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Phlebotominae fauna in a recent deforested area with American Tegumentary Leishmaniasis transmission (Puerto Iguazú, Misiones, Argentina): Seasonal distribution in domestic and peridomestic environments

María Soledad Fernández[a,*], Eduardo Ariel Lestani[b], Regino Cavia[c], Oscar Daniel Salomón[d]

[a] Centro Nacional de Diagnóstico e Investigación en Endemo-epidemias, Paseo Colón 568, 1er piso, Ciudad de Buenos Aires, and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[b] Centro de Investigaciones Entomológicas de Córdoba, Av Vélez Sarsfield 1611, (X5016 GCA) 7 Córdoba, and CONICET, Argentina
[c] Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Intendente Güiraldes 2160 Pabellón 2, Laboratorio 106bis, Ciudad Universitaria, Ciudad de Buenos Aires, and CONICET, Argentina
[d] Instituto Nacional de Medicina Tropical, Jujuy y Neuquén, Puerto Iguazú, and CONICET, Argentina

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ABSTRACT

Phlebotominae sand flies have been involved as vectors of *Leishmania*. In Argentina, *Nyssomyia neivai* and *Nyssomyia whitmani* are involved as the main vectors of American Tegumentary Leishmaniasis (ATL). In the northeastern border of the country, an outbreak of ATL during 2004–2005 was associated with deforestation and subsequent settlement of farmers close to the edge of the forest. The aim of this work was to study the community composition of sand flies along time in farms located near primary and secondary forest in two environments: houses and pigsties. The association of abundance with temperature and precipitation was also evaluated for the most prevalent species. A total of 23,659 Phlebotominae belonging to the genera *Nyssomyia*, *Migonemyia*, *Pintomyia*, *Evandromyia*, *Micropygomyia*, *Sciopemyia*, *Dampfomyia*, *Psathyromyia* and *Brumptomyia* were captured. *Ny. whitmani*, which was the most abundant species, and *Migonemyia migonei*, which was the second most abundant species, were present throughout the year. Both species were positively associated with temperature, mostly up to 31–47 days, and with precipitation at 31 days before the sampling day. The abundance was higher in pigsties than in houses, but the time pattern was positively associated between both environments. These results confirm that *Ny. whitmani* is the dominant species in the study area and its presence throughout the year indicates a potential long period of ATL transmission. The presence of *Mg. migonei* as the second species in abundance is relevant, because it has been described as a secondary vector of the parasites of ATL and a putative vector of the agent of American Visceral Leishmaniasis. We discuss the role of the pigsty as the environment that attract more sandflies, taking into account the number of sand flies captured there, the distance from the home, and the association of sand fly abundance with each of the two environments.

1. Introduction

In Argentina, Phlebotominae have been incriminated as the vectors of *Leishmania braziliensis*, the causal agent of American Tegumentary Leishmaniasis (ATL), endemic in Argentina mainly in the northern border, over a region of 250,000 km^2. Two Phlebotominae species have been identified as primary vectors: *Nyssomyia neivai* (Salomón et al., 2008) and *Nyssomyia whitmani* (Salomón et al., 2009). Since 1985, several outbreaks have been reported in different environmental scenarios from forest to periurban environments, associated mainly with *Ny. neivai* (Salomón et al., 2008). In the Argentinean northeastern border, which is a recently deforested area related to ATL foci and naturally infected with *Le. braziliensis*, *Ny. whitmani* has been found to be the most abundant species (Salomón et al., 2009). Differently, in an old human settlement 50 km from this border, *Ny. neivai* was the main species trapped during an ATL outbreak (Salomón et al., 2001).

Ny. neivai and *Ny. whitmani* have also been incriminated as vectors of *Le. braziliensis* in many foci of Brazil, and reported to have anthropophilic behavior in peridomestic environments (Cerino et al., 2009; Sampaio et al., 2009; Teodoro et al., 2003). Further, the sympatric presence of *Ny. whitmani* and *Nyssomyia intermedia*, species close related to *Ny. neivai* (Galati et al., 2009), has also been reported in Brazil, but with *Ny. intermedia* prevalent in peridomestic–domestic habitats (da Costa et al., 2007; Rangel and Lainson, 2009).

