

The sandfly fauna, anthropophily and the seasonal activities of *Pintomyia spinicrassa* (Diptera: Psychodidae: Phlebotominae) in a focus of cutaneous leishmaniasis in northeastern Colombia

Fredy Galvis Ovallos^{1/+}, Yanis Ricardo Espinosa Silva², Nelson Fernandez³, Reynaldo Gutierrez³, Eunice Aparecida Bianchi Galati¹, Claudia Magaly Sandoval³

¹Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, SP, Brasil ²Instituto de Física de Líquidos y Sistemas Biológicos, Universidad Nacional de La Plata, Buenos Aires, Argentina ³Laboratorio de Entomología Médica, Instituto de Investigación en Ciencias Biomédicas, Universidad de Pamplona, Pamplona, Norte de Santander, Colombia

*This study was conducted to identify the sandfly fauna and the anthropophilic species in a coffee-growing area of Villanueva, Norte de Santander, Colombia, a focus of American cutaneous leishmaniasis, and to analyse the relationship between the most frequent species and rainfall, relative humidity and temperature, with the aim of contributing to epidemiological surveillance in the area. Sandfly collections were performed fortnightly between February 2006-September 2007 using automatic light traps, Shannon traps, protected human bait and aspiration in resting places. A total of 7,051 sandflies belonging to 12 species were captured. *Pintomyia spinicrassa* (95.7%) predominated. *Pintomyia oresbia* and *Lutzomyia* sp. of *Pichinde* were found in the state of Norte de Santander for the first time. *Pi. spinicrassa*, *Pintomyia nuneztovari*, *Micropygomyia venezuelensis*, *Lutzomyia* (*Helcocyrtomyia*) *scorzai* and *Lu.* (*Helcocyrtomyia*) sp. were captured on the protected human bait. A significant association between *Pi. spinicrassa* abundance and the total rainfall and the average temperature and humidity 10 days before the collection was observed. The dominance of *Pi. spinicrassa*, a recognised vector of *Leishmania braziliensis*, especially during the dry periods, indicates that the risk of parasite transmission may increase.*

Key words: Phlebotominae - *Pintomyia spinicrassa* - vector ecology - leishmaniasis

Leishmaniasis are considered to be re-emerging diseases of great significance to public health due to the severity of their clinical manifestations. In Colombia, during the period 2006-2011, 62,843 cases of American cutaneous leishmaniasis (ACL) were reported, with an annual average rate of 8,926 new cases (INS 2012). Currently, 148 species of *Lutzomyia* (Bejarano 2006, Ferro et al. 2011) are known to exist in Colombia, six of which are recognised as vectors of leishmaniasis agents. Among the species of sandflies registered in Colombia, the species grouped in the *townsendi* series *Pintomyia* (*Pifanomyia*) (Galati 2003) of the Andean Region are epidemiologically relevant due to their involvement in the transmission of agents of ACL and human bartonellosis (Bejarano et al. 2003, Cohnstaedt et al. 2011).

The *Leishmania* species most frequently related to ACL is *Leishmania* (*Viannia*) *panamensis*, followed by *Leishmania* (*Viannia*) *braziliensis*, which has the most extensive geographical distribution in Colombia, especially in coffee-growing areas (Corredor et al. 1990).

In the northeastern region of Colombia, the state of Norte de Santander (NS) (Fig. 1) is a mesoendemic area for ACL; in the past seven years, 1,524 cases of cutaneous leishmaniasis have been registered there. Cardenas

et al. (2006) reported that the increase in the number of cases is associated with the climatic fluctuations caused by the El Niño Southern Oscillation (ENSO), which affect the sandfly population.

The main objective of this study was to describe the sandfly fauna in Villanueva, where there is active transmission of ACL in a coffee-growing area in NS. This study also sought to identify the anthropophilic species and their endophagic habits and to analyse the relationships between the most frequent species and the rainfall regime, relative humidity and temperature, with the aim of contributing to epidemiological surveillance in this transmission area.

