

RESEARCH ARTICLE

Distribution of *Lutzomyia longipalpis* Chemotype Populations in São Paulo State, Brazil

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Abstract

Background

American visceral leishmaniasis (AVL) is an emerging disease in the state of São Paulo, Brazil. Its geographical expansion and the increase in the number of human cases has been linked to dispersion of *Lutzomyia longipalpis* into urban areas. To produce more accurate risk maps we investigated the geographic distribution and routes of expansion of the disease as well as chemotype populations of the vector.

Methodology/Principal Findings

A database, containing the annual records of municipalities which had notified human and canine AVL cases as well as the presence of the vector, was compiled. The chemotypes of *L. longipalpis* populations from municipalities in different regions of São Paulo State were determined by Coupled Gas Chromatography – Mass Spectrometry. From 1997 to June 2014, *L. longipalpis* has been reported in 166 municipalities, 148 of them in the Western region. A total of 106 municipalities were identified with transmission and 99 were located in the Western region, where all 2,204 autochthonous human cases occurred. Both the vector and the occurrence of human cases have expanded in a South-easterly direction, from the Western to central region, and from there, a further expansion to the North and the South. The (S)-9-methylgermacrene-B population of *L. longipalpis* is widely distributed in the Western region and the cembrene-1 population is restricted to the Eastern region.

Conclusion/Significance

The maps in the present study show that there are two distinct epidemiological patterns of AVL in São Paulo State and that the expansion of human and canine AVL cases through the Western region has followed the same dispersion route of only one of the two species of

the *L. longipalpis* complex, (S)-9-methylgermacrene-B. Entomological vigilance based on the routes of dispersion and identification of the chemotype population could be used to identify at-risk areas and consequently define the priorities for control measures.

Author Summary

Information on the geographical distribution, dispersal mechanisms and dispersion route of insect-borne diseases can help to identify ongoing transmission areas, new risk areas and guide surveillance and control activities. *Lutzomyia longipalpis*, the principal vector of American visceral leishmaniasis disease in the Americas, is a group of closely related species that can be separated according to the type of pheromone produced by male individuals. It is still unclear how many members there are in this complex, how they are related and if some are more important vectors than others. In the present study, we show that the expansion of human visceral leishmaniasis in the state of São Paulo, Brazil, has followed the same dispersion route of only one of the two related species of *L. longipalpis* found in the state. The maps that we produced in the present study will allow us to determine risk areas for the occurrence of human visceral leishmaniasis, and reinforces our hypothesis that within São Paulo state these chemotype populations could have different biological capacities to act as a vector.

Introduction

Recording the geographic distribution and identifying the possible routes of expansion of both arthropod-borne diseases and their associated vectors is essential information for surveillance as well as the execution and elaboration of control strategies [1].

In Brazil, the expansion of the geographic range of *Lutzomyia longipalpis* (Lutz & Neiva), the principal vector of *Leishmania (Leishmania) infantum chagasi* (Cunha & Chagas), and its adaptation to domiciliary habitats in the urban areas throughout Brazil has resulted in an increase in the incidence of both canine and human visceral leishmaniasis (VL) in the last 25 years [2–6]. According to the Brazilian Ministry of Health, in the period from 2009 to 2011, there were 251 municipalities classified as having moderate (mean number of human cases ≥ 2.4 and < 4.4) or intense (mean number of human cases ≥ 4.4) VL transmission in the country [7].

Before 1998, São Paulo State was considered free of autochthonous cases of this zoonotic disease and records of the vector's presence were restricted to some rural areas of municipalities in the Northeast region of the state [8]. Two human cases had been reported in Greater São Paulo, but possible reservoirs and vectors were not described [9]. The first record of *L. longipalpis* in an urban area in São Paulo State was in 1997 from Araçatuba in the West of São Paulo State [8]. Canine and autochthonous human cases occurred in the same municipality in 1998 and 1999 respectively [10]. Since then, the appearance of *L. longipalpis* in urban areas of other municipalities has been linked to an increase in both canine and human visceral leishmaniasis within the State [11, 12]. From 1999 to April 2013, São Paulo State recorded 2204 autochthonous human cases of disease, with 192 deaths [13]. In São Paulo State, 18 municipalities were classified as having moderate or intense transmission in the period from 2010 to 2012 [11].

Based on genetic and behavioural studies it is generally accepted that *L. longipalpis* is a species complex, but it is unclear how many members there are and how they are related [14, 15].

Chemical, behavioural and ecological analysis of male produced sex pheromones suggests that *L. longipalpis* is a complex of at least four different, reproductively isolated members [16–19]. In Brazil two of these are represented by members where the males produce either 3-methyl- α -himachalene [20], a novel bicyclic methylsесquiterpene (C16; mw 218) found in Jacobina, Bahia State, or (S)-9-methylgermacrene-B [21], a novel monocyclic methylsесquiterpene (C16; mw 218) that is widely distributed throughout Brazil but typically represented by *L. longipalpis* from Lapinha Cave, Minas Gerais State. The other two members of the complex produce novel diterpenes, cembrene-1 and cembrene-2 (C20; mw 272) and are represented by the Sobral-2S population from Ceara State and the Jaibas-1S population from Minas Gerais State [22]. Two of these chemotype populations, (S)-9-methylgermacrene-B and cembrene-1, have been identified in Sao Paulo State [23].

Considering the remarkable epidemiological differences between the two municipalities of Araatuba and Espirito Santo do Pinhal (mainly the number of human cases notified as well as the abundance, and chemotype of the *L. longipalpis* population present in each urban area), Casanova et al (2006) [23] suggested that the (S)-9-methylgermacrene-B and cembrene-1—chemotype populations had different vectorial capacities. If this is true then it is important to have more detailed information on the distribution of the chemotypes to produce more accurate risk maps and to direct more effective control programs. With this in mind, the present study is aimed at determining the chemotypes of a greater number of *L. longipalpis* populations from different regions of Sao Paulo State.

Methods

Study area

Sao Paulo State is located in Southeast region of Brazil, and shares borders with Minas Gerais to the North and Northeast, Parana to the South, Rio de Janeiro to the East and Mato Grosso do Sul to the West, and to the Southeast, the Atlantic Ocean (Fig. 1). It is divided into 645 municipalities totalling 248,209 km².

Its climate can be divided into seven distinct types, most are classified as humid. According to Koeppen’s climate classification, the predominant climate type is Cwa, which includes Central and Eastern Sao Paulo, defined as high-altitude tropical climate, where summer is the rainy season, winter is dry, and the average temperature in summer is above 22°C. In the West region (Aw climate type), the rainy season is delayed until autumn, the winters are dry (the precipitation for the driest month is less than 60mm) and the average temperature for the coldest month is above 18°C [24].

Human cases

In Brazil, including Sao Paulo State, American visceral leishmaniasis is a compulsory notifiable disease. Data used in the present study were obtained from Epidemiological Surveillance Centre of Secretary of Health of Sao Paulo State [13]

Canine cases

Since the beginning of visceral leishmaniasis surveillance and control activities in Sao Paulo State, all the municipalities are expected to notify the first- confirmed (laboratory based parasitological identification) occurrence of *L. i. chagasi*. Data were obtained from canine surveys carried out by the municipalities, Adolfo Lutz Institute and the Secretary of Health of Sao Paulo State.