* Corresponding author. Tel.: +54 11 4331 2536; fax: +54 11 4331 2536.
E-mail address: sfernandez79@gmail.com (M.S. Fernández).

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The abundance of *Ny. whitmani* has been reported to increase as the rate of deforestation or the closeness to primary forest increases (Souza et al., 2002; Zeilhofer et al., 2008). *Ny. whitmani* is a sylvatic species but with opportunistic feeding behavior including domestic animals, and a high capacity of adaptation (da Costa et al., 2007; Shaw, 2007; Teodoro et al., 2003). The habitats of *Ny. whitmani* range from forest to peridomestic environments in different geographical areas. This species has also been associated with different mitochondrial lineages or a complex of cryptic species, but all equally anthropophilic, probably related to inter-breeding populations (Campbell-Lendrum et al., 1999; Ishikawa et al., 1999). However, different capability of adaptation to human-modified areas (Rangel et al., 1996) or specific transmission cycles related to parasite genetic polymorphism between lineages cannot be discarded (Rocha et al., 2010). It has also been suggested that after a deforestation, *Ny. intermedia*, *Ny. neivai* and *Ny. whitmani* can share the modified habitat, and that as the environmental disturbance progresses in time or intensity, *Ny. intermedia* s.l. prevails (Rangel and Lainson, 2009). However, this dominance is not evident in all the seasons, or after ten years (Medina JC, personal communication).

In Northeastern Argentina, in the deforestation area mentioned above, a sampling to study the composition of the community in different environments was conducted only once (Salomón et al., 2009). The temporal dynamics of Phlebotominae and its relationship with environmental characteristics are still unknown. The aim of this work was to describe the composition and abundance of sand flies two years after deforestation, and to study the changes in their abundance in time, in both the domicile and peridomicile of recently settled farms, and their association with weather variables.

2. Materials and methods

2.1. Study area

The study area was located in a rural zone with a high deforestation rate, adjacent to the south of the urban area of the city of Puerto Iguazú, Misiones, Argentina. The place, which is an area donated by the National State to the town council, is known as "2000 hectáreas" (the Spanish for "2000 hectares"). The area was originally a native forest, but after its partial deforestation, many farms have established there since 2003, with a peak in 2004 (Salomón et al., 2009). Farmers breed pigs and chickens and plant crops for subsistence. As a result, a mosaic composed of primary and secondary forest, pigsties, chicken houses, crops and houses (farms) compose the landscape of the area. This area is surrounded by natural protected forests on the south and east edges, and the Paraná river in the west (Fig. 1).

In this site, ATL outbreaks were recorded between 2004 and 2005: 31 cases in the study area and 9 in the Uruguaí dam zone, 50 km to the south. After these outbreaks, the area has suffered low but continuous incidence of ATL (Mastrángelo and Salomón, 2009; Salomón et al., 2008, 2009).

2.2. Phlebotomine sampling and identification

Phlebotomine captures were made in three farms of approximately 100 m × 400 m separated at least by 1.25 km and located near the forest. Two of the farms were selected because they had ATL antecedents of human cases whereas the other was chosen because of the high sand fly abundance obtained during the preliminary samplings.

Phlebotomine were captured within three farms during the time of the day of higher activity of sand flies (Alessi et al., 2009; Rangel and Lainson, 2009; Teodoro et al., 2003), from afternoon to the following morning with light CDC traps (from 17.00 to 9.00 h). In each farm one trap was located at the pigsty and another at one of the windows of the house, in both cases hanging 1.5 m above the soil (Sudia and Chamberlain, 1962). We chose these two environments in each farm due to the high abundance previously found in the pigsty (Salomón et al., 2009) and for the epidemiological interest of the house (Mastrángelo and Salomón, 2010). Traps were settled every other week (average 15.6 days, SD 3.8 days) from June 2006 to March 2008, except during January and February 2008, when no samplings were carried out. The sampling was repeated in each environment in each farm from one to three nights, according to the climatological conditions. The captures were performed only during the first and last quarter phases of the moon in order to assure equivalent light intensity. During November 2007 we have a gap in the sample due to logistic and heavy and continuous rainfall. In order to increase the success of capture and so the robustness of the analysis, after the beginning of the sampling in the two farms with recent ATL cases in June 2006 we added a third farm starting the captures in November 2006 due to high abundance of phlebotomine known from previous works in the area. The total sampling effort, considering the three farms, the two environments and repetitions of nights was 78 traps for pigsties and 73 for houses.

