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Co-occurrence and seasonal and environmental distributions of the sandflies *Lutzomyia longipalpis* and *Nyssomyia whitmani* in the city of Puerto Iguazú, northeastern Argentina

M. S. SANTINI[1,2,3], M. S. FERNÁNDEZ[2,3,4], R. CAVIA[2,4]
and O. D. SALOMÓN[2,3,5]

[1]Centro Nacional de Diagnóstico e Investigación en Endemo-epidemias (CeNDIE), ANLIS, Ministerio de Salud de la Nación, Buenos Aires, Argentina, [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina, [3]Leishmaniasis Investigation Network of Argentina (ReDILA), Buenos Aires, Argentina, [4]Departamento de Ecología, Genética y Evolución, FCEN, UBA e Instituto de Ecología, Genética y Evolución de Buenos Aires, UBA-CONICET, Buenos Aires, Argentina and [5]Instituto Nacional de Medicina Tropical (INMeT), Ministerio de Salud de la Nación, Puerto Iguazú, Argentina

Abstract. The aim of this work was to study the distribution of Phlebotominae (Diptera: Psycodidade) abundance in time and space in an area in northeastern Argentina with vector transmission of visceral and tegumentary leishmaniasis. For this, 51 households were selected using a 'worst scenario' criterion where one light trap was set during two consecutive nights in peridomiciles in the transitions between the four seasons, and the environment was surveyed simultaneously. The relationships of phlebotomine assemblage structure and the most abundant species with seasonality and environmental variables were evaluated using a canonical correspondence analysis and generalized linear mixed models, respectively. A total of 5110 individuals were captured. *Lutzomyia longipalpis* (Lutz & Neiva, 1912) and *Nyssomyia whitmani* (Antunes & Coutinho, 1939) were the most abundant species captured in all samplings (98.3% of the total capture). The period of highest abundance of *Lu. longipalpis* was early autumn, and it was distributed in the most urbanized areas. *Nyssomyia whitmani* occupied mainly the less urbanized areas, showing peaks of abundance in early spring and summer. Other species were captured in low numbers and showed seasonal–spatial variations similar to those of *Ny. whitmani*. We confirmed *Leishmania* spp. vector persistence throughout the year in spatial patches of high abundance even during the less favorable season.

Key words. Phlebotominae, seasonal dynamics, spatial dynamics, urban area.

Introduction

Leishmaniasis is a growing public health problem in many parts of Latin America. Several species of dipteran of the subfamily Phlebotominae are involved in the transmission of *Leishmania* spp., etiological agents of visceral leishmaniasis (VL) and tegumentary leishmaniasis (TL).

Lutzomyia longipalpis is the main vector of *Leishmania infantum* (Nicolle, 1908) (syn. *Leishmania chagasi*), the agent of VL in America, while the domestic dog is its principal reservoir in urban areas (Lainson & Rangel, 2005). In Argentina, the first records limited *Lu. longipalpis* to a scarce capture in rural areas (Salomón *et al.*, 2001), but after 2004 this species was found more frequently, and was more prevalent and abundant in urban

Correspondence: María Soledad Santini, Centro Nacional de Diagnóstico e Investigación de Endemo-epidemias – A.N.L.I.S – Ministerio de Salud de la Nación, Av. Paseo Colón 568, 1°, CP 1063, Buenos Aires, Argentina. Tel.: +54 11 4342 0216; E-mail: mariasoledadsantini@gmail.com

and peri-urban areas (Salomón & Orellano, 2005; Santini *et al.*, 2013).

Nyssomyia whitmani (Antunes & Coutinho, 1939), which has been proposed as the major vector of TL in large endemic areas of Brazil (Rangel & Lainson, 2009), was also implicated in Argentina as the main vector of *Leishmania braziliensis* (Vianna, 1911) (agent of TL) in a rural area of Puerto Iguazú, on the border with Brazil (Salomón *et al.*, 2009). In Argentina, despite reports of animal infections, the reservoirs of TL have not yet been identified.

Lutzomyia longipalpis and *Ny. whitmani* show spatial and temporal variations in their abundances (Brandão-Filho *et al.*, 2011; Donalisio *et al.*, 2012; Fernández *et al.*, 2012, 2013), and there are also studies of the distribution of these species at the village/city spatial scale (Oliveira *et al.*, 2008; de Souza *et al.*, 2014). However, studies simultaneously examining the seasonal–village abundance distributions of both species, with a focus on TL/VL, are scarce.