Fig 1. Study area. São Paulo and neighboring states.

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Lutzomyia longipalpis data

L. longipalpis distribution data was obtained from both published data and, principally, from entomological collections carried out by Secretary of Health of São Paulo State, during the performance of their epidemiologic surveillance activities [8, 11, 23, 25–27]. These activities included annual or biannual collections, with CDC light traps, in a minimum of 4 dwellings (more where possible) of all the municipalities considered silent—i.e. without canine or human cases, non-receptive—i.e. where *L. longipalpis* has not yet been found—and those considered vulnerable—i.e. those municipalities that are located near to or that connected because of intense transportation of goods and people by road and railway with municipalities with transmission [25]. Annual entomological collections were also carried out in at least 42 dwellings in areas where proven or suspected human or canine transmission occurs but where the vector has not yet been registered.

Chemotype populations

Male *L. longipalpis* from different municipalities were collected manually with an aspirator or CDC electric light trap from peridomiciliary habitats within urban, peri-urban and rural areas, always with permission from local homeowners. The attempts to collect males were made in at least three evenings in four peridomicilies of each sampled municipality. Samples from western

São Paulo State were obtained in 11 municipalities that were selected so as to represent all the vector distribution area. For the eastern area, where only 25 municipalities have registered the presence of the vector, nine municipalities, including those with canine transmission were sampled. The sampled municipalities, the geographic coordinates, the number of males chemically analysed and the collection year were respectively: Araçatuba (21°12'14" S; 50°42'61" W), >100, 2005 and 2009; Promissão (21°32'18" S; 49°51'28" W), 35, 2009; Bauru (22°18'55" S; 49°03'41" W), 23, 2009; Dracena (21°29'00" S; 51°32'01" W), 22, 2009; Adamantina (21°40'32" S; 51°03'47" W), 13, 2011; Oswaldo Cruz (21°47'38" S; 50°53'08" W), 17, 2011; Jales (20°16'06" S; 50°32'56" W), 10, 2013; Presidente Prudente (22°07'39" S; 51°23'08" W), 13, 2010; Marília (22°13'15" S; 49°56'55" W), 9, 2012; Salmourão (22°13'15" S; 49°56'55" W), 2, 2013; Lourdes (20°58'01" S; 50°13'27" W), 2, 2013; Espírito Santo do Pinhal (22°10'60" S; 46°45'45" W), >20, 2004 and 2009; Socorro (22°35'50" S; 46°31'04" W), 1, 2012; Salto (23°12'10" S; 47°17'11" W), 2, 2012; São Pedro (22°36'00" S; 47°52'31" W), 28, 2009; Indaiatuba (23°5'18" S; 47°13'24" W), 3, 2012; Campinas (22°54'23" S; 47°03'42" W), 10, 2009; Águas da Prata (21°56'18" S; 46°42'54" W), 4, 2013; Sorocaba (23°30'22" S; 47°27'21" W), 13, 2013; Votorantim (23°32'26" S; 47°26'38" W), 5, 2013. All males were observed under a stereomicroscope to identify to the species level by examination of external morphological characteristics (pale spots on the 4th or 3rd and 4th abdominal tergites and a pair of spikes on the paramere).

All males were killed by placing them in a freezer at -20° C for 10 minutes. They were then placed individually in a glass vial and then covered with hexane (ca. 20 µl). Analysis of male sex pheromone extracts was on a HP-5MS capillary column, 30 m x 0.25 mm i.d., 0.25 µm film thickness (Agilent, Stockport, Cheshire) in a Hewlett Packard 5890 II+ Gas Chromatograph coupled to a Hewlett Packard 5972A bench-top mass spectrometer (electron impact, 70 eV, 180°C). Injection and chromatography conditions were as previously described [19].

Results

Before 1997, *L. longipalpis* had been found only in the rural areas of six municipalities of São Paulo State, all of which are in the East and Northeast regions of the state. The first report of the vector in an urban area was in 1997 in the municipality of Araçatuba, in the Western region near the border with Mato Grosso do Sul State (Fig. 2). From 1998 to June 2014, *L. longipalpis* has been reported in another 164 municipalities (Fig. 2, S1 Database). During this period, between 2 and 21 new municipalities per year reported the presence of *L. longipalpis*, with more than 45 reporting the presence of the vector in the last 3 years (Fig. 3). The biggest expansion in the distribution of *L. longipalpis* happened in the western part of Sao Paulo where 146 municipalities have recorded their presence in urban areas during this 17.5 year period.

The spatial and temporal distribution of *L. longipalpis* and human and canine cases in general shows that the presence of the vector preceded the canine cases, and these in turn preceded the human cases (Figs. 2, 4, 5, S1 Database). Up until 2014, there have been 105 municipalities with canine and/or human VL transmission. The majority of these municipalities (93.3%), are placed in the Western part of São Paulo State, and the cases show an expansion route in a Southeasterly direction, towards the Central region, and from there, an expansion both to the North and the South (Figs. 4 and 5). In 71 of these municipalities, there has been both human and canine cases, in 23 only canine transmission and in five only human cases (Figs. 4 and 5). It is interesting to note that in the East region, *L. longipalpis* has been found in 25 municipalities only, with canine cases reported in seven, and no known human cases.

The chemical analysis of all samples of *L. longipalpis* males from 11 municipalities in the West region (Araçatuba, Promissão, Bauru, Dracena, Adamantina, Oswaldo Cruz, Jales, Presidente Prudente, Marília, Lourdes and Salmourão) have been shown to contain (S)-9-methylgermacrene-B