All specimens were identified according to the keys of Galati (2003), separated by sex and counted. The generic abbreviations were used as it was proposed by Marcondes (2007). The gravid females of *Ny. whitmani* were also recorded because they constitute an indirect risk indicator (Salomón et al., 2004).

2.3. Data analysis

To compare the average number of Phlebotominae captured in houses and pigsties we used a Wilcoxon test. To compare the community composition between houses and pigsties, we summarized all individuals captured in each environment and conducted an independence test between them. In order to study the association between the abundance of Phlebotominae species in the houses and the pigsties, in each capture ("sampling session"), we computed a Spearman correlation analysis between the abundance in the houses and the abundance in the pigsties. This analysis was carried out computing the average abundance using the captures of the three farms and of each farm separately.

To evaluate which environmental factors could be related to the changes in phlebotomine abundance, we studied the association of the average abundance of sand flies collected in each sampling session with the meteorological data available from Iguazú airport provided by the Servicio Meteorológico Nacional: minimum, average and maximum daily temperature, and values of accumulated daily precipitation. We also studied these associations by applying a delay to the meteorological data of 16 days, 31 days, and so on up to 156 days (corresponding to 10 sampling sessions). To this end, we used simple and partial Spearman correlation analysis, using the average abundance of phlebotomine species of each sampling session from June 2006 to December 2007. This analysis was conducted for the most abundant species of the community of sand flies, and all analyses were considered significant when p-values were <0.05.

3. Results

We captured 23,659 phlebotomines in 151 traps (78 traps from pigsties and 73 traps from houses). The genera *Nyssomyia* and *Migonemyia* represented almost all the captures, and the genus *Brumptomyia* completed the captures with less than 0.50% (Table 1). *Ny. whitmani* dominated the captures, but also an important number of individuals of *Migonemyia migonei* were collected (Table 1).

