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ECOSYSTEMS

Identification of phlebotomine sand fly (Diptera: Psychodidae) in Atlantic forest fragments and their dispersal to urban area

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Abstract: The geographical distribution of sand flies in Brazil has been the subject of some studies, yet there is no information about the phlebotomine fauna in João Pessoa, State of Paraíba, Brazil. The aim of this work is to evaluate the occurrence and distribution of sand flies in the Atlantic forest fragments and to evaluate a possible dispersion in 06 nearby districts. Light traps were used during three consecutive nights, supplemented by an aspirator during the dry period and rainy season. A total of 222 sand flies were found, 143 (130 males and 13 females) in the Atlantic forest, and 79 in urban areas. During the entire dry season, three species of phlebotomine sand flies were recorded in 11 forest fragments, *Lutzomyia longipalpis*, *Lu. migonei* and *Lu. whitmani*. During the rainy season, only *Lu. longipalpis* was found. This was the only species identified in the studied neighborhoods during both seasons. The differences in diversity of sand flies encountered between natural habitats and urban areas may thus be correlated mostly with adaptations to particular habitats and availability of food. One species (*Lu. longipalpis*) appears to be rapidly adapting to urban areas because of deforestation.

Key words: Ecosystem, entomology, psychodidae, Phlebotomus.

INTRODUCTION

Sand flies (Diptera, Psychodidae, Phlebotominae) are winged, hematophagous insects, that host the protozoans *Leishmania* and *Bartonella*, as well as several arbovirus, such as *Orbivirus*, *Vesiculovirus* and *Phlebovirus* (Forattini 1973, Young & Duncan 1994, Shimabukuro et al. 2011). These vectors are responsible for the transmission of tegumentary (or cutaneous) leishmaniasis and visceral leishmaniasis (Shimabukuro et al. 2011, WHO 2017), in silvatic or peridomestic cycles between human beings and domestic or wild animals. Infected female sand flies transmit these vectors during the suction of blood (WHO 2017).

Among sand flies that transmit visceral leishmaniasis, outstanding species are *Lutzomyia longipalpis* (Lutz & Neiva, 1912) and *Lu. migonei* (França, 1920). *Lu. whitmani* (Antunes & Coutinho, 1939) and *Lu. migonei* transmit tegumentary leishmaniasis (Shimabukuro et al. 2011). *Lu. longipalpis* occurs in the following countries: Argentina, Bolivia, Brazil, Colombia, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Paraguay, Uruguay, and Venezuela. The species *Lu. migonei* was recorded in Argentina, Bolivia, Brazil, Colombia, Paraguay, Peru, Trinidad and Tobago, and Venezuela. *Lu. whitmani* occurs in Argentina,

Bolivia, Brazil, French Guiana, Paraguay, Peru, and Suriname (Shimabukuro et al. 2017).

There are about 1000 described species in the world, of which circa 530 species of sand flies are reported from the Neotropics (Shimabukuro et al. 2017). Of these, 230 species are recorded from the regions of the Amazon, Cerrado, and Atlantic Forest (Dantas-Torres 2011, Shimabukuro et al. 2017). Identification and quantification of these insects is essential in order to elaborate public policies for the control of these tropical diseases (Dantas-Torres 2011).

Tegumentary and visceral leishmaniasis are documented from five regions in Brazil (Brazil 2014, 2017).

Northeastern Brazil has one of the highest rates of human visceral leishmaniasis (Cavalcante & Vale 2014). According to the Informatic Department of the Unified Health Superintendent (DATASUS), the Information System of Notifications (SINAN), recorded 348 cases of visceral leishmaniasis in the State of Paraíba and 13 cases in the city of João Pessoa, during the period from 2007 to 2015. During the same period, 600 cases of tegumentary leishmaniasis were recorded for the State, while 72 cases were recorded for the municipality of João Pessoa (Brazil 2016).