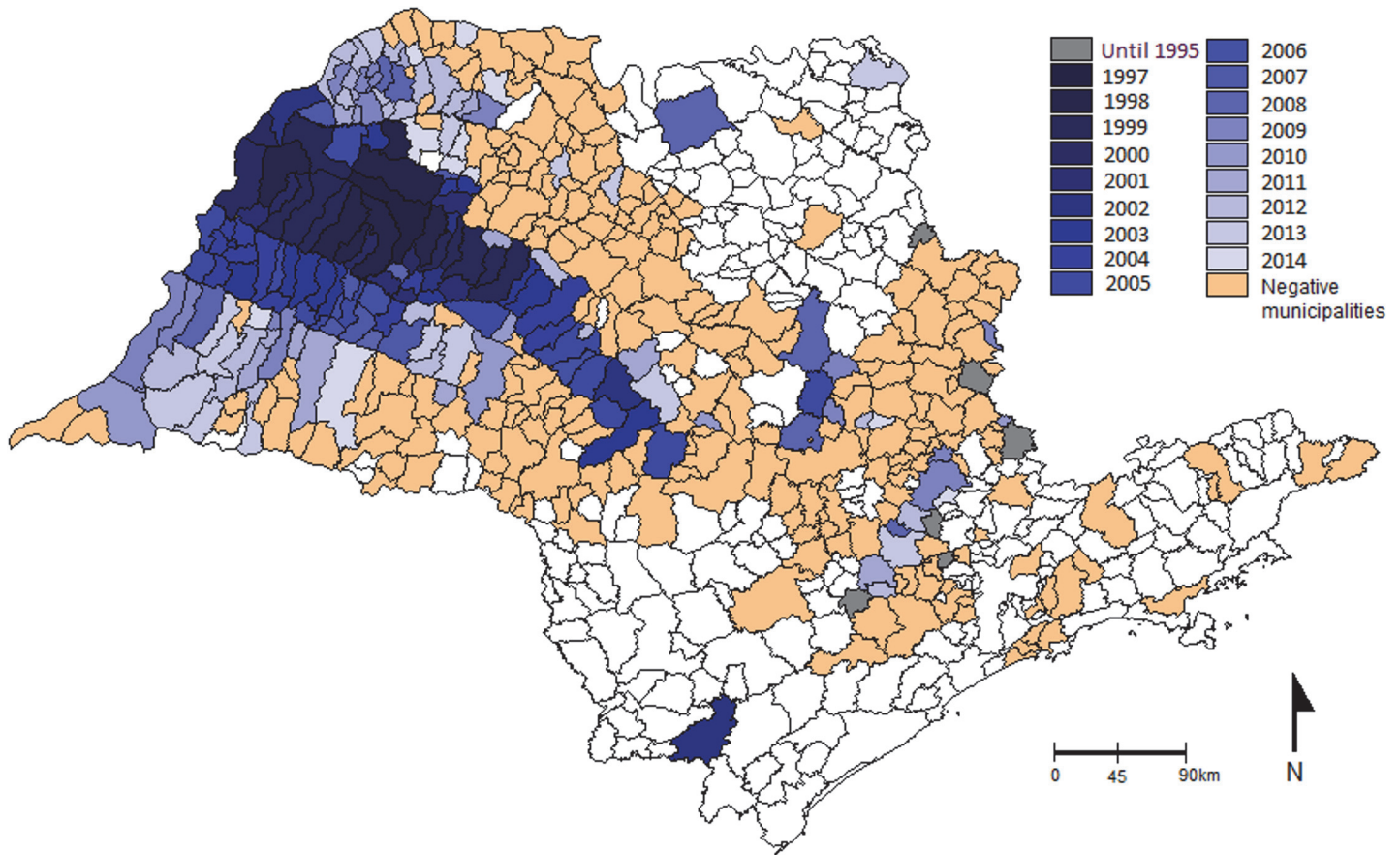


Fig 2. Expansion route of *Lutzomyia longipalpis*. Distribution of *Lutzomyia longipalpis*, in municipalities of São Paulo State, according to the first record, from the 1970's to June 2014. In negative municipalities, the entomological collections have not recorded the presence of *L. longipalpis*.

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(Fig. 6). On the other hand, all samples of male *L. longipalpis* collected in eight municipalities of the Eastern region (Espírito Santo do Pinhal, Socorro, Salto, Indaiatuba, Campinas, Águas da Prata, Sorocaba and Votorantim) contained cembrene-1 (C-20) (Fig. 6). In the municipality of São Pedro, situated in Central region of the State, it was found a (S)-9-methylgermacrene-B producing population and in addition, two flies that produced both (S)-9-methylgermancrene-B, and cembrene-1.

Discussion

The argument in favour of the hypothesis of the recent introduction of *L. longipalpis* into the Western region of São Paulo State can be supported by its absence, for decades, from various sporadic rural collections of sand flies [28–30]. These collections were done in areas where autochthonous cases of cutaneous leishmaniasis, caused by *Leishmania (Viannia) braziliensis*, had been reported. The contrary hypothesis, that *L. longipalpis* has always been there, hidden in the primitive natural vegetation habitat, could be supported because of the existence of several areas where collections have never been done [28–30]. Therefore, there are gaps in our knowledge of its distribution and in future, it would be interesting to collect samples from the few natural vegetation areas of São Paulo State.

It is very difficult to pinpoint the year when *L. longipalpis* first reached the urban areas of the municipalities in the Western region, however it is likely that, when detected for the first

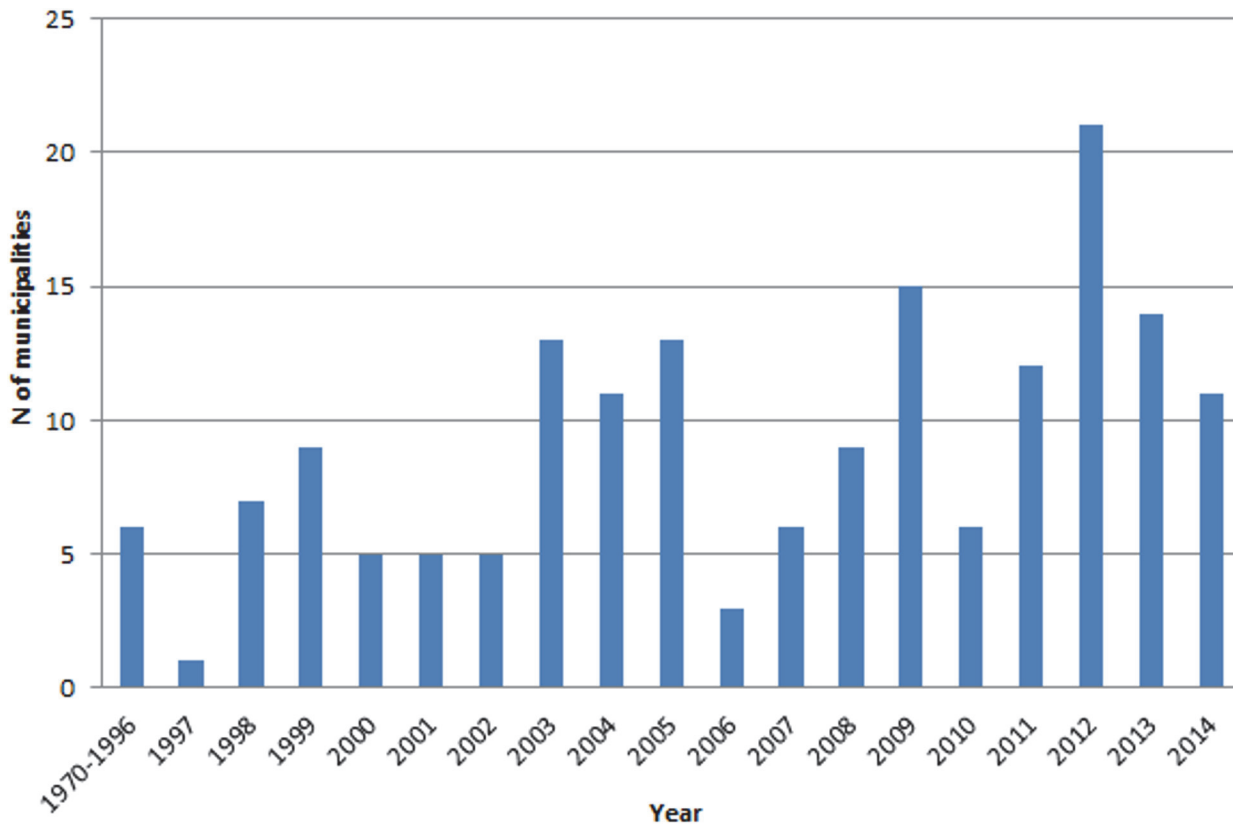


Fig 3. Annual new records of *Lutzomyia longipalpis*. Annual distribution of municipalities which reported *Lutzomyia longipalpis* for the first time in São Paulo State, from 1970's to June 2014.