In the last few decades, *Lu. longipalpis* has adapted to be an urban or peri-urban species, but in some places it is still found in rural environments (Salomón *et al.*, 2015). *Nyssomyia whitmani* is a species found mainly in rural areas and it is also common in areas with primary or secondary vegetation (Souza *et al.*, 2002; Rangel & Lainson, 2009; Salomón *et al.*, 2009; Santini *et al.*, 2013), with probable trends towards urban adaptation (Rangel & Lainson, 2009; Santini *et al.*, 2013).

Many studies have reported the association of these phlebotomine species with environmental characteristics. The distribution of *Lu. longipalpis* was associated with climate conditions such as temperature and humidity (Oliveira *et al.*, 2008). Further, peridomestic features such as the presence of chickens or dogs (Alexander *et al.*, 2002; Dias *et al.*, 2003), and the type and amount of vegetation cover, street type, distance to water bodies and landscape features were also related to the distribution of this species (de Oliveira *et al.*, 2012; Fernández *et al.*, 2013; Santini *et al.*, 2015). The abundance of *Ny. whitmani* was also correlated with weather conditions (Souza *et al.*, 2002; Fernández *et al.*, 2012) and it was captured in high numbers in pig and chicken sheds (Salomón *et al.*, 2009). The distribution of *Ny. whitmani* was also associated with several landscape features (Souza *et al.*, 2002; Costa *et al.*, 2007; Missawa *et al.*, 2008; Zeilhofer *et al.*, 2008; Salomón *et al.*, 2009).

Knowledge of the population dynamics and seasonal–spatial distribution modulators of the main *Leishmania* spp. vectors is required to improve current prevention and control strategies, for instance, to prioritize areas for intervention on appropriate scales of time and space, or to delimitate intensified surveillance. Therefore, the aim of this work was to study the distribution of phlebotomine abundance in time and space at a city spatial scale, focussing on records of both VL and TL vector transmission. This approach will contribute to the development of differential health-related messages, recommendations and interventions, and to the proper allocation of resources according to VL or TL risk in urbanized areas with leishmaniasis co-occurrence, increasing their efficacy and efficiency.

Materials and methods

Study area

The study was carried out in the city of Puerto Iguazú, in the province of Misiones, Argentina (25° 36′ S, 54° 35′ W), on the three-country border with Brazil and Paraguay (Fig. 1). Puerto Iguazú shows an environmental gradient from urban to rural landscapes with remaining patches of natural forest. Being one of the most important tourist areas of Argentina as a consequence of its proximity to Iguazú waterfalls, it is visited by over 1 million people every year, and has 82 227 residents (National Institute of Statistics and Censuses, 2010). Currently, both the urban area and the population of Puerto Iguazú are increasing, but it is still a smaller city than its neighbours Ciudad del Este (Paraguay) and Foz do Iguaçu (Brazil).

The area belongs to the Paraná forest ecoregion. The weather is humid subtropical (‘Cfa’ according to Köppen) with hot summers and without dry seasons (Peel *et al.*, 2007). The coldest months are June and July, with a mean minimum temperature of 11.6 °C and 10.8 °C, respectively, while the warmest month is January, with a mean maximum temperature of 31.8 °C. Rainfall is abundant throughout the year, with average annual values of 1919 mm (data from years 1980 to 2012; National Meteorological Service).

Entomological sampling

The city of Puerto Iguazú was divided into 51 squares of 400 by 400 m. In each square, one household peridomicile (sample site) was selected using the ‘worst scenario’ criterion (Santini *et al.*, 2013). The ‘worst scenario’ is a functional definition to denote a site within the study patch with the greatest probability of Phlebotominae presence as a consequence of habitat conditions, such as the presence of dense vegetation which provides shadow, humidity and detritus; soil rich in organic material, and access to blood sources, without the interference of external light. In each sample site, we placed one REDILA-BL light trap (Fernández *et al.*, 2015) which was operative overnight, from 5:00 pm to 9:00 am. The traps were placed 1.5 m above the ground in a henhouse, if there was one; if there was no henhouse, the traps were located in a dog’s outdoor resting place.

The traps were set four times over a 1-year period for two consecutive nights without rain or strong wind. The study was conducted during the transitions of the four seasons: ‘early spring’ from 24 to 27 September 2011 (just one night of sampling because of logistical problems), ‘early summer’ from 14 to 17 December 2011, ‘early autumn’ from 19 to 22 March 2012, and ‘early winter’ from 25 to 28 June 2012.