The Campus I of the Federal University of Paraíba (UFPB) has fragments of Atlantic forest and is located very close to several urban neighborhoods. These areas thus are ideal for monitoring patterns of transmission of leishmaniasis in the urban and periurban areas of the city of João Pessoa (Menezes et al. 2016).

This study aims to identify and analyze the species of Sand flies living in the forested areas of the campus of the Federal University of Paraíba and in six neighborhood areas, correlating the recorded patterns with climatic and environmental factors. Such results should serve as a warning for inhabitants or people that circulate in these areas of the risks of being infected by visceral and cutaneous leishmaniasis by these vectors.

MATERIALS AND METHODS

Study area

The present study involves ecological, entomological, and public health issues, analyzed both qualitatively and quantitatively. We evaluated ecological changes in *Lutzomyia* sp. in selected forest fragments of Atlantic Forest in Campus I of Federal University of Paraíba, João Pessoa and in neighborhood urban areas in João Pessoa, State of Paraíba (Castelo Branco, Bancários, São Rafael, Torre, Mangabeira, and Miramar) (Fig. 1).

According to demographic data from Instituto Brasileiro de Geografia e Estatística (IBGE), the human population in the studied neighborhoods is distributed as follows: Mangabeira, 75.988 individuals, Torre, 15.193 individuals, Bancários, 11.863 individuals, Castelo Branco and São Rafael together with a population of 11.642 individuals, and Miramar with 9.500 individuals. The choice of neighborhoods was defined by their demographic importance, as well as by the existing conditions for the transmission of Leishmaniasis.

According to the Geographic Atlas of the Federal University of Paraíba, Campus I has an inner area of 43,98 hectares, distributed into eight fragments, and 43,70 ha surrounding the campus (Rosa & Rosa 2013). Eleven forested areas were selected for this study. We used the following criteria to select study areas: a) Availability of food in the form of wild vertebrates (such as sloths, marmosets, rats, birds, capivaras, and others) for female sand flies; b) Presence or organic matter (litter); c) Presence of humid and shaded areas; d) Areas close to intense movement of human beings.