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time in the municipality of Araçatuba, in 1997 [8], *L. longipalpis* was already present in the urban areas of surrounding municipalities, as the species was found there in the first collections of the following year. From that point on, the spatial and temporal distribution leaves no doubt as to the west-east progression (as far as the central region of the state). This progression can be inferred from the annual urban entomological collection results, which showed that in various municipalities the vector detection only occurred after successive annual negative collections. It is possible that factors related to the economic development of the country, such as the increase in transportation of goods and people by road and railway, could have been responsible for the dispersion of the vector and, consequently, the expansion in the vector's range in the West.

The higher number of municipalities from the West of the state which reported the presence of *L. longipalpis* for the first time since 1997 indicates a rapid inter-municipality dispersion rate. The greatest number of municipalities reported in 2012 and 2013 (21 and 14, respectively) is a further indication of this ongoing, fast expansion. The fact that the expansion of canine and human cases through the Western São Paulo has followed the same dispersion route as that of the vector with a temporal delay cannot be considered to be merely coincidental because it has long been observed that in VL epidemiology the vector precedes canine and subsequently human cases [31]. The lower number of municipalities notifying the presence of the vector in the East region of the state, in contrast to the West, does not show a recognisable dispersion route. Spread of the disease is more likely therefore to be due to the expansion of urban areas into rural or wild areas. This hypothesis is further supported by the observation that *L.*

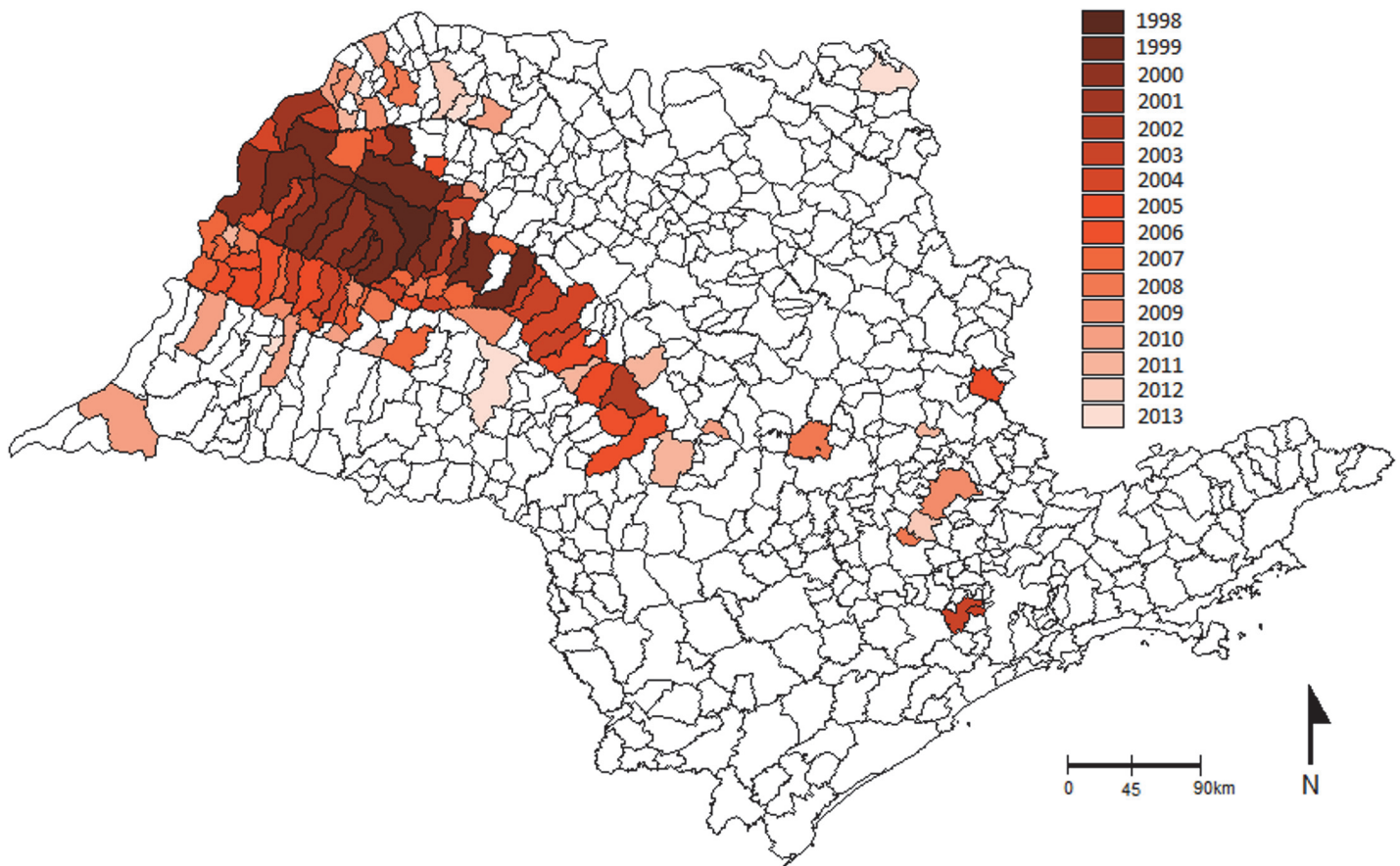


Fig 4. Expansion route of canine cases. Distribution of American visceral leishmaniasis in São Paulo State, according to the record of the first canine case, from 1998 to 2013.

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longipalpis was only found exclusively in rural habitats in nine of the 25 studied municipalities. In the other 16 municipalities, *L. longipalpis* was found in both rural and urban areas in four, and in periurban areas (i.e. those that have the characteristics of rural areas) in 12 municipalities.

It is clear that there are two distinct epidemiological patterns of VL in these two regions of São Paulo State. In the western region it is defined by the occurrence of human cases [12], frequent high prevalence of canine cases [32, 33], and a greater number of municipalities where *L. longipalpis* is present. Generally, a great number of flies is frequently found in both manual and CDC light trap collections carried out in peridomiliary environments associated with food sources, such as chickens and dogs [33, 34]. In this area, all the males analysed have been shown to be the (S)-9-methylgermacrene-B chemotype population, including those collected in the 6 of the 18 municipalities currently classified as having moderate or intense transmission in the period from 2010 to 2012. In contrast, the eastern region, can be characterized by the absence of notified human cases—even where the presence of *L. longipalpis* and canine cases have been reported for at least 12 years—low prevalence in dogs and a smaller number of municipalities where the vector is present. The populations of sand flies are generally low in abundance in manual and CDC light trap collections carried out in peridomiliary and rural environments associated with the feeding sources, such as chickens and dogs [35]. All samples of males analysed were the cembrene-1 chemotype.

