



ECOSYSTEMS

Environmental effects on phlebotominae sand flies (Diptera:Phychodidae) and implications for sand fly vector disease transmission in Corrientes city, northern Argentina

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Abstract: We evaluated species richness, abundance, alpha diversity, and true diversity of Phlebotominae sand flies temporal changes in domiciles within the northern Argentina city of Corrientes. A total of 16 sampling nights were conducted seasonally throughout the years 2012-2014 through light traps supplemented with CO₂. Meteorological and remote sensing environmental factors were used to assessed for vectors implications in disease transmission through Generalized Mixt Models. *Lutzomyia longipalpis* was the most abundant and common species, followed by *Nyssomyia neivai* and *Migonemyia migonei*. *Lutzomyia longipalpis* was more abundant in urban areas, *Ny. neivai* was associated with vegetation in periurban areas, both were found all sampling years with higher abundance during the rainy season. Positive association of *Lu. longipalpis* with precipitation and relative humidity and negative association with temperature were observed. Models showed humidity and vegetation as making effects on *Lu. longipalpis* abundance. Precipitation was significant for *Mg. migonei* models, with higher abundance in periurban and periurban-rural environments. For *Ny. neivai* models, relative humidity was the most important variable, followed by precipitation frequency. Our findings led to identify high risk areas and develop predictive models. These are useful for public health stakeholders giving tolls to optimized resources aim to prevent leishmaniasis transmission on the area.

Key words: Species abundance, species richness, modeling, public health entomology, remote sensing.

INTRODUCTION

Argentina has reported leishmaniasis cases from the two clinical forms, the visceral (VL) form which is the more serious form, and the tegumentary (TL) form which is the most common and presents manifestations in the skin and mucous (Salomón et al. 2008a, Locatelli et al. 2014). VL is caused by *Leishmania (Leishmania) infantum* Nicolle and TL is caused by *Le. (Viannia) braziliensis* Vianna. The latter is the most frequent species of *Leishmania* isolated in

Argentina (Salomón et al. 2006b). Other detected *Leishmania* species that cause tegumentary manifestations are *Le. (V.) guyanensis*, *Le. (L.) amazonensis*, and *Le. (V.) panamensis* (Marco et al. 2005, 2006, 2012, Salomón et al. 2012a, b).

In Argentina, different species of phlebotominae sand flies were found to be involved in the cycle of transmission of leishmaniasis. *Nyssomyia neivai* (Pinto), *Micropygomyia quinquefer* (Dyar) and *cortezii* complex have been found infected with *Leishmania* sp. by “Leishmania DNA

detection" (Córdoba Lanús et al. 2006, Rosa et al. 2012). *Lutzomyia longipalpis* (Lutz and Neiva), *Migonemyia migonei* (França) and *Ny. whitmani* (Antunes & Coutinho) have been found infected with *Le. infantum* by "Leishmania DNA detection" (Acardi et al. 2010, de Carvalho et al. 2010, Moya et al. 2015). Likewise *Evandromyia cortelezzii* (Brethes) has been found infected with *Le. infantum* DNA in Brazil (Saraiva et al. 2010). *Lutzomyia longipalpis* was detected in 2004 for the first time in Clorinda city, Formosa province, northern Argentina (Salomón & Orellano 2005), and the first VL human case as well as canine VL was reported by 2006 in Posada city (Salomón et al. 2008b). In 2008, *Lu. longipalpis* was first reported in Corrientes city (Salomón et al. 2009a, b).

Vector-borne diseases are influenced, along with the insects' population dynamics, by environmental factors that could be characterized and studied by meteorological and remote sensing (RS) data (Estallo et al. 2015, 2016). In the last decade, the use of RS data for vector-borne diseases has increased as a tool to estimate the environmental conditions where vectors could breed and develop (Estallo et al. 2016, 2018). Remote sensing allows environmental factor characterization in different ways such as vegetation quantification through vegetation indices like the Normalized Difference Vegetation Index (NDVI) and the Enhanced Vegetation Index (EVI) or through land surface temperatures (LST) (Estallo et al. 2016, Artun & Kavur 2017).

There have been studies on several species of phlebotomine sand flies aimed at investigating environmental factors and urban areas associated with VL (Santini et al. 2012). Likewise, different studies have been carried out to know the influence of modified environments and urbanized areas in the domestic and peridomestic TL transmission (Salomón et al.

2006a, b, 2008a). Many of these previous studies had focused primarily on Misiones province as a risk area for Argentina. Relevant studies of phlebotomine sand flies communities in new risk areas of VL of the country such as Corrientes city are lacking however as only a few studies have begun analyzing macro and microenvironmental factors mainly on a spatial scale (Berrozpe et al. 2017, 2018). Herein, we aim to evaluate the species richness and abundance of phlebotomine sand flies (Diptera: Psychodidae) in Corrientes city, temporal changes assessed by 16 sampling events over two years of sampling, and the relationship between meteorological factors and remote sensing environmental factors for vector implications in disease transmission.

MATERIALS AND METHODS

Study area

The study area was in Corrientes province, located in the Neotropical Region of northeastern Argentina. The soil permeability is moderately slow, which allows the formation of flooded areas (Bruniard 1978). Corrientes city (27°28'08" S, 58°49'50" W) is located in the biogeographic Chacoan province, the floodplain area of the Paraná and Paraguay rivers (Secretaría de Desarrollo Sustentable y Política Ambiental 1999). The mean annual temperature is 23°C and the mean annual rainfall is 1280 mm. In the summer, the mean temperature is 27°C, with temperatures as high as 43°C recorded. The mean temperature in winter is 15°C, with -1.1 °C as the lower recorded. The rainy season is between November and April, with peaks of rainfall in spring (September-October) and autumn (March- April) (Bruniard 1978).

