

Anthropogenic influence on the distribution, abundance and diversity of sandfly species (Diptera: Phlebotominae: Psychodidae), vectors of cutaneous leishmaniasis in Panama

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In Panama, species of the genus Lutzomyia are vectors of American cutaneous leishmaniasis (ACL). There is no recent ecological information that may be used to develop tools for the control of this disease. Thus, the goal of this study was to determine the composition, distribution and diversity of Lutzomyia species that serve as vectors of ACL. Sandfly sampling was conducted in forests, fragmented forests and rural environments, in locations with records of ACL. Lutzomyia gomezi, Lutzomyia panamensis and Lutzomyia trapidoi were the most widely distributed and prevalent species. Analysis of each sampling point showed that the species abundance and diversity were greatest at points located in the fragmented forest landscape. However, when the samples were grouped according to the landscape characteristics of the locations, there was a greater diversity of species in the rural environment locations. The Kruskal Wallis analysis of species abundance found that Lu. gomezi and Lu. trapidoi were associated with fragmented environments, while Lu. panamensis, Lutzomyia olmeca bicolor and Lutzomyia ylephiletor were associated with forested environments. Therefore, we suggest that human activity influences the distribution, composition and diversity of the vector species responsible for leishmaniasis in Panama.

Key words: phlebotomines - leishmaniasis - deforestation - ecology - species distribution

Leishmaniasis in Panama was first recorded in 1910. The staff of the Gorgas Memorial Laboratory has researched the ecology and epidemiology of leishmaniasis since that time. Cutaneous leishmaniasis is the most common form of leishmaniasis reported in the Republic of Panama; its clinical manifestations range from minor lesions to severe skin ulcers (Christensen et al. 1972, 1983, 1984). In humans, the disease is mainly caused by *Leishmania panamensis*, with a distribution extending from Belize-Argentina (Young & Duncan 1994, Miranda et al. 2009). However, other species such as *Leishmania colombiensis*, *Leishmania mexicana*, *Leishmania amazonensis* and *Leishmania braziliensis* have been reported; *Leishmania aristidesi* and *Leishmania hertigi* have both been isolated from mammals (Herrer 1971, Herrer et al. 1971, Christensen et al. 1972). Another strain, *Leishmania naiffi*, has recently been found in populations of *Lutzomyia gomezi* and *Lutzomyia trapidoi*, but it has not been found in human infections and its reservoirs remain unidentified (Azpuruá et al. 2010).

Leishmaniasis is a primarily zoonotic disease. The sloth *Choloepus hoffmanni* is the primary reservoir of the parasite, but other wild mammals, such as *Brady-*

pus variegatus, *Saguinus geoffroyi*, *Nasua nasua*, *Potos flavus*, *Bassaricyon gabbi*, *Proechimys semispinosus*, *Hoplomys gymnurus* and *Coendou rothschildi*, have also been identified as reservoirs and their role as secondary hosts has been established (Christensen et al. 1984, Méndez 1999).

Phlebotomine sandflies are the vectors of leishmaniasis and are present in almost every country in tropical and subtropical regions. Central and South America have the greatest concentration of these vectors (Young & Arias 1992, Seccombe et al. 1993, Young & Duncan 1994). About 74 species of sandflies have been reported in Panama (Christensen 1972, Herrer & Christensen 1976), including *Lutzomyia ylephiletor*, *Lutzomyia sanguinaria*, *Lutzomyia panamensis*, *Lu. trapidoi*, *Lu. gomezi* and *Lutzomyia olmeca bicolor*. These species were identified as the transmission vectors of the American cutaneous leishmaniasis (ACL) agent in Panama in the 1950s when *L. braziliensis sensu lato* was isolated from the vectors' digestive tracts (Herrer et al. 1971, Tesh et al. 1971, Herrer & Christensen 1973).

The distribution of phlebotomine sandflies in Panama correlates with the occurrence of ACL in the endemic regions of the provinces of Bocas de Toro, Veraguas, Coclé, Colón, Panama East, Panama West (MINSa 2005), especially in forest areas. Phlebotomine sandflies develop in humid locations, such as nests, rock crevices, animal burrows and tree bark (Christensen et al. 1983). However, with the depletion of forest and the disappearance of their natural habitat, some species seem to have adapted to degraded habitats, contributing to the spread of ACL (Jiménez et al. 2000, Travi et al. 2002).

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Few recent studies have appeared on the diversity, geographical distribution and abundance of the different species of phlebotomine sandflies as vectors of leishmaniasis agents in Panama. Thus, the aims of the present study were to determine the composition, geographical distribution and diversity of ACL vectors in the endemic regions of Panama. Additionally, this study sought to verify the association between the characteristics of the areas studied and the communities of phlebotomine sandflies inhabiting them.

MATERIALS AND METHODS

Study area - Sandflies were captured in Panama between the coordinates 7°11'-9°39'N and 77°10'-83°03'W. The most prevalent climatic regime in Panama is tropical humid, with the summer season (January-March) presenting an average temperature of 30°C and relative humidity of 75%. In other months (April-December), the average temperature is 27°C and the relative humidity averages 90%.

