# Eco-Epidemiology of American Visceral Leishmaniasis with Particular Reference to Brazil

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## Early History: Studies in the States of Sergipe, Pará and Ceará

After the first description of the sand fly *Lutzomyia longipalpis* Lutz and Neiva, 1912, in an indeterminate locality in Brazil, interest in this insect remained largely entomological until the mid-1930s. In 1934, however, Henrique Penna used the viscerotome to examine liver samples from persons who were suspected to have died from yellow fever in various rural localities in Brazil (Penna, 1934). In fact, 41 of these deaths were due to visceral leishmaniasis. His results suggested the major foci of the disease to be in the northeastern states, particularly in Ceará. Carlos Chagas, at that time the director of the Instituto Oswaldo Cruz in Rio de Janeiro, sent his son, Evandro Chagas, to investigate the epidemiology. His first study was made in Sergipe where, in addition to giving the first clinical description of a living case of American visceral leishmaniasis (AVL) in Brazil, he made the important observation that the most frequent blood-sucking insect in and around the patient's house was the phlebotomine sand fly *Lu. longipalpis* (Chagas 1936).

Evandro Chagas was appointed head of a commission set up in 1936 to continue his studies, and in view of the higher prevalence of AVL in the Northeast, it was there that he wished to work. Perversely, the only state governor who offered the

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necessary financial and logistic back-up was Da Gama Malcher of Pará in the North of Brazil, where the number of recorded cases was low, and a huge old colonial-style mansion was made available for conversion into the commission's laboratories, which received the imposing name of "The Institute of Experimental Pathology for the North" (IPEN).

Working in the rural areas of Abaetetuba and Moju, where cases of AVL had been recorded by Penna, the commission uncovered more cases of the disease in both humans and dogs (Chagas et al. 1938). Once more, *Lu. longipalpis* was shown to be the principal human-biting insect in and around the houses of the infected persons, and this sand fly became the major suspect as the vector. It was concluded that the disease was essentially rural and only occurred in the close vicinity of forest or copses. For this reason, it was suggested that the origin of the causative parasite, named *Leishmania chagasi* by Cunha and Chagas (1937), was in some wild animal. The commission's hope of confirming the role of *Lu. longipalpis* as the vector and indicating the wild-animal reservoir were dashed in 1940, however, when the tragic death of their leader Evandro Chagas in a mid-air plane collision put an abrupt end to their epidemiological studies. Although the IPEN was renamed "Instituto Evandro Chagas" in his honour, his small band of dedicated workers never recovered fully from the loss of their brilliant and colourful leader, and research on the epidemiology of visceral leishmaniasis in Brazil went into a steady decline.

A rude awakening to the real importance of AVL in Brazil did not occur until 1953, when >100 inhabitants of the small town of Sobral, Ceará, died in a severe outbreak, which jolted the health authorities into activity. Another epidemiological enquiry was organized involving three prominent figures in Brazilian tropical medicine—J. E. Alencar and the married couple L. M. Deane and M. P. Deane—who had formed part of the Evandro Chagas team in Pará. In Ceará, they made two vitally important findings: (1) heavy flagellate infections of what they considered to be promastigotes of *L.* (*L.*) *chagasi* in wild-caught specimens of *Lu. longipalpis* (Deane & Deane 1954a); and (2) the natural infection of foxes with that same parasite (Deane and Deane 1954b). The foxes were identified as *Lycalopex vetulus,* but evidence exists that they were more likely to have been *Cerdocyon thous* (Courtenay et al. 1996). Infections in *Lu. longipalpis* were readily obtained when these sand flies were experimentally fed on an infected fox (Deane and Deane 1954c).

By 1955, Alencar and the Deanes had recorded nearly 1000 new cases of human AVL in Ceará and neighbouring northeastern states. They noted that these occurred in the humid, wooded foothill valleys (boqueirões) and not in the dry lowland plains (sertões) nor on the exposed slopes of the hills where the arid conditions and strong winds were unfavourable for *Lu. longipalpis*. Dogs suffered as badly as humans from the infection: That they were the major reservoir of the human disease was clearly indicated by the high rate of canine infection and the ease with which *Lu. longipalpis* could be infected when fed on infected dogs. In contrast, it was found that humans were a somewhat poor source of the parasite for *Lu. longipalpis* when they were fed on infected patients (Deane 1956).

**Fig. 1** Areas of AVL. (**a**) Sobral (Ceará, Brazil), rural profile. (**b**) Araguaína (Tocantins, Brazil), urban profile. (Credit: Afonso MMS)

## Distribution of American Visceral Leishmaniasis

Human visceral leishmaniasis was soon shown to have a very wide distribution throughout Latin America extending from Mexico in the north to Argentina in the south. However, until 1984 it was estimated that >90% of the recorded cases in the New World were from Brazil, and of a total of 8959 cases registered in this country, 7882 were from the Northeast and 992 from the Southeast (Deane and Grimaldi 1985). Considering the inadequacy of diagnosis and a general reluctance in permitting autopsies in the more remote rural communities, these figures are likely to have been considerably greater. AVL has been recorded in 12 Latin American countries, and 90% of the cases occurred in Brazil. In most cases, the geographical expansion of *Lu. longipalpis* precedes that of AVL in Brazil, and its expansion is responsible for the establishment of the disease in urban areas of medium and large cities (da Costa et al. 1997; Brasil 2014) (Fig. 1). However, there have been outbreaks in cities, such as Porto Alegre, RS, where AVL is transmitted in the absence of *Lu. longipalpis* (Bianchi et al. 2016, SBMT 2017). Autochthonous human cases have been recorded in most Brazilian states except for Acre, Amapá, Amazonas and Rondônia (Brasil 2014; Sinan 2017; BBC Brasil 2017; Ministério da Saúde 2018 [personal communication]), and infected dogs were absent only in Acre and Amazonas (Maziero et al. 2014; Steindel et al. 2013; Ministério da Saúde 2018 [personal communication] (Fig. 2).