Sampling sites

The study was conducted seasonally for two years, from March 2012 to May 2014, attaining a

total of 16 nights of collection, simultaneously on each sampling site. Within the urban area of the city of Corrientes, 8 sites were selected for phlebotomine sand flies sampling, six of them (Figure 1: sites 1, 2, 3, 4, 6, 8) corresponded to houses that fulfilled the criterion of worst epidemiological scenario (Table I). A house fulfilling such criterion is an inhabited dwelling with favorable conditions for vector development, shady trees, hens or poultry in the peridomicile, and forest patches not less than 100m from the dwelling (Salomón et al. 2009a). One of the sampling sites met the conditions but was without a history of VL (Figure 1: site 5), and one site did not meet this criterion and had a high concentration of people throughout the day due to the Argentine Army Regiment (Figure 1: site 7). Sites 1, 2, 6, 7 and 8 were located in the urban area, which higher human density,

while sites 3, 4 and 5 they were in the suburban area with less human density (Figure 1). The urban sites have higher population density and well-developed infrastructure, and periurban sites are within an area with houses located in a green matrix (Berrozpe et al. 2018). Each sampling site was geo-referenced using a GPS (Global Positioning System) (Garmin GPSMAP® 60GSx).

Entomological sampling

Sampling was done 16 months at each sampling site, during the sampling period (from March 2012 to May 2014) at list ones in each climatic season. Phlebotomine sand flies were captured using 1 CDC-like light trap per site, supplemented with CO₂, and located in peridomestic environments. The traps were set at a height of 1 to 1.5 m above the ground and were active for one night from

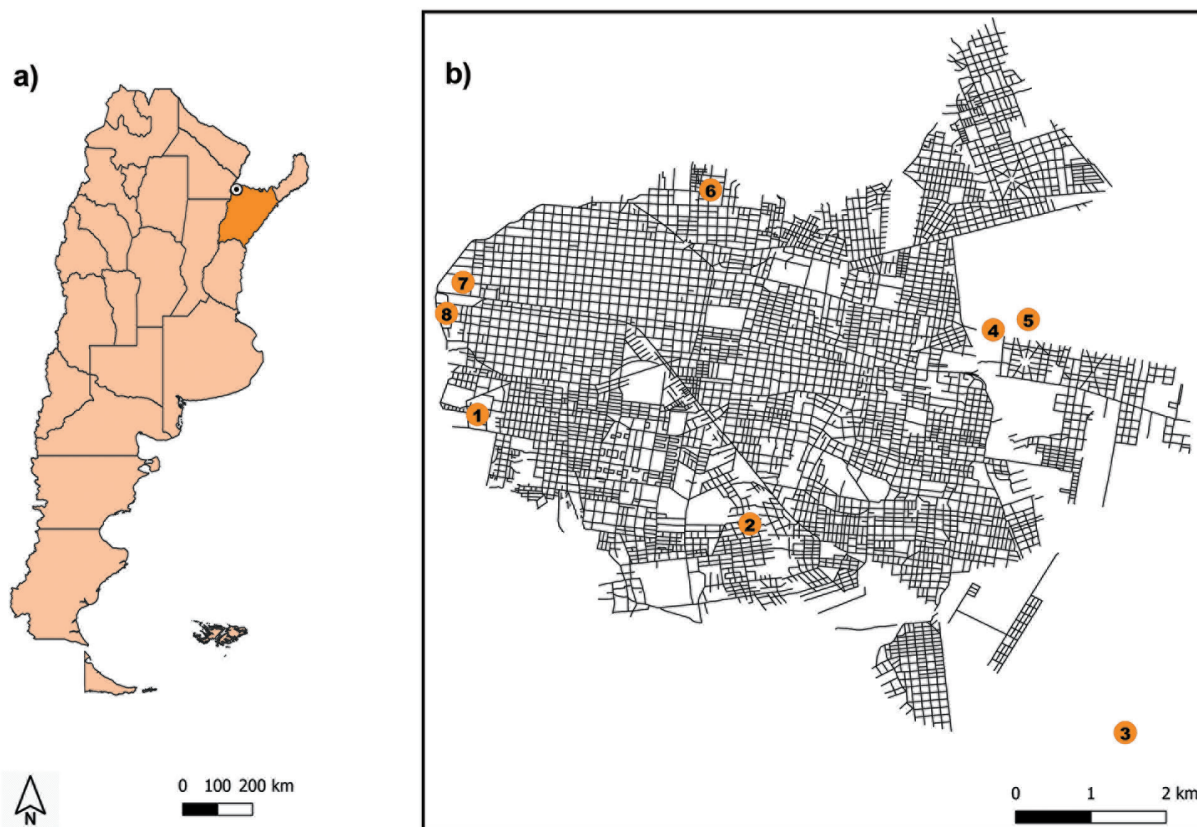


Figure 1. Study site. a: Map of Argentina showing the Corrientes province (northeast) and city of Corrientes. b: Map of the study locality: city of Corrientes indicating where the sampling points were located.

3 p.m. to 10 a.m. during each sampling date of the month.