There are three different regions in Panama: the Atlantic region, where it rains all year long and there is no clearly defined dry season, the Pacific region, characterised by moderate to heavy rainfall and a well defined dry season, and the central region, that occupies most of the continental part of the country and is subjected to moderate to heavy rains and strong winds due to the thermal contrast between the other two regions.

The vegetation in Panama varies according to the climate zones and consists mainly of tropical humid forest or savannahs resulting from agricultural activity (ANAM 2000). The data on temperature, precipitation and humidity from 2007-2010 were obtained from the weather stations of the Empresa de Transmisión Eléctrica Panameña, located at different points in the country.

This study was conducted at 43 locations throughout eight regions or provinces of Panama (Fig. 1). The landscapes of the locations were heterogeneous with a variety of diverse macro habitats created by human activity. Each site was classified according to its landscape characteristics as one of the following: (i) forest environment,

an extension of land with abundant primary or secondary vegetation (flora, fauna and climatic conditions typical of tropical rainforest), (ii) fragmented forest environment, areas degraded by human activity (timber extraction, cattle farming, agriculture etc.), presenting small patches of secondary vegetation close to the degraded areas, or (iii) rural environment, a province or settlement without any great concentration of people or commercial activity, limited urban growth and area used for agriculture. The traps were distributed at specific collection points in each landscape. In the fragmentary forest and rural landscapes, the traps were placed in houses that lacked window covering were in close proximity to domestic animals and ornamental vegetation. For each forest locality, the traps were placed near rock crevices and tree bark.

Sandflies were collected during six months in 2010, January-April (dry season) and May-June (early rainy season) at 43 locations where human cases of ACL had been recorded from 2006-2009, according to the Epidemiology Department of the Ministerio de Salud de Panama.

Adult sandflies were collected using CDC light traps (Sudia & Chamberlain 1962) and octanol solution (used to attract haematophagous insects) exposed at a height of 1.5 m. At least nine light traps were installed in each locality at approximately 50-m intervals. The collection period was 12 h (06:00 pm-06:00 am) for two consecutive days. The specimens were sacrificed with chloroform and stored in 95% ethanol at -20°C.

Identification of the collected specimens - Sandflies were classified according to the methods of Young and Duncan (1994). The nomenclature of the subgenus *Psychodopygus* described by Carvalho et al. (2006) was used in this study.

Analysis - The total abundance (P_i) ($= n_i/N$) and diversity (H') ($= -\sum p_i \ln p_i$) of sandfly species at each site was calculated in accordance with the methods described by Magurran (1988). To test the differences in sandfly species abundance at each type of landscape, the Kruskal Wallis test was used ($p \leq 0.05$) in conjunction with the multiple comparison test ($\alpha = 0.05$). Explora-

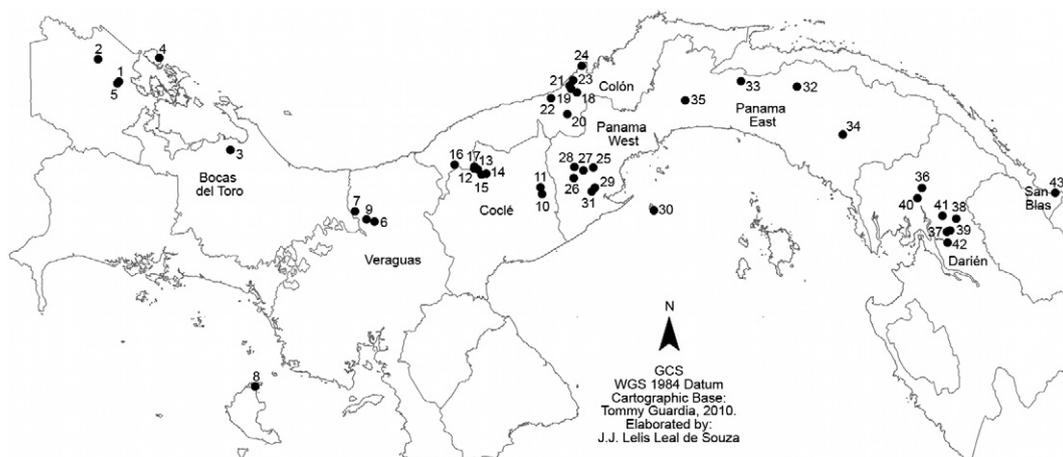


Fig. 1: geographical distribution of locations (see Table I) sampled of phlebotomine sandflies, vectors of American cutaneous leishmaniasis in Panama.

tory data (simple correspondence analysis) was tested to evaluate the association of the species with the characteristic landscape. In this analysis, only anthropophilic sandfly species were considered as ACL vectors in Panama (Herrer et al. 1971, Tesh et al. 1971, Herrero & Christensen 1973). Furthermore, other index of diversity (H') and dominance (D) ($= \sum p_i^2$) were conducted and Sørensen's similarity index (I_s) ($= 2c/a+b$) (Magurran 1988) was calculated to define the differences between the three landscapes.