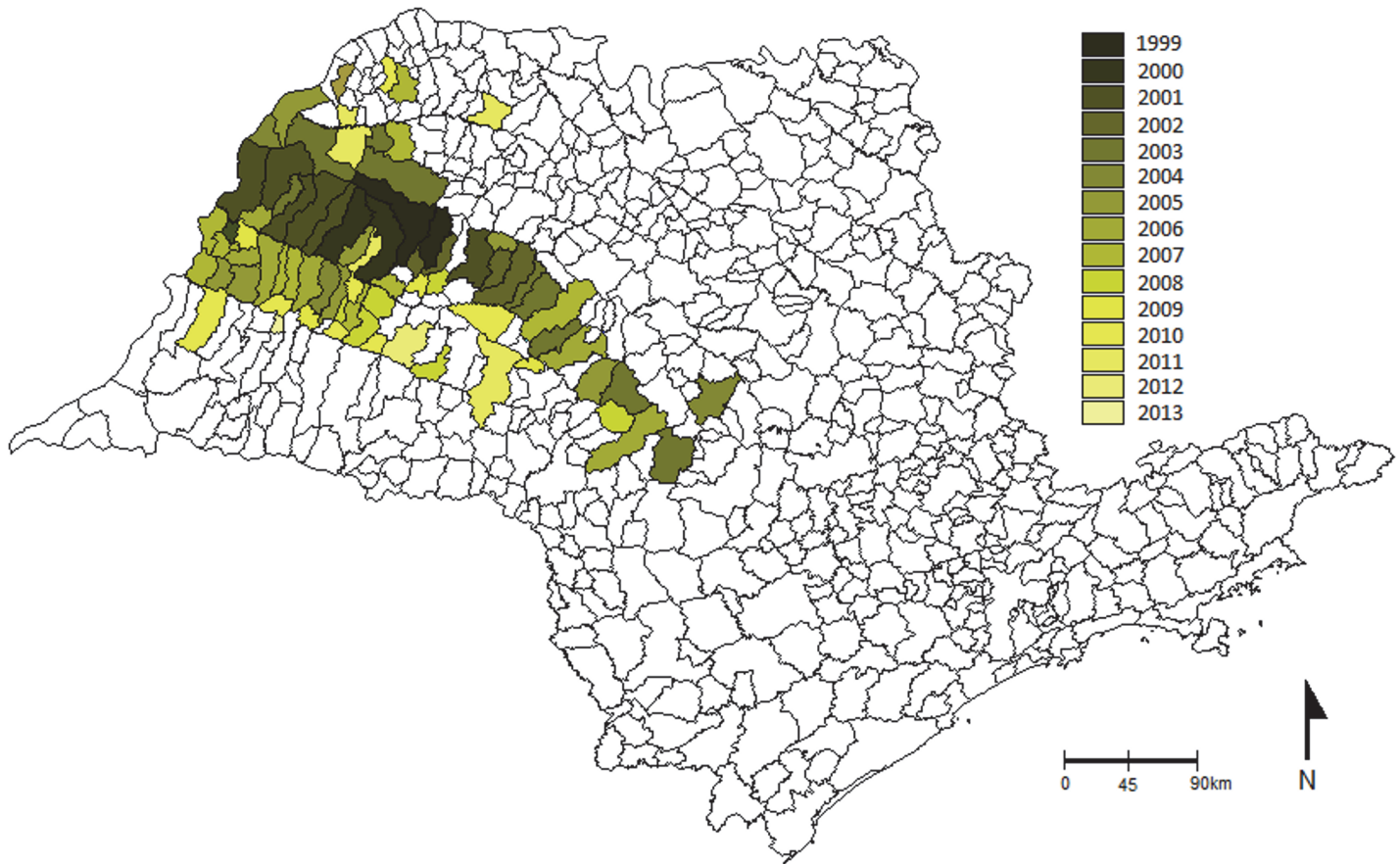


Fig 5. Expansion route of human cases. Distribution of American visceral leishmaniasis in São Paulo State, according to the record of the first human case, from 1999 to April 2013.

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These observations support the hypothesis of Casanova et al [2006] [23] which proposes that the (S)-9-methylgermacrene-B chemotype population has a greater vectorial capacity than the cembrene-1 chemotype in São Paulo State. Differences in ecological parameters of the vector capacity (e.g. vector abundance, survival, host feeding pattern and blood feeding rate) could vary between the two chemotype populations. Furthermore, susceptibility and coevolutionary interactions with *Leishmania* genotypes, which can influence *Leishmania* transmission, are parameters involved in vectorial competence and can vary between different species of *L. longipalpis* complex [15, 36, 37]. It is interesting to note that the two main genetic clusters of *L. i. chagasi*, identified by multilocus microsatellite typing, isolated from dogs from Northwest and Southeast regions of the São Paulo State [38] show distribution coinciding with the two *L. longipalpis* chemotype populations distribution presented here. The present study indicates that the chemotype of *L. longipalpis* populations as well as the effect of spatial and temporal environmental heterogeneity (reviewed in Belo et al 2013 [39]), should also be considered when explaining the variety of eco-epidemiological transmission scenarios in São Paulo State. However, it is also important to mention that the available data for pheromone types populations from municipalities of other states of Brazil and classified by the Brazilian Ministry of Health as having moderate and intense transmission of VL in the period from 2009 to 2011, shows that in three of these municipalities (Marajó-PA, Natal-RN, Pancas-ES) the chemotype population is cembrene-1, in two (Terezina-PI, Campo Grande-MS) it is (S)-9-