All phlebotomine sand flies were euthanized via freezing (-20°C) and preserved in 70% alcohol solution prior to processing. The specimens were cleared in lactophenol and identified according to Galati (2003).

Females of *Evandromyia cortelezzii* and *Ev. sallesi* cannot be distinguished by morphological characteristics, so female specimens were included within the *Cortelezzii* complex (Szelag et al. 2018).

Environmental factors

Meteorological data were provided by the National Meteorological Service weather station from the city of Corrientes Aero station (27°27' S 58° 50' O). Mean temperature (°C), relative humidity (%), accumulated precipitation (mm) and precipitation frequency (days) from 2012 to 2014 were obtained. The environmental characterization through remote sensing (RS) data was registered by the Moderate-resolution imaging spectroradiometer (MODIS) satellite products that are useful for vector-borne disease studies in Argentina (Estallo et al. 2016). MODIS products: MOD13Q1 (NDVI and EVI) vegetation satellite products at 250 meters and

16-day compositing periods and LST MOD11A2 satellite product at 8-day compositing period and 1km spatial resolution were used. The NDVI saturates in high biomass regions which provides continuity with NOAA's AVHRR NDVI time series record for historical and climate applications, while EVI remained sensitive to canopy variations because it minimizes canopy-soil variations and improves sensitivity over dense vegetation conditions (Huete et al. 2002).

Statistical analysis to evaluate the phlebotomine sand fly community structure

To compare the species abundance in the 8 sampling sites, the Index of species abundance (*ISA*) was calculated for individual species according to Roberts & Hsi (1979): ($ISA = a + R_j/K$).

The nonparametric richness estimator *ICE* (Abundance-based coverage estimator, Chao & Lee 1992) was calculated to measure the completeness of the phlebotominae sand flies inventory (Colwell & Coddington 1994). This analysis was performed using the free Estimate Sv9.1.0 software (<http://purl.oclc.org/estimates>; Colwell 2013). Alpha diversity was estimated by specific richness (*S*) and the method proposed by Jost (2006).

Table I. Characterization of the sampling sites.

Characteristic	Collected sites							
	1	2	3	4	5	6	7	8
Type of environment	U	U	SU	SU	SU	U	U	U
Pets presence	dog	chiken dog	dog chiken pig	chike dog	chiken pig dog	dog	dog	chiken duck dog
Soil Vegetation	g	wv	wv	g,b	g,b	g,b	wv	wv
Trees	a	s	s	a	a	a	s	s

U: urban; SU: semi-urban; g: grasses; b: bushes; wv: without vegetation; a: abundant; s: scarce.

Statistical analysis of meteorological and remote sensing environmental factors for vector implications in disease transmission

We selected the three most abundant phlebotomine sand flies species (*Lu. longipalpis*, *Mg. migonei*, and *Ny. neivai*), with response variables to develop the models to investigate how the environment affects relative abundance of the species sampled. First, correlations were obtained between each species abundance and meteorological (mean temperature, relative humidity, accumulated precipitation) and remote sensing environmental factors (EVI and LST) with and without time lags (1- and 2-months' time lags). This way, the best-correlated lag explanatory variable was selected for each species to model the species abundance. We used precipitation frequency (FrecPrec) without time lags.

In repeated measures data, individuals typically display a high degree of similarity in responses over time. Therefore, we used generalized linear mixed models (GLMM) known as multilevel models to evaluate the relationship between the observed species of Phlebotominae sand fly abundance (*Lu. longipalpis*, *Mg. migonei* and *Ny. neivai*) and environmental factors (meteorological and remote sensing variables).

Phlebotominae sand fly abundance (*Lu. longipalpis*, *Mg. migonei* and *Ny. neivai*) and environmental factors (meteorological and remote sensing variables)

We use a negative binomial distribution instead of Poisson because the exploratory analysis revealed that variance was much higher than the mean. To select the best model we tested two families of negative binomial distributions: family=nbinom1, where the variance linearly increases with the mean: $\sigma^2 = \mu(1 + \alpha)$, with $\alpha > 0$; and family=nbinom2, where the variance is a

quadratic function of the mean as $\sigma^2 = \mu(1 + \alpha\mu)$, with $\alpha > 0$.

These kinds of models are adequate for counting with overdispersion (where variance is higher than medium values) because of the number of null frequencies. Exploratory analysis showed that abundance depends on the year, therefore in every case the years were used as random factors. At the second step, three different models were compared for each species:

1-Negative binomial distribution (for family=nbinom1 and family=nbinom2) without considering the variable that determines overdispersion;

2-Negative binomial distribution (for family=nbinom1 and family=nbinom2) considering months as overdispersion variable;

3-Negative binomial distribution (for family=nbinom1 and family=nbinom2) zero-inflated model, considering month as z_i (the variable where the presence of zeros could depend).

A total of 6 models (the above describe models for two different families each one) were developed for each species abundance in order to see which environmental variables affect the species abundance, and selection of the best model was based on the Akaike's information criterion (AIC- Akaike 1974). The model with the lowest AIC was selected as the best one of the 6 compared models. The selected model was implemented and developed in each case for each species, in order to evaluate the effects of the explanatory variables.

For each species, at third step, the selected model was implemented with groups of explanatory variables to avoid collinearity. Therefore for each species model, explanatory variables were grouped as:

A-mean EVI (EVI_m), precipitation frequency (FrecPrec) and medium relative humidity (HR_m);

B- mean land surface temperatures (LSTm), FrecPrec, HRm and precipitation (prec).