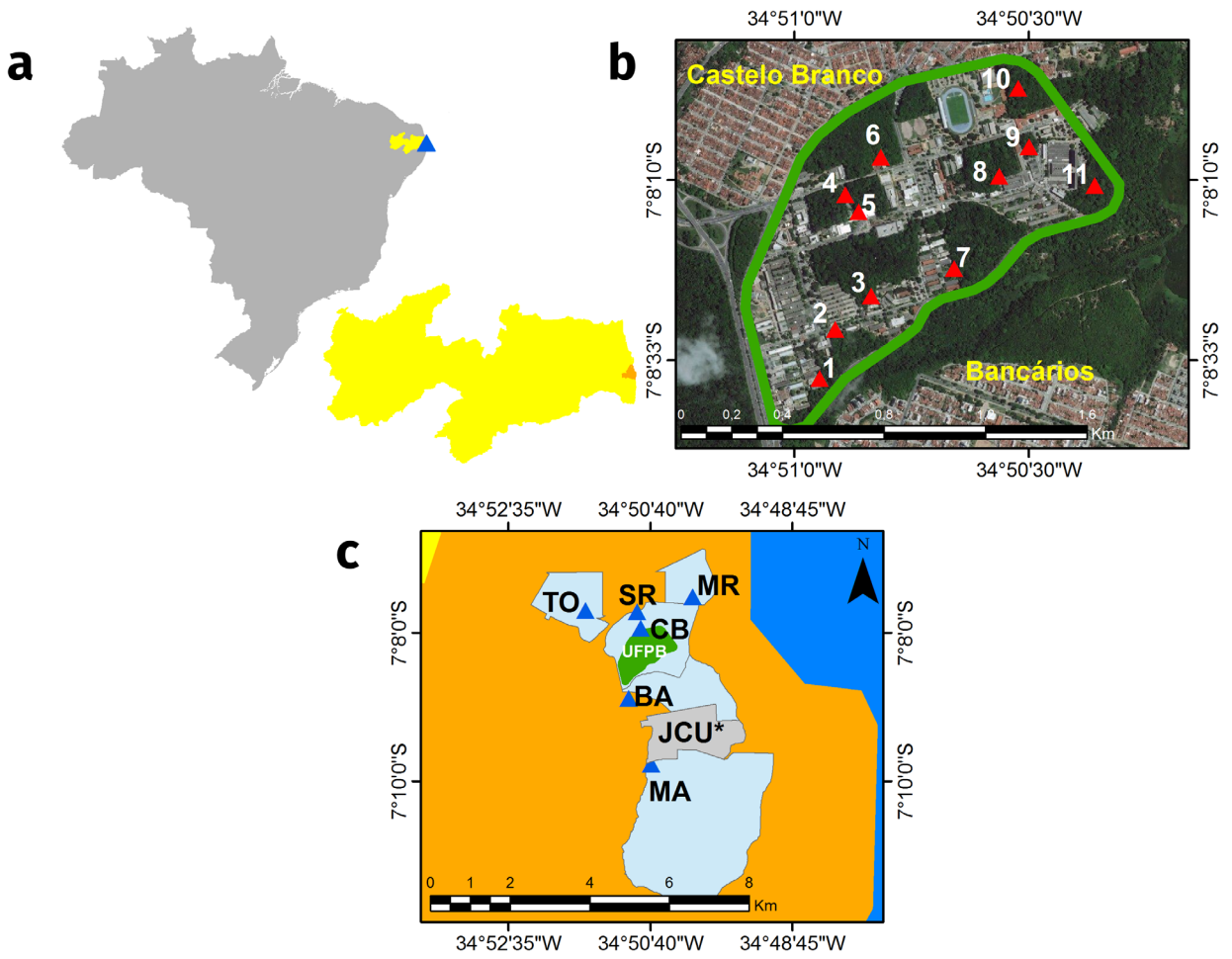


Figure 1. a. Map of Brazil, with the city of João Pessoa, capital of the State of Paraíba, in detail (in orange). b. Map of Federal University of Paraíba (Delimited in green) and the 11 study points (red triangle). c. Map indicating the sampled surrounding neighborhoods (CB: Castelo Branco, BA: Bancários and SR: São Rafael) and the more distant neighborhoods (TO: Torre, MA: Mangabeira and MR: Miramar) (blue triangle).

In later years, these forested fragments are also being used by domestic animals (dogs and cats).

In order to evaluate dispersal, neighborhoods surrounding the campus were selected (Fig. 1): Castelo Branco (trap installed 1 km from University), Bancários (trap at a distance of 2,5 km) and São Rafael (distance of 1,5 km), as well as neighborhoods lying at some distance from campus: Torre (trap installed at a distance of 5 km), Mangabeira (6,8 km) and Miramar (5,5 km). The choices of these neighborhoods were made taking into account their relative closeness to the University, as well as to demographic, socioeconomic and environmental characteristics that favor the cycle of *Leishmania* sp.

Considering the occurrence of cases of human and canine leishmaniasis in the State of Paraíba and in the Municipality of João Pessoa, and taking into account the low number of studies related to the vectors of leishmaniasis, sand flies inquiries were conducted in the area of the Atlantic Forest within the university campus and in six neighborhoods in order to determine the diversity of sand flies in two different ecotopes, besides evaluating the possibility of dispersion.

Climate conditions and Capture of Sand Flies

Flying mosquitos were captured in the Campus forested areas and in the surrounding urban areas. During the dry season, 12 samples were obtained in the months of November and December, and in January and February. Twelve further samples were obtained during the rainy season (March to May).

The climate in the city of João Pessoa is tropical and humid, with a median temperature around 25°C. The dry season occurs between September and February, while the rainy season occurs from March to August. Climatological Data (AESA) indicate that annual precipitation varies from 900 to 1800 mm, while the mean annual relative air humidity is 80% (Lima & Heckendorff 1985).

