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Aspects of the bionomics of three phlebotomine vector species (Diptera: Psychodidae) at El Carrizal, an endemic focus of cutaneous leishmaniasis (CL) in the Venezuelan Andean Region

Bionomía de tres especies de flebótomos vectores (Diptera: Psychodidae) en El Carrizal, un foco endémico de leishmaniasis cutánea en la región Andina Venezolana

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SUMMARY

We studied some aspects of the bionomics of phlebotomine vectors of cutaneous leishmaniasis (CL) at El Carrizal, Municipality Tovar, Venezuela: *Lutzomyia youngi*, *Lutzomyia spinicrassa* and *Lutzomyia migonei*. Monthly catches were performed throughout 2008 using CDC and Shannon traps. We investigated: (i) species richness, (ii) relationships richness-traps-habitats (domestic, peri-domestic and forest), (iii) monthly fluctuations and relationship with climatic variables, (iv) relationship climatic factors-cutaneous leishmaniasis cases at El Carrizal and Municipality Tovar (2000-2008). Females in the Verrucarum group and males of *Lu. youngi* prevailed. Catches with CDC revealed the predominance of *Lu. migonei* outdoors where probably they maintains cutaneous leishmaniasis transmission, while females in the Verrucarum group would contribute to maintain it indoors. The greatest abundance of the three species occurred in November after the heaviest peak of rains. The correlation species-climate for *Lu. migonei* indicated that temperature seems a limiting factor and precipitation a favouring factor, which agreed with results obtained through Ecological Niche Modelling of this species over 23 records at national level. The correlation cutaneous leishmaniasis incidence-climate showed that the greatest number of cases tends to occur, with a month lag, in the months of most rainfall. These data may support the Ministry of Health, since they provide robust information about "when", "where" and "how" to implement target phlebotomine vectors control methods in the Andean Region of Venezuela.

Key words: *Lutzomyia youngi*, *Lutzomyia spinicrassa*, *Lutzomyia migonei*, bionomics, climate, cutaneous leishmaniasis.

RESUMEN

Se estudiaron algunos aspectos de la bionomía de vectores de leishmaniasis cutánea en la localidad El Carrizal, Municipio Tovar, Venezuela: Lutzomyia youngi*,* Lutzomyia spinicrassa *y* Lutzomyia migonei. *Las capturas mensuales se realizaron a lo largo del año 2008 usando trampas CDC y Shannon. Se investigó: (i) riqueza de especies, (ii) relaciones riqueza-trampa-hábitat (doméstico, peri-doméstico y selvático), (iii) fluctuaciones mensuales y relación con variables climáticas, (iv) relación factores climáticos-casos de leishmaniasis cutánea en El Carrizal y Tovar (2000-2008). Predominaron las hembras del grupo Verrucarum y los machos de* Lu. youngi. *Las capturas con CDC revelaron el predominio de* Lu. migonei *en el habitat peridoméstico donde probablemente mantienen la transmisión, mientras que las hembras del grupo Verrucarum contribuirían a mantenerla dentro de las casas. La mayor abundancia de las tres especies ocurrió en noviembre después del pico más alto de lluvias. La correlación especie-clima para* Lu. migonei *indicó que la temperatura parece ser un factor limitante y la precipitación un factor favorecedor, lo cual coincidió con los resultados obtenidos a través de la Modelación de Nichos Ecológicóos de esta especie sobre 23 registros a nivel nacional. La correlación de incidencia de leishmaniasis cutánea-clima mostró que el mayor número de casos tiende a ocurrir, con un mes de retraso, en los meses de mayor precipitación. Estos datos pueden ser utiles al Ministerio de Salud ya que proveen información sólida sobre "cuándo", "dónde" y "cómo" implementar métodos de control de vectores en la Región Andina de Venezuela.*

Palabras clave: Lutzomyia youngi*,* Lutzomyia spinicrassa*,* Lutzomyia migonei*, bionomía, clima, leishmaniasis cutánea.*

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INTRODUCTION

Leishmaniases are parasitic diseases caused by Protozoa belonging to *Leishmania* genus. In the New World, 15 out of 22 *Leishmania* species, from wild and domestic reservoirs animals, are transmitted to humans by the bite of infected plebotomine sand flies of the genus Lutzomyia whose outcome may give rise to clinical forms of cutaneous (CL) and visceral leishmaniasis (VL).

WHO (http://www.who.int/leishmaniasis/burden/en/ (Consulted 03 Aug 2016) reports that each year an average of 60,000.00 new CL cases are diagnosed. During 2013-2014, Colombia, Brazil and Venezuela have been recognized among the nine countries with high, intense and very intense *Leishmania* transmission. In relation to phlebotomine sandflies, 56 out of about 500 species are suspected or proven vectors in Latin America and 12 of about 100 have been related to CL transmission in Venezuela (Maroli *et al.*, 2013).

In Venezuela, the Control Program of leishmaniases, under the direction of the Institute of Biomedicine of the Ministry of Health (MPPS), is entirely focused on the diagnosis and treatment of patients. In relation to CL the main weaknesses are late diagnosis and underreporting that are difficult to be estimated. Moreover, for logistic problems, like shortage of personnel, the program does not reach remote rural areas (De Lima *et al.*, 2010). On the other hand, the vector control of insects of medical importance is competence and responsibility of the General Direction of Health and Environment of the MPPS, but, aside other priorities like Malaria, Dengue and Chagas' disease, regarding *Leishmania spp.* vectors, the greatest weakness in establishing a Program, is due to the fact that several aspects on their bionomics are quite scarce and scattered throughout the country in different ecological and epidemiological scenarios (Feliciangeli, 2014). Considering the above, an entomological survey was carried out in 2008 with the aim of studying aspects of the bionomics of three CL vector species, *Lutzomyia youngi* Feliciangeli & Murillo, *Lu. spinicrassa* Morales, Osorno-Mesa, Osorno & Muñoz and *Lu. migonei* (França) at El Carrizal village, an ancient endemic focus of CL in the Muncipality Tovar, Mérida state, in the Venezuelan Andean region which includes also Trujillo state and Táchira state and only small parts of two municipalities of Barinas State. Infection by *Leishmania braziliensis* was found in *Lu. youngi* (= *Lu. townsendi*) in Trujllo (Scorza *et al.*, 1984; Añez *et al.*, 1994), and in *Lu. spinicrassa* in Táchira State (Perruolo *et al.*, 2006). Moreover, it had been found infected by the same parasite near Las Arboledas, in the Department of Norte de Santander, Colombia (Young *et al.*, 1987). In Venezuela *Lu. migonei* was found infected by promastigotes thought to be *Le. braziliensis* by Pifano & Ortiz (1952) in Yaracuy State. As far as we know, no more records of *Leishmania* natural infection of *Lu. migonei* was incriminated as potential vector of *Le. mexicana* and *Le. guyanensis* (Feliciangeli *et al.*, 2011). In Brazil ancient records have been reviewed by Rangel & Lainson (2009), including the molecular identification of *Le. braziliensis* in this species by Pita Pereira *et al.* (2005). Moreover, *Lu. migonei* has been associated to CL transmission in Argentina (Salomón. *et al.*, 2008, 2009).