The phlebotomines captured were dried and preserved prior to processing. The specimens were diaphanized with lactophenol. The identification of species was performed according to Galati (2003) under a compound microscope (Zeiss, Oberkochen, Germany; 400×).

As *Evandromyia cortelezzii* (Brètes, 1923) and *Evandromyia sallesi* (Galvao & Coutinho, 1941) females cannot be discriminated at species level by morphology, individuals are reported as *Ev. cortelezii-sallesi* complex.

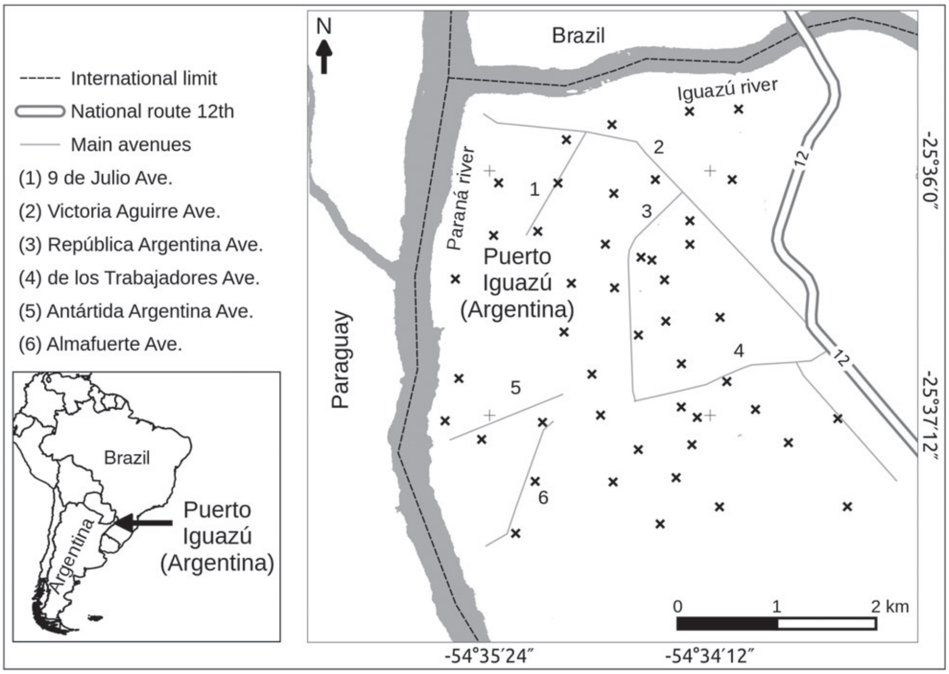

Fig. 1. Location of the city of Puerto Iguazú, province of Misiones, Argentina (study area), on the three-country border with Brazil and Paraguay.

Environmental survey

The peridomestic and neighborhood environments were surveyed in each sampling period simultaneously with the entomological trapping, with an *ad hoc* questionnaire for the householders or direct observation. On the peridomestic scale, we recorded: the surface covered by unused materials ('unused material'); the surface that usually floods with rain ('flooded area'); the number of trees in a 10-m-diameter buffer zone around the trap; and the number of chickens, dogs and pigs. On the neighborhood scale, we recorded the availability of piped potable water ('tap water') and electric power ('electric power'), and the existence of a public waste collection at least two times a week ('waste collection'), an unpaved street as access to the household ('unpaved street'), street lights within 50 m of the sampled site ('street lighting'), and a dump within 300 m of the sampled site ('dump').

Temperature (T) and relative humidity (RH) were recorded with digital thermo-hygrometers (THs; TFA Dostmann, Wertheim, Germany) during the trapping period in a random subset of sampling sites. Between one and 16 THs (usually at least six) were placed in position each night. To estimate T and RH in a sampling site where no TH was placed, the average records for THs placed in other sampling sites were used.

Data analysis

Phlebotomine species occurrence at each sample site was defined as the capture of at least one individual during the sampling period, while the abundance was estimated as trap success = number of individuals captured/number of sampled nights (Seber, 1982). As sampling traps were active for only one night in early spring, we analyzed whether the estimation of the occurrence could be underestimated for this sampling in relation to the other three samplings where the traps were active for two nights. For this, we computed the percentage of increase in the estimations with a two-night protocol compared with a single-night protocol for the three samplings for which these data were available. The trap success of the more abundant phlebotomine species and the occurrence of the less abundant ones were mapped for each sampling period to describe their spatial distribution within the city of Puerto Iguazú.