For each collecting sample, data on temperature and air humidity were obtained, including other information relative to the environment in which the traps were installed.

Light traps equipped with 12A batteries were exposed from 17h00 to 5h00, during three consecutive nights, with intervals of 20 to 35 days between sampling. Manual collections were also conducted with an aspirator in the studied environments.

Samples were fixed in alcohol at 70%. Phlebotomine sand flies were separated from other species under a dissecting microscope in the lab (UFPB).

Identification of Phlebotomine sand flies

For identification, specimens of sand flies were clarified following the technique described in Forattini (1973). The identification key of Young & Duncan (1994) was used.

Analysis of data

Data normality was verified with the Shapiro-Wilk test. Comparison of results was then obtained with the use of Program PAST, version 3.15.

RESULTS

During the study period, 222 phlebotomine sand flies were obtained. Of these, 143 came from forested areas, remnants of primary Atlantic Forest in the Campus of the Federal University of Paraíba. One hundred and thirty (93%) were males and 13 females (9%). In the urban areas, 79 individuals were collected (all males), distributed among the neighborhoods of Castelo Branco (07), Bancários (19), São Rafael (09), Torre (15), Mangabeira (19) and Miramar (10).

During the entire dry season, three species of phlebotomine sand flies, *Lu. longipalpis*, *Lu. migonei* e *Lu. whitmani* were recorded in the 11 forest fragments. *Lu. longipalpis* predominated in all sampled areas, with 95 individuals (86 males and 09 females), followed by *Lu. migonei*, with 10 individuals (06 males and 04 females) and *Lu. whitmani,* with 02 male individuals. Of the 11 sampling points, only Point 1 contained all three species, while Point 10 was the only point in which no species of phlebotomine sand flies were collected.

During the rainy season, only *Lu. longipalpis* was collected in the forested areas. A total of 36 individuals came from the 11 sampling points within Campus I. Points 3, 5, and 8 had a higher abundance, with 08 individuals each. No phlebotomine sand flies were obtained from Points 2, 6, and 9 (Fig. 2).

Temperature and humidity were correlated with the phlebotomine sand flies collected in the 11 forest fragments in order to determine differences between the dry and wet seasons. The Shapiro-Wilk test was applied to verify the normality of the data on mean temperature in the dry and rainy seasons. The data were significantly correlated ($p<0,05$), demonstrating