The aspects considered in our study have been: (i) species composition and richness; (ii) their population dynamics from the forest, their primary habitat, to the peri-domestic and domestic habitats and the relationship with each of them; (iii) the relationship species-climatic factors. Since species composition and richness, as well as monthly fluctuations in different habitats, and the occurrence of CL are in turn deeply influenced by such factors, an attempt was also made to relate the occurrence of CL cases with climatic factors during the period 2000-2008 in the Municipality Tovar. It is important to say that subsequent visits and phlebotomine catches at the village have highlighted that no substantial ecological and social alterations have occurred in the last years. This situation is common in the Andean region in small villages of high altitude, where people are much attached to their traditions and lands where, in addition to coffee, they can produce their own food through small harvests of vegetables, bananas, beans, corn and the breeding of domestic animals. The exodus from the field to the big cities in general has manifested itself in the past years in the plains, more arid and less productive.

MATERIAL AND METHODS

Study area

The entomological monitoring was carried out at El Carrizal village (08°17´63"N, 7°45'75"W),

Municipality of Tovar, in Merida state (Fig. 1). From 2003 to 2007, Mérida state accounted for 785 CL cases with an incidence rate of 19.52 cases x 100,000 inhabitants, the highest number of CL cases after Trujillo State (De Lima *et al.*, 2010). Of the total CL cases, 93 (11.85%) were recorded by the Service of Dermatology of the Municipality Tovar, the majority of them (24.73%) were from El Carrizal. This village is located at 1,300 m a.s.l. in a rural area. In 2007 it consisted of 152 houses and 624 inhabitants. Basic services such as medical ambulatory, primary school, church, electricity, piped drinking water and a sewer system and poor roads (narrow, unpaved, impaired) characterized the village.

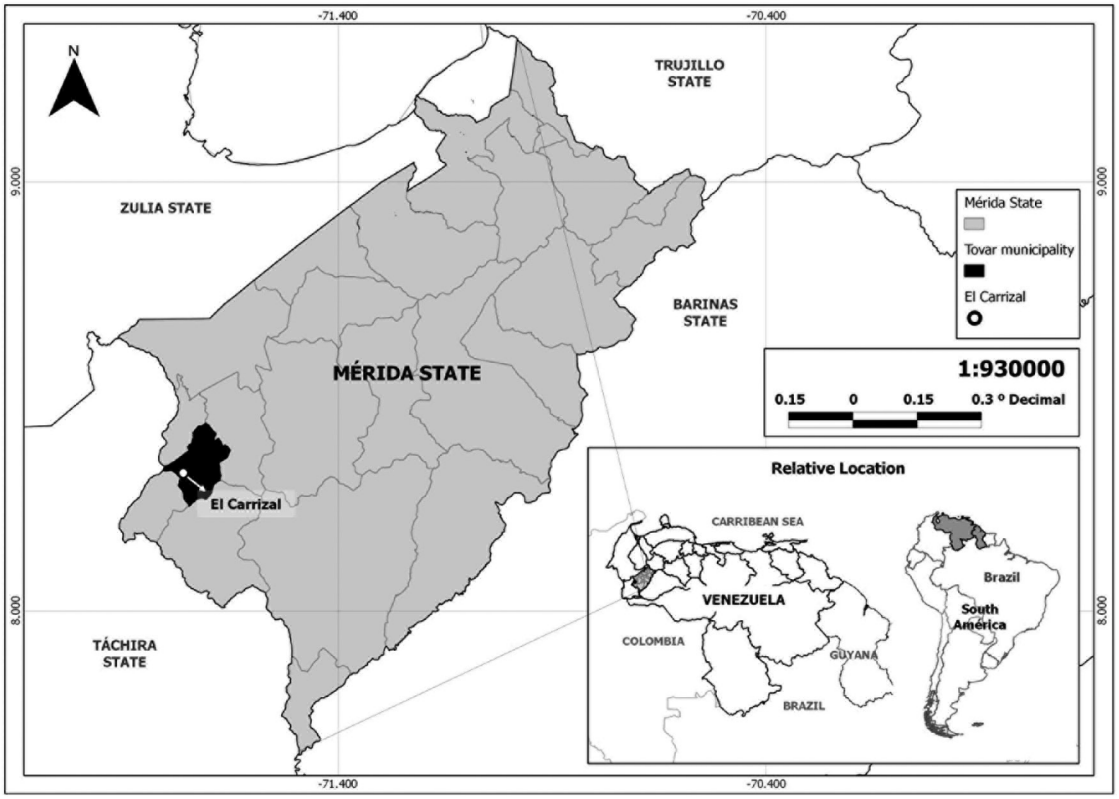
Being the landscape similar along all the village, for the entomological study five houses spaced throughout it, were selected at random in between the ones at the extremes (08° 16' N; 71°, 45'W - 08° 17'N; 71° 45' W and 1,211 to 1,452 m a.s.l.).

Phlebotomine collections and species identification

Monthly phlebotomine collections were carried out from January to December 2008. Catches were performed by using miniature CDC light traps (John W. Hock Company, USA) and Shannon traps. Three CDC light traps were placed overnight (from18:00-19:00 to 6:00-7:00) the last week of each month (Monday to Thursday) in each of the enrolled houses. It took about one hour to place them and in the same order the traps were collected the day after. Domestic, peri-domestic and sylvatic habitats were monitored for the sand fly presence. The monthly catches were correlated with climatic variables throughout the year 2008.

Domestic habitat. In agreement with the homeowners, one CDC light trap was placed in the sleeping room or, when people preferred, in a contiguous room, given that all the internal walls did not reach the ceiling.

Fig 1. Map showing the relative location of El Carrizal, Municipality Tovar, Mérida state and Venezuela in South America.