The relationship of the phlebotomine assemblage structure, based on the species trap success, with environmental variables and the sampling period (explanatory variables) was evaluated using a canonical correspondence analysis (CCA). The association of the occurrence and abundance of each of the most abundant species with environmental variables and the sampling period was assessed using multiple regression analysis. When necessary, explanatory variables were transformed. To avoid including correlated explanatory variables in CCA and multiple regression analysis, the association between environmental variables was studied by Pearson correlation analysis and principal component analysis (PCA) based on a correlation matrix.

Rare species (with an incidence lower than four) were excluded from the CCA analysis. The significance of CCA individual axes was evaluated using 999 Monte Carlo permutations. Variables that contributed to explaining phlebotomine abundances were evaluated using a backward selection procedure. These analyses were conducted using the *vegan* package for R 3.0.1 (R Core Team, 2013).

Multiple regression analyses were performed using a forward stepwise procedure with generalized linear mixed models (GLMMs) (Zuur *et al.*, 2009). Because the same site was sampled four times throughout the study, it was incorporated into the analysis as a random factor. Variations in the occurrence were analyzed using binomial distribution errors and a logit link function; and variations in trap success were analyzed using negative binomial distribution errors considering the dispersion parameter when Poisson distribution errors were used (Zuur *et al.*, 2009). When differences between sampling periods were observed, a Tukey test was performed to evaluate the differences. The order in which the explanatory variables entered the model was according to the explained deviance of the predictors when fitted individually, starting with the explanatory variable that produced a greater change in deviance. When correlations between pairs of explanatory variables were observed, the variable that produced a greater change in deviance was kept, and the other was dropped. Interaction effects between explanatory variables were also evaluated. When the model could not be improved any further, the accuracy of the occurrence models was evaluated using the kappa index, the proportion of correct classifications (PCC), and their sensitivity and specificity with threshold values that maximized the kappa index (Freeman & Moisen, 2008); and for trap success models, we calculated the percentage of deviance explained by the model (according to Zuur *et al.*, 2009). These analyses were conducted using *lme4*, *glmmADMB* and *PresenceAbsence* packages for R 3.0.1 (R Core Team, 2013). We used an alpha level of 0.05 for all statistical tests.

Ethics statement

The study was conducted with the approval of all the neighbors who collaborated in the study: they were previously informed about the methods of the study and signed an informed consent form. The study fulfilled the ethical requirements of the funding agencies (Ministry of Sciences and Technology and Ministry of Health, Argentina).

Results

A total of 5110 individuals of nine phlebotomine species were captured on 347 trap nights (Fig. 2). *Lutzomyia longipalpis* and *Ny. whitmani* were the most abundant species, together representing 98.3% of total capture (3727 *Lu. longipalpis*, with 2991 males and 736 females; 1295 *Ny. whitmani*, with 767 males and 528 females). The maximum quantity of individuals captured during a single night was 638 for *Lu. longipalpis* and 412 for *Ny. whitmani*. The other species captured were *Migonemyia migonei* (França,1920) ($n = 50$; 26 males and 24 females), *Micropygomyia quinquefer* (Dyar, 1929) ($n = 13$; eight males and five females), *Ev. cortelezzii/sallesi* ($n = 11$; only females), *Brumptomyia* spp. ($n = 7$; three males and four females), *Pintomyia pessoai* (Couthino & Barretto, 1940) ($n = 4$; three males and one female), *Nyssomyia neivai* (Pinto, 1926) ($n = 2$; only females) and *Sciopemyia sordellii* (Shannon & Del Ponte, 1927) ($n = 1$; female). Despite the diversity found, only four species were present in all the sampled seasons: *Ny. whitmani*, *Lu. longipalpis*, *Mg. migonei* and *Ev. cortelezzii-sallesi* (Fig. 2).

In relation to peridomestic environmental features, six sampling sites were removed from these analyses because of a lack of complete environmental information. The presence of dogs and chickens was common, even though their presence was used as an *a priori* criterion to select a household as representing the 'worst scenario'. The numbers of dogs and chickens were variable, and pigs were only present in two sites (Table S1A in File S1).