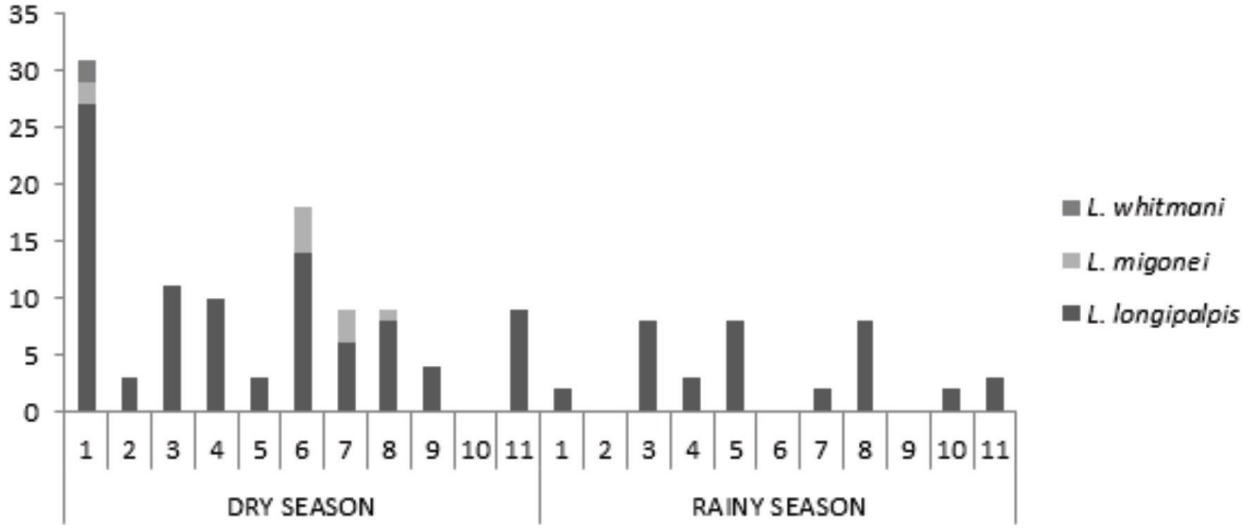


Figure 2. Abundance of the three species of Lutzomyia captured capturadas in 11 fragments of Atlantic Forest in the Campus of Federal University of Paraíba during the dry and rainy seasons.

that distribution is not normal. As a consequence, the non-parametric Wilcoxon test was applied, and a significant difference was observed between the mean temperature of the dry and rainy seasons (W=8428; p<0,05).

Considering the number of sand flies, the data do not present a normal distribution (p<0,05). Thus the Wilcoxon test was applied, and a significant difference was verified (W=58; p<0,05) between the dry and rainy seasons. It is possible to conclude that the quantity of phlebotomine sand flies is significantly larger in the dry season (Table I).

Regarding humidity between the two periods, data are also not normal (p<0,05). The Wilcoxon test indicated that there is a significant difference (W=66; p<0,05) between the dry and rainy seasons. From the dry season to the rainy season, mean temperature decreases while humidity increases (Table I).

To evaluate if the climatic factors resulted in changes in the number of phlebotomine sand flies, a graph is presented correlating these factors to the number of phlebotomine sand flies. As the data or not normal, as concluded above, a Spearman parametric correlation was made. This indicated a significant positive correlation between the number of phlebotomine sand flies and mean temperature (S= 0,45; p=0,036). In other words, as the temperature diminished from the dry to the rainy season, the number of phlebotomine sand flies also decreased. On the other hand, it is possible to observe a significantly negative correlation between the number of phlebotomine sand flies and the humidity of the air (S= -0,48; p=0,023). As humidity increases from the dry to the rainy season, the number of phlebotomine sand flies decreases (Fig. 3).

The academic community of the Federal University of Paraíba has 39.283 enrolled undergraduate students, 2.766 teachers (primary, secondary, and university levels) and 3.572 administrative and technical servers (UFPB 2016). These numbers indicate a large number of people exposed at risk of acquiring Leishmaniasis.

Traps in surrounding and more distant neighborhoods recorded only the species *Lu. longipalpis* in both the dry and the rainy seasons. The species was more abundant in Bancários, with 08 individuals, followed by Torre,

Table I. Wilcoxon test: temperature and humidity during the dry and rainy seasons.

Wilcoxon Test				
	Mean Standard Deviation		W	p-value
Mean temperature (dry)	26,49	1,57	8428	**< 0,001***
Mean temperature (rain)	23,55	0,77	66	**< 0,003**
Humidity (dry)	66,91%	2,50		
Humidity (rain)	78,82%	1,27		
Wilcoxon Test				
	Mean Standard Deviation		**W**	**p-value**
Nr. of phlebotomine sand flies (dry)	9,73	8,61	58	**0,026**
Nr. Of phlebotomine sand flies (rain)	3,27	3,23		

with 06 individuals, Miramar, with 04 individuals, and Mangabeira with 03 individuals. No vectors were collected in Castelo Branco and São Rafael during the dry season (Fig. 4).

On the other hand, during the rainy season, more phlebotomine sand flies were collected: 16 individuals in Mangabeira, 11 in Bancários, 09 in Torre and São Rafael, 07 in Castelo Branco, and 06 individuals in Bairro Miramar (Fig. 4). These data demonstrate an increase in the distribution of *Lu. longipalpis* during the rainy season, a period in which the species was identified in all studied neighborhoods.