Peri-domestic habitat. In the peri-domestic habitat, the CDC light trap was sited within 5-20 m from the house, close to the resting places of domestic animals, mainly chickens and dogs and in less proportion, pigs and cats. The inhabitants also reported the occasional presence of rodents and opossums (*Didelphys marsupialis*) around dwellings.

Sylvatic habitat. The 3rd CDC light trap was placed into the forest in a minimum radius of 100 m from the house avoiding interference with the CDC light trap. As in many villages in the Andean region primary vegetation close to the dwellings, has been heavily intervened to establish coffee crops. The remaining large trees still provide protected microhabitats for mature and small and thin trees, like Inga edulis, are grown to provide shadow for coffee plantations whose fruits are an important source of sugars for *Lu. youngi* (= *Lu. townsendi*) (Scorza *et al.*, 1985), while leaf litter appeared to be a good substrate for breeding sites (Alexander *et al.*, 1992; Perruolo, 2004).

When the weather was propitious, in the same days of CDC light traps collection, one Shannon trap was also used for two hours, between 7-8 to 9-10 pm. The trap was placed in the forest in a minimum radius of 100 -150 m from a house.

Phlebotomine identification - The day after each capture, some batches of live specimens from abundant catches were kept in plastic boxes inside polystyrene containers for further studies (natural infection and morphometry). Dead specimens were stored in vials containing 100% ethyl alcohol. In the field, each batch was coded by house, date, trap and habitat. When in the laboratory, males and females were separated in Petri dishes and a reliable method that had shown to be practical for a quick sand fly identification in abundant and long-term entomological studies, was used (Feliciangeli *et al.*, 1999). Briefly, each batch was passed from ethyl alcohol in distilled water and then each specimen was put individually in a small drop on a slide (usually ten per slide) of diluted phenol to allow a quick clarification. Genitalia, genital pump and filaments and the aedeagus in males; pharynx, cibarium, horizontal and vertical teeth, spermathecae and spermathecal ducts in females, were observed under 250x and 400x magnifications, using keys by Young & Duncan (1994). New batches were then performed and stored by species, sex and all the data of each collection.

Meteorological data - Monthly meteorological data, minimum, mean and maximum temperature in ºC (Min, Mean, Max T), precipitation as total rainfall in mm (P) and minimum, mean and maximum percentage of relative humidity (Min, Mean, Max RH) from the nearest meteorological station (08º36' N; 71º11' W; 1,479 m a.s.l.) were provided by the Venezuelan Air Force (FAV).

CL cases reports

The occurrence of CL cases during the period 2000-2008 in the Municipality Tovar, as usually reported by the Services of Dermatology at national level, was assigned to the month based on the date referred by the patients about the appearance of the lesion plus one month lag as for the period of incubation.

Data analysis

Absolute and percentage compositions of the phlebotomine fauna by species, sex, trap, habitat and month of capture were analyzed. A frequency analysis of catches per each variable, which included the descriptive analysis of frequency and / or proportions, was performed and the chi-square test was applied to verify whether there was a significant relationship between the frequencies observed and expected for each variable. In those cases in which the association was significant, the bi-plot graph of simple correspondence analysis was built up to characterize the association found.

In addition, using the chi-square test, it was verified if the population distribution of the catch was homogeneous throughout the year. The significance level of 5% was selected. The intervals were constructed at 95% confidence for proportions. If the catch was not homogeneous, the linear correlation analysis using the Pearson correlation coefficient between the frequency of catches and climatic variables, temperature (average, maximum, minimum), relative humidity (average, max, min) and accumulated precipitation, would be carried out. Significance level of 10% was used for Pearson correlations.

Data were analyzed by using StatXact 8.0 for Windows, Minitab and SPSS 14.3 software 15.0 for windows.

RESULTS

Phlebotomine species richness and relationship species-traps-habitats - During the whole study 698 sandfly catches with CDC traps (227 indoors, 235 outdoors and 236 in the forest) and 40 with Shannon traps were made, which yielded a total of 6,827 females and 3,060 males. At least 10 sandfly species were present at El Carrizal (Table I). This was revealed by the occurrence of males of three cryptic species in the Verrucarum group, series *townsendi*: *Lu. youngi*, *Lu. spinicrassa* and *Lu. sauroida*, whose females are morphologically indistinguishable. These constituted an overwhelming amount in relation with the rest of the females. Among males in the Verrucarum group (n=1,838), *Lu. youngi* accounted for 90.64%, *Lu. spinicrassa* 9.25% and of *Lu. sauroida* 0.11%. The sex ratio of *Lu. migonei* was 2.7 ♂: 1♀.

In Table II we show the frequency distribution of catches of all species by sex and trap in each habitat. Among species in the Verrucarum group, only one male and no females of *Lu. sauroida* were caught. Therefore, as females in the Verrucarum group with males of *Lu. youngi* and *Lu. spinicrassa* and males and females of *Lu. migonei*, represented the 98.85% of the total population, the statistical analysis was focused on these three vector species.

Because of their different behavior, the chi-square test of independence was applied separately for females and males. In relation to the catches with the CDC traps in the three habitats, for females, the test showed significant association between the species and the environment where the trap was placed ($\chi^2 = 238.06$, d.f. = 2, $P < 0.0001$). The bi-plot graph (Fig. 2A) shows that those in the Verrucarum Group can be found in any of the three habitats although with this trap there is a greater association to the indoor habitat (1,259/2,888; 43.59%). In contrast, females of *Lu. migonei* are strongly associated with the peri-domestic habitat (283/432, 65.51%) and in less extent to the forest (128/432, 29.63 %). As for males, the chi-square test also showed significant association for each species and the environment in which the trap was placed (χ^2= 511.99, d.f.= 3; P <0.0001) (Fig. 2B) with a clear relationship of males of the species *Lu. migonei* and the peri-domestic habitat. In fact the comparison of proportions between species pairs (Table III) showed no significant differences between the proportions of males of *Lu. youngi* with those of *Lu. spinicrassa* in the three habitats. In contrast, *Lu. migonei* clearly predominated outdoors in comparison with the other two species, since significant less numbers of males of this species were caught indoors and in the forest in relation to males of *Lu. youngi* and *Lu. spinicrassa*.

When comparing the attraction of CDC and Shannon traps in the forest, the chi-square test

Table I. Species composition and abundance of phlebotomine sandflies collected during January-December 2008 at El Carrizal, Mérida State, Venezuela.

