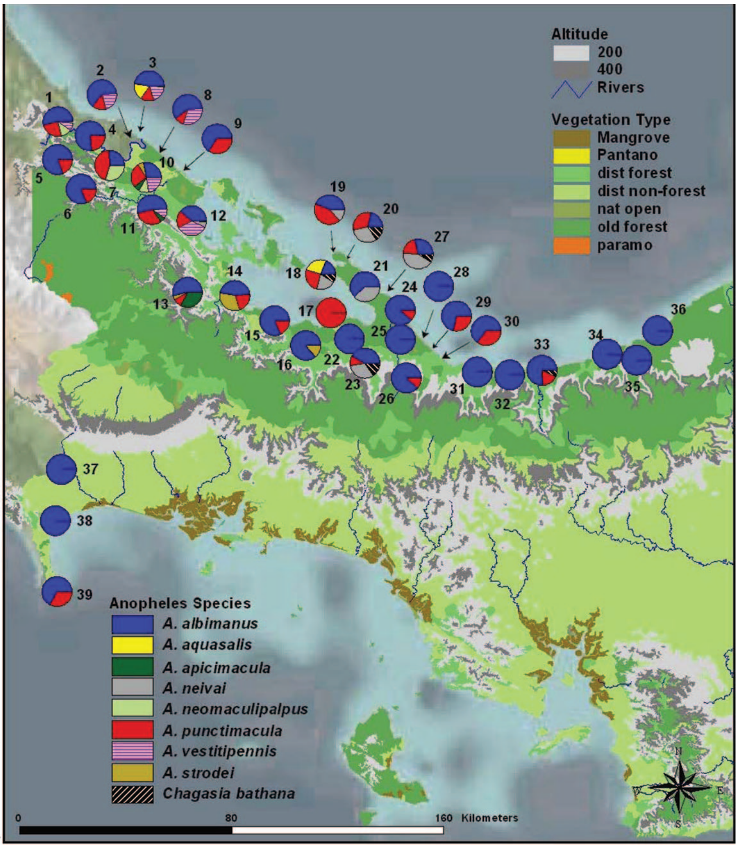

Fig. 3. *Anopheles* collecting sites, western Panama.

have resulted in a lower HBR overall and by species with respect to other studies (Table 4). However, the range of average HBR of *An. albimanus* in 31 Panamanian localities (1.8–18.5; Table 2; this study) does not differ substantially from a study in three villages in Chiapas, Mexico (monthly range, wet season, 1.0–18.8, compared with dry season, 0–10.7; Bown et al. 1991) or from the highest biting rate (7.1) for *An. albimanus* in coastal urban Buenaventura, Colombia (Olano et al. 1997).

Table 4. Overall and seasonal avg human-biting rate (HBR ± SE) of *Anopheles* species collected in Panama

Anopheles species	HBR (mean[a] ± SE)[b] dry season	HBR (mean[a] ± SE)[b] rainy season	HBR (mean[a] ± SE)[b] overall
1. *An. albimanus*	2.4 ± 1.11a	10.2 ± 0.91b	9.8 ± 0.08a
2. *An. punctimacula*	1.3 ± 0.70a	8.9 ± 1.12b	6.2 ± 0.13b
3. *An. aquasalis*	6.1 ± 0.23b	0.2 ± 0.10a	5.1 ± 0.03a
4. *An. vestitipennis*	1.2 ± 0.91a	6.1 ± 0.25b	2.3 ± 0.72b
5. *An. neivai*	3.9 ± 1.94a	5.4 ± 0.83b	1.3 ± 1.12b
6. *An. pseudopunctipennis*	1.3 ± 0.21a	1.2 ± 0.56a	1.5 ± 1.26b
7. *An. triannulatus* s.l.	3.1 ± 2.45a	3.3 ± 0.17a	1.1 ± 0.19b
8. *An. apicimacula*	0.8 ± 0.12a	0.6 ± 1.11a	0.8 ± 0.11b
9. *An. neomaculipalpus*	1.1 ± 0.30a	1.5 ± 0.72a	0.6 ± 0.09b
10. *An. albitarsis* s.l.	<0.5	<0.5	<0.5
11. *An. oswaldoi* s.l.	<0.5	<0.5	<0.5
12. *An. strodei* s.l.	<0.5	<0.5	<0.5
13. *An. malefactor*	<0.5	<0.5	<0.5
14. *An. squamifemur*	<0.5	<0.5	<0.5
15. *Chagasia bathana*	<0.5	<0.5	<0.5