DISCUSSION

In Sistema de Informação de Agravos de Notificação (SINAN), from 2013 to 2017, 221 cases of Visceral Leishmaniasis were identified in the State of Paraíba. Fifty-two (23.53%) of these cases refer to the city of João Pessoa. During the same period, 234 cases of American Cuticular Leishmaniasis cases were recorded in the State, of which 49 cases (21%) refer to João Pessoa. Fernandes et al. (2016) worked with a population of 1.043 dogs in the State of Paraíba and identified 81 seropositive animals for *Leishmania* spp. The canine prevalence for this parasitoses for the Municipality of João Pessoa turned out to represent 5,9% of those cases.

The comparison of data between the two studied environments, the Atlantic Forest within the University and the urban sites represented by the 06 neighborhood areas indicated a clear difference in the diversity of phlebotomine sand flies. In the wild environments three species were found (*Lu. longipalpis*, *Lu. migonei* and *Lu. whitmani*), while only *Lu. longipalpis* was found in urban areas. This difference is due to ecological interferences occurring in the anthropic environment. A similar low diversity in urban areas was demonstrated by Barbosa et al. (2009) for the phlebotomine sand flies in Angra dos Reis, Rio de Janeiro, Brazil.

The record of a single species of Phlebotomus (*Lu. longipalpis*) in the urban environment indicates that this species has become adapted to areas influenced by humans. The species *Lu. longipalpis* has been accommodating to the growing loss of native vegetation such as of the Primary Atlantic Forest. This would explain the populational increase of this species in urban areas, elevating the risk of dissemination of Visceral Leishmaniasis in these areas (Martins 2011). The presence of this species in urban environments results from the opportunistic

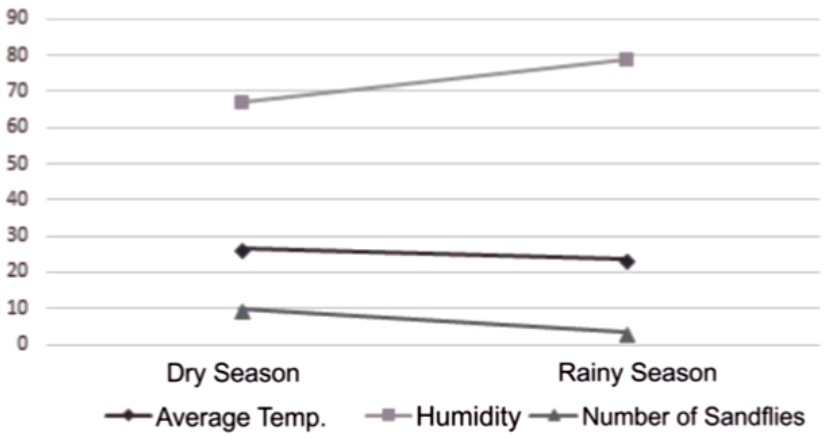


Figure 3. Correlation between the number of phlebotomine sand flies and the climatic factors (humidity and temperature) during the dry and rainy seasons.

SPEARMAN CORRELATION	Mean Temperature (°C)	Humidity (%)
N° of Phlebotomine sand flies	0,45	-0,48
p-value	0,036	0,023