RESULTS

A total of 1,923 sandflies of the *Lutzomyia* genus were captured. Of these, 66.2% were anthropophilic species and vectors of *Leishmania* species in Panamá: *Lu. panamensis* 24.2%, *Lu. gomezi* 18.6%, *Lu. trapidoi* 15.6%, *Lutzomyia ovallesi* 3%, *Lu. sanguinaria* 1.5%, *Lu. olmeca bicolor* 1.2%, *Lu. ylephiletor* 0.9% and *Lutzomyia carrerai* 0.6%. The other 33.8% of sandflies captured were zoophilic species: *Lutzomyia triramula* 3.2%, *Lutzomyia dysponeta* 10.5%, *Lutzomyia camposi* 6.8%, *Lutzomyia vesicifera* 2.7%, *Lutzomyia sordellii* 0.5%, *Lutzomyia vespertilionis* 0.2%, *Lutzomyia carpenteri* 1.3%, *Lutzomyia serrana* 0.6%, *Lutzomyia gorbitzi* 0.3%, *Lutzomyia aclydifera* 4.2%, *Lutzomyia trinidadensis* 2%, *Lutzomyia cruciata* 0.5%, *Lutzomyia odax* 0.5% and *Lutzomyia barretoii* 0.6%, all of which are not responsible for transmitting the disease to humans as they are part of the wildlife cycle of leishmaniasis. *Lutzomyia longipalpis*, the main vector of visceral leishmaniasis, also was collected in two localities, Limón and in Boná Island.

A detailed analysis of the anthropophilic species per location is shown in Table I. *Lu. gomezi*, *Lu. panamensis* and *Lu. trapidoi* were wide-spread and present in 35, 27 and 26 out of the 43 locations sampled, respectively, and were the most predominant species at 15, 13 and seven of the locations, respectively. In contrast, *Lu. sanguinaria*, *Lu. olmeca bicolor* and *Lu. ylephiletor* were scarce and found at a small subset of locations. *Lu. ovallesi* is the most common vector of *L. braziliensis* in Venezuela and Guatemala, and although it contributed only a small percentage to the total number of sandflies captured, it was the predominant species identified at two locations in the Darién and in Ollas Arriba (provinces of Panama). *Lu. carrerai* was collected only in Charagre, in Bocas del Toro; information for this species not is shown in Table I because it was isolated in low numbers and at few locations. Nine specimens of *Lu. aclydifera* and six of *Lu. dysponeta* were captured in the Parque Altos de Campana.

The male to female ratio of captured sandflies was highest in Puerto Obaldía, El Llanillo and Unión Piña. In the other locations, more females than males were collected (Table I). Cutevilla, Vaquilla, Chirigui Arriba, Donoso, Piña and Ollas Arriba had high numbers of females (> 50) and only one male was isolated in Arimay.

Cutevilla, Chirigui Arriba, Vaquilla and Piña yielded the highest relative abundance of anthropophilic sandflies (0.11, 0.09, 0.06 and 0.06, respectively). The abundance of anthropophilic sandflies at the other locations varied from 0.004-0.052. Parque Altos de Campana was the only locality where no vector species was collected. In

35% of the sites sampled, the Shannon-Weiner H' varied from 1.03-1.49 and, in 42% of the sites, this index varied from 0.30-0.96 (Table I).

The number of cases of leishmaniasis registered in 2009 in the regions sampled is shown in Table I. Bocas del Toro, Colón and Coclé experienced the highest rates of leishmaniasis incidence, while the San Blas regions had the lowest incidence of leishmaniasis. Temperature, precipitation and humidity data are shown in Table I.

The abundance of *Lu. panamensis*, *Lu. gomezi* and *Lu. trapidoi* varied significantly between the three types of environments ($p < 0.05$). This difference, however, was not verified for *Lu. ylephiletor*, *Lu. sanguinaria*, *Lu. olmeca bicolor*, *Lu. ovallesi*, *Lu. carrerai* and *Lu. longipalpis* (Table II).

The analysis of simple correspondence between the species and the type of environment showed a strong association of *Lu. gomezi* and *Lu. trapidoi* with fragmented environments. *Lu. olmeca bicolor*, *Lu. panamensis* and *Lu. ylephiletor* were most strongly associated with forest environments and *Lu. sanguinaria*, *Lu. ovallesi*, *Lu. carrerai* and *Lu. longipalpis* did not show any association with any type of environment (χ^2 : 177,761; $df = 16$; $p < 0,05$) (Fig. 2).

When the species diversity at each type of landscape was examined, the Shannon-Weiner index showed that the rural environments contained a higher diversity ($H' = 1.51$) than the areas with fragmented vegetation ($H' = 1.47$) and the forested areas ($H' = 1.29$). The Simpson index, in turn, showed that forest environments ($D = 0.35$) frequently had one dominant species, contrary to our observations in the fragmented ($D = 0.27$) and rural environments ($D = 0.25$). When the zoophilic species were included in the analysis, diversity was higher in the forest environments ($H' = 2.41$) than fragmented vegetation ($H' = 2.01$) and rural environments ($H' = 1.99$). We also observed that the Simpson index showed higher dominance in fragmented environments ($D = 0.20$), in rural environments ($D = 0.18$) and in forest environments ($D = 0.12$), contrary to the results with only anthropophilic vector species.