We used Generalized Linear Mixed Model throughout the GLMMTMB package on R software 3.5.0 (04-23-2018).

RESULTS

In all trapping periods, 1310 phlebotomine sand flies were captured belonging to 7 species from 5 genera. *Lutzomyia longipalpis* was the most abundant ($n = 1,121$) and most common species from the study area (ISAs=0.925), followed by *Ny. neivai* (ISAs=0.625) and *Mg. migonei* (ISAs=0.525). Site 1 showed the highest abundance of sand flies over the capture period. *Cortelezzii* complex females were scarce although they were captured in 4/8 sites (ISAs = 0.225). The rest of the species showed ISAs=0.05 (Table II).

Diversity and richness

According to the richness estimator calculated, representation of the sampling was 71.57% (ICE mean= 9.78). While the greatest species richness was found in site 4 ($S = 6$), followed by sites 3 ($S = 5$), 2 ($S = 4$) and 8 ($S = 4$) (Table III), site 4 also had the least abundance of sandflies. The effective number of species was higher in environment 4 (${}^1D = 5.29$ effective species), followed by site 3 (${}^1D = 3.97$ effective species). On the contrary, site 1 presented a reduction of 76% in phlebotomine sand flies diversity compared to the most diverse site and the most abundant of sand flies with a high predominance of *Lu. longipalpis*. This was a large open area (1/4 ha approx.), with a house and a large open space with some scattered trees (Table I). Collections at sites 3 and 5 consisted largely of *Ny. neivai*.

Seasonal distribution of phlebotomine sand flies abundance

Lutzomyia longipalpis and *Ny. neivai* were collected during all sampling years, while *Mg.*

Table II. Species collected during the trapping period with their abundance, the trapping site of the species and the Index of species abundance (ISA).

Species	Male	Female	Gravid female	Male /Female ratio	Total	Sites	ISA
<i>Evandromyia cortellezzi-sallesi</i>	2	3	3	0.66	8	2,3,5,8	0.275
<i>Evandromyia sallesi</i>	1	-	-	-	1	4	0.05
<i>Lutzomyia longipalpis</i>	764	356	1	2.17	1121	1,2,3,4,5,6,7, 8	0.925
<i>Migonemyia migonei</i>	71	26	1	2.73	98	1,2,3,4,6,8,	0.525
<i>Nyssomyia neivai</i>	41	35	1	1.66	76	1,2,3,4,5,6,7, 8	0.625
<i>Nyssomyia whitmani</i>	-	3	-	-	-	3	0.05
<i>Psathyromyia bigeniculata</i>	1	-	-	-	1	4	0.05
Total	881	423	6	-	1310	-	-

migonei was collected from March 2012 to May 2013 (sampling was done during June-July-August-September 2012 that correspond to south hemisphere winter time) (Figure 2). Females of *Ev. cortellezzi-sallesi* were found only two times in autumn of 2012 and ones at summer of 2013. *Nyssomyia whitmani* was captured once in December 2012 and *Psathyromyia (Psathyromyia) bigeniculata* (Floch & Abonnenc) once in April of the same year.

The highest overall species richness was recorded twice, one in the beginning of autumn 2012 (March) and the highest at the end of 2013 summer season (February). During the winter of 2013 (June and August), only two species were recorded, *Lu. longipalpis* and *Ny. neivai*. The two species presented similar patterns of temporal distribution throughout the year, with greatest

abundance in autumn and winter, followed by summer and then spring. *Migonemyia migonei* was most abundant during autumn.

Meteorological and remote sensing environmental factors for implications in vector-borne disease transmission

The GLMM considered the sampling sites as the random factor for each model developed. Explanatory variables were selected according to the best lag correlation with each phlebotomine sand flies species (Table IV), and the fluctuation for temperature, mean rainfall and relative humidity for *Lutzomyia longipalpis*, *Ny. neivai* and *Mg. migonei* are shown also in Figure 3.

Table III. Species abundance by trapping sites.

Species	Collected sites							
	1	2	3	4	5	6	7	8
<i>Ny. neivai</i>	45	3	11	1	9	1	4	11
<i>Lu. longipalpis</i>	999	51	4	3	15	11	19	31
<i>Mi. migonei</i>	20	81	6	1	-	2	-	9
<i>Ny. whitmani</i>	-	-	3	-	-	-	-	-
<i>Ev. cortellezzi-sallesi</i>	-	6	1	1	5	-	-	2
<i>Ev. sallessi</i>	-	-	-	1	-	-	-	-
<i>Psathyromyia bigeniculata</i>	-	-	-	1	-	-	-	-
Total	1065	143	28	11	34	20	30	61
Thru diversity	1.3	2.46	3.97	5.29	2.73	1.92	1.58	2.9
Dominance	0.8837	0.4631	0.2928	0.2188	0.3936	0.6429	0.7127	0.4155
Simpson index	0.1163	0.5369	0.7072	0.7813	0.6064	0.3571	0.2873	0.5845
Fisher's alpha	0.3776	0.7662	1.879	10.91	0.8404	1.171	0.5263	1.004