## Origin and Taxonomic Position of American Visceral Leishmaniasis's Aetiologic Agent

The name "*Leishmania chagasi* Cunha and Chagas, 1937" long remained in use despite considerable debate regarding the origin of the parasite and its taxonomy. Lainson and Shaw (1987, 1998) considered the parasite as being indigenous to the

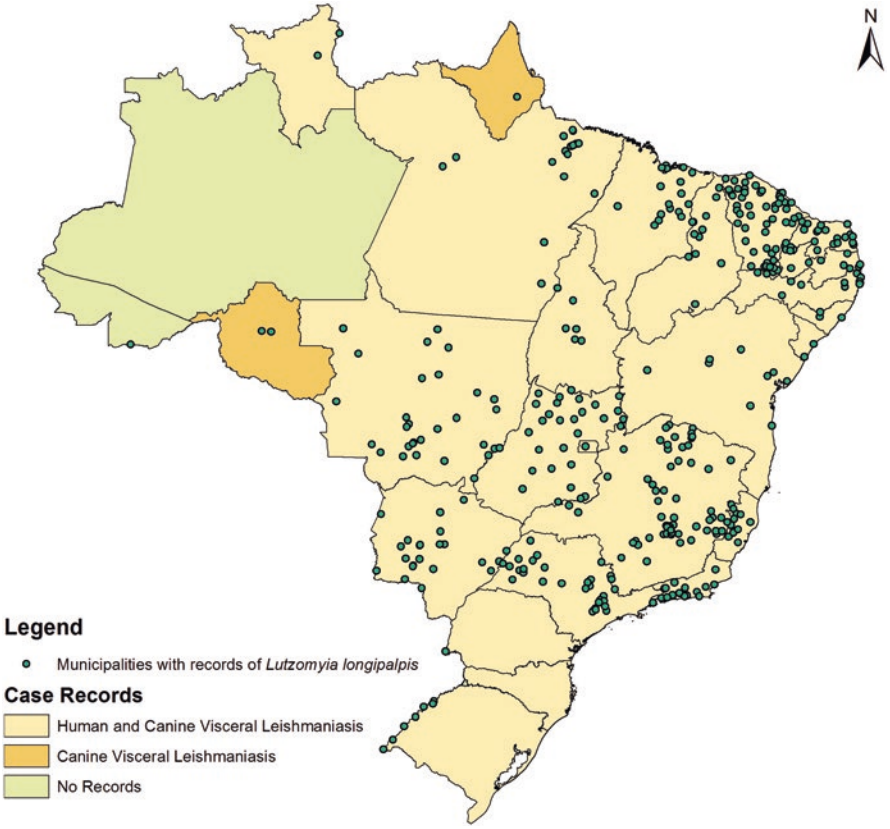

**Fig. 2** Geographic distribution of *Lu. longipalpis*: Human and canine visceral cases of AVL in Brazil

Americas. In contrast, Killick-Kendrick (1985) and Rioux et al. (1990) favoured the view that *L.* (*L.*) *infantum* had been imported into Latin America during the Portuguese and Spanish colonization. Another investigator (Lukes et al. 2007), using molecular methods, also considered that AVL had been imported from the Old World. Today there is little support of the parasite's presence in Latin America before human occupation. In a recent paper, Valdivia et al. (2017) estimated that American and Spanish *L.* (*L.*) *infantum* separated around 500 years ago. This coincides with the Iberian colonization of South America. In contrast, *L.* (*L.*) *amazonensis* strains from eastern Brazil and Amazonia had separated some 82,000 years ago, thus indicating their indigenous origin.

The rapid spread of the parasite is almost certainly due to the installation of foci due to the introduction of infected dogs. However, it is possible that in remote newly settled areas, dogs could become infected from sylvatic flies that had become infected after feeding on such wild animals as foxes. In addition, the presence of the parasite in so many different Latin American countries is most likely due to the importation of infected dogs from neighbouring countries or from abroad. It also seems likely that the parasite had been introduced from the Old World on more than one occasion. Evidence for this is the occurrence of infections in many USA states in dogs that have travelled abroad. In some states, a CVL foci is maintained by vertical transmission (Petersen and Barr 2009). Recent studies have shown how rapidly a population of *Lu. longipalpis* can disseminate within a state. After a mere 20 years, the fly expanded some 500 kl in São Paulo State (Casanova et al. 2015).

Similarities of enzyme profiles of stocks identified as *L.* (*L.*) *chagasi* and *L.* (*L.*) *infantum* led Lainson et al. (1981) to suggest that the taxonomic separation of the two parasites "…would best be at subspecific level." Although Mauricio et al. (1999) considered that there were no grounds for any separation, differences have been found between Old and New World *L. infantum* (Jackson et al. 1982, 1984; Decker-Jackson & Tang 1982; Santoro et al. 1986; Martinez et al. 2003; Valdivia et al. 2017). In consideration of these and eco-epidemiological differences, some investigators (Shaw 2002, 2006; Lainson and Rangel 2003a; Lainson and Shaw 2005) adopted the use subspecific names *L.* (*L.*) *infantum* and *L.* (*L.*) *i. chagasi*. We prefer to use the latter name for the parasite circulating in Latin America's endemic visceral leishmaniasis areas but not for the parasite that has recently been imported, such as in the USA.

## The *Lutzomyia longipalpis* Complex

Mangabeira (1969) first drew attention to small morphological differences between male examples of *Lu. longipalpis* from Ceará, Northeast Brazil, and others from Pará, North Brazil, and Lainson and Shaw (1979) suggested that the presence of "(…) a *Lu. longipalpis* complex of very similar sand flies (…) may account for certain anomalous situations" and that "a taxonomic revision is needed of (…) *Lutzomyia longipalpis*."

Ward et al. (1983) confirmed Mangabeira's finding, namely, that the male flies from Pará had a pair of white spots on the fourth abdominal tergite, whereas those from Ceará had two pairs of spots on the third and fourth tergites. Furthermore, they showed that the two forms were sexually isolated suggesting that they represented two cryptic species. It was also suggested that this might account for epidemiological differences in AVL in the two geographic areas (Ward et al. 1985).

Using electron microscopy, Lane et al. (1985) showed that the tergal spots were the site of pheromonal glands. Further studies (Ward et al. 1988; Hamilton et al. 1996) showed that the pheromones were specific to a *Lu. longipalpis* population and that the female flies could differentiate the correct one. Successful mating also depends on recognition of the "song" produced by the vibrating wings of the male (de Souza et al. 2002).