MATERIALS AND METHODS

Area of study - The municipality of Gramalote is located in the central region of NS. The urban area was obliterated in 2011 by a natural landslide; it was located between 7°50' 8°3'N-72°45' 72°55'W. The population of the municipality was estimated at 7,964 inhabitants in 2005 and the local economy is based on coffee and cattle (CORPONOR 2012). The climate in Gramalote includes two dry seasons, one from January-February and the other from July-September, that are interspersed with rainy seasons, representing a typical bi-modal pattern. The annual averages are 23°C for temperature, 2,500 mm for rainfall and 80% for relative humidity.

Collection sites - Three houses, one on each of three farms (Buenavista, El Almendro and Villa-Antigua), were selected in the Villanueva neighbourhood, a typical coffee-growing region located between 1,300-1,650

+ Corresponding author: fgalvis@usp.br

Received 12 August 2012

Accepted 12 November 2012

masl. This region is characterised as premontane rainforest (CORPONOR 2012). The criteria for the selection of the houses were the notification of confirmed ACL cases and a previous verbal agreement with the owners.

Sandfly collections - The sandfly captures were performed at fortnightly intervals between February 2006-October 2007 with Shannon and automatic light traps, human bait and collection in resting places.

Shannon traps - The trap was operated by a person using a Castro aspirator and a flashlight on two successive nights at fortnightly intervals, from 07.00 pm-09.00 pm, in the peridomicile area (a coffee plantation) on Buenavista farm from February 2006-February 2007.

Protected human bait - Simultaneously with the operation of the Shannon trap, also on Buenavista farm, flies landing on two researchers who were clothed in dark pants and long-sleeved shirts and who were stationed in the intra and peridomicile areas were captured using a Castro aspirator and a flashlight.

Resting places - Collections were also performed among the rocks found on the coffee plantations and on the trunks of the interspersed trees on two successive days fortnightly from February 2006-February 2007. The collections were performed between 02:00 pm-04:00 pm using a Castro aspirator.

Automatic light traps - The three selected houses were all sampled. One CDC-type automatic light trap was placed indoors (bedroom) and another in the peridomicile area (coffee plantation). The traps were located 1.5 m above the ground and 25 m apart. The collections were performed fortnightly between October 2006-September 2007 on two successive nights from 06:00 pm to 06:00 am. In January and February 2007, two traps were installed in the forest 3 km from Buenavista farm for one night.

The specimens collected were preserved in 70% alcohol and taken to the Laboratory of Medical Entomology of the Biomedical Sciences Institute of Pamplona

University, Pamplona (NS) for identification and slide mounting. The identification was performed according to Galati's (2003) classification and the species name abbreviations follow Marcondes (2007).

The climatic data for temperature, relative humidity and rainfall were obtained from climatological station 1602503 of the Instituto de Hidrología, Meteorología y Estudios Ambientales (ideam.gov.com), Colombia, located at 07°47'N 72°49'W, 860 masl.

The frequencies of the most abundant species were calculated according to Williams' average (Haddow 1960). A Spearman test (PASW v.17) was used to analyse the strength of the correlations between the most abundant species and several climatic parameters: total rainfall, the average temperatures and humidities for the 15 and 10 days prior to the capture date and the temperature and humidity on the day of the collection. A Mann-Whitney *U* test was used to compare the frequencies in the intra and peridomicile areas.

RESULTS

The number of sandflies captured for each species, according to the collection method and collection site, is presented in Table I.

The phlebotomine fauna was represented by 12 species: *Evandromyia* (*Aldamyia*) *dubitans* (Sherlock, 1962), *Lutzomyia* (*Helcocyrtomyia*) *hartmanni* (Fairchild & Hertig, 1957), *Lutzomyia* (*Helcocyrtomyia*) *scorzai* (Ortiz, 1965), *Lu. (Hel.)* sp., *Lu. (Hel.)* sp. of Pichinde Young 1979, *Micropygomyia* (*Sauromyia*) *venezuelensis* (Floch & Abonnenc, 1948), *Psathyromyia* (*Psathyromyia*) *shannoni* (Dyar, 1929), *Pintomyia* (*Pifanomyia*) *nuneztovari* (Ortiz, 1964), *Pintomyia* (*Pifanomyia*) *oresbia* (Fairchild & Hertig, 1961), *Pintomyia* (*Pifanomyia*) *sauroida* (Osorno-Mesa, Morales & Osorno, 1972), *Pintomyia* (*Pifanomyia*) *serrana* (Damasceno & Arouck, 1949) and *Pintomyia* (*Pifanomyia*) *spinicrassa* (Morales, Osorno-Mesa, Osorno & Hoyos, 1969). *Pi. spinicrassa* was the most frequent species (95.7%) and was predominant for all the collection methods used and at all the sites sampled.