Four out of five environmental variables at neighborhood level were strongly correlated. The availability of tap water and the existence of waste collection, street lighting and an unpaved street were correlated, and in the PCA were associated with the first principal component (1st PC; $|r| > 0.70$ for all variables) that retains 50% of the variance of the environmental variables and where positive values represent more urban services. The availability of tap water and the existence of waste collection and street lighting were associated positively with this component, whereas the existence of an unpaved street was negatively associated with it. For the environmental analyses, the 1st PC was included as an explanatory variable in order to reduce the number of explanatory variables. Proximity to a dump was the only variable associated with the second axis, so it was tested in the environmental analyses at the original scale. Electric power showed low variability, being present in almost all sample sites (Table S1B in File S1). In relation to weather conditions recorded, the warm period extended from the early spring to the early autumn. In the sampling conducted in early autumn, the highest values for daily minimum and maximum average temperatures were recorded, and in the early winter the lowest value for daily maximum average temperature was recorded. In all sampling periods, the average daily maximum relative humidity recorded exceeded 90% (Table S1C in File S1). According to these results, for the CCA and the regression analysis, the following explanatory variables were considered: sampling period, amount of unused material, flooded area, number of chickens, number of dogs, number of trees, 1st PC, proximity to a dump, minimum temperature, maximum temperature, minimum RH and maximum RH.

During the study, the highest abundances of *Lu. longipalpis* were recorded in the northwest of the city and in a few sites in the southeast. However, during the peak of this species in early autumn, it was observed distributed in a larger area within the city (Fig. 2B). In contrast, *Ny. whitmani* was abundant in the northwest (near to the Paraná river) and the southeast, including the southernmost sites sampled. In early spring and early summer, this species peaked its occurrence not exceed 40% of the sampled sites (Fig. 2C). These segregated distributions observed in space were maintained throughout the study period for both species. Regarding the distribution pattern of the less abundant species, it was similar to that observed for *Ny. whitmani* (Fig. 2D). The minimum number of species was observed in early winter and the maximum in early summer (Fig. 2D).

For the phlebotomine assemblage structure analyses (CCA), six species were included after the removal of species that occurred in a low number of sites. A total of 46.3% of the variability in phlebotomine abundance was explained by the