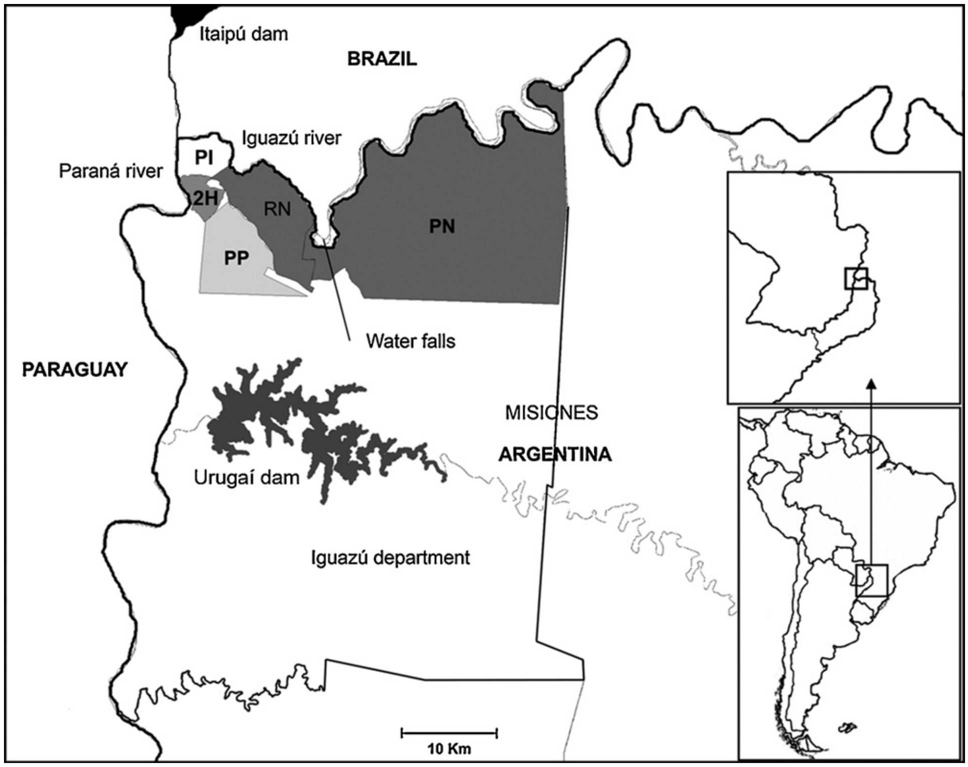

Fig. 1. Study area and its location in South America. 2H: "2000 hectáreas"; PI: Puerto Iguazú town; PP, RN and PN: protected natural areas ("Puerto Península", "Iguazú Reserve" and "Iguazú National Park" respectively).

Table 1
Phlebotominae caught in houses (73 traps-night) and pigsties (78) from three farms in Puerto Iguazú by species, sex and environments, and relative abundance for species (×100).

Species	Houses			Pigsties			Total (%)
	Females	Males	Total	Females	Males	Total	
Ny. whitmani	1164	661	1825	13,893	6009	19,902	91.8340
Mg. migonei	25	53	78	572	604	1176	5.3003
Pi. fischeri	3	1	4	161	164	325	1.3906
Ps. shannoni	2	1	3	36	60	96	0.4184
Pi. pessoai	5	5	10	24	18	42	0.2198
Ny. neivai	3	2	5	21	22	43	0.2029
Ev. cortelezzii/sallesi	1	0	1	10	5	15	0.0676
Pi. monticola	1	0	1	3	5	8	0.0380
Ps. punctigeniculata	1	1	2	0	0	0	0.0085
Mi. quinquefer	0	0	0	2	0	2	0.0085
Sci. sordelli	1	0	1	1	0	1	0.0085
Mi. oswaldoi	1	0	1	0	0	0	0.0042
Da. firmatoi	0	0	0	0	2	2	0.0085
Pi. damascenoi	0	0	0	1	0	1	0.0042
Ps. pascalei	0	0	0	1	0	1	0.0042
Pi. bianchigalatiae	0	0	0	1	0	1	0.0042
Brumptomyia spp.	5	11	16	37	60	97	0.4776
Total	1212	735	1947	14,763	6949	21,712	

The remaining species (except the genus *Brumptomyia*) accounted for less than 2.50% of the captures, being eight species represented by only one or two individuals.

The abundance of phlebotomines was higher in pigsties than in houses ($p < 0.0001$, with 91.26% of all specimens in the pigsties). The average of individuals by trap was 278.30 and 26.76 for pigsties and houses respectively.

Ny. whitmani was the dominant species in both environments, followed by *Mg. migonei*. In pigsties, *Pintomyia fischeri* was the third most abundant species while the remaining species were less represented in both environments. Species were not equally distributed between houses and pigsties ($X^2 = 50.05$; $p < 0.0001$): *Pi. fischeri* and *Mg. migonei* were more abundant in pigsties, while *Pintomyia pessoai*, and all the other species pooled were more abundant in houses (independence test, $p < 0.05$ in all cases). *Ny. whitmani*, *Ny. whitmani* gravid females, *Ny. neivai*, *Psathyromyia shannoni* and *Brumptomyia* spp. resulted homogeneously distributed in both environments (independence test, $p > 0.05$ in all cases).

Ny. whitmani was present in pigsties and houses during all the seasons, although higher abundances were observed during the warm months (September to December-Spring, December to March-Summer and March to June-Fall, Fig. 2). Gravid females of *Ny. whitmani* were recorded in all seasons, and the percentage of