Values of <0.5 were not analyzed.
Dry season: Jan. to April (avg monthly rainfall of 34.1 mm).
Rainy season: May to Dec. (avg monthly rainfall of 507 mm).
[a] Average obtained from three collectors in between 5–10 d/h per day replicates.
[b] Values followed by different letters are significantly different (P <0.05; PROC GLM; Mean comparison by LSD).

In this study, *An. albimanus* and *An. punctimacula* were the most abundant and prevalent species showing the highest overall HBR over 35 yr in Panama. *An. albimanus* is considered the most important malaria vector across its distribution (Rubio-Palis and Zimmerman 1997), whereas *An. punctimacula* is considered a secondary vector in Costa Rica (Kumm and Ruiz 1939) and Colombia (Olano et al. 2001). The fact that both species have been previously incriminated as vectors in Panama (Darling 1910, Rozeboom 1935) combined with their considerable degree of association with human dwellings is very suggestive of their involvement in malaria transmission. The highest HBR in *An. punctimacula* was in DR, which has >70% forest cover (ANAM 2003). This finding supports Service (1989), who noted that the larval habitats of *An. punctimacula* occur in deep or partial shaded areas.

Malaria outbreaks have occurred in KYC, where we found most *An. aquasalis,* at times when this species was the most abundant or even the only anopheline present (SNEM, unpublished data). Darling (1910) considered *An.* (*Nyssorhynchus*) *tarsimaculatus* (*Syn. An. aquasalis)* as the second most important malaria vector in Panama based on salivary gland dissections, and *An. aquasalis* is a known regional malaria vector in eastern Venezuela and Brazil (Berti et al. 1993, Póvoa et al. 2003). However, its more restricted distribution in Panama indicates that it is likely important only in local malaria transmission.

Anopheles vestitipennis and *An. neivai* are confirmed malaria vectors in Belize (Grieco 2001) and Colombia (Carvajal et al. 1989) and *An. vestitipennis* in Chiapas, Mexico (Loyola et al. 1991). Both species were predominantly collected from western Panama where they seem to cluster with specific types of vegetation and land use practices (ANAM 2003). In the Pacific coast of Colombia, *An. neivai* is presumed to be involved in malaria transmission both during the daytime and the evening because of its multiple hemorrhagic peaks (Solarte et al. 1996). This species breeds along the seashore, mainly associated with lowland tropical forest, where it develops in several species of epiphytes (Carvajal et al. 1989). *An. neivai* was frequently collected from NBC where transportation between villages is mostly by foot, increasing the human contact with this species and perhaps the likelihood of malaria transmission. Because our data consist only of evening collections, the lower HBR of *An. neivai* may underestimate its actual role as a vector in Panama. In contrast, *An. vestitipennis* was collected more inland in BC, overlapping with banana plantations and woody areas. In Belize, *An. vestitipennis* is considered a more efficient vector than *An. albimanus* even though it is usually less abundant (Grieco 2001). The proposed explanation is a marked preference for human blood (anthropophagic), endophagic habit, and higher infection rate (Achee et al. 2000). The lower relative abundance of *An. vestitipennis* in Panama could suggest poor vectorial competence or it may reflect bias in our sampling protocol (i.e., collecting mosquitoes only outdoors). It is not possible to discern the transmission role of *An. vestitipennis* and *An. neivai* based only on the present dataset. To date, *An. vestitipennis* and *An. neivai* have not been found naturally infected with *Plasmodium sp.* in Panama, nor have vector competence evaluations been undertaken.