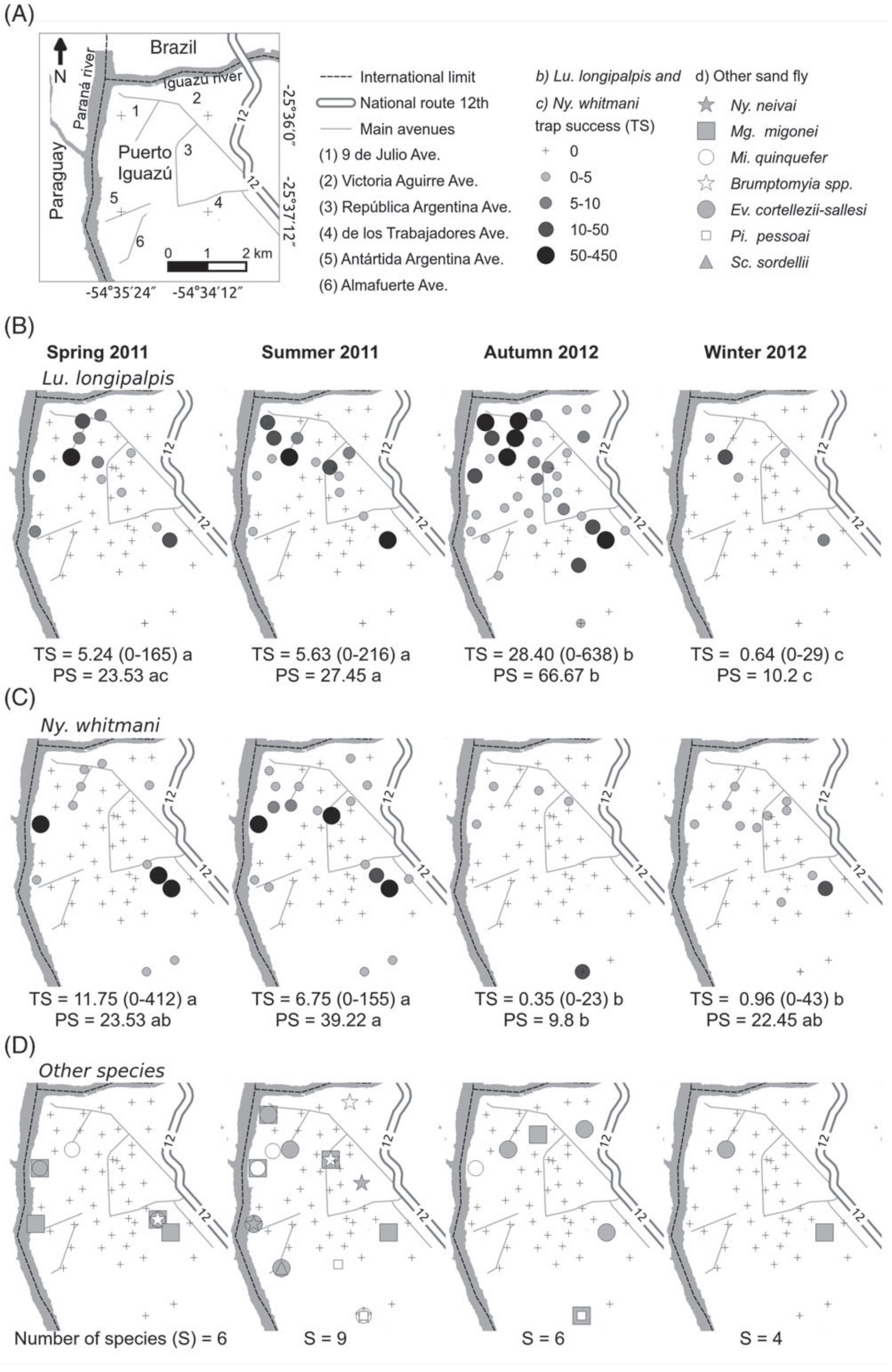

Fig. 2. (A) Location of the city of Puerto Iguazú, province of Misiones, Argentina (study area), on the three-country border with Brazil and Paraguay. (B, C) Spatial distribution of and trap success (TS) for *Lu. longipalpis* (B) and *Ny. whitmani* (C) in each sampling period in Puerto Iguazú. For each species and sampling period, mean TS (and minimum and maximum numbers of individuals recorded in one night in one sample site) and percentage of sampled sites where species occurred (PS) are reported. Different letters for TS and PS for each species indicate significant differences between sampling periods (Tukey test; $P < 0.05$). (D) Occurrence spatial distribution of other phlebotomine species and the number of species recorded (S) in each sampling period in Puerto Iguazú. Spring: 24–27 September 2011; summer: 14–17 December 2011; autumn: 19–22 March 2012; winter: 25–28 June 2012.

Table 1. Phlebotomine species abundances and environmental characteristic scores according to the canonical correspondence analysis (CCA) in Puerto Iguazú, Argentina, September 2011 to June 2012.

	CCA 1
Species scores	
Lu. longipalpis	0.39
Ny. whitmani	−1.30
Mg. migonei	−1.14
Mi. quinquefer	−0.85
Brumptomyia spp.	−1.40
Ev. cortelezzii/sallesi	−0.93
Environmental scores	
1st PC	0.75
Early spring	−0.80
Early summer	−0.97
Early autumn	0.75
Early winter	−0.35

The environmental characteristics explained 46.3% of the variance of phlebotomine species abundances. The first axis (CCA 1) retained 98.9% of it. Only constraining environmental variables retained by the backward selection procedure are reported: 1st PC (first axis of the principal component analysis conducted previously with variables related to the degree of urban services; positive values represent more urban services) and sampling periods.
PC, principal component.

environmental variables analyzed. Almost all the constrained variance was explained by the first axis (CCA1; $P = 0.001$) (Table 1). The backward selection procedure retained two explanatory variables: the sampling period ($P = 0.025$) and the 1st PC ($P = 0.030$). This analysis showed an inverse relationship between *Lu. longipalpis*, with higher abundances in early autumn and in the areas with more urban services, and the rest of phlebotomine species, which were more abundant in early spring and early summer and in areas with less urban services (Table 1).

As *Lu. longipalpis* and *Ny. whitmani* were the most abundant species and are of epidemiological importance, we performed a regression analysis for each of these species. Similar to observations at community level, these analyses showed that the occurrence and abundance of both species significantly changed over the year, with a different pattern for each species (Fig. 2B and C, Table 2 and Table S2 in File S1). The occurrence in peridomiciles in early spring could have been underestimated by the single-night sampling in this season. We observed that the estimate of this variable increased by between 25% and 76% when we compared data from the single-night and two-night protocols. Both the occurrence and abundance of *Lu. longipalpis* increased with the 1st PC and the occurrence was related to a high number of dogs. This last variable explained a low percentage of deviance in relation to seasonality and the 1st PC, and its significance disappeared when a site with a large number of dogs was removed from the analysis (Table 2 and Table S2 in File S1). The *Lu. longipalpis* occurrence model showed good accuracy (kappa index 0.81; correctly classified cases 91.6%; sensibility 89.8%; specificity 92.4%), whereas for the trap success model the percentage of explained deviance was 12.9%. *Nyssomyia whitmani* occurrence and abundance increased with the number of chickens (Table 2); and this species occurrence model showed good accuracy (kappa index 0.77; correctly classified cases 92.1%; sensibility 75.6%; specificity 97.1%), while the percentage of explained deviance in the trap success model was 7.2%.