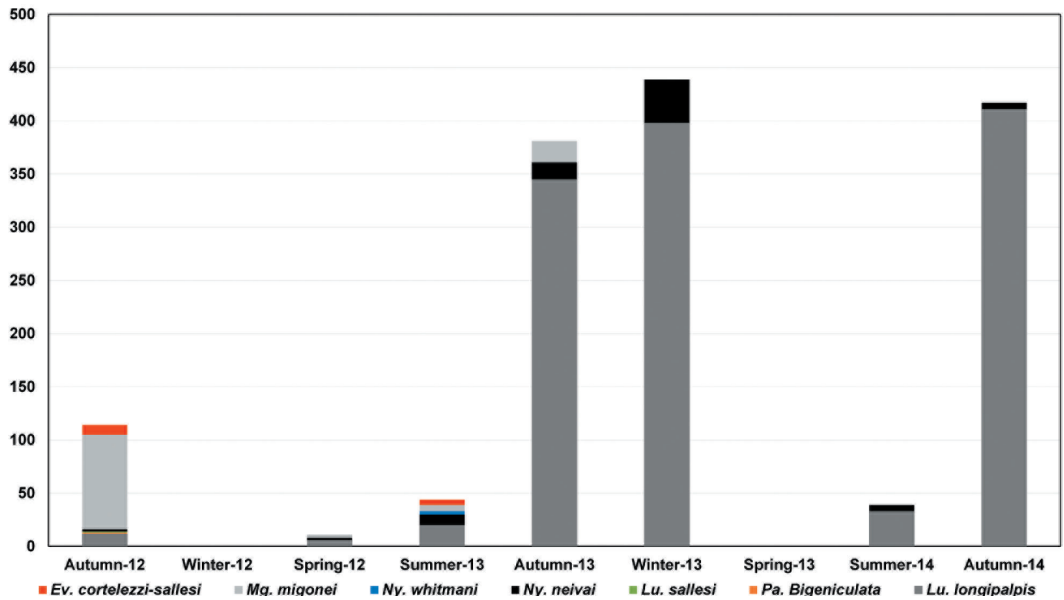


Figure 2. Seasonal distribution of phlebotomine abundance.

Table IV. Spearman correlation coefficients between the abundance of *Lu. longipalpis*, *Mi. migonei* and *Ny. neivai* with the meteorological and satellite variables: with and without time lags. In the table is showed the week with the best correlation value selected for the models.

Species	Variables	Correlation without time lag	Correlation with time lag	Time lags (months) with best correlation
<i>Lu. longipalpis</i>	Tm	-0.1519	0.2999	2
	HRm	0.3046	0.3849	1
	Prec	-0.1030	0.3526	1
	EVI _m	0.0463	0.2594	2
	NDBI _m	-0.1778	-0.4462	1
	LST _{mD}	-0.2377	0.2422	2
<i>Mi. migonei</i>	Tm	0.1605	0.3351	2
	HRm	0.0461	-0.2393	1
	Prec	0.0460	0.2020	2
	EVI _m	0.2321	-	-
	NDBI _m	-0.0919	-	-
	LST _{mD}	0.0190	0.3336	2
<i>Ny. neivai</i>	Tm	0.0026	0.1307	2
	HRm	0.0842	0.3611	1
	Prec	-0.1531	0.3779	2
	EVI _m	0.0332	0.1388	1
	NDBI _m	-0.0844	-0.2799	1
	LST _{mD}	-0.0140	0.0828	2

Tm: medium temperature, HRm: medium relative humidity, Prec: precipitation, EVI_m: medium vegetation index; NDBI_m: medium build up index; LST_{mD}: medium day surface temperature.