In the captures using protected human bait, five anthropophilic species were identified: *Pi. spinicrassa*, *Pi. nuneztovari*, *Mi. venezuelensis*, *Lu. scorzai* and *Lu. (Hel.)* sp. These species were collected in peridomicile and intradomicile areas, a result that could suggest an endophagic habit. The highest frequencies obtained using human bait were for *Pi. spinicrassa* in the peridomicile area.

In addition to *Pi. spinicrassa* and *Pi. nuneztovari*, specimens of the subgenus *Helcocyrtomyia* and a female of *Pi. oresbia* were collected using the Shannon trap. *Ev. dubitans*, *Pi. serrana* and *Pa. shannoni* were only attracted by the automatic light traps. Twenty-four females of *Pi. spinicrassa* and one of *Mi. venezuelensis* were collected from resting places on rocks.

The frequency of *Pi. spinicrassa* collected with protected human bait was higher in the peridomicile area than in the intradomicile area ($p < 0.003$), whereas the highest frequencies found for the automatic light trap (CDC) collections were observed in the intradomicile area ($p < 0.014$). The highest Williams' average (11.5 insects/trap) was obtained for the forest, followed by the



Fig. 1: study area: Villanueva, municipality of Gramalote, Norte de Santander, Colombia.

TABLE I
Sandfly species by sex and environment, captured in Villanueva, municipality of Gramalote, Norte de Santander, Colombia, using various trapping methods, between February 2006-September 2007

Collection method	Automatic light trap-CDC						Protected human bait						Shannon trap						Resting places						Total
	Intradomicile		Peridomicile		Forest		Intradomicile		Peridomicile		Peridomicile		Intradomicile		Peridomicile		Intradomicile		Peridomicile		Intradomicile		Peridomicile		
Environment	150		150		4		52		52		52		52		52		52		52		52		52		
Species	Sex	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	MF (%)	
<i>Evandromyia (Aldamyia) dubitans</i>		4	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	8	-	-	-	8 0.11	
<i>Lutzomyia (Helcocyrtomyia) hartmanni</i>		-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	1	-	-	-	1 0.01	
<i>Lutzomyia (Helcocyrtomyia) scorzai</i>		-	-	-	-	-	-	1	-	3	-	-	-	-	-	-	-	5	-	5	-	-	-	5 0.07	
<i>Lutzomyia (Helcocyrtomyia) sp.</i>		6	-	-	-	1	-	4	-	18	1	8	-	-	-	-	-	37	1	38	1	-	-	38 0.54	
<i>Lutzomyia (Helcocyrtomyia) sp. of Pichinde</i>		-	-	-	-	8	2	-	-	-	-	1	-	-	-	-	-	8	3	11	3	-	-	11 0.16	
<i>Micropsyomyia (Sauromyia) venezuelensis</i>		7	15	12	14	-	-	2	5	1	6	2	4	1	-	-	-	25	44	69	44	-	-	69 0.97	
<i>Psathyromyia (Psathyromyia) shannoni</i>		1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	2	-	-	-	2 0.03	
<i>Pintomyia (Pifanomyia) nuneztovari</i>		31	10	43	7	4	-	4	-	30	-	17	1	-	-	-	129	18	147	18	-	-	-	147 2.10	
<i>Pintomyia (Pifanomyia) oresbia</i>		-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	1	-	-	-	1 0.01	
<i>Pintomyia (Pifanomyia) sauroida</i>		-	1	1	2	-	5	-	-	-	-	-	1	-	-	-	-	1	9	10	9	-	-	10 0.14	
<i>Pintomyia (Pifanomyia) serrana</i>		3	-	2	2	-	-	-	-	-	-	-	-	-	-	-	-	5	2	7	2	-	-	7 0.10	
<i>Pintomyia (Pifanomyia) spinicrassa</i>		1,509	241	946	296	102	1	359	109	1,074	72	1,368	651	24	-	-	5,382	1,370	6,752	1,370	-	-	-	6,752 95.76	
Total by sex		1,561	267	1,009	321	115	8	370	114	1,124	79	1,400	658	25	-	-	5,604	1,447	-	-	-	-	-	-	
Percent		85.4	14.6	75.9	24.1	93.5	6.5	76.4	23.6	93.4	6.6	68	32	100	-	-	79.6	20.4	-	-	-	-	-	-	
Total both sexes		1,828		1,330		123		484		1,203		2,058		25			7,051		7,051					-	
Percent		25.9		18.9		1.7		6.9		17.1		29.2		0.3			100		100					100	