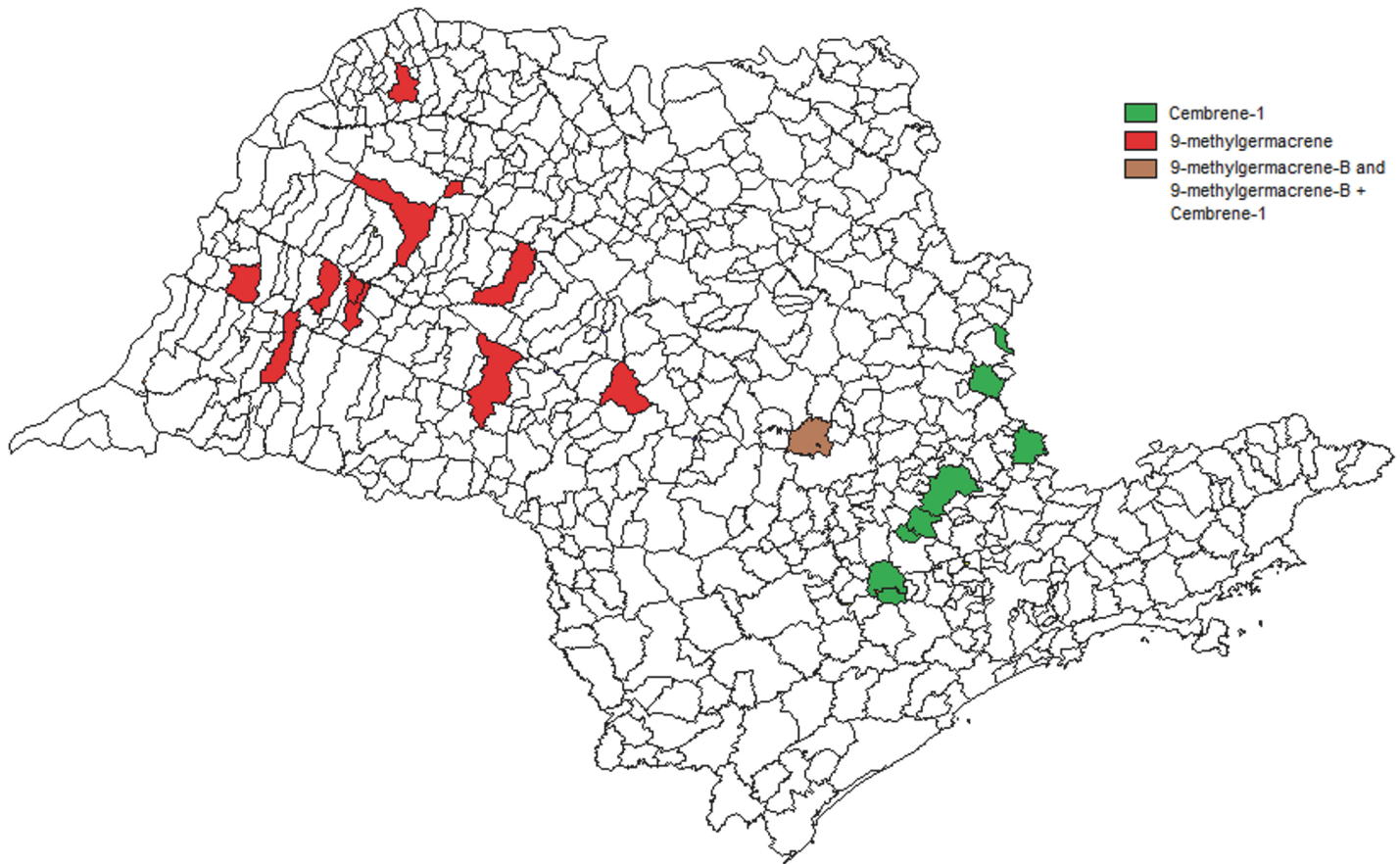


Fig 6. Spatial distribution of chemotype populations. Distribution of (S)-9-methylgermacrene-B and cembrene-1 pheromone producing population in São Paulo State. Map shows only municipalities sampled for chemical analysis.

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methylgermacrene-B chemotype, and in one (Sobral-CE) both occur [7, 15]. Further studies are required to access the parameters of vectorial capacity of the longipalpis species complex.

The (S)-9-methylgermacrene-B, distributed throughout São Paulo State's western region has previously been found and characterized in males from Lapinha Cave-MG [21] and later in populations from Sobral-CE, Terezina-PI, Campo Grande-MS, Barra de Guaratiba-RJ, Montes Claros-MG and Araçatuba-SP [15, 19, 23, 40]. The diterpene (C-20) found in populations from municipalities of the East region of the state was previously found and characterized as cembrene-1 in *L. longipalpis* from Sobral-CE, and later also found in populations from Marajó-PA, Natal-RN, Estrela-AL, Jaíba-MG, Pancas-ES and Espírito Santo do Pinhal-SP [15, 19, 23]. The presence of a cembrene-1 population in São Paulo State is the southernmost extension of this chemotype.

Based on the results of our present study we suggest that the *L. longipalpis* cembrene-1 populations are of rural origin and native of the Eastern region of the São Paulo State, while (S)-9-methylgermacrene-B is an introduced chemotype population. Although *L. longipalpis* has been found in urban areas of several municipalities of Mato Grosso do Sul State [41], we are not aware of any publications on *L. longipalpis* distribution through time in this State. However, although human cases are not a good space-time indicator of parasite circulation, the occurrence of the first human autochthonous case in a municipality usually indicates that the vector and the canine transmission were already established in the area. The West-to-East expansion of

human VL in Mato Grosso do Sul was properly demonstrated by Correa-Antonioli et al (2007) [42] who pointed to the construction of the Bolivia-Brazil gas pipeline as a possible cause for the VL time and space expansion. This same hypothesis was considered to explain the spread of the canine and human disease in the west region of São Paulo [12, 38]. Finding the same pheromone type in males in the same temporal expansion route from West to East in São Paulo State may also support the hypothesis that the (S)-9-methylgermacrene-B *L. longipalpis* chemotype has been introduced from Mato Grosso do Sul.

More samples of males from São Pedro, in the Central region of São Paulo State, should be analysed to allow a better understanding of the possible presence and distribution of sympatric populations of the two pheromone chemotypes. Molecular and behavioural analyses such as those done by Araki et al (2009, 2013) [15, 43] may also help to clarify this question.

Information on the dispersion route and distribution of *L. longipalpis* chemotype populations is essential to understand the epidemiological patterns observed in São Paulo State. It may be used to identify areas at risk and consequently define priorities for control measures. In addition, identifying the distribution of the different chemotype populations is helpful in the application of appropriate synthetic male sex pheromone, in pheromone-baited traps or other appropriate “attract-and-kill” approaches [40, 44–49].

Supporting Information

S1 Database. List of municipalities of São Paulo State, according to their epidemiological classification. Record of the first encounter of *Lutzomyia longipalpis* and the first registered canine and human cases.

(XLS)

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Author Contributions

Conceived and designed the experiments: CC JJS JGCH. Performed the experiments: CC FECJ JGCH. Analyzed the data: CC JGCH FECJ RPB JJS. Contributed reagents/materials/analysis tools: CC JGCH FECJ. Wrote the paper: CC FECJ JGCH RPB JJS.

References

1. Domanovic D, Giesecke J (2012) How to define an area where transmission of arthropod-borne disease is occurring? *Euro Surveill* 17(20): pii = 20171. PMID: [22642947](#)
2. Desjeux P (2004) Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis* 27: 305–318. PMID: [15225981](#)
3. Ministry of Health of Brazil (2006) Manual de vigilância e controle da leishmaniose visceral. Editora MS.
4. Maia-Elkhoury ANS, Alves WA, Sousa-Gomes ML, Sena JM, Luna EA (2008) Visceral leishmaniasis in Brazil: trends and challenges. *Cad Saude Publica* 24: 2941–2947. PMID: [19082286](#)
5. Werneck GL (2008) Forum: Geographic spread and urbanization of visceral leishmaniasis in Brazil. Introduction. *Cad Saude Publica* 24: 2937–2940. PMID: [19082285](#)
6. Romero GAS, Boelaert M (2011) Control of visceral leishmaniasis in Latin America—A systematic review. *PLoS Negl Trop Dis* 4(1): e584.
7. Ministry of Health of Brazil (2014) Casos confirmados de Leishmaniose Visceral, Brasil, Grandes Regiões e Unidades Federadas. 1990 a 2011 [http://portal.saude.gov.br/portal/arquivos/pdf/2012_11_casos_de_lv_entre_1990_e_2011_final.pdf]. Accessed 22 September 2014.
8. Costa AIP, Casanova C, Rodas LAC, Galati EAB (1997) Atualização da distribuição geográfica e primeiro encontro de *Lutzomyia longipalpis* em área urbana no Estado de São Paulo, Brasil. *Rev Saúde Publica* 31(6): 632–633.