Anopheles darlingi was collected biting just after sunset in the Colombian town of Capurgana, <15 km near the Panama–Colombia border. *An. darlingi* is found in part of Central America but never officially reported from Nicaragua, Costa Rica, or Panama, resulting in an apparent discontinuity in its distribution (Linthicum 1988). One hypothesis to explain its distribution is an introduction event from Colombia into Central America (Manguin et al. 1999, Mirabello and Conn 2006). Another possibility is that unsolved geological factors related to the uplift of the Panamanian Isthmus (Bermingham and Martin 1998) may have precluded its successful establishment in eastern Panama. The high malaria incidence in this region requires a more comprehensive mosquito survey to evaluate the potential presence of *An. darlingi.*

Because of their low density and limited distribution, it is unlikely that *An. pseudopunctipennis* s.l., *An. oswaldoi* s.l., *An. strodei* s.l., *An. triannulatus* s.l., *An. neomaculipalpus*, and *An. apicimacula* contribute significantly to malaria transmission in Panama. However, four of these taxa are species complexes that include proven malaria vectors in other Latin American countries (Rosa-Freitas et al. 1998, Coetzee et al. 1999, Marrelli et al. 2006, Quiñones et al. 2006). Moreover, *An. neomaculipalpus* was recently reported as a potential secondary vector of *P. vivax* in southern Venezuela, with an overall sporozoite rate similar to that for *An. darlingi* and higher than that reported for *An. marajoara* (Moreno et al. 2005). Previous incriminations of *An. neomaculipalpus* and *An. apicimacula* as vectors in Panama were supported by the presence of *Plasmodium* sporozoites in salivary glands (Table 1).

Even though only 41 specimens of *An. malefactor* (formerly *An. punctimacula*) were collected in four localities in eastern Panama, Aguas Claras, Canazas–Bayano, Ipeti Kuna, and Biroquera (Fig. 2), this species has been collected using human landing catches in Panama (Wilkerson 1990). However, it seems unlikely to be involved in malaria transmission because of its restricted distribution (Canal Zone and northwestern Colombia) (Wilkerson 1990). However, populations of *An. punctimacula* in southern Mexico have been shown to be mostly zoophagic, feeding primarily on cattle (Ulloa et al. 2006). These findings are in disagreement with our results where *An. punctimacula* was frequently caught feeding on people. Zimmerman et al. (2006) encountered significant differences in host selection patterns by several *Anopheles* species among adjacent villages, attributing this to host availability. Cattle farming is still an incipient activity in some areas of NBC, KYC, and DR; thus, the partial absence of large mammals as alternative blood sources may explain a more anthropophagic tendency by *An. punctimacula* in these Panamanian regions.

Anopheles albitarsis s.l. was collected biting people in areas where malaria cases have occurred in eastern Panama. *Anopheles* (*Nyssorhynchus*) *marajoara*

(Galvão and Damesceno), a member of the *An. albitarsis* complex (Lehr et al. 2005, Wilkerson et al. 2005), has been identified in Panama previously (Wilkerson and Strickman 1990). This species is a malaria vector in eastern Amazonian Brazil (Póvoa et al. 2000; Conn et al. 2002) and Venezuela (as revised in Rubio-Palis et al. 2003, Moreno et al. 2005; 2007); however, its role in Panama cannot be established until we determine the identity of our Panamanian specimens of *An. albitarsis* s.l. using molecular techniques (Li and Wilkerson 2005). Despite previous records in DR, Panama (Faran 1980), *An. nuneztovari* was not identified in this study, even though this species is considered a primary regional malaria vector in nearby Colombia (Brochero et al. 2005) and in Venezuela (Gabaldón 1981). Although highly trained personnel did mosquito identifications based on morphological characters and keys, molecular confirmation was not done at the time. We plan to use molecular approaches to determine whether species such as *An. nuneztovari* s.l., *An. (Nyssorhynchus) benarrochi*, and *An. (Nyssorhynchus) albitarsis* F (a new putative species in the *An. albitarsis* Complex) (Brochero et al. 2007) are distributed in Panama, using voucher specimens stored at ICGES.