Discussion

This work is a first approximation to the understanding of the variations of phlebotomine species in time (on a seasonal scale) and space (on a city scale) with presence of species of epidemiological interest for VL and TL transmission. *Lutzomyia longipalpis* and *Ny. whitmani* were the most abundant species of Phlebotominae in the urban environment of Puerto Iguazú. The temporal and spatial distributions patterns were different between these species.

Both species were present throughout the study period. The warm seasons, from early spring to early autumn, were the periods with a higher risk of transmission of VL and TL in the study area, as the competent vector abundance is taken as a risk surrogate. However, we recorded some high abundances even during the early winter period, so the possibility of human−vector contact throughout the year cannot be discounted, even though the winter probability is lower.

The period of highest occurrence and abundance of *Lu. longipalpis* was early autumn so, in view of the extrinsic period of incubation of the parasite, cases of VL could increase during this season. Regarding the distribution in space, the peridomicile farthest away from the forest edge had the highest proportion of *Lu. longipalpis*, as was found previously (Santini *et al.*, 2013; de Andrade *et al.*, 2014). In addition, in this study we found that urbanized landscapes or areas with more public urban services were more suitable for *Lu. longipalpis*. However, the definition of the urbanization range is relative, as there is not a very dense urbanized downtown in Puerto Iguazú. However, this species occurred over a large part of the study area during peaks of abundance, which is consistent with potential periodical colonization of these sites.

The records of occurrence and abundance of *Ny. whitmani* indicate that TL cases could appear throughout the warm season. As has been previously reported, this species was observed mainly in peri-urban and more rural areas (Souza *et al.*, 2002; Fernández *et al.*, 2012; Santini *et al.*, 2013). Only a few sites with a high abundance of *Ny. whitmani* were observed in the most urbanized areas, but all these were close to primary vegetation or dense vegetation patches. *Nyssomyia whitmani* also expanded its distribution through the city during its peaks of abundance, but it was found throughout the year only in less urbanized areas.

Variations in the occurrence and abundance of *Lu. longipalpis* and *Ny. whitmani* were better explained by seasonality than by temperature changes. The explanation for this could be the fact that seasonal dynamics is a complex process resulting from abiotic variables modulating biotic ones. Temperature, rainfall, and RH have previously been studied as possible factors associated with phlebotomine seasonal dynamics (Oliveira *et al.*, 2008, Zeilhofer *et al.*, 2008; de Oliveira *et al.*, 2013; de Andrade

Table 2. Multiple regression using generalized linear mixed models [parameter estimates, standard errors (SEs), and z and *P* values] for the occurrence and abundance of *Lu. longipalpis* and *Ny. whitmani* in Puerto Iguazú, Argentina, September 2011 to June 2012.

	Occurrence			Abundance				
	Estimate	SE	*z* value	*P* value	Estimate	SE	*z* value	*P* value
Lu. longipalpis								
Intercept	−4.16	1.23	−3.38	0.0007	−1.35	0.50	−2.69	0.0072
Early summer	0.43	0.63	0.69	0.4901	0.11	0.45	0.24	0.8113
Early autumn	3.49	0.85	4.08	0.0000	2.33	0.41	5.65	0.0000
Early winter	−1.99	0.84	−2.36	0.0183	−2.46	0.60	−4.07	0.0001
1st PC	0.90	0.31	2.96	0.0031	0.98	0.28	3.54	0.0004
log (dogs + 1)	1.53	0.71	2.16	0.0310				
Ny. whitmani								
Intercept	−2.97	0.83	−3.56	0.0004	−2.47	0.88	−2.82	0.0048
Early summer	1.19	0.59	2.00	0.0450	0.58	0.51	1.14	0.2529
Early autumn	−1.58	0.76	−2.07	0.0385	−2.32	0.76	−3.06	0.0022
Early winter	−0.21	0.65	−0.32	0.7500	−1.28	0.65	−1.98	0.0475
log (chickens +1)	0.63	0.27	2.37	0.0180	0.73	0.27	2.68	0.0073