Species	♀	%	♂	%
Verrucarum group*	6,292	92.16	--	--
*L. youngi***	--	--	1,666	54.44
*L. spinicrassa***	--	--	170	5.56
*L. sauroida***	--	--	2	0.07
L. migonei	446	6.53	1,199	39.18
L. lichyi	61	0.89	7	0.23
L. nuneztovari	25	0.37	13	0.42
L. venezuelensis	1	0.01	1	0.03
L. dubitans	1	0.01	1	0.03
L. sp.	1	0.01	0	0.00
B. beaupertuyi	0	0.00	1	0.03
Total	6,827	100	3,060	100

* Females in the Verrucarum group morphologically indistinguishable
** Males in the Verrucarum group present in the area.

Table II.Sandfly species caught at El Carrizal (January-December 2008) by traps and habitats.

Species	CDC traps									Shannon trap		
	Indoors			Outdoors			Field			Field		
	♂	♀	total	♂	♀	total	♂	♀	total	♂	♀	total
Verrucarum group	-	1,259	1,259	-	1,112	1,112	-	517	517	-	3,404	3,404
L. youngi	492	-	492	668	-	668	350	-	350	156	-	156
L. spinicrassa	53	-	53	62	-	62	42	-	42	13	-	13
L. sauroida	0	0	0	1	0	1	0	0	0	1	0	1
L. migonei	29	21	50	962	283	1,245	168	128	296	40	14	54
L. lichyi	2	6	8	1	7	8	4	14	18	0	34	34
L. nuneztovari	1	7	8	10	13	23	1	3	4	1	2	3
L. venezuelensis	0	0	0	1	0	1	0	1	1	0	0	0
L. dubitans	0	0	0	1	0	1	0	1	1	0	0	0
B. beaupertuyi	0	0	0	1	0	1	0	0	0	0	0	0
L. sp.	0	0	0	0	0	0	0	0	0	0	1	1
Total	577	1,293	1,870	1,707	1,415	3,122	565	664	1,229	211	3,455	3,666

Table III. Tests of comparison of proportions by species - habitat between males of CL sandfly vector species caught by CDC light traps at El Carrizal, Merida State, Venezuela.

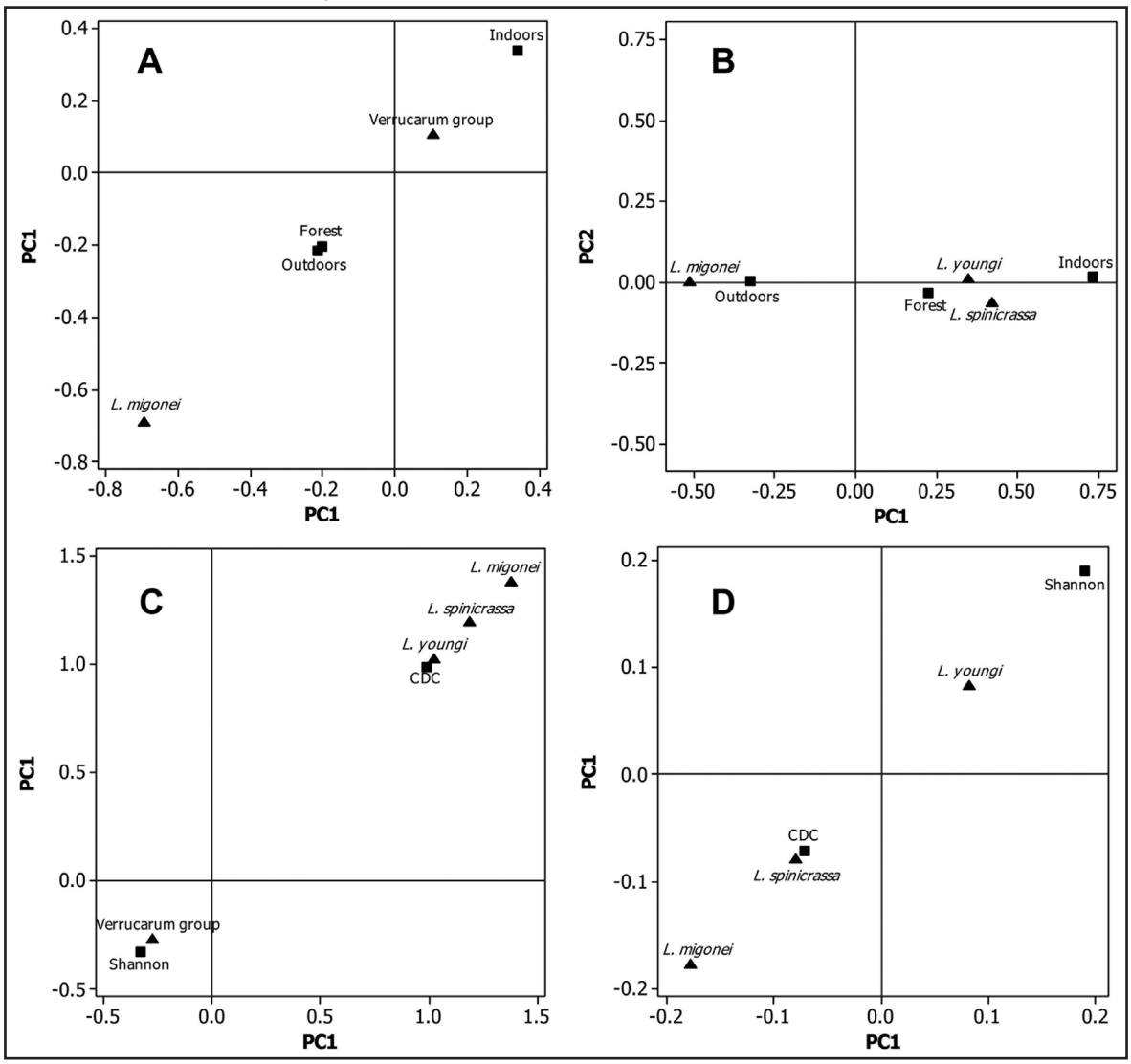
Species	Habitat	n	Total /habitats	%	χ^2	d.f.	*P*
* *L. youngi*	indoors	492	1,510	32.58	*° 0.09	1	0.765127
° *L. spinicrassa*	" "	53	157	33.76	*# 377.67	1	0.000000
# *L. migonei*	" "	29	1,159	2.50	°# 231.19	1	0.000000
* *L. youngi*	outdoors	668	1,510	44.24	*° 1.30	1	0.253764
° *L. spinicrassa*	" "	62	157	39.49	*# 414.45	1	0.000000
# *L. migonei*	" "	962	1,159	83.00	°# 151.53	1	0.000000
* *L. youngi*	forest	350	1,510	23.18	*° 1.01	1	0.315067
° *L. spinicrassa*	" "	42	157	26.07	*# 31.61	1	0.000000
# *L. migonei*	" "	168	1,159	14.50	°# 15.49	1	0.000008