More evidence of the existence of a species complex of *Lu. longipalpis* s.l. was offered by Crampton et al. (1989), who prepared a DNA probe specific for a Bolivian population of this sand fly, and Lanzaro et al. (1993), who compared examples from

Costa Rica, Colombia and Brazil by enzyme electrophoresis and cross-breeding experiments. They concluded that these populations were of three distinct species, but they refrained from using any new specific names. Mutebi et al. (2002) added support to this conclusion by demonstrating genetic differentiation of these populations, and this was also demonstrated in populations of the sand fly in Venezuela (Arrivillaga et al. 2000). Further evidence for the presence of cryptic species within a *Lu. longipalpis* complex has been provided by numerous other investigators (Dujardin et al. 1997; Lampo et al. 1999; Uribe 1999; Yin et al. 1999; Arrivillaga and Feliciangeli 2001; Soto et al. 2001; Arrivillaga et al. 2002, 2003).

The existence of such cryptic species in Brazil was disputed by Mukhopadhyay et al. (1998), Mutebi et al. (1999), Azevedo et al. (2000) and Arrivillaga et al. (2002, 2003), all of whom considered that there is only a single species in that country based on a study of several widely separated populations for genetic variability in biochemical characters. They believed that the reasons for any epidemiological variations in AVL should be sought elsewhere. In favour of this view, a recent study of *Lu. longipalpis* populations from six locations in a transect across eastern Brazil by mitochondrial cytochrome-b gene-sequence analysis suggested that sequence divergence also did not adequately indicate cryptic species (Hodgkinson et al. 2003). In contrast, differences in the song patterns between Brazilian populations of *Lu. longipalpis* were consistent with levels of molecular divergence at the *cacophony* locus (Bottecchia et al. 2004; Souza et al. 2004). These groups of investigators suggest that their findings, together with other evidence, supports the existence of a cryptic species-complex under the name of *Lu. longipalpis* in Brazil with as many as four sibling species (Souza et al. 2004). In addition, Jacobina (Bahia), Lapinha (Minas Gerais) and Natal (Rio Grande do Norte) populations had been differentiated on genetic grounds (Bauzer et al. 2002). Maingon et al. (2003) produced genetic evidence of the existence of two sibling species of *Lu. longipalpis* that produce distinct male sex pheromones in Sobral, Ceará, Northeast Brazil. Finally, Watts et al. (2005) correlated microsatellites and the male pheromones of 11 populations from Brazil and Venezuela. They concluded that "Temporal genetic differentiation was mostly not significant at the same site. Spatial genetic differentiation was, however, strong, although there was only a weak relationship between genetic differentiation and the geographic distance separating the samples. (…) Geographic separation explained a much greater (…) percentage of the genetic differences among populations when samples with the same pheromone type were analyzed separately." A cluster analysis showed five groups: *Lu. cruzi* (Brazil) and *Lu. pseudolongipalpis* (Venezuela) as separate species; two Venezuelan and Brazilian groups; and a distinct cluster of Brazilian cembrene populations.

Most investigators have cautiously refrained from giving names to "cryptic species," and it has rightly been asked if these different populations might not simply indicate the initiation of a speciation process rather than the existence of valid species (Bottecchia et al. 2004). It has also been questioned as to whether *Lu. longipalpis* "(…) is a highly polymorphic and geographically variable species, but not a species complex" (Bauzer et al. 2002).

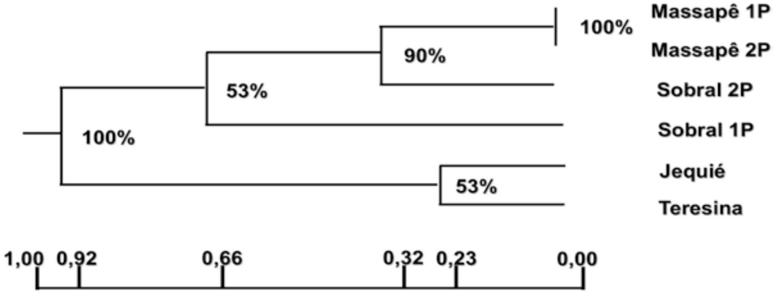

**Fig. 3** Dendrogram based on the values of genetic distance inferred from morphometric data of *Lu. longipalpis* populations from different locations of Ceará State. (Source: Afonso MMS 2009)

Considering the observations by Mangabeira (1969) and Azevedo et al. (2000), where morphological differences were clearer, *Lu. longipalpis* males from Jequié (BA), Teresina (PI), Sobral (CE) and Massapê (CE) showed no difference in the spot pattern. In morphometric analyzes, the Jequié population was different from the others by analysis of variance (significant difference in >80% of features) and by Student-Newman-Keuls test (separation based on 37% of analyzed features). Among the four populations from Ceará state, there was a high level of homogeneity; however, the dendogram revealed an isolated branch for the Sobral population with one pair of spots, thus concluding that the populations with one and two pairs of spots would be under speciation, whereas the populations of Massapê are 100% similar (Afonso 2009) (Fig. 3).

Arrivillaga and Feliciangeli (2001), however, gave the name of *Lu. pseudolongipalpis* to a sand fly in Venezuela. The adults are apparently morphologically indistinguishable from those of *Lu. longipalpis*, but the larvae are morphologically distinct. In addition, the adult fly's biting activity was shown to be continuous throughout the night, unlike that of two populations of Venezuelan *Lu. Longipalpis*, which was greatest before 23:00 and steadily decreased from that time onward (Feliciangeli et al. 2004). Arrivillaga et al. (2003) performed phylogenetic analyses of 31 populations of *Lu. longipalpis* s.l. originating throughout this species' geographic range using 7 isozyme loci and mitochondrial genes. The analyses revealed four distinct clades which, it was considered, supported the existence of four species. These had distinct geographic ranges defined as (1) Brazil (*Lu. longipalpis* sensu stricto); (2) Laran (Northwestern Venezuela populations); (3) cis-Andean-Colombia; and (4) *trans*-Andean-Central American populations. The Brazilian clade was represented by 11 populations sampled throughout this country including areas in which *Lu. longipalpis* was originally described: the sand fly of the Laran clade = *Lu. pseudolongipalpis* from Northwest Venezuela; the cis-Andean clade consisted of Colombian populations in Bucaramanga, Palo Gordo, Neiva, Durania and a population from Pacaraima, North Brazil (a mountainous area in Roraima, on the borders of Venezuela and Guyana); and the *trans*-Andean clade included 11 populations from various parts of Central America. The investigators proposed to prepare descriptions and new specific names for the sand flies of the latter two clades.