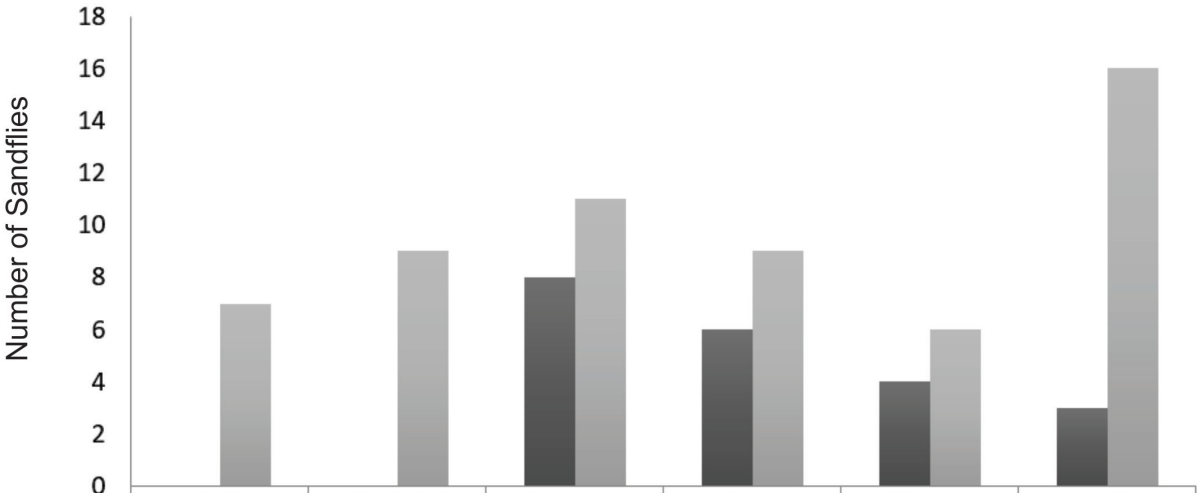
behavior of this species when in seach of sources of carbohydrates and blood.

Despite the low diversity of phlebotomine sand flies in the native areas studied, it is important to stress that they are nevertheless capable of transmitting the two distinct types of Leishmaniasis (Visceral and Tegumentary).

The species *Lu. migonei* and *Lu. whitmani* are associated with the transmission of American Tegumentary Leishmaniasis (ATL). On the other hand, *Lu. migonei* has already been found naturally infected with *Leishmania infantum*, in São Vicente Ferrer, State of Pernambuco (Guimarães et al. 2012), and in the State of Rio de Janeiro (Pita-Pereira et al. 2005).

Cardoso et al. (2009) identified *Lu. whitmani* as the second most prevalent species in the Municipality of Seropédica, State of Rio de Janeiro. In our study Point 1, where we found the three species of phlebotomine sand flies, *Lu. whitmani* was the least prevalent species. Notwithstanding its low densities, this species is the main vector of ATL, transmitting mainly *Leishmania* (*Viannia*) *braziliensis* (Queiroz et al. 1991), although it is also associated with the transmission of *Leishmania* (*Viannia*) *guyanensis* and *Leishmania* (*Viannia*) *shawi* (Lainson et al. 1989).

Male/female proportions found in this study was 9:1 in the Forest Areas, but attained 100% of males in the urban areas. This difference is due to the phototaxic behevior of females, associated with their opportunistic behavior when searching for sources of carbohydrates and blood. They thus become easily attracted to homes in their search for human and domestic animal blood. Cardoso et al. (2009) identified four species of phlebotomine sand flies in the Municipality of Seropédica, Rio de Janeiro (*Lu. intermedia*, *Lu. whitmani*, *Lu. migonei* and *Lu. oswaldoi*), with a proportion of males to females close to 9:1. That research group justifies their results by explaining that males are born before



	Castelo Branco	São Rafael	Bancários	Torre	Miramar	Mangabeira
Dry Season	0	0	8	6	4	3
Rainy Season	7	9	11	9	6	16

Figure 4. Prevalence of phlebotomine sand flies in the studied neighborhoods (Castelo Branco, São Rafael and Bancários being close to the University, Torre, Miramar and Mangabeira being more distant), during the dry and rainy seasons.

females and tend to group together in order to chase females for mating (Cardoso et al. 2009).