of independence showed a significant association of the species with the trap used (χ^2 = 1,449.94, d.f,= 3; *P* <0.0001). This evidenced a strong attraction of females in the Verrucarum group (3,404/3,921; 86.81%) towards the Shannon trap (χ^2 = 607.69; d.f.= 1; *P* <0.0001) (Fig. 2C) with an indirect relative risk OR = 60.1978 indicating that it is much more likely to capture an individual of this species group in the field with Shannon trap rather the CDC trap. In relation to males, *Lu. migonei* and *Lu. spinicrassa* were significantly more trapped by CDC trap, while *Lu. youngi* showed an independent behaviour as to the traps (Fig. 2D, Table IV).

Phlebotomine species - climatic factors relationship at El Carrizal (2008) and monthly CL cases-climatic factors relationship at the Municipality Tovar (2000-2008)

Because of the bias created by the Verrucarum group females, with overwhelming numbers collected using the Shannon trap, and with the aim of this work to focus the intrusion of vector species from their wild primary habitat to the anthropogenic ones (outdoors and indoors), we show in Fig. 3 the monthly species fluctuations of collections by CDC traps. The graph illustrates that females in the Verrucarum group displayed the same

Fig 2. Bi-plot graph showing: (1) the relationship between sandflies caught by the CDC trap per species and habitat: A, females, B, males; (2) between sandflies caught by CDC and Shannon traps in the forest C, females and males, D, only males (El Carrizal, Mérida State, Venezuela).



trend in all the habitats with three peaks of greatest abundance along the year.

Results of the homogeneity chi-square test for the frequency of monthly catches across the species by trap and sex showed non homogeneous frequency distribution throughout the year, being all $P < 0.0001$. Significant correlations (positive and negative) between catch rates per species [individuals / trap / month] with climatic variables: temperature in ºC (Min, Mean, Max T), precipitation , and percentage of relative humidity (Min, Mean, Max RH) are shown in Table V. It is to be noticed that they were obtained at the 10% level. This level of significance was considered instead of the usual 5% due to the small sample size used in the correlation analysis as the monthly catch rates were considered by species, habitat and trap.

Because no cases were recorded in the village El Carrizal in 2008, we attempted to correlate the monthly incidence of cases reported in El Carrizal and in the municipality Tovar, with climatic factors during the period 2000 – 2008. Fig. 4 shows that,

Table IV. Tests of comparison of proportions of males of sandfly vector species caught by CDC traps and Shannon traps in the sylvatic habitat at El Carrizal, Merida State, Venezuela.

Species	Trap	n	total n	%	Trap	n	total n	%	d.f.	χ^2	*P*
L. youngi	CDC	350	560	62.50	Shannon	156	209	74.64	1	9.97	0.001591
L. spinicrassa	"	42	560	7.50	"	13	209	6.22	1	0.38	0.540047
L. migonei	"	168	560	30.00	"	40	209	19.14	1	9.10	0.002557

Table V. Values of Pearson correlations that were significant to 10% between rates of monthly catches [individuals / trap] and climatic variables.

Traps	Species	Habitat	MinT	MeanT	MaxT	*P*	MinRH	MaxRH
CDC	*L. migonei*	- all		-0.601				
		- indoors	-0.587	-0.660				
		- outdoors		-0.530				
		- forest	-0.701	-0.689				
	L. spinicrassa	- all					0.557	
		- indoors					0.546	
		- forest				0.501	0.551	
	L. youngi	- all					0.537	
		- indoors					0.510	
		- outdoors					0.516	
		- forest					0.523	
	Verrucarum g.	- all			-0.556			0.548
		- indoors						0.532
		- outdoors		-0.517	-0.501			
Shannon	*L. migonei*	- forest	-0.594	-0.566		0.498		
	L. spinicrassa							
	L. youngi			-0.531				
	Verrucarum g.							0.499

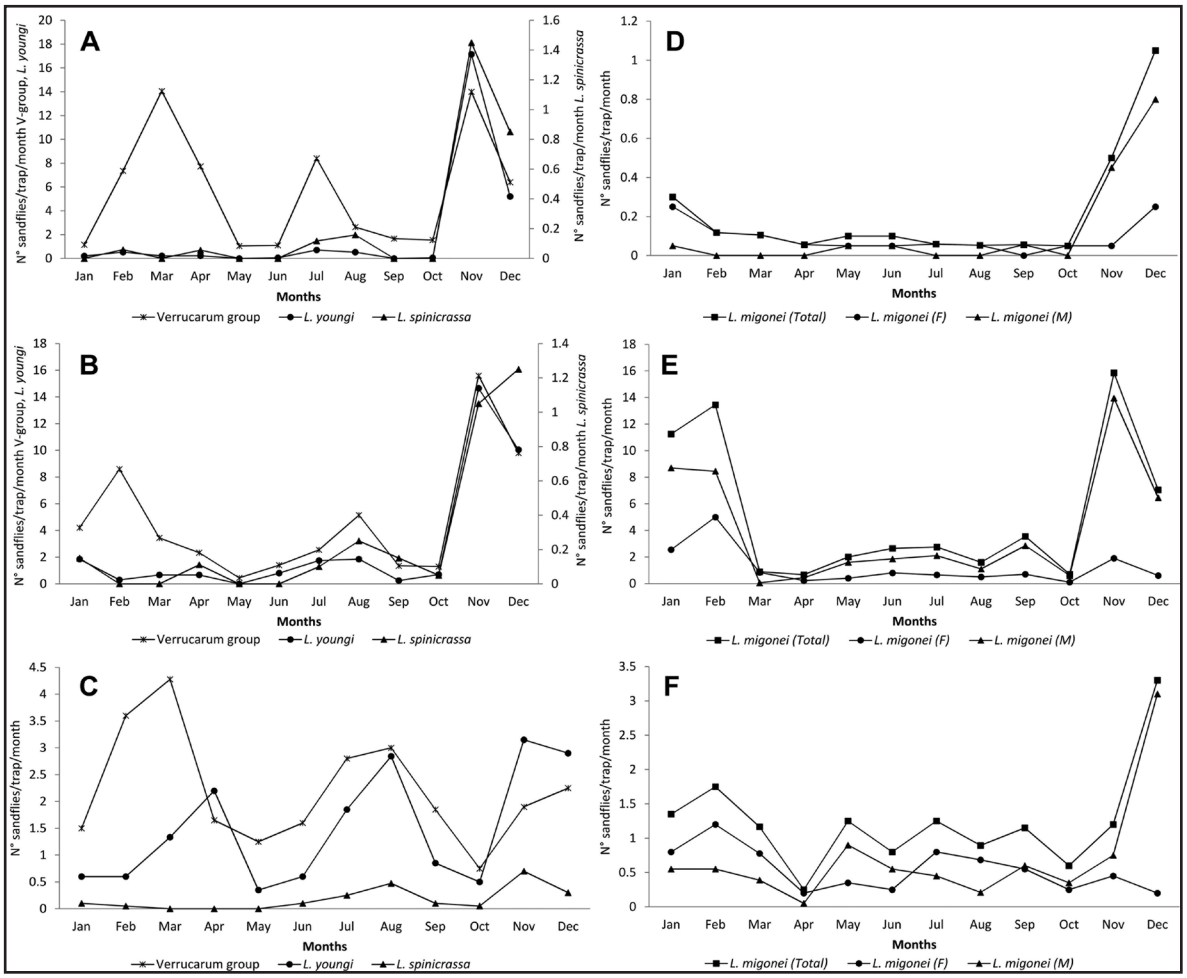
at El Carrizal the number of CL cases was 27.15% (41/151) of the total recorded in Tovar. The monthly incidence was not homogeneous throughout the period considered (2000-2008) in fact, among 2002 – 2004, occurred 69.54% (105/151) of cases in the municipality Tovar and 63.41% (26/41) in the town of El Carrizal. In both, the number of CL cases showed an approximate match with the maximum precipitation.