The similarity in sandfly species was 68.4% between the forest and rural environments; 55.7% between the rural and fragmented environments and 43.9% between the fragmented and forest environments. When zoophilic species are included, the similarity between rural and fragmented environments reached 57.1%.

DISCUSSION

There are 7,476,836 ha of land in Panama. Of these, 43.36% are still covered by forest, but 33,531.61 ha are destroyed every year by human activity (ANAM 2009). This loss in vegetation results in the migration and adaptation of some vector species to degraded environments, increasing their spatial distribution (Service 1991, Walsh et al. 1993, Grillet 2000, Aguiar & Medeiros 2003).

The data obtained in this study shows the impact of human activity and deforestation on *Lu. gomezi*, *Lu. panamensis* and *Lu. trapidoi*, abundant species with wide distributions in Panama. Earlier studies on sandflies reported the presence of these species in areas with leishmaniasis outbreaks, but showed that their geographical

TABLE I
Abundance absolute and relative, Shannon diversity index (H'), sex ratio of sandfly species transmitter of leishmaniasis in the locations sampled in Panama

Regions	Locations	Landscape	Cases										H'	Weather			
			<i>Lutzomyia gomezi</i>	<i>Lutzomyia trapidoi</i>	<i>Lutzomyia ylephiletor</i>	<i>Lutzomyia sanguinaria</i>	<i>Lutzomyia olmeca bicolor</i>	<i>Lutzomyia ovallesi</i>	♂ (n)	♀ (n)	♂/♀	Pi					
Bocas del Toro	1: Nance Valle Risco	Fr	437	36	2	3	-	1	-	-	-	11	31	0.4	0.03	0.55	Ta = 28
	2: Charagre	Fr		2	-	14	1	4	-	-	-	9	24	0.4	0.03	1.26	Pa = 3,000
	3: Bisira	R		5	5	-	-	-	-	-	-	2	8	0.3	0.01	0.69	Ha = 87
	4: Isla Colón	R		11	-	-	-	-	-	-	-	1	10	0.1	0.01	0	
Veraguas	5: Parque Nacional Palo Seco	Fo		-	-	1	-	-	-	-	0	1	0.0	0.001	0		
	6: Altos de Piedra	Fr	73	-	7	1	-	-	-	-	1	7	0.1	0.01	0.38	Ta = 25	
	7: Llanillo	Fr		4	-	6	-	-	3	-	-	7	6	1.2	0.01	1.06	Pa = 2,550
	8: Parque Nacional Coiba	Fo		-	7	-	-	-	1	-	-	1	7	0.1	0.01	0.38	Ha = 83
Coclé	9: Parque Nacional Santa Fé	Fo		-	-	1	-	-	-	-	0	1	0.0	0.001	0		
	10: Chirigui Arriba	R	296	80	8	21	-	3	-	-	54	58	0.9	0.09	0.84	Ta = 26	
	11: Vaquilla	Fr		19	40	17	3	1	-	-	12	68	0.2	0.06	1.2	Pa = 2,117	
	12: Coclesito-community	R		-	6	-	-	-	-	-	4	2	2.0	0.01	0	Ha = 88	
Colón	13: Coclesito-forest	Fo		6	1	2	2	-	-	-	5	6	0.8	0.01	1.17		
	14: Cutevilla	Fr		70	28	44	-	-	-	-	39	103	0.4	0.11	1.03		
	15: Molejón	Fr		14	9	7	-	-	-	-	7	23	0.3	0.02	1.06		
	16: Donoso	Fr		36	-	29	-	-	-	-	8	57	0.1	0.05	0.69		
	17: Villa del Carmen	R		8	3	-	-	-	-	-	3	8	0.4	0.01	0.59		
	18: Providencia	R	376	4	11	1	-	-	-	-	2	14	0.1	0.01	0.78	Ta = 30	
	19: Achioté	R		1	10	-	-	-	-	-	1	10	0.1	0.01	0.3	Pa = 2,550	
	20: Cuipo	R		1	9	-	-	-	-	-	4	6	0.7	0.01	0.33	Ha = 86	
	21: Piña	Fr		38	26	13	1	-	-	-	16	62	0.3	0.06	1.07		
	22: Quebrada Leona	Fr		34	7	6	-	3	-	-	12	38	0.3	0.04	0.96		
	23: Unión Piña	Fr		8	8	24	-	-	-	-	24	16	1.5	0.03	0.95		
Panama West	24: Parque Nacional San Lorenzo	Fo		42	9	4	-	1	2	-	15	43	0.3	0.05	0.89		
	25: Ollas Arriba	R	197	4	12	13	3	1	-	30	8	55	0.1	0.05	1.38	Ta = 27	
	26: Cacao	Fr		16	6	20	2	-	8	-	7	45	0.2	0.04	1.39	Pa = 1,704	
	27: Valdeza	R		1	9	3	1	-	8	-	4	17	0.2	0.02	1.29	Ha = 60	
Panama West	28: Vista Alegre	Fr		6	11	13	-	2	1	-	4	29	0.1	0.03	1.32		
	29: Limón	Fr		9	6	3	2	-	-	-	6	14	0.4	0.02	1.49		
	30: Boná	Fo		-	-	-	-	-	-	-	-	-	-	-	-	-	
31: Parque Altos de Campana	Fo		-	-	-	-	-	-	-	-	-	-	-	-	-	-	