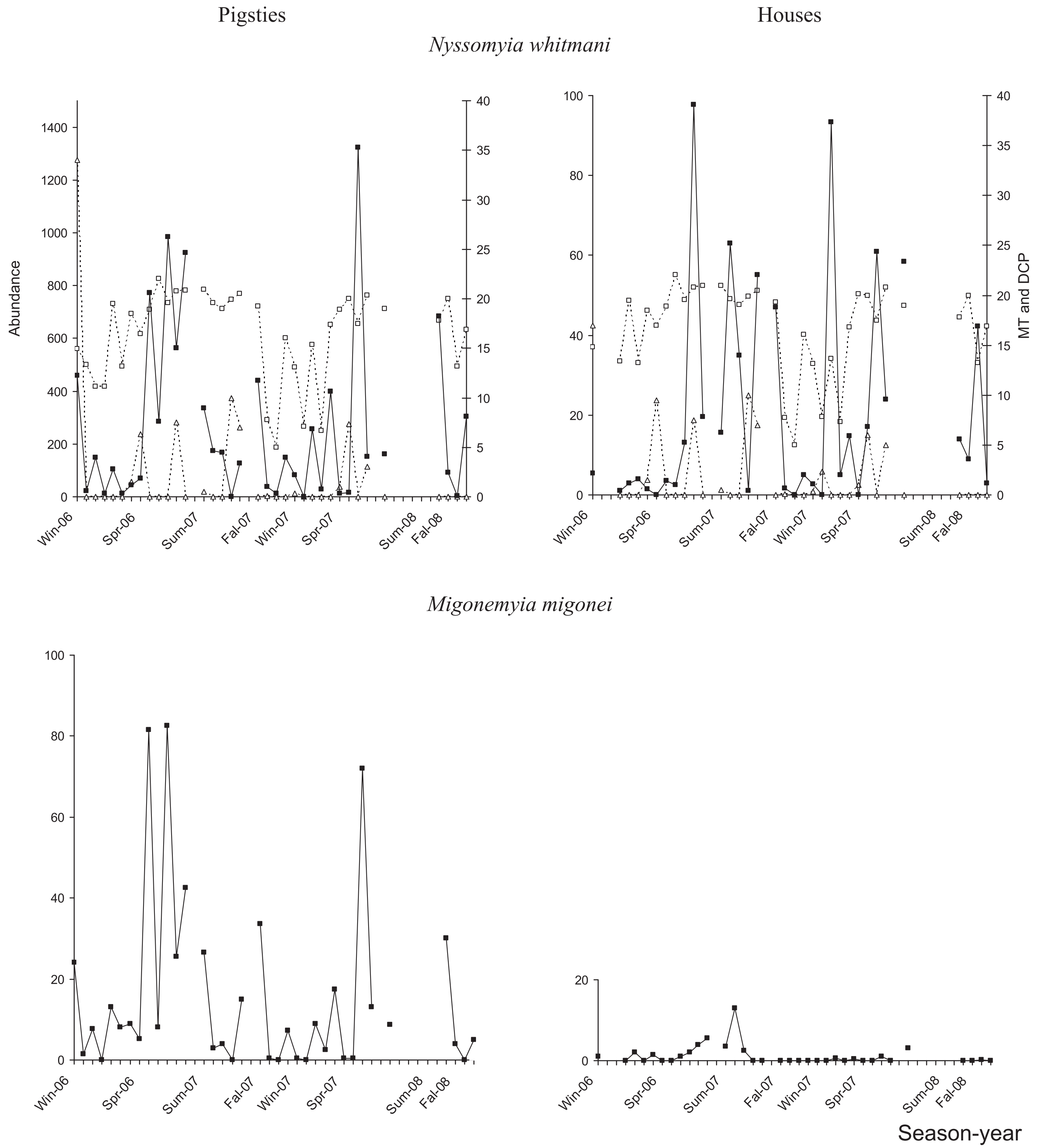

Fig. 2. Abundance of *Ny. whitmani* and *Mg. migonei* (continuous line), and daily cumulated precipitation (triangles) and minimum temperature (squares) in pigsties and houses. Win: winter; Spr: spring; Sum: summer; Fal: fall; 06, 07 and 08: years 2006, 2007 and 2008. The discontinuities in the sample are shown without points on the *x* axis.

gravid females in both environments was similar (1.46% pigsties, 1.49% houses), but with differences in the total numbers: 17 and 207 total gravid females in houses and pigsties respectively. *Mg. migonei* was present in pigsties during all seasons, although its abundance dropped markedly in houses during winter (Fig. 2). The abundance of *Pi. fischeri*, *Pi pessoai*, *Ny. neivai* and *Brumptomyia* sp. increased mainly in spring and summer, whereas that of *Ps. shannoni* remained present mostly all around the year with 1.23 individuals by trap in pigsties (data not shown).