9. Iverson LB, Pires RBR, Ribeiro MA, Escrivão-Júnior A, Toleano JE, et al. (1982) Investigação epidemiológica de um novo caso de leishmaniose visceral ocorrido na Grande São Paulo, Brasil. *Rev. Saúde Pública* 16: 205–219.
10. Galimberti MZ, Katz G, Camargo-Neves VLF, Rodas LAC, Casanova C, et al. (1999) Leishmaniose visceral americana no Estado de São Paulo. *Rev Soc Bras Med Trop* 32(Supl 1): 217–218.
11. Rangel O, Hiramoto RM, Henriques LF, Taniguchi HH, Ciaravolo RMC, et al. (2013) Classificação epidemiológica dos municípios segundo o Programa de Vigilância e Controle da Leishmaniose Visceral Americana no estado de São Paulo, para 2013. *BEPA* 10(111): 5–16.
12. Cardim MFM, Rodas LAC, Dibo MR, Guiraldo MM, Oliveira AM, et al. (2013) Introduction and expansion of human American visceral leishmaniasis in the state of São Paulo, Brazil, 1999–2011. *Rev Saúde Pública* 47(4): 1–9.
13. Secretary of Health of São Paulo State (2014) Dados estatísticos da Leishmaniose Visceral Americana de 1999 a 2013 [http://www.cve.saude.sp.gov.br/htm/zoo/leishv_dados.html]. Accessed 22 September 2014.
14. Bauzer LG, Souza NA, Maingon RD, Peixoto AA (2007) *Lutzomyia longipalpis* in Brazil: a complex or a single species? A mini-review. *Mem Inst Oswaldo Cruz* 102: 1–12. PMID: [17293992](#)
15. Araki AS, Vigoder FM, Bauzer LG, Ferreira GE, Souza NA, et al. (2009) Molecular and behavioral differentiation among Brazilian populations of *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae). *PLoS Negl Trop Dis* 3: e365. doi: [10.1371/journal.pntd.0000365](https://doi.org/10.1371/journal.pntd.0000365) PMID: [19172187](#)
16. Ward RD, Phillips A, Burnet B, Marcondes CB (1988) The *Lutzomyia longipalpis* complex: reproduction and distribution. In *Biosystematics of Haematophagus Insects*, Service Oxford University Press, Oxford, p. 257–269.
17. Ward RD, Morton IE (1991) Pheromones in mate choice and sexual isolation between siblings of *Lutzomyia longipalpis* (Diptera: Psychodidae). *Parasitologia* 33: 527–533.
18. Souza NA, Ward RD, Hamilton JG, Kyriacou CP, Peixoto AA (2002) Copulation songs in three siblings of *Lutzomyia longipalpis* (Diptera: Psychodidae). *Trans R Soc Trop Med Hyg* 96: 102–103. PMID: [11925981](#)
19. Hamilton JGC, Maingon RDC, Alexander B, Ward RD, Brazil RP (2005) Analysis of the sex pheromone extract of individual male *Lutzomyia longipalpis* sandflies from six regions in Brazil. *Med Vet Entomol* 19: 480–488. PMID: [16336313](#)
20. Hamilton JGC, Hooper AM, Mori K, Pickett JA, Sano S (1999) 3-Methyl- α -himachalene confirmed, and the relative stereochemistry defined, by synthesis as the sex pheromone of the sandfly *Lutzomyia longipalpis* from Jacobina, Brazil. *Chem Commun* (4:): 355–356.
21. Hamilton JG, Ibbotson HC, Hooper AM, Mori K, Pickett JA, et al (1999) 9-Methylgermacrene-B confirmed by synthesis as the sex pheromone of the sandfly *Lutzomyia longipalpis* from Lapinha, Brazil, and the absolute stereochemistry defined as 9S. *Chem Commun* 8: 2335–2336.
22. Hamilton JGC, Brazil RP, Maingon R (2004) A fourth chemotype of *Lutzomyia longipalpis* (Diptera: Psychodidae) from Jaíba, Minas Gerais State, Brazil. *J Med Entomol* 41(6): 1021–1026. PMID: [15605640](#)
23. Casanova C, Hamilton JGC, Trigo JR, Costa AI (2006) Identification of sex pheromones of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) populations from the state of São Paulo, Brazil. *Mem Inst Oswaldo Cruz* 101: 113–115. PMID: [16699721](#)
24. CEPAGRI (2014) Clima dos Municípios Paulistas: a classificação climática de Köppen para o Estado de São Paulo. [<http://www.cpa.unicamp.br/outras-informacoes/clima-dos-municípios-paulistas.html>]. Accessed 22 September 2014.
25. Secretary of Health of São Paulo State (2006) Manual de Vigilância e Controle da Leishmaniose Visceral Americana do Estado de São Paulo. 158p.
26. Cutolo AA, Camargo DA, Cutolo AA, Von Zuben CJ, Galati EAB (2008) *Lutzomyia longipalpis* (Diptera, Psychodidae) em Cuesta Basáltica, na bacia hidrográfica do Rio Corumbataí, Região Centro-leste do Estado de São Paulo. *Rev Bras Epidemiol* 11(2): 336–339.
27. Galati EAB, Marassá AM, Fonseca MB, Gonçalves-Andrade RM, Consales CA, et al. (2010) Phlebotomines (Diptera, Psychodidae) in the speleological province of the Ribeira Valley: 3. Serra district—area of hostels for tourists who visit the Parque Estadual do Alto Ribeira (PETAR), state of São Paulo, Brazil. *Rev Bras Entomol* 54(4): 665–676.
28. Barretto MP (1943) Observações sobre a Biologia em Condições Naturais dos Flebotomos do Estado de São Paulo (Diptera:Psychodidae), PhD Thesis, Universidade de São Paulo, São Paulo, 162 pp.
29. Gomes AC, Galati EAB, Casanova C, Domingos MF, Marques GRAM, et al. (1995) Analysis of the geographical distribution of leishmaniasis vectors in the state of São Paulo Brazil. *Bol Dir Malariol San Amb* 35(Supl. 1): 143–146.