Regions	Locations	Landscape	Cases										Weather				
			<i>Lutzomyia panamensis</i> (n)	<i>Lutzomyia gomezi</i> (n)	<i>Lutzomyia trapidoi</i> (n)	<i>Lutzomyia ylephiletor</i> (n)	<i>Lutzomyia sanguinaria</i> (n)	<i>Lutzomyia olmeca bicolor</i> (n)	<i>Lutzomyia ovallesi</i> (n)	♂ (n)	♀ (n)	Pi		H'			
Panama East	32: Buenos Aires-Chepo	Fr	24	2	9	3	-	1	-	-	-	2	13	0.2	0.01	1.08	Tm = 27
	33: Gato Real	Fr	5	8	44	1	8	-	-	-	-	16	48	0.3	0.05	1.04	Pa = 1,704
	34: Torti	Fr	3	43	2	-	-	-	-	-	-	7	41	0.2	0.04	0.49	Ha = 87
	35: Cerro Azul	Fo	-	1	5	2	-	-	1	-	-	4	5	0.8	0.01	1.15	
	36: Arimay	R	81	-	1	-	-	-	-	-	-	1	0	-	0.001	0	Tm = 27
Darién	37: Bijagual	Fr	-	8	-	-	-	1	-	-	-	4	10	0.4	0.01	0.88	Pa = 1,704
	38: Buenos Aires	R	-	9	-	-	-	-	-	-	18	6	21	0.3	0.02	0.64	Ha = 86
	39: Parque Serranía Filo del Tallo	Fo	-	6	-	-	-	-	-	-	-	4	2	2.0	0.01	0	
	40: La Cantera	R	-	13	-	-	-	-	-	-	-	3	10	0.3	0.01	0	
	41: Nicanor	Fr	-	4	-	-	-	-	-	-	4	2	8	0.3	0.01	0.69	
San Blas	42: Rio Iglesia	R	-	8	-	-	-	2	-	-	1	10	0.1	0.01	0.76		
	43: Puerto Obaldía	Fr	2	-	8	-	-	-	-	-	6	2	3.0	0.01	0	Tm = 28 Pa = 2,650 Ha = 83	

Fo: forest; Fr: fragmented vegetation; Ha: average annual relative humidity; Pa: annual precipitation; Pi: abundance relative; R: rural; Ta: average annual temperature; ♂: male; ♀: female; ♂/♀: sex ratio.

distributions were restricted to high-humidity forest regions (Chaniotis et al. 1971a, Rutledge & Ellenwood 1975, Christensen et al. 1983). However, locations with recent outbreaks have been impacted by deforestation and there has also been an increase in the incidence of leishmaniasis in communities near the locations of the outbreaks.

The increase in leishmaniasis cases is due to a combination of multiple factors, involving changes in climate, human behaviours, vectors and reservoirs. The changing pattern of dispersal and the spatial distribution of sandflies to new locations are critical factors that may implicate an increased risk of human-vector contact. There are few similar studies that estimate the expansion of the vector species, but there are records from studies at the Gorgas Memorial Institute that were performed in the 1960-1980s that help us understand how the species distribution has changed. More current information could be used to alert health officials to what areas may be at risk for leishmaniasis and could help them evaluate what kind of intervention may be useful in those areas.

We observed that some species, such as *Lu. sanguinaria*, *Lu. ovallesi*, *Lu. olmeca bicolor*, *Lu. ylephiletor*, *Lu. carrerai* and *Lu. longipalpis*, exhibited a discontinuous distribution, i.e., they were identified in localities far from each other. It is probable, however, that these species are also present in the intermediate regions between the points where they were collected. Therefore, deforestation could also be contributing to the expansion of the geographic distribution of these species. A more intensive sampling and comparative data in the surrounding regions could be used to test this hypothesis.

Climatic variations, high humidity and the prolonged rainy season in Panama have fundamental roles in maintaining the diversity of habitats available to sandflies (Chaniotis et al. 1971a, Christensen et al. 1983). The destruction of the forest and sandfly habitats permits the establishment of vector species in fragmented zones, close to peridomestic zones. In the provinces of Panama, Bocas del Toro, Colón and Veraguas, the sampling locations are subjected to rain throughout most of the year, while the wet and dry periods in other regions are clearly defined. The overall temperature varies by two degrees between regions. We did not examine the link between climate variables and species diversity in this study because of the high variability in climate throughout the day and between days. However, we may consider performing a further analysis of variations in climate and sandfly abundance over time in order to observe temporal distribution patterns and ecological niches.