F: female, M: male.

Shannon trap (10.7 insects/trap) and protected human bait in the peridomicile area (9.9 insects/person). More females than males were captured by all the sampling methods. The lowest sex ratio (female/male) was obtained using the Shannon traps (2.0:1.0) and the highest was obtained in the forest using automatic light traps (102:1.0) (Table II).

The monthly frequency of *Pi. spinicrassa* collection by sampling method is presented in Figs 2, 3. The protected human bait gave the highest abundances during the dry periods (1st: January-February; 2nd: July-September), but lower abundances during the rainy periods (1st: March-June; 2nd: October-December). The highest abundance during the first dry period was observed in the intradomicile area. During the second dry period, the highest abundance was observed in the peridomicile area (Fig. 2). The highest frequencies found in the Shannon trap collections occurred throughout the second dry period in 2006 and the peak was observed in February 2007. For the automatic light trap, the peak of abundance was observed in the dry periods for both the intra and peridomicile areas.

The values of r and p obtained in the tests of the correlations between the abundance of *Pi. spinicrassa* and rainfall, temperature and relative humidity 10 days before the captures are presented in Table III. The correlation analysis showed a significant negative correlation between the abundances of this species collected using human bait (intra and peridomicile areas) and Shannon traps and the relative humidity. This analysis also revealed the same pattern for protected human bait and rainfall; however, the correlations between the abundances of flies collected using protected human bait (peridomicile) and Shannon traps and temperature were significant and positive. Non-significant correlations were observed between the abundance of *Pi. spinicrassa* and the rainfall, temperature and relative humidity 15 days before collection and on the collection day.

DISCUSSION

Pi. spinicrassa predominated among the 12 species identified in Villanueva, Gramalote. In this study, specimens of *Evandromyia walkeri* (Newstead, 1914), *Pintomyia pia* (Fairchild & Hertig, 1961), *Pintomyia ovallesi* (Ortiz, 1952), *Lutzomyia lichyi* (Floch & Abonnenc, 1950), *Lutzomyia gomezi* (Nitzulescu, 1931), *Trichopygomyia ferroae* (Young & Morales, 1987) and *Lutzomyia erwindonaldi* (Ortiz, 1978), found in a previous study (Alexander et al. 2001), were not collected. These differences in the faunal composition in Gramalote may be related to differences between the collection sites, given the variations in altitude in the northeastern Andean cordillera. With the identification of the Villanueva fauna, 19 species of sandfly have been identified in Gramalote, NS; the species *Pi. oresbia* and *Lutzomyia* sp. of Pichinde were recorded in this state for the first time.