The mean abundances recorded in pigsties and houses were positively associated for *Ny. whitmani* ($r_s = 0.67$; $p = 0.0003$) and *Mg. migonei* ($r_s = 0.51$; $p = 0.0052$). However, this association was lower when the analysis was carried out separately for both species ($0.003 < p < 0.06$).

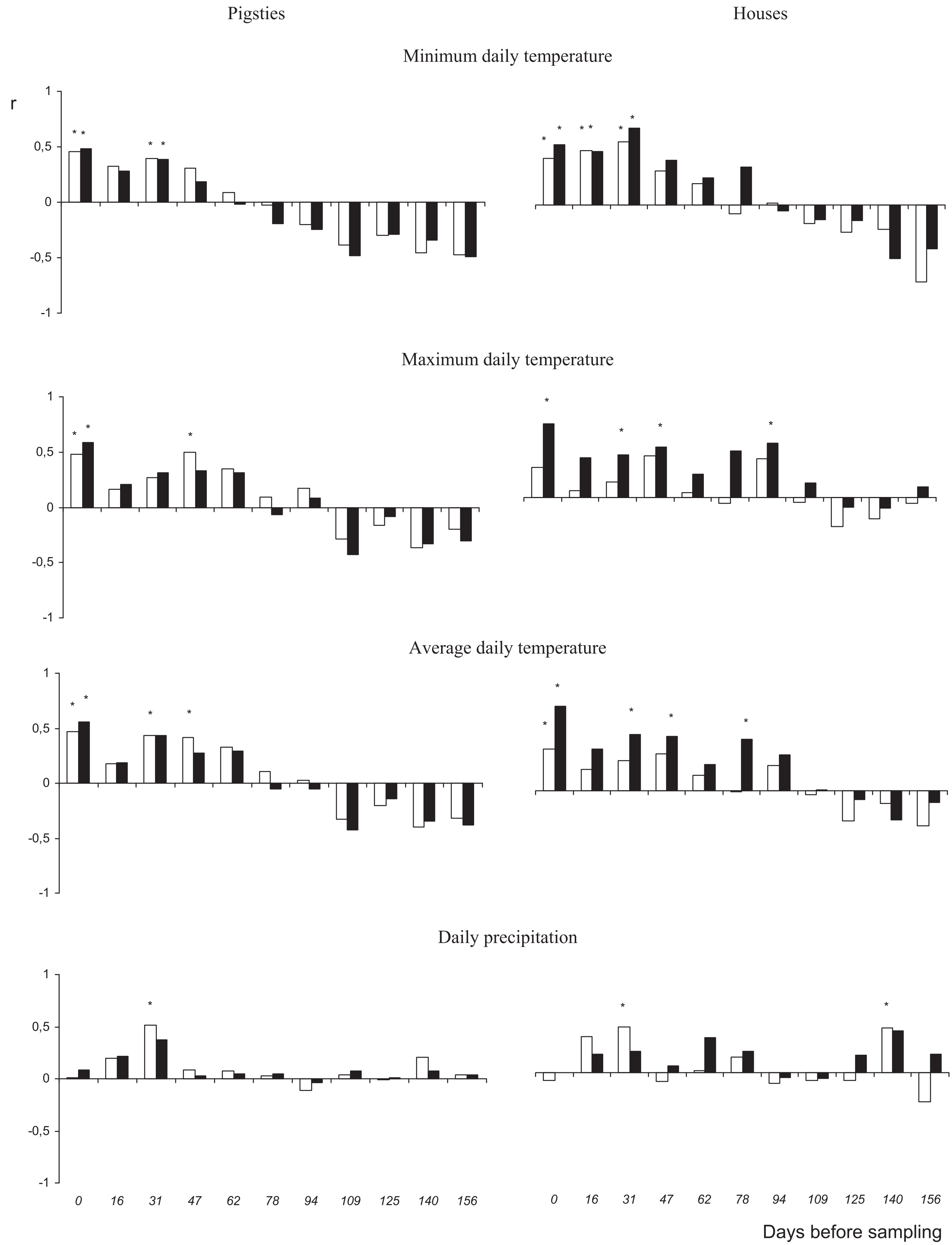

Fig. 3. Correlations between the abundance of *Ny. whitmani* (white bars) and *Mg. migonei* (black bars) with four climatic variables averaged, from day cero (same day of sampling) up to 156 days before (10 previous samples). *x*-axis: days before sampling, *y*-axis: Spearman *r* coefficient. The asterisk indicates positive association between abundance and the climatic variable showed.