As observed in the present study, the abundance of sandfly species was greater in locations with environments fragmented by human activities. Other authors have reported that *Lu. longipalpis* has a wide geographical distribution (Souza et al. 2009) and is abundant in anthropic environments (Oliveira et al. 2006). Other species, such as *Lutzomyia withmani*, *Lutzomyia evansi*, *Lutzomyia neivai* and *Lutzomyia olmeca olmeca*, vectors of ACL agents in different regions of Latin America, are also abundant in degraded areas or those that have been altered by agriculture and cattle (Travi et al. 2002, Andrade Filho et al. 2007, Salomón et al. 2008, Virgens et al.

2008, Pech-May et al. 2010). Therefore, it has been suggested that the creation of new habitats (stables, chicken ranges, orchards etc.), new food sources and the accumulation of organic material from domestic animals, in addition to the conditions of microclimates (temperature and humidity) of these ecotopes, favour the life cycle of sandflies and support their population growth in these habitats (Souza et al. 1999, Dias et al. 2003, Muniz et al. 2006, Monteiro et al. 2008).

The sex ratio analysis suggests that females are more prevalent than males in almost all locations. This is very important in terms of the population ecology because the growth and fitness of the sandfly population depends on the number of females. The estimated sex ratio should be considered when studying the best methods for efficient control of the sandfly population.

The diversity calculated for each individual location was low, especially considering that the values for the Shannon-Weiner index varied from 0-5. However, the results obtained in 35% of the locations indicated a remarkable coexistence of several vector species in different locations. A high diversity was also observed in rural and fragmented locations while a greater dominance was observed in the forest, indicating the prevalence of a single species in this type of environment.

When zoophilic species are included in the analysis, we observed an increase of diversity in the forest environment, greater dominance in the fragmented environment and similarities between the rural and fragmented environments. This may be because *Lu. camposi* and *Lu. dysponeta* were more abundant in those environments and therefore affected the weighting of the index. Based on our experimental design, we cannot say if these two species or other zoophilic species could be involved in transmission of leishmaniasis. Further research into the vector competence and anthropophily rates of these species is required in order to determine if they participate

in the epidemiology of the disease. We have not found any reports of visceral leishmaniasis despite the presence of *Lu. longipalpis* found in the community of Limon; the isolation of *Lu. longipalpis* is interesting because little is known about this species in Panama. We also did not find any reports of visceral leishmaniasis related to cases of cutaneous leishmaniasis.

Sandfly species, especially those that transmit leishmaniasis, have a high tendency to dominate degraded environments despite microclimatic changes, demonstrating their ability to adapt (Peterson & Shaw 2003). The data on diversity and dominance of species in fragmented and rural environments of Panama obtained in this study suggest that these sandfly species are in the process of adapting to the gradual changes in landscape.

Therefore, human activities had a positive effect on vector species for leishmaniasis in Panama. Considering that there is no information on the diversity of sandflies in Panama prior to forest fragmentation, the results obtained in this study may serve as a baseline for future monitoring of the effects of the changing environment on the incidence of ACL in Panama.

Because the frequency of *Lu. panamensis*, *Lu. gomezi* and *Lu. trapidoi* varies between the different types of environment, it seems likely that the reduction and fragmentation of forested landscapes influence the abundance and the composition of sandfly species. These changes in the sandfly species could be due to migration from forested to fragmented environments or through the adaptation of sandflies to new habitats. Additionally, the association of *Lu. gomezi* and *Lu. trapidoi* with fragmented environments suggests that these species may be better able to adapt to environmental alterations. The association of *Lu. panamensis*, *Lu. olmeca bicolor* and *Lu. ylephiletor* with the forested landscapes indicates that these species may prefer the conditions of this environment.

In conclusion, multiple factors affect the distribution, abundance and diversity of sandflies. Fragmentation of forests due to increased agricultural activity in Panama may be influencing the geographic distribution of three species of sandflies that are vectors of ACL agents in the country

TABLE II

Comparison mean of sandfly vector trapped in three different characteristic landscapes in Panama, January-June 2010

Species	Landscape		
	Forest	Fragmented	Rural
<i>Lutzomyia panamensis</i> ^c	0.93 ^b	2.27 ^a	1.11 ^b
<i>Lutzomyia gomezi</i> ^c	0.27 ^b	1.59 ^a	0.93 ^a
<i>Lutzomyia trapidoi</i> ^c	0.47 ^b	1.98 ^a	0.41 ^b
<i>Lutzomyia ylephiletor</i>	0.04	0.09	0.04
<i>Lutzomyia sanguinaria</i>	0.01	0.21	0.04
<i>Lutzomyia olmeca bicolor</i>	0.04	0.1	0.09
<i>Lutzomyia ovallesi</i>	0	0.05	0.54
<i>Lutzomyia carrerai</i>	0	0.13	0
<i>Lutzomyia longipalpis</i>	0.05	0.05	0

a, b: distinct letters on the same line indicate significant differences in multiple comparison (alpha: 0.05); c: significant differences in Kruskal Wallis test (alpha: 0.05).