Among the species collected on protected human bait, two species, *Pi. spinicrassa*, which has been found naturally infected with *L. (V.) braziliensis* and *Pi. nuneztovari*, which is susceptible to infection by this parasite under laboratory conditions (Young et al. 1987, Santamaria et al. 1999), should be highlighted. In terms of the eco-epidemiology of ACL, our results indicate that *Pi. spinicrassa* plays an important role in the transmission of *Leishmania* sp. in the coffee-growing areas of Gramalote in view of this species' high frequencies, anthropophilic habits and endophagic behaviour. An important role of *Pi. spinicrassa* was also observed by Alexander et al. (2009) in other localities in NS. In addition, this species has been found naturally infected by *L. (V.) braziliensis* in the coffee-growing regions of Colombia and Venezuela (Young et al. 1987, Perruolo et al. 2006).

The low frequencies of the other species of the subgenera *Pifanomyia* and *Helcocyrtomyia* may indicate their lesser importance in the transmission of *Leishma-*

TABLE II

Distribution of *Pintomyia spinicrassa* by site, method and environment, collected in Villanueva, municipality of Gramalote, Norte de Santander, Colombia, between February 2006-September 2007

Site	Method	Environment	F n (%)	M n (%)	F/M ratio	William's average
Buenavista farm	Human bait	Intradomicile	359 (76.7)	109 (23.3)	3.3:1.0	2.5
		Peridomicile	1074 (93.7)	72 (6.3)	14.9:1.0	9.9
	Shannon trap	Peridomicile	1368 (67.8)	651 (32.2)	2.1:1.0	10.7
		resting places	Peridomicile	25 (100)	-	-
	CDC	Forest	102 (99)	1 (1)	102.0:1.0	11.5
		Intradomicile	1509 (86.2)	241 (13.8)	6.3:1.0	3.5
El Almendro farm	CDC	Peridomicile	946 (76.2)	296 (23.8)	3.2:1.0	2
		Intradomicile	392 (87.3)	57 (12.7)	6.9:1.0	4.1
Villa-Antigua farm	CDC	Peridomicile	325 (82.5)	69 (17.5)	4.7:1.0	1.8
		Intradomicile	117 (76)	37 (24)	3.2:1.0	1.4
		Peridomicile	147 (77.3)	43 (22.7)	3.4:1.0	1.2

F: female; M: male.

nia in the study area. However, the capture of specimens of *Lutzomyia* sp. of Pichinde, a species not yet formally described in NS expands the knowledge of its geographical distribution. Further, the presence of this taxon in this region, in sympatry with *Lu. scorzai* and other species of the subgenus *Helcochyrtomyia*, may provide data to elucidate the taxonomy of *Lutzomyia* sp. of Pichinde.

For the captures made on protected human bait, the high frequencies in the peridomicile area can be explained by these species' close contact with the host (the natural shelters of these females are close to the capture area). Although the highest Williams' average and sex ratio (female/male) were obtained in the forest (Table II), these values could have been influenced by the period and the number of collections made (only 1, during the dry season). The similarity between the Williams' averages for the Shannon trap (10.7 insects/trap) and for the protected human bait in the peridomicile area (9.4

insects/person) is reasonable because the two collections were performed in the same environment and because the presence of humans was required by both methods. The high frequencies observed in the automatic light traps in the intradomicile area suggest entomophilic behaviour and a high risk of infection due to *Leishmania* sp. in this environment; therefore, the use of mosquito nets could have an important role as a prophylactic method in this region. Of the species captured on human bait, only *Mi. venezuelensis* does not appear to be anthropophilic because the number of males collected was greater than the number of females. In fact, the females of this species feed on reptiles (Young & Duncan 1994).

The abundance and monthly distribution of *Pi. spinicrassa* in Gramalote show that this species predominates in the dry periods. These results differ from those of Alexander et al. (1992) obtained on coffee plantations in the municipality of Arboledas, where high frequencies were observed during the rainy seasons. The differences between the results of these two contrasting studies are reasonable because the captures in Arboledas were made in resting places, where the sandflies tend to remain sheltered during the rainy seasons. Moreover, high abundances of *Pi. spinicrassa* during the dry season have been