The Pearson correlation analysis showed that cases of the municipality Tovar one month lag gave 10% significant positive correlation with the variables Max RH (r = 0.5002,*P* = 0.0977), Mean RH (r = 0.5927, *P* = 0.0423), Min T (r = 0.5557, *P*= 0.0607) and *P* (r = 0.8023, *P* = 0.0017); being the strongest association with the precipitation. However it should be noted that the variable Min RH showed significant positive correlation with the frequency of cases with no lag in the municipality Tovar (r = 0.5359, *P* = 0.0726). For the village El Carrizal only was found significant positive correlation with one month lag between the number of CL cases and the precipitation (r = 0.5718, *P* = 0.0521).

DISCUSSION

Lutzomyia youngi and *Lu. spinicrassa* are cryptic species in the Verrucarum group, series townsendi (Feliciangeli *et al.*, 1992, Young &

Fig 3. Monthly fluctuations of species vectors: Verrucarum group females and males: A, indoors, B, outdoors, C, forest; *Lu. migonei:* D, indoors, E, outdoors, F, forest (El Carrizal, Mérida State, Venezuela).

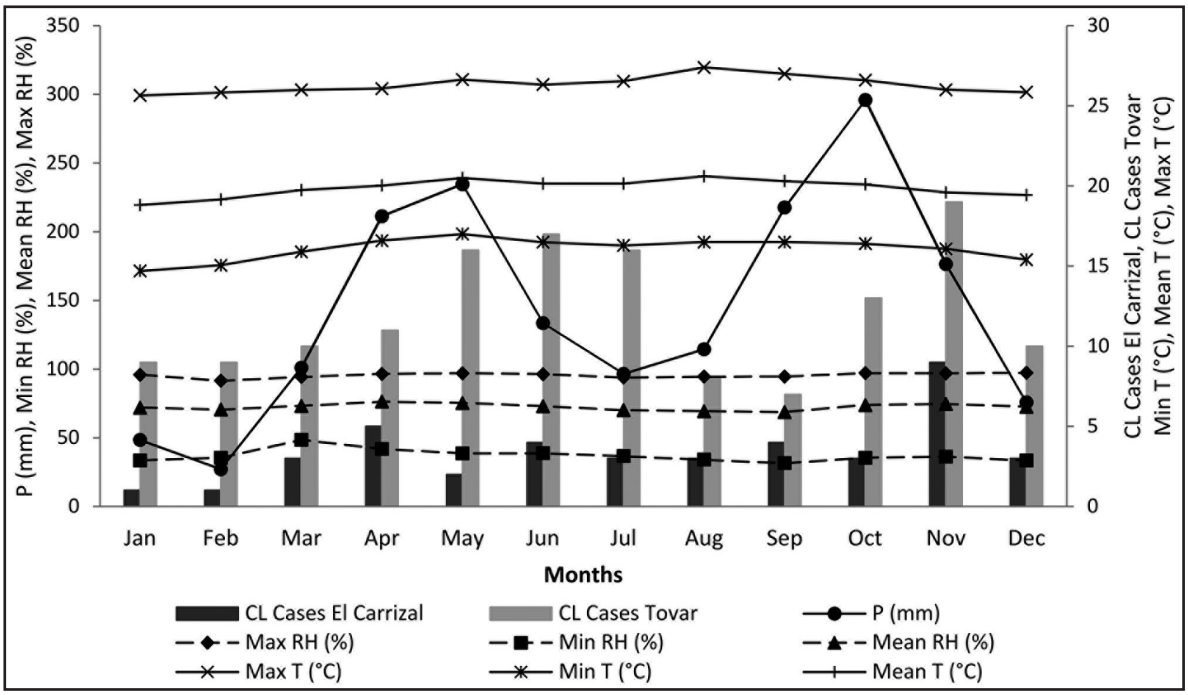


Duncan, 1994). Attempts have been made to identify them by morphometry (Añez *et al.*, 1997), isoenzymes (Kreutzer *et al.*,1990) and molecular tools (Adamson *et al.*, 1993; Testa *et al.*, 2001), but they have not reached conclusive results in terms of their application for the identification of large entomological samples.

So far *Lu. youngi* has been recorded in Venezuela, Colombia and Costa Rica, while *Lu. spinicrassa* seems to be restricted to Colombia and Venezuela (Young & Duncan, 1994, Bejarano & Estrada, 2016). In Colombia, Ferro *et al.* (2015) report that *Lu. youngi* is distributed over the Andean region, with some records observed through the Pacific region, while *Lu. spinicrassa* has been only registered in the north-eastern Andean Region, close to Venezuela.