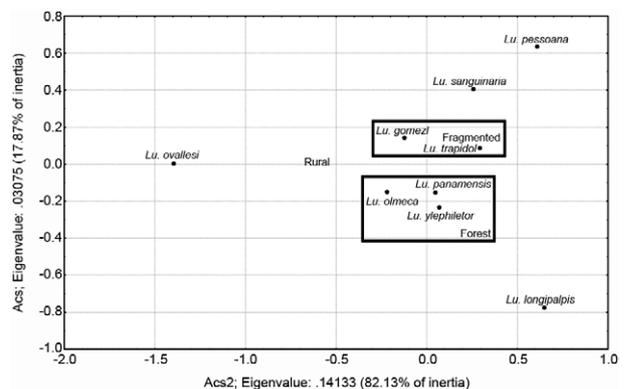


Fig. 2: simple correspondence plot showing the association of number of sandfly vector species (.) trapped for each characteristic landscape (forest, fragmented, rural) in Panama. Acs: analyses correspondence simple.

(*Lu. panamensis*, *Lu. gomezi* and *Lu. trapidoi*). Analysis of species abundance and diversity showed that more than one vector species is often present in rural and fragmented locations where there have been cases of leishmaniasis. Although there is an absence of historical data that would allow us to compare the past and present sandfly populations in a quantitative manner, this study allows us to describe the current sandfly population and will enable us to evaluate the long-term effects of deforestation on the sandfly population and transmission of leishmaniasis.

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REFERENCES

- Aguar GM, Medeiros WM 2003. Distribuição regional e habitats das espécies de flebotomíneos do Brasil. In EF Rangel, R Lainson, *Flebotomíneos do Brasil*, Fiocruz Rio de Janeiro, p. 207-255.
- ANAM - Autoridad Nacional del Ambiente 2000. *Primer informe de la riqueza y estado de la biodiversidad de Panamá*, ANAM, Panamá, 174 pp.
- ANAM - Autoridad Nacional del Ambiente 2009. *Informe del Estado del ambiente*, ANAM, Panamá, 155 p.
- Andrade Filho JD, Galati EAB, Falcão AL 2007. *Nyssomyia intermedia* (Lutz & Neiva, 1912) and *Nyssomyia neivai* (Pinto, 1926) (Diptera: Psychodidae: Phlebotominae) geographical distribution and epidemiological importance. *Mem Inst Oswaldo Cruz* 102: 481-487.
- Azpurua J, de la Cruz D, Valderrama A, Windsor D 2010. *Lutzomyia* sandfly diversity and rates of infection by *Wolbachia* and an exotic *Leishmania* species on Barro Colorado Island, Panama. *PLoS Negl Trop Dis* 4: e627.
- Carvalho GML, Falcão AL, Andrade Filho JD 2006. Taxonomic revision of phlebotomine sandfly species in the series *davisi* and *panamensis* of the subgenus *Psychodopygus* Mangabeira, 1941 (Diptera: Psychodidae: Phlebotominae). *Mem Inst Oswaldo Cruz* 101: 129-136.
- Chanotis BN, Correa MA, Tesh RB, Johnson KM 1971a. Daily and seasonal man-biting activity of Phlebotominae sandflies in Panama. *J Med Entomol* 8: 415-420.
- Christensen H 1972. Check list of the Phlebotominae sandflies (Diptera: Psychodidae) of Panama including two species not previously reported. *Mosq News* 32: 89.
- Christensen H, Fairchild GB, Herrer A, Johnson C, Young D, de Vásquez A 1983. The ecology of cutaneous, leishmaniasis in the Republic of Panama. *J Med Entomol* 20: 463-484.
- Christensen H, Johnson C, Vasquez AM 1984. Leishmaniasis cutánea en Panamá: un breve resumen. *Rev Med Panama* 9: 182-1987.
- Christensen HA, Herrer A, Telford SR 1972. Enzootic cutaneous leishmaniasis in eastern Panama. II. Entomological investigations. *Ann Trop Med Parasitol* 66: 55-66.
- Dias FO, Lorosa ES, Rebêlo JM 2003. Blood feeding sources and peridomiliation of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Psychodidae, Phlebotominae). *Cad Saude Publ* 19: 1373-1380.
- Grillet ME 2000. Factors associated with distribution of *Anopheles aquasalis* and *Anopheles oswaldoi* (Diptera: Culicidae) in a malarious area, northeastern Venezuela. *J Med Entomol* 37: 231-238.
- Herrer A 1971. *Leishmania hertigi* sp.n. from the tropical porcu-spine, *Coendou rothschildi*. *J Parasitol* 57: 626-629.
- Herrer A, Christensen HA 1973. Epidemiological patterns of cutaneous leishmaniasis in Panama. *Am J Trop Med Hyg* 25: 54-58.
- Herrer A, Christensen HA 1976. Natural cutaneous leishmaniasis among dogs in Panama. *Am J Trop Med Hyg* 25: 59-63.
- Herrer A, Telford S, Christensen HA 1971. Enzootic cutaneous leishmaniasis in eastern Panama I: investigation of the infection among forest mammals. *Ann Trop Med Parasitol* 65: 349-358.
- Jiménez AE, Rojas JC, Vargas F, Herrero MV 2000. Temporal and spatial variation of phlebotomine (Diptera: Psychodidae) community diversity in a cutaneous leishmaniasis endemic area of Costa Rica. *J Med Entomol* 37: 216-221.
- Magurran AE 1988. *Ecological diversity and its measurement*, Princeton University Press, Princeton, 179 pp.
- Méndez E 1999. *Insectos y otros artrópodos de importancia médica y veterinaria*, Impresora Pacífico, Panama, 45 pp.
- MINSA - Ministerio de Salud del Perú 2005. *Situación Nacional de Salud, Dirección Nacional de Políticas de Salud*, MINSA, Lima, 57 pp.
- Miranda A, Carrasco R, Paz H, Pascale JM, Samudio F, Saldaña A, Santamaría G, Mendoza Y, Calzada J 2009. Molecular epidemiology of American tegumentary leishmaniasis in Panama. *Am J Trop Med Hyg* 81: 565-571.
- Monteiro WM, Neitzke HC, Lonardon MV, Silveira TG, Ferreira ME, Teodoro U 2008. Distribuição geográfica e características epidemiológicas da leishmaniose tegumentar americana em áreas de colonização antiga do estado do Paraná, sul do Brasil. *Cad Saude Publ* 24: 1291-1303.
- Muniz LHG, Rossi RM, Nietzke HC, Monteiro WM, Teodoro U 2006. Estudo dos hábitos alimentares de flebotomíneos em área rural no sul do Brasil. *Rev Saude Publ* 19: 1087-1093.
- Oliveira AG, Galati EAB, Oliveira O, Oliveira GR, Espindola IAC, Dorval MEC, Brazil RP 2006. Abundance of *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae) and urban transmission of visceral leishmaniasis in Campo Grande, state of Mato Grosso do Sul, Brazil. *Mem Inst Oswaldo Cruz* 101: 869-874.
- Pech-May A, Escobedo-Ortegón FJ, Berzunza-Cruz M, Rebollar-Téllez EA 2010. Incrimination of four sandfly species previously unrecognized as vectors of *Leishmania* parasites in Mexico. *Med Vet Entomol* 24: 150-161.
- Peterson AT, Shaw JJ 2003. *Lutzomyia* vectors for cutaneous leishmaniasis in southern Brazil: ecological niche models, predicted geographic distributions and climate change effects. *Int J Parasitol* 33: 919-931.
- Rutledge LC, Ellenwood DA 1975. Production of Phlebotominae sandflies on the open forest floor in Panama: phytologic and edaphic relations. *Environm Entomol* 4: 83-89.
- Salomón OD, Quintana MG, Zaidenberg M 2008. Urban distribution of Phlebotominae in a cutaneous leishmaniasis focus, Argentina. *Mem Inst Oswaldo Cruz* 103: 282-287.
- Secombe AK, Ready PD, Huddleston LM 1993. *A catalogue of Old World Phlebotominae sandflies (Diptera: Psychodidae: Phlebotominae)*, The Natural History Museum, London, 57 pp.
- Service MW 1991. Agricultural development and arthropod-borne diseases: a review. *Rev Saude Publ* 25: 165-178.