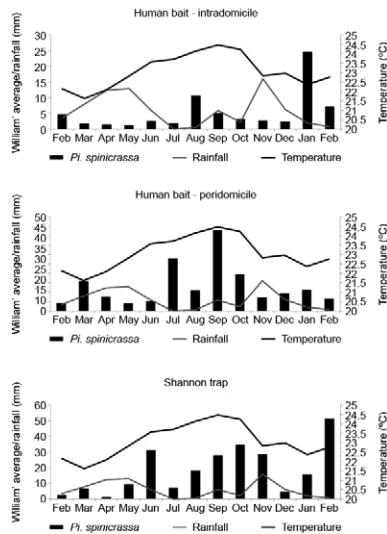


Fig. 2: monthly abundance of *Pintomyia spinicrassa* captured with protected human bait in intradomicile and peridomicile and Shannon trap from February 2006-February 2007.

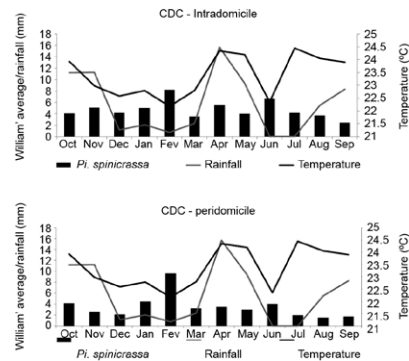


Fig. 3: monthly abundance of *Pintomyia spinicrassa* captured with automatic light traps (CDC) in intradomicile and peridomicile from October 2006-September 2007.

TABLE III

Spearman correlation values between the abundance of *Pintomyia spinicrassa* and the average rainfall, temperature and relative humidity 10 days before the collections in Villanueva, municipality of Gramalote Norte de Santander, Colombia

Method/environment	Rainfall		Humidity		Temperature	
	r	p	r	p	r	p
Human bait/intradomicile	-0.48	0.01 ^a	-0.52	0.01 ^a	0.06	0.78
Human bait/peridomicile	-0.19	0.36	-0.39	0.05	0.43	0.03 ^a
Shannon trap/peridomicile	-0.01	0.95	-0.53	0.01 ^a	0.50	0.01 ^a
CDC/intradomicile	0.00	0.9	-0.15	0.49	0.36	0.10
CDC/peridomicile	-0.30	0.16	-0.22	0.31	-0.18	0.40

a: values statistically significant; b: value not absolute zero.

described in an endemic area of the Venezuelan Andes (Perruolo 2004). Recently, Ferro et al. (2011) described a similar distribution of *Pintomyia longiflocosa* (townsendi series) in the Andean region of Colombia. Thus, our observations in NS indicate that the dry seasons are periods of great risk of ACL transmission given the more intense activity of the anthropophilic species of the townsendi series, particularly *Pi. spinicrassa*, a suspected vector of *L. (V.) braziliensis*, during these periods.

The dynamics of the diseases transmitted by vectors are influenced by the weather, other natural phenomena and human activities, all of which affect the vectors, parasite reservoirs and blood sources (Patz et al. 2000). Thus, knowledge of the seasonal distribution of insect vectors and the relationship between this distribution and climatic variables are important tools in entomological surveillance, providing the information necessary for the development of prevention and control measures. Analysis of the correlation between the climatic variables and the *Pi. spinicrassa* frequencies revealed that the relative humidity (closely related to the rainfall) limits the density of the sandfly population in this region. Further, an increase in the temperature led directly to an increase in the density of *Pi. spinicrassa*, which might intensify the vectorial capacity of this species during the hot and dry periods of the year. These findings are consistent with the continuous occurrence of El Niño during the study period, from August 2006-January 2007, including the entire dry season from mid-year (NOAA 2010) on. Our results are in agreement with those of Cardenas et al. (2006), who observed an increase in the number of ACL cases during the ENSO events in NS, Colombia, which are characterised by a decrease in rainfall and a lengthening of the dry periods with an increase in temperature. These temperature and rainfall changes could decrease the duration of the immature stages and lead to an increase in the adult sandfly population (Endris & Young 1984), thus reducing the duration of the gonotrophic cycle and the extrinsic incubation period of the parasite (Meyer 1989), both of which are important factors in the transmission of parasites.

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