Lutzomyia migonei is widely spread in Latin America (Young & Duncan, 1994, Bejarano & Estrada, 2016). In Venezuela it has been so far recorded in ten out of 24 Federal Entities (Sanchez *et al.*, 2015). It calls the attention that in Colombia the distribution of *Lu. migonei* is limited only to five out of 32 Departments (Bejarano & Estrada 2016) and it is not reported in the list of the 21 incriminated vectors of leishmaniases (Ferro *et al.*, 2015). An extensive review on the eco-epidemiology of *Lu. migonei* in Brazil where it is largely distributed, has been reported by Rangel &

Fig 4. Monthly CL cases and monthly means of climatic variables during the years 2000-2008 (El Carrizal, Mérida State, Venezuela).



Lainson (2009). Here we discuss some aspects on the bionomics of these species in relation to these and other subsequent works.

Species richness, sex ratio and relationship species-trap-habitat ar El Carrizal, Municipality Tovar, Mérida State

For this study we selected CDC and a Shannon trap. In fact, phototropism is strictly related to antropophily, which is the 1st requisite for a sandfly to be a vector (Maroli *et al.*, 1997). Moreover, CDC traps are actually the only method that allows the follow-up of sandfly dynamics from the forest to the houses in order to know which species would contribute to domestic and peri-domestic transmission, that is one of the more important epidemiological changes of CL reported in Latin America (Campbell-Lendrum *et al.*, 2001, Maroli *et al.*, 2013). On the other hand, Shannon trap were used because they allow the collection in the field of larger numbers of phototropic-antropophilic species for additional biological studies (natural infection, food sources, breeding etc.).

Therefore, we are aware that, due to the limited collecting methods used, the 10 sandfly species caught at El Carrizal would not reflect the species composition of the sandfly fauna in this CL focus. As said above, because of their predominance and epidemiological importance, we focused this study on the species in the Verrucarum group, *Lu. youngi* and *Lu. spinicrassa*, sympatric in the Merida State, and *Lu. migonei*.

Using CDC and Shannon traps at El Carrizal, females in the Verrucarum group were 92.34% of the total females. *Lu. youngi* males accounted for the 54.25 % of the total males and were 10 times more abundant than *L. spinicrassa*. The clearest relationship between Verrucarum group females and *Lu. youngi* males was noticed in the forest, while, with the exception of the months of highest capture, *Lu. spinicrassa* males presented catch rates near zero almost throughout the year. The relationship in between females and males of *Lu. migonei* was also clear in the field and in the peri-domestic habitat, where the counts were greatest. On the other hand, it draws attention the fact that while the behavior of the three species looks distinctive, all reached the maximum capture rate in November, indicating that during that month the sandfly population is highest in the study area.

In an allopatric population of *Lu. youngi*, in a CL focus in Lara State, Las Maticas, (1,320 m.a.s.l.) *Lu. youngi* (n= 9,831), represented 95.4% of the total phlebotomine sandflies caught and the sex ratio males: females was 1: 7,07 (Traviezo, 2006). On the other hand, in an allopatric population of *Lu. spinicrassa* in the village of Catárnica (1,300 m.a.s.l.) in Táchira state, *Lu. spinicrassa* (n=7,322), was the predominant species (80.4%) with a sex ratio males: females 1: 1.41 (Perruolo, 2004). This information seems to indicate that in allopatric conditions, the populations of *Lu. youngi* and *Lu. spinicrassa* are predominant over the other species present in the area, but as for the sex ratio, *Lu. youngi* females would overwhelm males, while for *Lu. spinicrassa*, this proportion would be about the same. So, among the sympatric populations at El Carrizal, where *Lu. spinicrassa* males accounted only for about 10% of the amount of *Lu. youngi*, we would expect that *Lu. spinicrassa* females would be present in a similar proportion.

As documented above, because of ecological–anthropologic environmental fragmentation and degradation, mainly due in the Andean region to deforestation for coffee economical exploitation, sandfly vector species have been adapted to peri-domestic and domestic habitats. With CDC traps, it was evidenced that, as expected, because of phototropism and search of blood sources *Lu. youngi*, *Lu. spinicrassa* and *Lu. migonei*, from the sylvatic habitat invade peri-domestic and domestic habitats. At El Carrizal significantly more females in the Verrucarum group (*Lu. youngi* and *Lu. spinicrassa*) were caught indoors. In contrast, the highest catches of *Lu. migonei* females as males were clearly associated with outdoors. The behavior of males helped to clarify the situation, in fact, indoors and in the forest, both, males of *Lu. youngi* and *Lu. spinicrassa*, were significantly more numerous in relation to *Lu. migonei* but not significantly different between them. In contrast, in the peri-domestic habitat, *Lu. migonei* males significantly exceeded those of the two species in the Verrucarum group. This would indicate that *Lu. migonei* might have adapted to this habitat probably because they have established their breeding sites there. This hypothesis might also be supported by the fact that males, which emerge before females, would easily find them to mate in the peri-domestic area. That is why they are caught indoors in significantly smaller numbers in relation to males in the Verrucarum group, who, more attracted by pheromones of females, would enter indoors in order to mate. A greatest propensity of *Lu. youngi* to enter houses was also observed by Alexander *et al.* (1995) in the CL focus of La Guaira in the Colombian department of Valle del Cauca throughout high catches by CDC traps and oil sticky papers.

As phlebotomine breeding sites are terrestrial, they are widely dispersed (Feliciangeli, 2004). Therefore, knowledge on this matter in general is limited and, as far as we know, no findings of immature stages of these two species in the Verrucarum group have been reported. In contrast, recent efforts on the search of breeding sites made in Argentina and Brazil have given useful information that supports our results in relation to *Lu. migonei*. In the forest of dry Chaco, Argentina, using emergency traps, only one specimen was recovered from 127 soil samples in areas of CL, at the base of a bromeliad (100 m from the houses) (Parras *et al.*, 2012). However, in Southeastern Brazil, Vieira e *et al.* (2012) found breeding sites of *Lu. migonei* between 20 and 60 m around houses and estimated and predicted the maintenance of a large population of this species in this habitat, independent from the forest. In Colombia, Vivero *et al.* (2015) recovered. *Lu. migonei* from soil samples collected at the base and tubular roots of trees at the Natural reserve Coraza, in tropical dry forest, but not in the Natural reserve Rio Claro in the Andean region, a tropical humid forest. On the other hand, Reinhold-Castro *et al.* (2015) found one *Lu. migonei* out of 21 adults emerged from 122 incubated soil samples in Recanto Amarista, state of Paraná, Brazil, from the roots of trees, but none from soil samples collected from hen houses. It was suggested that keeping the surroundings of hen houses free from chicken faces, leftover food, and excess moisture in the soil, might have been the reason of the small amount of adults recovered outdoors in relation with previous abundant adult sandfly collections in this habitat (Reinhold-Castro *et al.*, 2015).