Variables and their interactions were included by a forward stepwise selection procedure ($P < 0.05$). The sampled site was included as a random factor in all models. Parameters estimated are shown in the linear predictor scale. 1st PC, first axis of the principal component analysis conducted previously with variables related to the degree of urban services (positive values represent more urban services). Numbers of dogs and chickens were logarithmicaly transformed. *Lutzomyia longipalpis* occurrence: null deviance = 220.4; degrees of freedom (df) = 176; residual deviance = 144.8; df = 171; abundance: null deviance = 614.4; df = 175; residual deviance = 536.3; df = 171. *Nyssomyia whitmani* occurrence: null deviance = 182.5; df = 176; residual deviance = 158.2; df = 172; abundance: null deviance = 383.6; df = 175; residual deviance = 355.8; df = 171.

et al., 2014), as well as photoperiod (Rivas *et al.*, 2014). However, climatic variables not only have effects on current activity and therefore the success of trapping, but also have delayed effects mediated by breeding site availability and population size-cohort progression (Salomón *et al.*, 2004).

All the sites had dogs and/or chickens as this was a site selection criterion, and the numbers of these animals were related to *Lu. longipalpis* and *Ny. whitmani* distributions, respectively. Previous studies of feeding patterns of *Lu. longipalpis* reported feeding on blood of birds and dogs, and reports for *Ny. whitmani* included feeding on blood of birds, dogs and pigs (Afonso *et al.*, 2012), although both species are considered opportunistic (Dias-Sversutti *et al.*, 2007; Afonso *et al.*, 2012).

Although environmental characteristics substantially explained *Lu. longipalpis* and *Ny. whitmani* occurrence, abundance was only partially explained by these characteristics. This result might suggest the existence of stochastic factors related to an early colonization of the urban area of Puerto Iguazú by these species. Also, environmental variables not considered or the ones used but recorded in other scales, could improve the prediction of the distribution of these species. In other studies, the abundance of *Lu. longipalpis* was positively related to vegetation cover within urban areas (Fernández *et al.*, 2013). This association for *Ny. whitmani* has not been investigated in detail yet, although previous studies have related *Ny. whitmani* abundance to the type of vegetation (Costa *et al.*, 2007; Missawa *et al.*, 2008; Zeilhofer *et al.*, 2008). In this study, only the estimation of tree density at the household scale was directly related to vegetation cover, but, as no relationship was found with phlebotomine abundance, more precise land cover information is required to examine this factor. Further, this study was conducted only in 'worst scenarios' according to the main objective, so this issue must be considered in the interpretation of the environmental models.

Early summer was the period with the highest species diversity, and the remaining species were < 2% of the total phlebotomine capture, maybe as a consequence of the kind of environments sampled, which were more favorable for urban/peri-urban species (Rangel *et al.*, 2012). Among these species we found *Mg. migonei*, *Mi. quinquefer*, *Ev. cortelezzii/sallesi*, *Pi. pessoai*, and *Ny. neivai*, which are of epidemiological interest as eventual competent vectors of *Leishmania* spp. (de Pita-Pereira *et al.*, 2005; Szelag *et al.*, 2016).

In the analysis of the assemblage of phlebotomine species, we found that high abundances of *Lu. longipalpis* were opposed to high abundances of the other phlebotomine species on the ordination axis. This result supports the difference between urbanized *Lu. longipalpis*, which peaks in early autumn, and the other species, which are still more commonly found in less urbanized areas and are more abundant in spring and summer.

We confirmed the presence of vectors in spatial patches of high abundance even during cold unfavorable periods, which suggests their probable persistence throughout the year. The vectors could spread from there to other environmentally suitable patches when the weather or seasonal conditions improve. Hence, this pattern of source and sink populations can be modulated in time by the weather/season and in space by landscape fitness and obstacles as 'least-cost paths' on a city scale (Fischer *et al.*, 2011). Therefore, if the source population sites are characterized and the source attributes sustained throughout long-term studies, a control intervention strategy could be designed during the most critical seasonal phase for vector growth, in order to avoid further spread to other areas of the city and so to interrupt vector dispersal. Despite the fact that the environmental modification and vector control they are preventive measures might be performed by the community or programmatic agents, the messages and activities they should be focused on these potential source sites, and in moments with a potential vector increase as in winter/early spring, in order to improve intervention effectiveness.

Finally, in view of the location of Puerto Iguazú city, the risk scenario described here could be extrapolated to the border territory shared by Argentina, Brazil and Paraguay, and so it would be advisable for the three countries involved to coordinate prevention and control measures in the region.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/mve.12283

File S1. Supporting information files.

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