- Souza GD, Santos E, Andrade Filho JD 2009. The first report of the main vector of visceral leishmaniasis in America, *Lutzomyia longipalpis* (Lutz & Neiva) (Diptera: Psychodidae: Phlebotominae), in the state of Rio Grande do Sul, Brazil. *Mem Inst Oswaldo Cruz* 104: 1181-1182.
- Souza RN, Lima JWO, Souza FV, Gadelha AC, Braga VSS 1999. Estabelecimento de um criadouro natural de *Lutzomyia longipalpis*. *Rev Soc Bras Med Trop* 32: 214-215.
- Sudia WD, Chamberlain RW 1962. Battery operated light trap, an improved model. *Mosq News* 22: 126-129.
- Tesh RB, Chanotis BN, Aronson MD, Johnson KM 1971. Natural host preferences of Panamanian Phlebotominae sandflies as determined by precipitin test. *Am J Trop Med Hyg* 20: 150-156.
- Travi BL, Alder GH, Lozano M, Cadena H, Montoya-Lerma J 2002. Impact of habitat degradation on Phlebotominae (Diptera: Psychodidae) of tropical dry forest in northern Colombia. *J Med Entomol* 39: 451-456.
- Virgens TM, Santos CB, Pinto IS, Silva KS, Leal FC, Falqueto A 2008. Phlebotominae sandflies (Diptera: Psychodidae) in an American tegumentary leishmaniasis transmission area in northern Espírito Santo state, Brazil. *Cad Saude Publ* 24: 2969-2978.
- Walsh JF, Molyneux DH, Birley MH 1993. Deforestation: effects on vector-borne disease. *Parasitol* 106: 55-75.
- Young DG, Arias JA 1992. *Flebótomos: vectores de leishmaniasis en las Américas*, Technical Report 33, Organización Pan-Americana de Salud, Washington, 28 pp.
- Young DG, Duncan MA 1994. Guide to the identification and geographic distribution of *Lutzomyia* sandflies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). *Mem Amer Ent Inst* 54: 1-881.