In both environments, the abundance of *Ny. whitmani* and *Mg. migonei* was associated positively with all the temperature variables when no delay was applied in the analysis. In general, this association decreased when it was compared with the temperatures of 16 days before the captures and then increased, reaching a maximum value of association (r_s coefficient), generally at 31 days of delay, but also at 47 days of delay (Fig. 3). Inverse associations between the abundance and temperatures started generally after 78 days of delay. The minimal daily temperature at which phlebotomine were recorded was 6 °C, with a daily maximum of 19.5 °C. The abundance of *Ny. whitmani* in both environments was positively associated with the daily accumulated precipitation of the last 31 days previous to the sampling session. For *Mg. migonei*, this association was marginal ($p < 0.10$) and only in pigsties (Fig. 3). However, significant associations between *Ny. whitmani* and *Mg. migonei* with precipitation (at 31 days of delay) became not significant when the effect of temperature variables was removed using partial Spearman correlation analysis ($p > 0.05$ for all cases).

4. Discussion

Just after the main deforestation in the ATL focus of “2000 hectáreas”, Misiones, Argentina, *Ny. whitmani* was the dominant Phlebotominae species followed by *Mg. migonei* (Salomón et al., 2009), four years later the relative abundance of these species was similar. *Ny. whitmani* is also the dominant species (up to 98.9%) in many foci of ATL in Brazil, where it has been suggested to be in the process of adaptation to modified environments, present in domestic (endophilic and endophagic) and peridomestic habitats, and feeding on humans, and domestic and synanthropic animals (Cerino et al., 2009; Colla-Jacques et al., 2010; Fonteles et al., 2009; Paiva et al., 2010; Rebêlo et al., 2009; Sampaio et al., 2009; Teodoro et al., 2003). Although this species could be persistent after the deforestation (Alessi et al., 2009; Azevedo et al., 2002; Missawa et al., 2008; Zeilhofer et al., 2008), its replacement by *Ny. intermedia* in peridomestic habitats has also been suggested (Rangel and Lainson, 2003). However, in the study area, *Ny. neivai*, a species closely related to *Ny. intermedia*, remains in much lower abundance than *Ny. whitmani* in domicile and peridomicile, despite the prevalence of *Ny. neivai* in the ATL focus in a rural neighborhood 50 km from “2000 hectáreas” (Salomón et al., 2001).

The phlebotomine abundance and species richness in domicile were lower than those obtained in pigsties, except *Psathyromyia punctigeniculata* and *Micropygomyia oswaldoi*, which was represented by two and one individuals in domicile respectively. Although the abundance of *Ny. whitmani* and *Mg. migonei* in both environments was different, their peaks in abundance were associated. The relative abundance between these habitats has been previously described for *Ny. whitmani* (Sampaio et al., 2009; Souza et al., 2002). Since this is an anthropophilic species, but also an opportunistic feeder, pigsties and hen houses could provide blood, shelter and breeding places for this vector (Campbell-Lendrum et al., 1999; Rodrigues et al., 2003; Zeilhofer et al., 2008). Therefore, any zoo-prophylactic strategy for ATL should be evaluated taking into account the risk associated with the increased size of the population due to animal dwellings, the spatial disposition of these structures in relation to the house, and the parasite-dilution effect of the different available hosts. The fact that the association between peridomicile and domicile abundances of all the farms computed together turns to a weak association and the fact that there was no association when the farms were analyzed individually could be explained by the differences in environmental management (weeding, regular removing the manure from pigsties, pigsties structure) and insecticide application by the householders between farms (MSF and ODS, personal observation). Thus, even if the zoo-prophylactic strategies are effective, they should be also integrated to other measures to avoid an actual increase in human–vector effective contacts.