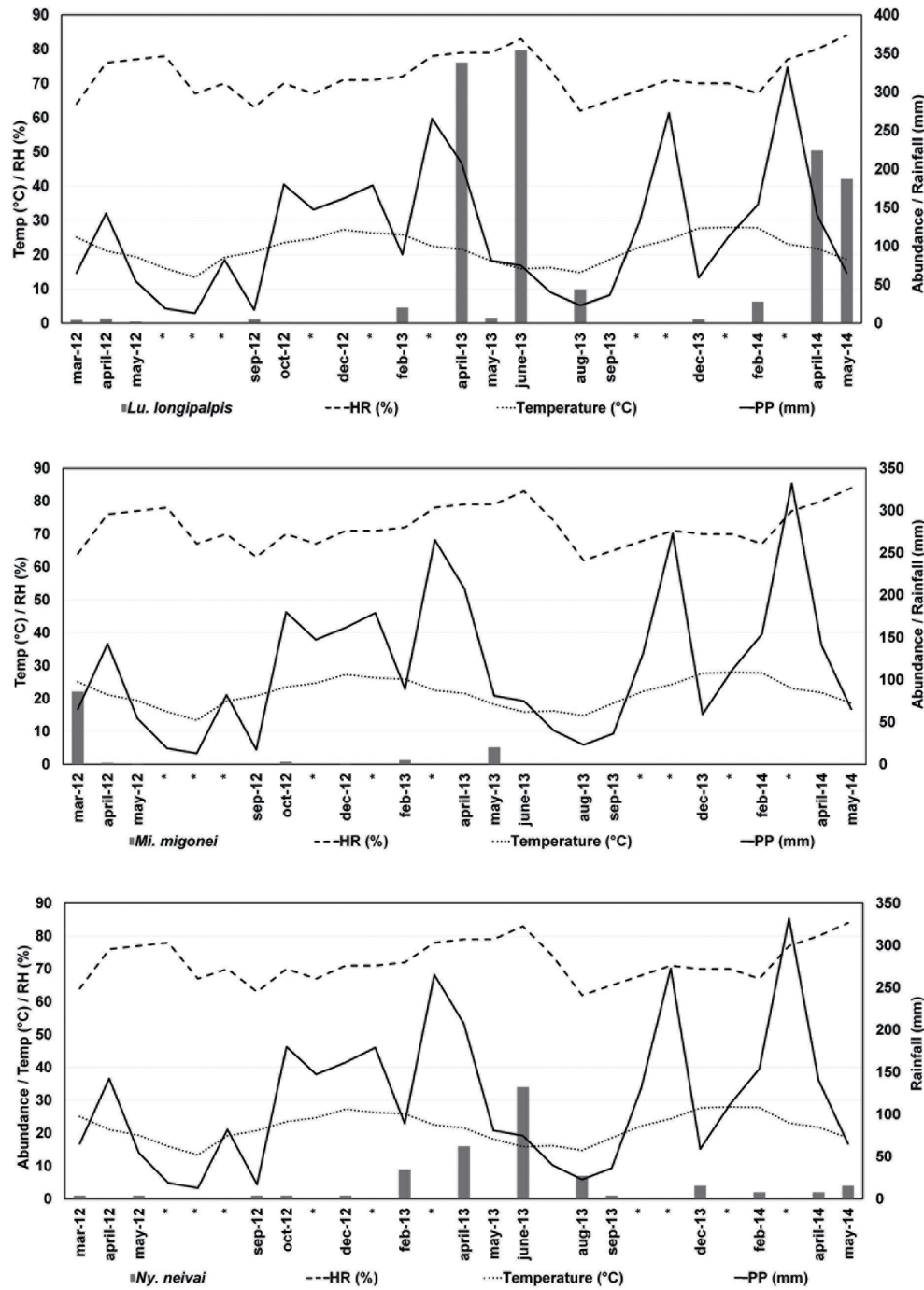


Figure 3. Abundance of *Lu. longipalpis*, *Mg. migonei* and *Ny. neivai*, mean temperature, mean rainfall, and relative humidity data.

***Lutzomyia longipalpis* models**

Explanatory variables EVIm2, FrecPrec, and HRm1

Comparing the goodness of fit (AIC) from the 6 models developed as a first step, we found the

negative binomial distribution (family=nbinom2: where dispersion parameter as a quadratic function of the mean) zero-inflated model, as the best one.

Explanatory variables LSTmN2, FrecPrec, HRm1, and prec1

In this particular case, the negative binomial with the dispersion parameter as a linear function of the mean (family=nbinom1) without considering the variable that gives overdispersion was the best.

For both models developed for this species (Table Va, b), we found that relative humidity with 1-month time lag (HRm1) was the only significant variable. Also we saw that the intercept variance of the random factor for both models was very small, indicating that *Lu. longipalpis* abundance doesn't change between years.

Migonemyia migonei models

Explanatory variables EVIm1, PrecFrec, and RHm1

Comparing the goodness of fit (AIC) from the 6 models developed as a first step, we found the negative binomial distribution (family=nbinom1) without dispersion parameter model as the best one to use.

We found no evidence of the existence of a significant effect of any of the variables considered on *Mg. migonei* abundance (Table Vc).

Explanatory variables LSTmN2 PrecFrec, RHm1, and prec2

The best model to apply was a mixed model without dispersion parameter using a negative binomial distribution as a quadratic of the mean (family=nbinom2).

The only variable that has a positive significant effect over the abundance of *Mg. migonei*, in this case, was the precipitation ($p = 0.0152$) (Table Vd).

Nyssomyia neivai models

Explanatory variables EVIm1, FrecPrec, and HRm1

We found the mixed model that considered zero-inflation with negative binomial distribution as a quadratic of the mean (family=nbinom2) as the best one to apply in this particular case (Table Ve). The relative humidity at 1 month time lag ($z = 4.52$; $p = 0.00000618$) and the precipitation frequency (without time lags) ($z = 2.65$; $p = 0.00817$) were both significant variables. There is no evidence that *Ny. neivai* abundance could be influenced by EVI because this variable showed not significance ($z = -0.052$; $p = 0.95867$).

Explanatory variables LSTmN2, FrecPrec, HRm1, and prec2

We found as the best model for these variables the mixed model with zero-inflation and negative binomial distribution as a quadratic of the mean (family=nbinom2) and dispersion parameter (Table Vf).

From the GLMM developed here only the relative humidity at lag 1-month (RHm₁), was significant ($z = 4.070$; $p = 0.000047$).

DISCUSSION

Previous studies on the distribution of Phlebotomine sand flies fauna in Argentina mentioned 12 species for the province of Corrientes (Salomón et al. 2008a, Quintana et al. 2012).