Our study provides evidences that, as in Argentina and Brazil, *Lu. migonei* might play an important role in the peri-domestic transmission of the CL at El Carrizal, which is favored by the habit of the inhabitants to stay outdoors from the twilight hours until night, a very common practice among Latin America people, at the time that sandfly females seek for blood sources. Therefore, the relationship of this species with this habitat,

suggests that it would be important and useful to promote the community participation in order to implement such methods in this environment for the control of CL transmission outdoors, while bed-nets will be appropriate to reduce indoors transmission (Alexander & Maroli, 2003).

Sandfly species - climatic factors relationship at El Carrizal (2008) and monthly CL cases-climatic factors relationship at the Municipality Tovar (2000-2008)

Literature on the monthly dynamics of sandfly vector populations of CL and its correlation with climatic factors is very abundant and has been recognized worldwide by several authors. However, the comparison of data obtained for the same sandfly species in different foci is not ever straightforward since abiotic and biotic factors may vary among the foci and therefore this interaction can influence the results and interpretation thereof.

At El Carrizal, with CDC traps, at greatest Min RH, the rate of catches of males of *Lu. youngi* would increase in all habitats. Males of *Lu. spinicrassa* showed positive correlations with Mean RH indoors and Mean RH with rainfall in the forest, therefore, humid environments would favor catches of this species. *Lutzomyia migonei* showed significant negative correlations with Min T and Mean T indoors, outdoors and in the forest, so at cooler T, greatest collections of this species are expected in all the habitats. Greatest catches of females in the Verrucarum group would occur in anthropogenic habitats when T decreases and RH increases. With the Shannon trap, in the forest, catches of *Lu. spinicrassa* and *Lu. youngi* increased when T diminished, while *Lu. migonei* was favoured by precipitation. Females in the Verrucarum Group are expected to increase with highest RH.

With the same trap, the allopatric population of *Lu. youngi* (= *Lu. townsendi*) in Las Calderas, Trujillo State (1,340 m a.s.l.), showed a bimodal pattern of greater abundance of *Lu. youngi* which also matched with the heaviest precipitation in the locality (Marquez & Scorza, 1984). In Las Maticas, Lara State, only one annual peak of this species was registered at the end of the dry season and after the peak of rain with Min RH, and the Max T (Traviezo, 2006). As for the allopatric species of *Lu. spinicrassa* in Táchira State, the population density increased with the P in the focus Santa Anita (Perruolo, 1984), while negative significant correlation was found with the P and RH in the village Catárnica (Perruolo, 2004).

In Brazil, in their review, Rangel & Lainson (1999) reported that *Lu. migonei* is absent in the dry and colder months of the year, but in Sao Vicente, Pernambuco, Brazil, where *Lu. migonei* was the prevalent and very abundant species, the greatest catches were recorded before and after the rainy season and no significant correlation was found between population density and climate variables (Guimaraes *et al.*, 2012).

In recent years potential applications of Ecologic niche modelling (ENM) are used to explore the complex influences of several environmental variables (e.g. climate, landscape, land use change, topographic variables etc.) on species vectors distributions, representing a powerful tool for the characterization of ecologic and in turn predictive geographic distributions of species and their translation into disease transmission patterns and epidemiological changes (Peterson, 2006).

Results obtained through a first approach using this method (ENM) over 23 records of *Lu. migonei* in Venezuela suggested that precipitation (of driest quarter and driest month) was the most important variable in the prediction of *Lu. migonei* distribution (Sanchez *et al.*, 2015).This agreed with the focal data obtained at El Carrizal. In fact here, for *Lu. migonei*, T seems to be a limiting factor, as shown by the negative correlation in all habitats with the CDC traps collections, but at the same time the positive correlation with precipitation in catches with the Shannon trap would collate with this result, since at more rainfall, T would diminish. Quintana *et al.* (2013) also showed that seasonal and warmest quarter precipitation were the variables that most explained its distribution of *Lu. migonei* in Argentina. On the other hand, Ferro *et al.* (2015) in Colombia, using ENM, found that temperature seasonality and the slope of the terrain were the variables associated to the distribution of *Lu. youngi* and *Lu. spinicrassa*, respectively. Moreover, land use indicated that 76% of the points of data collection corresponded to transformed ecosystems, which, as said above, was observed in Venezuela (Scorza *et al.*, 1985) where, like in Colombia, *Lu. youngi* was recognized as one of the species more related to coffee plantations.

When the CL incidence was correlated with climate data for the period 2000-2008 in Tovar, it was found that the greatest number of cases tends to occur in the months of most precipitation, with approximately a month lag, which also agreed with the results obtained when the relationship species-climatic variables at El Carrizal was analysed.

In summary, the data obtained from this study leads to conclude that CL transmission at the monitored village in the Andean region of Venezuela, may occur indoors, outdoors and in the forest. Among the sandfly females in the Verrucarum group, *Lu. youngi* probably prevails and may be primarily responsible for the indoors transmission, while *Lu. migonei* would preferably stop outdoors where they may have established breeding sites and keep the *Leishmania* epidemiological cycle among dogs and humans (Feliciangeli *et al.*, 2011). The abundance of the three species is mainly regulated by the precipitation with the greatest peak in November. The correlation of climate variables and the CL cases which occurred during 9 years (2000-2008) in the CL focus studied, revealed that the greatest number of cases tends to occur with approximately a month lag, after the months of most rainfall. As no substantial ecological and social changes have not occurred in the village during the last years, our data would provide to the Ministry of Health helpful information to promote cooperation among multilateral institutions (Feliciangeli, 2014) in order to implement target control methods in the Venezuelan Andean region. These, preceded by educational health programs to be further evaluated, could properly be applied at the lowest administrative level in the health system (Bates *et al.*, 2015). Basic epidemiological data jointly with further studies with ENM (Peterson, 2006) including a greatest number of variables at largest scale, would be helpful for the prediction of outcome of the disease in Venezuela and elsewhere.

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