A study of the period gene (Lima Costa et al. 2015) of populations from Sobral, Ceará state, and Pernambuco again supported the presence of two sibling species in Sobral known as the one- and two-spot populations (1S & 2S, respectively). It was shown that the two Sobral sibling species had expanded into other areas of these two states. These investigators also draw attention to the importance of the abdominal spots in diagnosing the presence of sympatric species. Previously it was suggested (Coutinho-Abreu et al. 2008) that the 1S and 2S populations had been separated by the original course of the San Francisco river. Then its change in course led to a re-encounter of the 1S populations, which could be responsible for their present-day genetic diversity.

Differing opinion will doubtless continue concerning the criteria needed before a considered “cryptic species” is given specific rank, but intrinsic reproductive isolation, as demonstrated by cross-breeding experiments, must surely be high on the list. In this connection, work on the sand-fly’s mating “song” is particularly interesting because the vocalization of the males of a number of insects appears to be the most important barrier isolating the different species (Imms 1964; Perdeck 1957).

In a study with five populations of *Lu. longipalpis* from Mato Grosso do Sul State (Campo Grande, Três Lagoas, Aquidauana, Miranda and Bonito), in one from Alagoas State (Estrela de Alagoas) and one population of *Lu. cruzi* from Corumbá (MS), microsatellite analyzes revealed divergence among populations from the two states (due to the large geographical isolation), thus supporting the *Lu. longipalpis* complex. In addition, *Lu. longipalpis* and *Lu. cruzi* showed an introgression between species (Santos et al. 2013).

The high level of the variety of evidence, including chemical, behavioural and molecular characteristics, suggests very recent speciation and complex population structure in the *Lu. longipalpis* species complex. Although significant progress has been achieved to date, the knowledge about recent epidemiological changes, such as urbanization, are essential for the use of effective strategies to control this species (Souza et al. 2017). The finding that the rapid spread of visceral leishmaniasis in São Paulo state was due to the expansion of a population of one chemotype (Casanova et al. 2015) suggests that variations in levels of transmission may be related to specific cryptic species. It also indicates that control measures should concentrate on cryptic species that are epidemiologically important.

The existence and identification of cryptic species, under the name of *Lu. longipalpis* s.l., helps in explaining why different clinical manifestations of AVL exist in Latin America, especially when this is considered in the light of studies on the nature of the saliva of *Lu. longipalpis* s.l. from widely separated geographical areas.

The actual reasons for the drastic changes in the distribution of *Lu. longipalpis* s.l. distribution, especially urbanization, which we will discuss later, are unknown; however, one possibility is climate changes related to global warming. A recent study by Peterson et al. (2017), using ecological-niche models, showed variations that ranged from stability to expansion and even decline. There were indications of expansion in southern Brazil and Argentina and in Amazonia. In this region, the presence of *Lu. longipalpis* was predicted in the northeastern regions Pará state and Amapá state, which are both north of the Amazon River. So far it has only been recorded once in Amapá State (Galardo et al. 2013), and to date no autochthonous cases of human or canine VL have been reported in the region. Data from other areas of Amazonia support the reliability of predictions in this area. However, the models of Peterson et al (2017) failed to predict this species' presence in Uruguay. This may be due to climate changes that occurred after 2000 being too recent to include in the model or due to human introduction.

## Influence of *Lutzomyia longipalpis s.l.* Saliva on Infection of Humans with *Leishmania* (*Leishmania*) *infantum chagasi*

Although infection with *L.* (*L.*) *i. chagasi* is predominately associated with a visceral disease, the same parasite has been shown to produce only non-ulcerative cutaneous lesions in Costa Rica (Zeledón et al. 1989), whereas in Honduras it may cause both visceral and cutaneous leishmaniasis in the same focus (Ponce et al. 1991). The saliva of *Lu. longipalpis* contains a potent vasodilatory peptide, "maxadilan" (Lerner et al. 1991). In experiments investigating the possible influence of the sand fly's saliva on the course of human infection with *L.* (*L.*) *i. chagasi*, Warburg et al. (1994) fed *Lu. longipalpis* s.l. of Brazilian, Colombian and Costa Rican origin on the arms of volunteers. They found that the measurements of the resulting erythema at the sites of the bites correlated well with the levels of maxadilan in the sand flies from the three geographical areas. Saliva from the Brazilian colony was the most potent, and that from the Colombian flies was less so. Saliva from the Costa Rican specimens had very little maxadilan, had a very low vasodilatory activity and produced negligible erythema: When mixed with promastigotes of *Leishmania major* and inoculated into the foot-pads of mice, it strongly enhanced proliferation of cutaneous lesions. In contrast, similar inoculations of mixtures of promastigotes and saliva from Colombian and Brazilian *Lu. longipalpis* exacerbated the development of cutaneous lesions to a lesser degree. It was suggested that some of the variability in the clinical presentations of *L.* (*L.*) *i. chagasi* infections may be due to the different composition of the saliva of the sand fly, presumably accounting for the manifestation of *L.* (*L.*) *i. chagasi* infection in humans as either a visceral or a cutaneous disease. The significance of these findings regarding the nature of infections in wild- or domestic-reservoir hosts in foci of human cutaneous and/or visceral leishmaniasis due to this parasite remains to be studied. A cutaneous lesion due to *L.* (*L.*) *i. chagasi* has been reported in a patient from the state of Rio de Janeiro, Brazil (Oliveira et al. 1986). Unlike those described in Costa Rica and Honduras, however, the lesion was ulcerative, and cutaneous manifestations of infection with this parasite in Brazil would appear to be a rarity.

## *Lutzomyia longipalpis:* The Major Vector of American Visceral Leishmaniasis

The overall coincidental distribution of *Lu. longipalpis* and AVL throughout most of Central and South America greatly strengthened the Deanes' conviction that this was the major vector of the disease. Strangely enough, however, although *Lu. longipalpis* is perhaps the most easily colonized of all sand flies in the laboratory, repeated attempts to experimentally transmit the parasite by the bite of this insect failed. Appropriately enough it was in the Instituto Evandro Chagas, where so much of the early history of AVL began, that the chain of evidence incriminating this sand fly was finally completed when five separate transmissions to hamsters were obtained by the bites of experimentally infected laboratory-bred *Lu. longipalpis* (Lainson et al. 1977). The same laboratory (Lainson et al. 1984, 1985) studied a serious outbreak of AVL in the outskirts of Santarém, Pará, where they found this sand fly to be the only species consistently present in and around houses with human and canine infections. Large numbers were captured in the backyard of one house and were fed on clean hamsters, four of which subsequently develop fulminating infections. Dissections of the sand flies used in this experiment indicated an infection-rate of 7%, and 16 isolates were identified as *L.* (*L.*) *chagasi* on enzyme profiles and by monoclonal antibodies. This transmission by the bites of naturally infected *Lu. longipalpis* provided the most conclusive proof possible of the role of this sand fly as a major vector of AVL.