The abundance of *Mg. migonei* also has epidemiological importance, as it has been incriminated as a vector of ATL in Brazil and Argentina (Rangel and Lainson, 2003; Salomón et al., 2008). This species, abundant in animal dwellings, could be the link between the zoonotic and anthropo-zoonotic ATL cycles (Chaves and Añez, 2004). Further, *Mg. migonei* is also epidemiologically important for Visceral Leishmaniasis (caused by *Leishmania infantum* (*syn chagasi*)), because it has been suggested as a putative vector (Salomón et al., 2010a; de Carvalho et al., 2010). *Le. infantum* is already circulating in the urban area of Puerto Iguazú, where *Lutzomyia longipalpis*, the primary vector of this parasite, is also present, but up to now there are no human cases of VL reported in the Puerto Iguazú area, but canine cases were recorded (Salomón et al., 2011).

In relation to the remaining Phlebotominae community, the species found here have been reported in previous captures (Salomón et al., 2009), except for the absence of *Pintomyia misionensis* and the new records for Argentina of *Mi. oswaldoi*, *Pintomyia bianchigelatiae*, *Dampfomyia firmatoi* and *Pintomyia damascenoi* (Salomón et al., 2010b).

The association between the abundances of *Ny. whitmani* and *Mg. migonei* and temperature with no delay (time 0) may be related to the increase in the metabolism of adults, even the flying-food searching activity. The association between the abundance of this species and the temperature recorded at 31 and/or 47 days before may be related to the increase in metabolism in all the other stages of the biological cycle. Precipitation may be related to the humidity of the soil and the survival only of the larvae, as the lag is consistent with the Phlebotominae larval period (Alexander et al., 1994; Brazil and Gomes Brazil, 2003). However, according to our results, at 31 days of delay, temperature explained the changes observed in abundance better than precipitations, probably because precipitations are relatively abundant along the year in the study area where the climate is subtropical without dry season (IGM, 1998), therefore not being a limiting factor. These hypotheses should be further tested. Since the data of environmental variables were obtained at a weather station a few miles from the study area, the relationships with this factor at a micro-scale level should be further analyzed. In other foci *Ny. whitmani* was also present all year round and its abundance is modulated by the precipitation, and temperature as in our results (Rangel and Lainson, 2009; Alessi et al., 2009; Souza et al., 2002; Teodoro et al., 2003), the delayed association between rainfall-soil humidity and larval development suggested here was also observed by the peaks after the rainy season, after the soil water balance surplus, with temperatures above 20 °C (Cerino et al., 2009; Colla-Jacques et al., 2010). On the other hand, *Ny. whitmani* is reported more abundant in the coolest season not in the warmth months, difference that could be explained by local meteorological conditions as rainfall pattern, humidity and winds, the last one variable not recorded in our study (Alessi et al., 2009; Souza et al., 2002; Teodoro et al., 2003).

In summary, the most abundant species, *Ny. whitmani*, including gravid females, used as an indirect indicator of risk although parous females would be more accurate, was present all the year round in domicile and peridomicile. Thus, this species is well adapted to human-modified environments, where it finds micro-environmental conditions that allow it to avoid extreme adverse climate conditions (animal dwellings). This species thus generates a long period of risk to humans, which is extended practically without interruption throughout the year, with increased risk of human–vector contact during the warm months. Therefore, in order to make recommendations in ATL similar risk scenarios related to deforestation and immediate human settlements, we are currently assessing the vector abundance associated with the distribution of dwellings at a micro-spatial scale and designing community participation based on interventions with environmental management. During the warm periods and for at least 45 days after the warm period, personal protective care should be maximized. Similarly, since occasional warm days of winter may also constitute optimal conditions for the vector, this should also be taken into account. However, from the control strategy designing point of view, the winter proved to be the worst season for sand fly populations, besides the fact that short favorable periods during exceptional winters are usually related to short life span of adults and so with uncompleted parasite cycles.

It is of interest to evaluate in the future the relationship between the abundance of sandflies in animal enclosures and nearby remnant vegetation patches, considering environmental variables at macro- and micro-scales. This may provide knowledge about which are the breeding sites in the study area and have a notion of the actual risk of humans to enter the forest or to remain on the edge of animal enclosures. Lastly, the lag between climate variables and vector abundance encourages the exploration of forecasting models with climate indicators to be used in early warning systems.

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