30. Shimabukuro PH, Silva TR, Fonseca FO, Baton LA, Galati EA (2010) Geographical distribution of American cutaneous leishmaniasis and its phlebotomine vectors (Diptera: Psychodidae) in the state of São Paulo, Brazil. *Parasit Vectors* 3: 121. doi: [10.1186/1756-3305-3-121](https://doi.org/10.1186/1756-3305-3-121) PMID: [21171969](https://pubmed.ncbi.nlm.nih.gov/21171969/)
31. Costa DNCC, Codeço CT, Silva MA, Werneck GL (2013) Culling Dogs in Scenarios of Imperfect Control: Realistic Impact on the Prevalence of Canine Visceral Leishmaniasis. *PLoS Negl Trop Dis* 7(8): e2355. doi: [10.1371/journal.pntd.0002355](https://doi.org/10.1371/journal.pntd.0002355) PMID: [23951375](https://pubmed.ncbi.nlm.nih.gov/23951375/)
32. Nunes CM, Lima VM, Paula HN, Perri SH, Andrade AM, et al (2008) Dog culling and replacement in an area endemic for visceral leishmaniasis in Brazil. *Vet Parasitol* 153: 19–23. doi: [10.1016/j.vetpar.2008.01.005](https://doi.org/10.1016/j.vetpar.2008.01.005) PMID: [18314275](https://pubmed.ncbi.nlm.nih.gov/18314275/)
33. Holcman MM, Sampaio SMP, Rangel O, Casanova C (2013) Spatial and seasonal distribution of *Lutzomyia longipalpis*, a city in the western region of the State of São Paulo, Brazil, that is endemic with visceral leishmaniasis. *Rev Soc Bras Med Trop* 46: 704–712. doi: [10.1590/0037-8682-0188-2013](https://doi.org/10.1590/0037-8682-0188-2013) PMID: [24474011](https://pubmed.ncbi.nlm.nih.gov/24474011/)
34. Casanova C, Andrighetti MTM, Sampaio SMP, Macoris MLG, Colla-Jacques FE, et al. (2013) Larval breeding sites of *Lutzomyia longipalpis* (Diptera: Psychodidae) in visceral leishmaniasis endemic urban areas in Southeastern Brazil. *PLoS Negl Trop Dis* 7(9): e2443. doi: [10.1371/journal.pntd.0002443](https://doi.org/10.1371/journal.pntd.0002443) PMID: [24069494](https://pubmed.ncbi.nlm.nih.gov/24069494/)
35. Colla-Jacques FE, Casanova C, Prado AP (2010) Study of sand fly fauna in an endemic area of American cutaneous leishmaniasis and canine visceral leishmaniasis in the municipality of Espírito Santo do Pinhal, São Paulo, Brazil. *Mem Inst Oswaldo Cruz* 105(2): 208–215. PMID: [20428683](https://pubmed.ncbi.nlm.nih.gov/20428683/)
36. Watts PC, Hamilton JGC, Ward RD, Noyes HA, Souza NA, et al. (2005) Male sex pheromones and the phylogeographic structure of the *Lutzomyia longipalpis* species complex (Diptera: Psychodidae) from Brazil and Venezuela. *Am J Trop Med Hyg* 73: 734–743. PMID: [16222018](https://pubmed.ncbi.nlm.nih.gov/16222018/)
37. Maingon RD, Ward RD, Hamilton JG, Bauzer LG, Peixoto AA (2008) The *Lutzomyia longipalpis* species complex: does population sub-structure matter to Leishmania transmission. *Trends Parasitol* 24: 12–17. PMID: [18023260](https://pubmed.ncbi.nlm.nih.gov/18023260/)
38. Motoie G, Ferreira GEM, Cupolillo E, Canavez F, Pereira-Chioccola VL (2013) Spatial distribution and population genetics of *Leishmania infantum* genotypes in São Paulo State, Brazil, employing multilocus microsatellite typing directly in dog infected tissues. *Infection, Genetics and Evolution* 18: 48–59. doi: [10.1016/j.meegid.2013.04.031](https://doi.org/10.1016/j.meegid.2013.04.031) PMID: [23665466](https://pubmed.ncbi.nlm.nih.gov/23665466/)
39. Belo VS, Werneck GL, Barbosa DS, Simões TC, Nascimento BWL, et al (2013) Factors associated with visceral leishmaniasis in the Americas: A systematic review and meta-analysis. *PLoS Negl Trop Dis* 7(4): e2182. doi: [10.1371/journal.pntd.0002182](https://doi.org/10.1371/journal.pntd.0002182) PMID: [23638203](https://pubmed.ncbi.nlm.nih.gov/23638203/)
40. Bray DP, Bandi KK, Brazil RP, Oliveira AG, Hamilton JGC (2009) Synthetic sex pheromone attracts the leishmaniasis vector *Lutzomyia longipalpis* (Diptera: Psychodidae) to traps in the field. *J Med Entomol* 46: 428–434. PMID: [19496409](https://pubmed.ncbi.nlm.nih.gov/19496409/)
41. Almeida PS, Sciamarelli A, Batista PM, Ferreira AD, Nascimento J, et al. (2013) Predicting the geographic distribution of *Lutzomyia longipalpis* (Diptera: Psychodidae) and visceral leishmaniasis in the state of Mato Grosso do Sul, Brazil. *Mem Inst Oswaldo Cruz* 108(8): 992–996. doi: [10.1590/0074-0276130331](https://doi.org/10.1590/0074-0276130331) PMID: [24402151](https://pubmed.ncbi.nlm.nih.gov/24402151/)
42. Correa-Antonialli SA, Torres TG, Paranhos-Filho AC, Tolezano JE (2007) Spatial analysis of American visceral leishmaniasis in Mato Grosso do Sul state, Central Brazil. *J Infect* 54: 509–514. PMID: [16979241](https://pubmed.ncbi.nlm.nih.gov/16979241/)
43. Araki AS, Ferreira GEM, Mazzoni CJ, Souza NA, Machado RC, et al. (2013) Multilocus analysis of divergence and introgression in sympatric and allopatric sibling species of the *Lutzomyia longipalpis* complex in Brazil. *PLoS Negl Trop Dis* 7(10): e2495. doi: [10.1371/journal.pntd.0002495](https://doi.org/10.1371/journal.pntd.0002495) PMID: [24147172](https://pubmed.ncbi.nlm.nih.gov/24147172/)
44. Ward RD, Morton IE, Brazil RP, Trumper S, Falcao AL (1990) Preliminary laboratory and field trials of a heated pheromone trap for the sandfly *Lutzomyia longipalpis* (Diptera: Psychodidae). *Mem Inst Oswaldo Cruz* 85: 445–452.
45. Dye C, Davies CR, Lainson R (1991) Communication among phlebotomine sandflies—a field study of domesticated *Lutzomyia longipalpis* populations in Amazonian Brazil. *Anim Behav* 42: 183–192.
46. Kelly DW, Dye C (1997) Pheromones, kairomones and the aggregation dynamics of the sandfly *Lutzomyia longipalpis*. *Anim Behav* 53: 721–731.
47. Kelly DW, Mustafa Z, Dye C (1997) Differential application of lambda-cyhalothrin to control the sandfly *Lutzomyia longipalpis*. *Med Vet Entomol* 11: 12–24.
48. Bray DP, Alves GB, Dorval ME, Brazil RP, Hamilton JGC (2010) Synthetic sex pheromone attracts the leishmaniasis vector *Lutzomyia longipalpis* to experimental chicken sheds treated with insecticide. *Parasites & Vectors* 3: 16.

49. Bray DP, Carter V, Alves GB, Brazil RP, Bandi KK, et al; (2014) Synthetic Sex Pheromone in a Long-Lasting Lure Attracts the Visceral Leishmaniasis Vector, *Lutzomyia longipalpis*, for up to 12 Weeks in Brazil. PLoS Negl Trop Dis 2014, 8(3): e2723. doi: [10.1371/journal.pntd.0002723](https://doi.org/10.1371/journal.pntd.0002723) PMID: [24651528](https://pubmed.ncbi.nlm.nih.gov/24651528/)