## The Ecology of *Lutzomyia longipalpis*: A Sylvatic Origin

Most early studies on AVL in Brazil were conducted in the sparsely forested northeastern states or in other parts of the country that have suffered considerable deforestation: As a result, there developed a tendency to think of the disease only as one that involves a dog and *Lu. longipalpis* in a domestic environment. Observations in the Amazon region of Brazil (Chagas et al. 1938; Lainson et al. 1986; Ryan et al. 1986c), however, indicated that *Lu. longipalpis* is primordially a sylvatic species and that it can still be captured in remote primary forest far from human habitation. In Northern Brazil, this is particularly evident along the length of newly opened roads that pass through forested areas. Primitive houses, inevitably with dogs, chicken houses and other animal shelters, are rapidly thrown up along their length in very close proximity to the forest edge. *Lu. longipalpis* females have catholic feeding habits and quickly invade such habitations; thus, in an epidemiological investigation of cases of AVL along the forest-fringed Igarapé Miri-Tucuruí highway, this sand fly was found in the chicken houses of numerous widely separated houses only 18 months after the road had been opened (Lainson, Shaw, Silveira, & Souza, unpublished observations) (Fig. 4). Finally, even more conclusive evidence came from studies in the municipality of Salvaterra, Island of Marajó, Pará, in a

**Fig. 4** Focus of AVL in Pará. Igarapé Miri-Tucuruí highway crossing the primary forest. Dwellings were built close to the road, and within 18 months of their construction they contained *Lu. longipalpis*

focus of AVL (Lainson et al. 1990). Using CDC light-traps variously placed over caged chicken, a fox and sawdust impregnated with the urine and faeces of a fox, attempts were made to capture *Lu. longipalpis* in a pocket of residual primary forest, in the backyard of a house some 500 m distant, and in neighbouring open

**Fig. 5** Primary forest in the Serra dos Carajás, Pará State, Brazil, in which very small numbers of *Lu. longipalpis* were captured

savanna. During the dry season, 80 trapping-nights in the forest produced a total of 47 of these sand flies consisting of 22 males and 25 females: None were caught after 14 captures in the savanna, and 2 captures in the backyard of the house provided only 1 male and 4 females. During the wet season, the results were much more impressive: 32 trapping-nights in the forest provided 1161 (463 males and 698 females); 26 captures in the savanna gave a total of 4 (1 male and 3 females); and 24 captures in the backyard of the house produced a total of 1274 (572 males and 702 females). From this and other studies, it was clear that the natural savanna is an unattractive breeding site for *Lu. longipalpis*. In contrast, the large numbers of this sand fly caught in the patch of forest, and the marked association of males and females during both the dry and wet seasons, strongly suggested this to be an important breeding site (Figs. 5 and 6). Galati et al. (2003) reported the capture of *Lu. longipalpis* in the forest environment in the state of Mato Grosso do Sul, Brazil. It remains to be determined, however, if the peri-domestic accumulation of this sand fly is entirely due to their migration from the sylvatic habitat or, at least in part, to the establishment of a secondary peri-domestic breeding site.

The discovery of the latter would be a significant step forward in the control of AVL, but to date all available evidence suggests that the immature stages of *Lu. longipalpis* are thinly dispersed and not concentrated in any particular microhabitat (Deane 1956). In Salvaterra, on the Island of Marajó, the results of an examination of soil removed within and around a small, heavily infested chicken house suggested that the sand flies were not breeding in that microhabitat but had migrated to the chicken house from elsewhere (Dye and Quinnell 1994, personal communication). It has been shown in the laboratory that the male produces a pheromone that

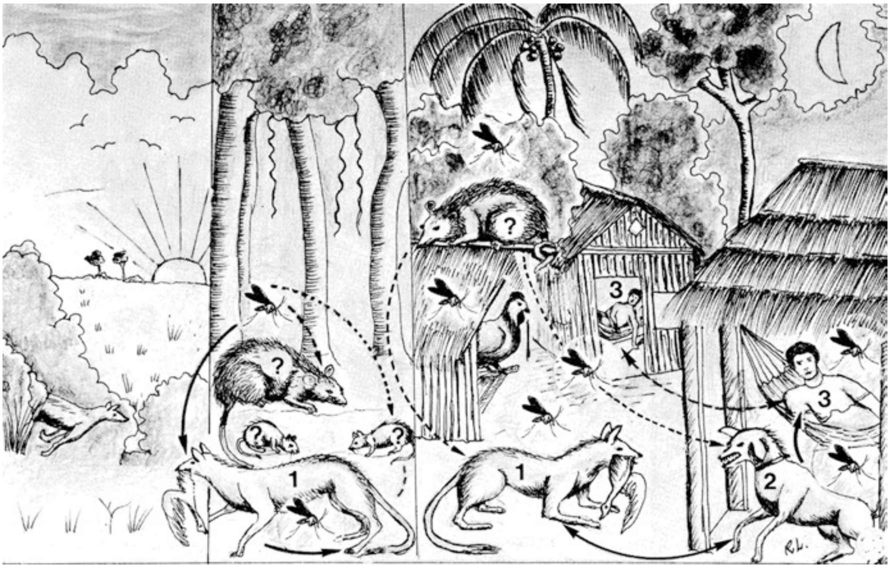

**Fig. 6** Eco-epidemiology of AVL in Pará State, Brazilian Amazonia. Full lines = definite transmission lines. Interrupted lines = possible alternative transmission lines, involving other animals as well as humans, as a source of infection for *Lu. longipalpis*. (Source: Lainson and Rangel 2003b)

attracts the female from a substantial distance (Morton and Ward 1989), thus leading these investigators to suggest that the attraction of host odour and male pheromone worked together synergistically at the same time. In contrast, after their observations on the progressive infestation of newly constructed chicken houses by *Lu. longipalpis*, Dye et al. (1991) and Quinnell and Dye (1994a) were led to the conclusion that the females, accompanied by some males, are at first attracted by host odour and later by the pheromone. It was noted, however, that whereas the males tended to remain longer in the chicken houses, most of the females did not rest there during the day.