Psathyromyia (*Psathyromyia*) *bigeniculata* (Floch and Abonnenc) was previously considered a junior synonym of *Pa. shannoni*. However, its taxonomic status was revalidated, and based on the morphological differences presented by Sábio et al. (2014) to distinguish *Pa. bigeniculata* from *Pa. shannoni*, the presence of *Pa.*

Table V. GLMM parameter estimates for group of selected explanatory variables for *Lutzomia longipalpis* model.

a Parameter	Estimate	Std. Error	z-value	p-value
Intercept	-23.34851	4.68542	-4.983	<0.001
EVIm (2 time lags)	15.49852	9.02544	1.717	0.0859
FrecPrec	-0.02678	0.09982	-0.268	0.7885
HRm (1 time lag)	0.30378	0.05509	5.514	<0.001*

b Parameter	Estimate	Std. Error	z-value	p-value
Intercept	-35.982075	28.059493	-1.282	0.1997
LSTmN (2 time lags)	0.102475	0.093343	1.098	0.2723
HRm (1 time lag)	0.127094	0.060644	2.096	0.0361*
FrecPrec	-0.029004	0.089864	0.323	0.7469
Prec (1 time lag)	0.004572	0.004149	1.102	0.2704

GLMM parameter estimates for group of selected explanatory variables for *Migonemyia migonei* model.

c Parameter	Estimate	Std. Error	z-value	p-value
Intercept	0.26415	10.32004	0.026	0.980
EVIm	13.15870	14.38974	0.914	0.360
FrecPrec	0.04063	0.11746	0.346	0.729
HRm (1 time lag)	-0.04971	0.09887	-0.503	0.615

d Parameter	Estimate	Std. Error	z-value	p-value
Intercept	-171.82641	127.77380	-1.345	0.1787
LSTmN (2 time lags)	0.59599	0.41103	1.450	0.1471
HRm (1 time lag)	-0.17461	0.14453	-1.208	0.2270
FrecPrec	0.11165	0.23559	0.474	0.2270
Prec (2 time lags)	0.04078	0.01679	2.428	0.0152*

GLMM parameter estimates for group of selected explanatory variables for *Nyssomyia neivai* model.

e Parameter	Estimate	Std. Error	z-value	p-value
Intercept	-10.43569	2.47373	-4.219	<0.001
EVIm (1 time lag)	-0.21563	4.16106	-0.052	0.95867
FrecPrec	0.11326	0.04282	2.645	0.00817*
HRm (1 time lag)	0.14266	0.03156	4.520	<0.001

f Parameter	Estimate	Std. Error	z-value	p-value
Intercept	-13.874771	10.464336	-1.326	0.185
LSTmN (2 time lags)	0.012976	0.035011	0.371	0.711
HRm (1 time lag)	0.135955	0.033404	4.070	<0.001
FrecPrec	0.089704	0.056185	1.597	0.110
Prec (2 time lags)	0.001759	0.002551	0.690	0.490

An asterisk indicates significant p-value (<0.05).

bigeniculata in Corrientes city is confirmed for the present study. Rosa et al. (1999) recorded three *Pa. bigeniculata* (previously *Lutzomyia shannoni*) individuals in Corrientes city using Shannon traps. A few years later, Borda et al. (2002) presented a review of phlebotomine sand flies in 11 departments of Corrientes province where this species is mentioned without indicating the geographic location of capture. Also Salomón et al. (2008a) mention its presence, without mentioning the geographic location of capture in Corrientes province. Berrozpe et al. (2017, 2018) does not capture this species in the following years from these studies, therefore we considered that *Pa. bigeniculata* had not been registered in Corrientes city since the first detection by Rosa et al. (1999) during 1997 samplings. Sábio et al. (2014) mentioned the need for a review of the specimens identified as *Pa. shannoni* to determine the actual distribution of *Pa. bigeniculata* and the possible presence of other species of Shannoni complex in Argentina.

The present study contributes to the confirmation of 5 Phlebotomine sand flies species of medical importance in northeastern Argentina. Two of them, *Lu. longipalpis* and *Ny. neivai*, were collected throughout the sampling period with a wide spatial range in the city of Corrientes. *Migonemyia migonei* was the species in third highest abundance as was also registered by Berrozpe et al. (2018). These findings are in accordance with other studies in different places of northern Argentina like Santo Tomé (Corrientes), Puerto Iguazú (Misiones) and Corrientes city where *Lu. longipalpis* was the most abundant species (Santini et al. 2015, 2017, Berrozpe et al. 2017, 2018). It is important to note that in the present study a higher richness of phlebotomine sand flies species was found for the city of Corrientes, compared to previous studies (Rosa et al. 1999, Borda et al.

2002, Berrozpe et al. 2017). The sites of greatest species richness were found in the peri-urban city area, characterized by less constructions and lower human population density, with surrounding vegetation areas and presence of domestic animals, whereas Berrozpe et al. (2018) found lower phlebotomine sand flies richness in areas with low vegetation measure through NDVI values.

The sampling at site 1 made up 89.1% of the total collected *Lu. longipalpis*. This area is highly urbanized with a landscape appropriate for vector development represent by trees, bushes, and high grass as well as surrounding abandoned green spaces, similar characteristics of those suggested by Quintana et al. (2012), where they also associated high abundance of *Lu. longipalpis* at 100 meters around the sampling point with similar landscape. In Corrientes city urbanized area, the first VL human case was reported in March 2012 where our sampling site 1 was placed. This leads to thinking that this area could act as a hotspot for the disease, which is in accordance with several studies that showed the presence of *Lu. longipalpis* in areas with an urbanized structure (Quintana et al. 2012, Gómez-Bravo et al. 2017, Berrozpe et al. 2018, Santini et al. 2015). We found the highest abundance of *Lu. longipalpis* in urban areas (site 1) and less abundance in periurban areas as sites 3 and 5. Quintana et al. (2012) hypothesized that *Lu. longipalpis* populations of high and medium abundance in urban areas act like hotspots, as they are immersed in a matrix with low presence or absence of vector activity that act as sink populations. The results of the present study provide evidence that our site 1 could be acting as proposed by Quintana et al. (2012). *Lutzomyia longipalpis* was not only the most abundant species, but also the most widely distributed in the study area. The latter point is important to consider because of the

importance of multiple spots of transmission in the urban area. *Nyssomyia neivai* abundance has been associated with the presence of vegetation patches in a periurban area of the cities (Quintana et al. 2012) in accordance with places where we found the higher species abundance during the present study.