The distribution of phlebotomine sand flies through the eleven sampling points in the Atlantic Forest varied according to the environmental characteristics of each area. Point 01 was the only one to present all three species of phlebotomes. The presence of litter was greater in Point 01 than in the remaining areas, which, according to Quinnell & Dye (1994), may favor the life cycle of phlebotomine sand flies. It is important to stress that the density of vectors in forest areas is related to environmental characteristics such as presence of vegetation, roots, tree-trunks, and presence of organic material that provide shelter and are the site of reproduction (Camargo-Neves et al. 2001).

For this study, we selected a forested area, for which we already expected to find a greater diversity, and surrounding urban areas. In the selected neighborhoods, the highest diversity was found in Bancários and Mangabeira. These areas, as well as being very close, suffered an intense and rapid socioeconomic growth that resulted in environmental changes of previously forested areas. Such changes may have favored the dispersion of phlebotomine sand flies to human environments and may have favored the increase of cases of Leishmaniasis. The prevalence of *Lu. longipalpis* in these neighborhoods confirms this species as being the best adapted for anthropic settlements. This species is now largely synanthropic (Dantas-Torres et al. 2012).

Regarding climatic conditions, *Lu. longipalpis* prevailed and was the only species found in the wet season in forest fragments. These data are in accord with Machado et al. (2017), who identified a larger richness of species in forests and family units in the city of Palmas, during the month of July (dry month), when compared to the month of November (wet season). According to those authors, phlebotomes become reduced in numbers when the soil is drenched during the rainy season. Similar results regarding dry

versus wet seasons were reported by Pinheiro et al. (2013) in the State of Rio Grande do Norte and by Moraes et al. (2015) in the State of Maranhão.

Our evaluation of the neighborhoods lying outside of the rain forests found a larger abundance of *Lu. longipalpis* in the wet season. We recorded 16 individuals in Mangabeira, 11 in Bancários, 09 in Torre and São Rafael, 07 in Castelo Branco, and 06 individuals in Bairro Miramar. During the rainy season in which the traps were installed there occured a larger concentration of domestic animals, such as dogs and chickens, for example, which may have been responsible for attracting the phlebotomine sand flies.

In Northeast Brazil a dispersion of *Lu. longipalpis* has occurred away from originally forested habitats located in rural areas, where these phlebotyomids fed mainly on wild animals and plants (Ximenes et al. 2000). With the growth of urban zones in areas where these vectors of a serious tropical disease were endemic, a constant populational advancement of dipterans belonging to this genus has been observed (Ximenes et al. 2007). Our study corroborates the previous observations that the species *Lu. longipalpis* is dispersing from the forested areas located within the Federal University of Paraíba in the direction of neighboring urban areas, as a consequence of the adaptive behavior of these phlebotomine sand flies towards anthropic environments.

CONCLUSION

The characterization of the fauna of phlebotomine sand flies in a particular region is important from an entomological, an epidemiological, and a public health perspective. Areas may be identified as representing potential risks for the transmission of parasitoses. The results of this research detected the presence of phlebotomine sand flies in the Federal University of Paraíba Campus and in all surrounding neighborhoods.

Distinct differences in the diversity of phlebotomine sand flies were observed between the forested areas within the University Campus and the 06 surrounding urban areas. Three species were found in the wild areas (*Lu. longipalpis*, *Lu. migonei* and *Lu. whitmani*), while only one species was identified in the urban areas (*Lu. longipalpis)*.

In both forested and urban areas the values of mean temperature and humidity during the dry and rainy seasons were similar (26 and 23ºC, 66 and 78%, respectively); The differences in diversity of phlebotomine sand flies encountered between natural habitats and urban areas may thus be correlated mostly with adaptations to particular habitats and availability of food.

One species (*Lu. longipalpis*) appears to be rapidly adapting to urban areas because of the deforestation of native habitats. The protection of wild habitats may be important to avoid the dissemination of Leishmaniasis into urban areas. The monitoring of wild and domestic animals for the detection of *Leishmania* may represent an important step for the control of Visceral and Tegumentary Leishmaniasis.

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