That *Lu. longipalpis* females feed readily on domestic chicken suggests that wild birds are likely to be among their sylvatic hosts. This sand fly's concentration in chicken houses is of considerable epidemiological importance because it is not customary to spray these with insecticides during antimalarial campaigns, which still remain the principal indirect form of control of AVL.

After experimental studies on the peri-domestic distribution of *Lu. longipalpis* in Salvaterra, Island of Marajó, Quinnell and Dye (1994a, b) concluded that this sand fly tends to congregate at sites outdoors, including animal sheds, where leks can most easily form on abundant, stationary (sleeping) hosts. The flies are much less frequently encountered within houses and, because most dogs sleep outdoors, this probably accounts for a much higher infection rate of AVL in dogs than in humans. It was also suggested that human exposure to the bites of *Lu. longipalpis* was greatest in poorly constructed houses with abundant holes in the walls and the roof.

## Urbanization

Visceral leishmaniasis produces major impacts on human health as a consequence of environmental change, mainly through the possible expansion of transmission areas. Continuous environmental change processes—such as deforestation, fires, agriculture, mining, construction of dams and hydroelectric-power plants, migration, unplanned urbanization and lack of urban infrastructure—are examples of situations that have led to an increase in people at risk of infection and fostered the emergence of outbreaks of leishmaniasis in a new eco-epidemiological pattern (Rangel and Vilela 2008; Vilela 2012). AVL shows a persistent scenario in Brazil with most of the factors contributing to its endemicity residing in processes that are external to the health sector. This makes the strengthening of new strategies necessary.

In a study conducted at the state of Tocantins, the results demonstrated a correlation between deforestation and the possible emergence of outbreaks because AVL persists in areas with environmental changes. The increase of deforested areas remained constant in the state and also showed expansion in the record of human cases, especially in the municipality of Araguaína. Some municipalities remained classified as areas of intense transmission according to the methodology proposed by the Brazilian Control Program of Visceral Leishmaniasis (Brasil 2014), especially in the northern and central region, thus showing that the number of human cases remained high and constant throughout the years, principally in areas where there is great environmental impact (Fig. 7). The vector *Lu. longipalpis* was present in all land-use classes because it has adapted to all environments, including impacted areas. This information, coupled with the correlation between the incidence of the disease and urban areas, demonstrate once again the vector's adaptation to anthropic environments (Afonso et al. 2017) (Fig. 8). In the same municipality, a centrifugal dissemination pattern was suggested for AVL, in which the disease showed successive outbreaks in central and peri-urban areas from 2008 onward (Toledo et al. 2017).

The literature contains records of the eclectic behaviour of *Lu. longipalpis*, which feeds on a variety of mammals, including dogs, pigs, equines, bovines and birds (Afonso et al. 2012). Given favourable environmental conditions, the abundance of feeding sources is a determinant factor for the vector's population growth, especially in peri-urban and urban areas, which favours its approximation to man.

In Ceará state (Massapê), specimens that fed on more than one source of animal blood were detected, which illustrates the eclectic feeding behaviour of *Lu. longipalpis* and the general behaviour of sand flies of "tasting" different hosts before completing a blood meal, an important aspect of leishmaniasis transmission (Afonso et al. 2012).

In a study of *Lu. longipalpis* populations from Araguaína (TO), Fortaleza (CE), and Sobral (CE), all of which are areas of intense transmission of AVL, the vector fed mainly on humans (Afonso 2013; Brasil 2014). The anthropophilic behaviour of *Lu. longipalpis* has been demonstrated in many studies (Deane 1956; Lainson and