Even though sites 6 and 8, have the social conditions, domestic animals, chickens and pigs to support Phlebotomine sand flies development, the vector abundance was low throughout the sampling period. We do not found any explanation for this. We believe that other studies that include other environmental variables not considered in the present one, such as wind speed, should be taken into account.

The presence of avenue street lighting in sites 7 and 8 could also be a factor affecting the presence of Phlebotomine sand flies. As we see on the present study, the highest Phlebotomine species richness was observed where no water or even garbage service were available, considering also that this city sites have animal reservoirs presence (like pigs and chickens) with a high vegetation coverage, which is in accordance with Berrozpe et al. (2018).

Lutzomyia longipalpis was found during the entire sampling period with high abundance during autumn and winter, which is in opposition with the findings of Berrozpe et al. (2018) who found high abundance during spring and summer seasons for the same city. One potential explanation for this discrepancy in findings is that our sampling was done monthly during the sampling period and Berrozpe et al. (2018) sampled one occasion during autumn because of operational reasons. The remainder of the seasons in Berrozpe et al. (2018) were sampled on two occasions during each season, which may be insufficient to represent the real seasonal variation of the city. Additionally, Berrozpe et al. (2018) begin their research immediately after

the end of this study in Corrientes city, so the differences found could be due to changes in climatic conditions between seasons of the different years. In Brazil (Sobral, Ceará), Deane & Deane (1955) showed an association between sampling seasons and *Lu. longipalpis*, finding higher abundance during the rainy season. In our study area as we saw in results, two precipitation picks occurred, as we see one in autumn and the other in spring. Here we observed on this study the higher abundance of *Lu. longipalpis* in accordance with the autumn precipitation pick which could lead to vegetation growth, less insolation, higher soil humidity and thus better insect development.

Oliveira et al. (2008) in Campo Grande, Mato Grosso do Sul, Brazil, found high *Lu. longipalpis* abundance during autumn and winter after a one or two-month lag in precipitation as we found in our study with best correlations at one-month time lag followed by two month time lags. Our study also provides evidence for a positive association not only of *Lu. longipalpis* with precipitation but also with relative humidity and a negative association with temperature. The models developed showed that humidity at a one-month time lag and EVI (vegetation) at a two month time lag had significant effects on *Lu. longipalpis* abundance. Satellite vegetation measured with Landsat 8 images by Berrozpe et al. (2018) found that extreme values of vegetation index correspond with lower vector abundance.

The negative association with temperature could be an indication of a maximum threshold tolerance of this vector and emphasizes the need for further studies on this point that could be very useful for decision makers. Berrozpe et al. (2018) found that extreme temperature values (measure as LST from Landsat 8 satellite) corresponded to lowest *Lu. longipalpis* abundance.

For our *Mg. migonei* models, only precipitation at a 2-month lag time was significant. In a temperate area of central Argentina, Ontivero et al. (2018) found a positive correlation of the abundance of this Phlebotomine sand flies with precipitation from 15 to 45-day lag time periods, but no correlation was found with mean temperature or relative humidity.

Migonemyia migonei abundance was higher in periurban and periurban-rural environments (Sites 2 and 3) with the presence of chickens and pigs. In Santiago del Estero, *Mg. migonei* was the only species found in all sampling sites with autochthonous VL cases reports and was the most abundant in households with a history of canine VL (Salomón et al. 2010), so these authors propose *Mg. migonei* as a putative vector in La Banda, Argentina. These findings are in accordance with the results of the present study, where the predominance of this species was in a household with 2 confirmed canine VL cases (personal communication, Ministerio de Salud de la Nación).

This species was also associated with periurban-rural transition environments and with domestic animals in Córdoba city, and was associated with the peri-domiciliary as well for Misiones (Ontivero et al. 2018, Fernández 2012). It is necessary to note that *Mg. migonei* have been found infected with with *L. infantum* in Brazil by “Leishmania DNA detection” (de Carvalho et al. 2010).

For *Ny. neivai* models relative humidity was the most important variable with one-month time lag, followed by precipitation frequency. This showed the effect of relative humidity during the previous month and precipitation frequency due to the importance of moisture for adult and immature survival. This could be related to soil moisture and the effects of environment for larval survival. These findings

are in accordance with those of Salomón et al. (2008c).

Through the models developed here, we show the utility of knowing the relationship between meteorological and satellite environmental variables (with time lags) and the vector abundance and the importance of these relationships in anticipating and determining risk transition periods and the influence of the environment on those periods.

As final considerations, the study area showed a rich Phlebotomine sand flies of public health importance with a risk of pathogen transmission, suggesting the need for permanent surveillance studies on human and canine cases as well as additional entomological studies. Our findings emphasize the need to consider further studies on the effects of temperatures and maximum thresholds and the need of considering time lag effects in studies of these vectors. Such studies are important for developing predictive models for species distribution and abundance so that decision makers could use these findings to manage the vectors and prevent leishmaniasis transmission.

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