Anopheles albimanus and *An. punctimacula* were more abundant during the rainy season, implying that the highest risk of malaria transmission by these species in Panama occurs from May to December. This is not a novel finding for *An. albimanus,* but it is known to occur in places where alternative breeding sites are absent during the dry season (Frederickson 1993). On the contrary, the highest vector competence for *An. punctimacula* in southern Mexico has been proposed to occur during the dry season when this species is more abundant (Ulloa et al. 2006). Achee et al. (2005) encountered *An. punctimacula* in Belize with one peak of density in January at the beginning of the dry season and another peak in August during the rainy season, perhaps reflecting differences in larval habitat distributions determined by local climatic and ecological conditions. It is also possible that *An. punctimacula* (in the past Syn. *An. malefactor*) consists of more than two cryptic species. We also determined that *An. vestitipennis* was more abundant during the rainy season in Panama. In Belize, this species peaks in density in August and September at the end of the rainy season (Achee et al. 2005); however, its seasonal fluctuation seems to be ultimately determined by the presence of specific vegetation, *Typha domingensis,* at its main breeding sites (Grieco et al. 2006).

In Central America, the highest risk of malaria transmission by *An. albimanus* occurs from 1800 to 2200 hours when children and young adults gather outside houses (PAHO 1996), and our outdoor collections in localities where *An. albimanus* was the most prevalent species confirm this. Nevertheless, a high diversity of *Anopheles sp.* and six confirmed malaria vectors provide evidence for a more complex situation in Panama.

Panama has used residual insecticide spraying (RIS) since 1957 as the main method of vector control. Four discrete chemical groups have been used: dieldrin and DDT (organoclorate), propoxur (carbamate), fenitrothion (organophosphate), and deltamethrin and cyflutrin (pyrethroid), with insecticide shifts according to chronological appearance of resistance in populations of *An. albimanus* (Caceres 1999). The effectiveness of RIS in controlling malaria is acknowledged (Roberts et al. 2002); however, the exophagic and exophilic behavior displayed by *An. albimanus* in Central America (PAHO 1996) makes its control uncertain even in highly susceptible populations.

RIS disrupts malaria transmission by reducing female longevity, and depending on the insecticides, by irritating mosquito mechanoreceptors (i.e., pyrethroids), thus repelling them from inside houses and minimizing overall vector contact. However, avoidance behavioral responses against DDT displayed by Panamanian populations of *An. albimanus* may have lessened the successful contact between this species and sprayed surfaces (Trapido 1952). The effectiveness of RIS in controlling *An. albimanus* and other *Anopheles* species needs further assessment in Panama.

Recent studies in Thailand have shown that the seasonal migration of cross-border laborers is a leading cause of malaria transmission (Zhou et al. 2005). In Panama, malaria is largely endemic in rural areas, especially near the Panama–Costa Rica and Panama–Colombia borders, where transmission could be influenced by human migration. Furthermore, the fact that *P. falciparum* has been recorded traditionally from KYC (Panama–Colombian border), where *An. albimanus* and *An. punctimacula* are not as prevalent as *An. aquasalis,* suggests that its incidence may be related to differential transmission capabilities among vector species or between populations of the same species across the country. Moreover, the absence of large mammals as an alternative blood source for mosquitoes may enhance the transmission role of secondary and local vectors, especially in indigenous comarcas such as NBC and KYC.

These facts underscore the need for more in-depth studies to clarify the basic ecology of anopheline mosquitoes in western and eastern Panama. This report improves our understanding of malaria transmission in Panama, and also provides baseline information required for further research in *Anopheles* bionomics.

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