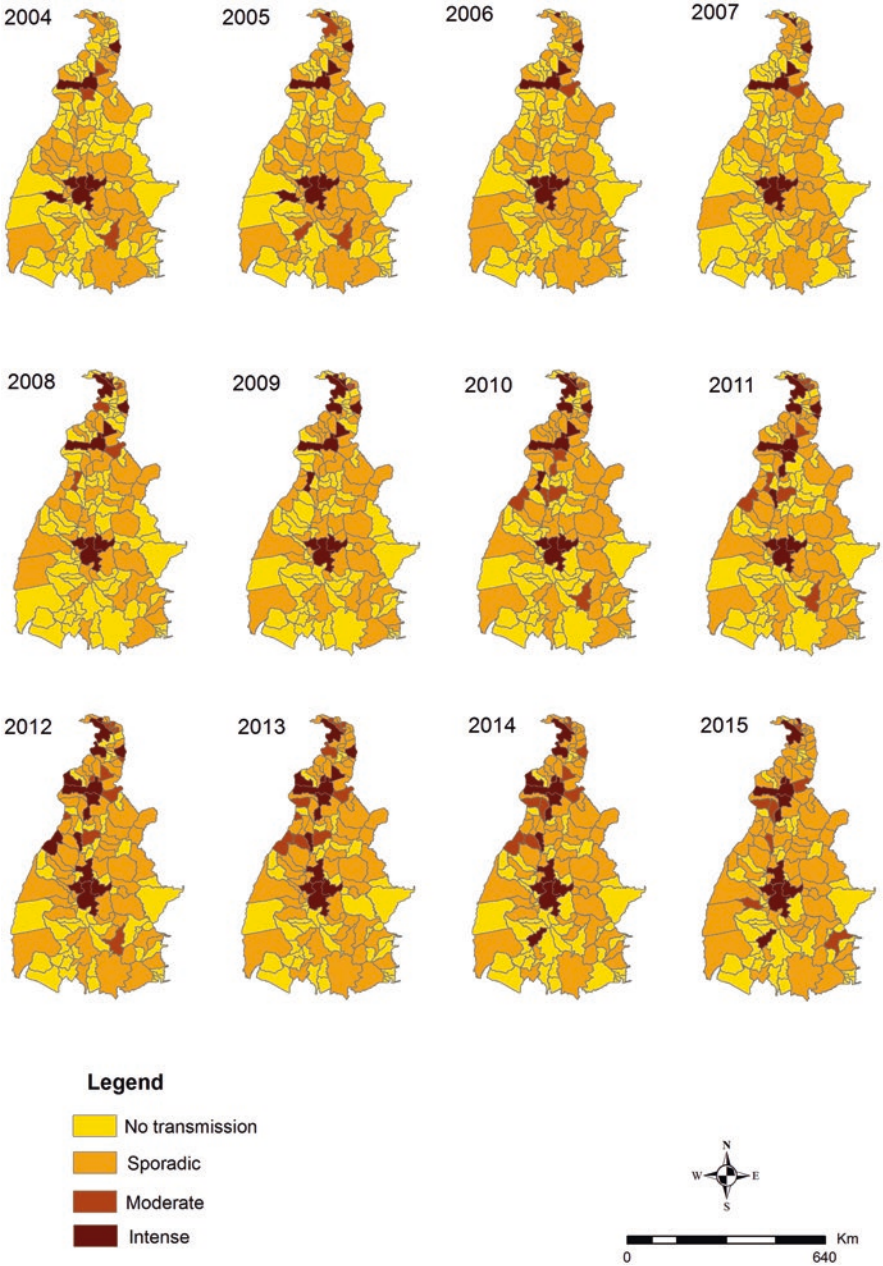

**Fig. 7** Spatio-temporal profile of the stratification of municipalities for AVL in Tocantins State, 2004–2015. (Source: Afonso et al. 2017)

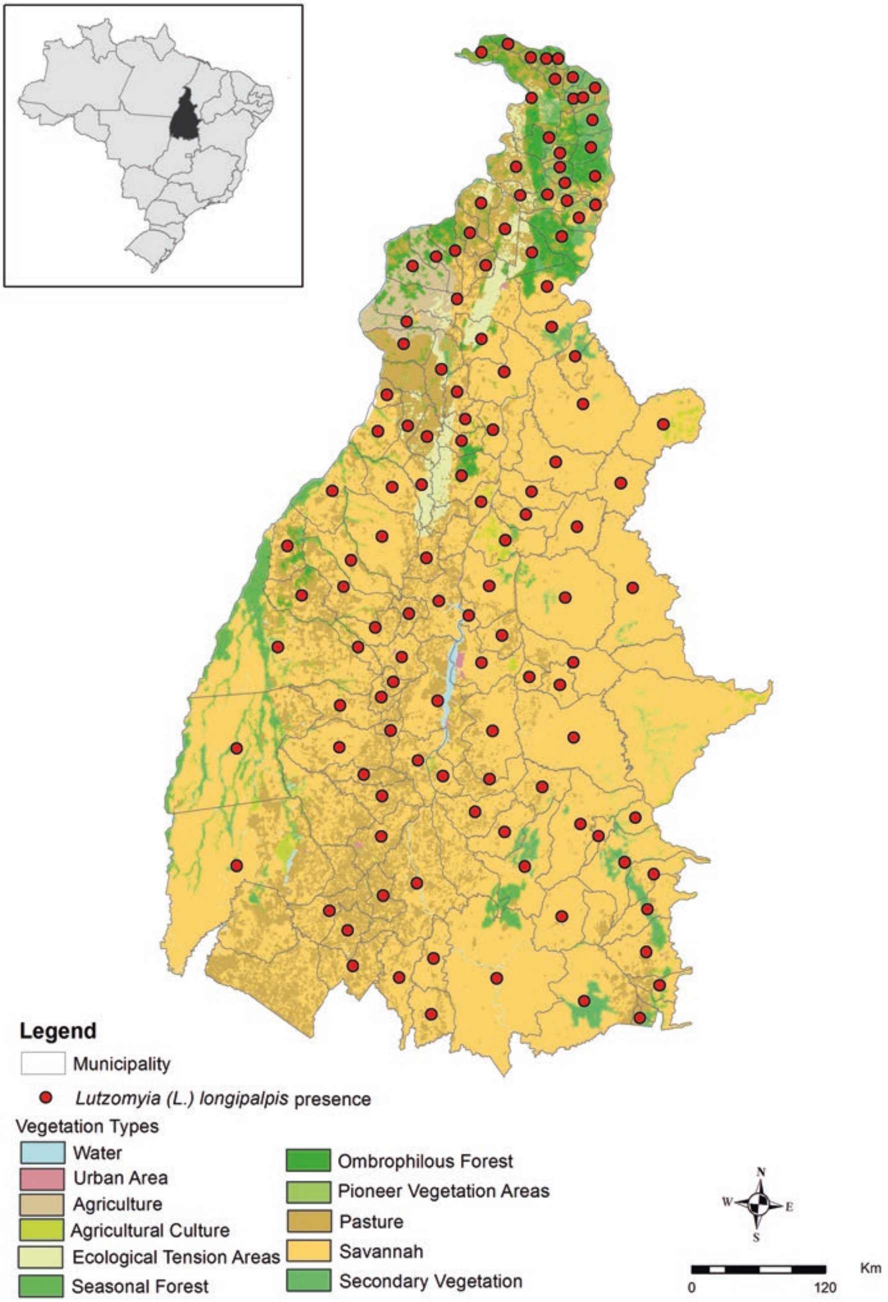

**Fig. 8** Map of Tocantins State with land-use classes in association with the presence of *Lu. longipalpis*. (Source: Afonso et al. 2017)

Rangel 2005), which is one of the essential criteria for vector incrimination according to Killick-Kendrick (1990).

The ability to feed on domestic animals (Alexander et al. 2002; Afonso et al. 2012; Brasil 2014) and on synanthropic animals, such as opossums and rodents (Sherlock et al. 1984, 1988; Schalling et al. 2007; Afonso et al. 2012; Lara-Silva et al. 2014), favours the adaptation of *Lu. longipalpis* to human-modified habitats. This allows the maintenance of the transmission cycle of AVL in rural areas and its expansion to urban areas, which characterize the two Brazilian transmission profiles (Brasil 2014).

To understand AVL's urbanization in Brazil, we must use different tools and analyses in entomological surveillance capable of detecting changes in the biology and behaviour of the vector species involved in its transmission. The principal AVL vector in the Americas is *Lu. longipalpis*, which is present in most Brazilian states except for Amazonas and Santa Catarina (Vilela et al. 2014; Borges et al. 2017, Fig. 2). AVL is expanding because of environmental changes and the adaptation of *Lu. longipalpis* to various habitats. To stop this, the planning and implementation of public policies must include measures that minimize the impact of anthropogenic environmental changes favouring AVL transmission. These integrated actions must be incorporated at regional, national and international levels (Salomón et al. 2015).

In the Central Western region of Brazil (Almeida et al. 2015), *Lu. longipalpis* has been shown to be present in all biomes, which corroborates with the results of other studies and demonstratesits adaptability to different environments (Deane and Deane 1962; Lanzaro et al. 1993; Sherlock 1996; Aguiar and Medeiros 2003; Galati 2003; de Almeida et al. 2013). *Lu. cruzi* is predominant in the states of Mato Grosso and Mato Grosso do Sul, thus confirming the findings of Missawa and Lima (2006). *Lu. longipalpis* presented a broader geographic distribution compared with *Lu. cruzi*, although the niche model revealed high climatic suitability for *Lu. cruzi* to the southern region of Goiás.

In the State of São Paulo, a study of spatial and temporal distribution detected that the presence of the vector *Lu. longipalpis* preceded the presence of both canine cases and thus human cases and that the transmission expanded to the central region and then to the north and south regions (Casanova et al. 2015).

## Other Possible Vectors of *Leishmania* (*Leishmania*) *infantum chagasi* in Brazil and Neighbouring Countries

The idea that sand flies, other than members of the *Lu. longipalpis* complex, transmit *L.* (*L.*) *infantum* in the Americas originated in epidemiological studies and recently has gained support from molecular studies. However, this must be viewed with caution due to technical limitations. In addition, finding parasite DNA in a sand fly does not relate to transmissibility.

The assumption that *Lu. longipalpis* s.l. was the sole sand-fly vector of *L.* (*L.*) *i. chagasi* throughout the whole geographical range of AVL persisted for >50 years. Suspicions were raised, however, that other species of sand flies might be involved in Venezuela when cases of the disease were recorded in the apparent absence of this sand fly.

Thus, Potenza and Anduze (1942) were unable to find *Lu. longipalpis* in two districts of the state of Bolivar, where two cases of infantile visceral leishmaniasis had been diagnosed, and Pifano and Romero (1964) suggested that *Pintomyia evansi* (Nuñez-Tovar) might be an alternative vector in a focus of AVL in the Turmiquire hills, state of Sucre, Venezuela, where *Lu. longipalpis* was seemingly absent. A further 26 years elapsed, however, before this suspicion was substantiated when Travi et al. (1990) showed that 87% of the sand flies captured in a focus of AVL in the Córdoba Department of Colombia were *Pi. evansi* and that one of these flies was infected with *L.* (*L.*) *i. chagasi* as identified by isolation of the parasite and its characterization by isoenzyme electrophoresis. In further studies in north Colombia, promastigotes were found in nine more specimens of *Pi. evansi* and the parasite again identified as *L.* (*L.*) *i. chagasi* on two occasions (Travi et al. 1996). Thus, the presence of *Pi. evansi* in peri-domestic and intra-domestic habitats throughout the year, as well as the apparent absence of *Lu. longipalpis*, led to the conclusion that *Pi. evansi* is the principal vector of AVL in that region of Colombia, although elsewhere the vector has been shown to be *Lu. longipalpis* (Ferro et al. 1995). Recently, in Carabobo state, Venezuela, Aguilar et al. (1998) recorded the presence of promastigotes in a single specimen of *Pi. evansi* captured in an area endemic for AVL, and—among 1757 sand flies caught in and around houses—72.9% were *Pi. evansi* and only 1.3% were *Lu. longipalpis*.

Finally, Feliciangeli et al. (1999) used k-DNA restriction analysis to show high homologies between the culture forms of the parasite from *Pi. evansi* and a standard stock of *L.* (*L.*) *i. chagasi*. These findings regarding *Pi. evansi* raise two major questions: (1) whether or not it may be an alternative vector of AVL in other parts of this sand fly's geographical range; and (2) whether there exist other alternative vectors. In addition to Colombia and Venezuela, *Pi. evansi* has been recorded in Costa Rica, Honduras, Nicaragua, El Salvador, Guatemala (Young and Duncan 1994) and Mexico (Ibáñez- Bernal et al. 2004). Evidence suggests that adaptation of *L.* (*L.*) *i. chagasi* to *Pi. evansi* is a relatively recent event that is still in progress. Thus, Montoya-Lerma et al. (2003) made a study of the infection-rates and development of *L.* (*L.*) *i. chagasi* in *Lu. longipalpis* and *Pi. evansi* under both natural and experimental conditions. Experimental infection rates and the cycle of *L.* (*L.*) *i. chagasi* in the two flies showed that parasite colonization, differentiation, attachment to the gut epithelium and migration to the foregut were all more frequent and uniform in *Lu. longipalpis* than they were in *Pi. evansi*.

As far as we can ascertain, *Pi. evansi* has not been found in Brazil; however, speculations have been made regarding the possible role of a variety of other sand-fly species in the transmission of *L.* (*L.*) *i. chagasi*. Oliveira et al. (1959) failed to find *Lu. longipalpis* in a village in Minas Gerais where there was a high incidence of AVL, and suspicion fell principally on *Lu. intermedia* and *Lu. whitmani*.

Coelho et al. (1965) were also unable to capture *Lu. longipalpis* in a focus of the disease in southwest Goiás where the most common sand flies were *Lu. intermedia, Lu. whitmani*, *Lu. shannoni* and *Lu.* (*Psychodopygus*) *davisi*. Ryan et al. (1984) recorded heavy promastigote infections in *Lu. antunesi* captured in a focus of AVL on the Island of Marajó, Pará. Although the organism remained unidentified, its supra-pylarian development in the sand fly raised the question as to whether it was *L.* (*L.*) *i. chagasi*. The same investigators (unpublished observations) found heavy infestations of *Lu. furcata* in pigsties in an area near Belém, Pará, where isolations of *L.* (*L.*) *i. chagasi* had been made from foxes but where *Lu. longipalpis* could not be found. *Lu. furcata* is not anthropophilic but attacks a variety of wild and domestic animals. It could possibly represent, therefore, an alternative vector among such reservoir hosts of *L.* (*L.*) *i. chagasi* as dogs and foxes: Experimentally, it has been shown to can transmit another species of *Leishmania*, *L.* (*L.*) *amazonensis* (Ryan et al. 1986a).

The female of the sand fly *Lu. cruzi* is morphologically indistinguishable from that of *Lu. longipalpis* (Martins et al. 1984), and the two species can only be reliably separated by small differences when comparing the males. To add to the confusion, a distribution overlap makes it difficult to incriminate either species as the vector of AVL in areas where the two are found together. Santos et al. (1998) dissected many sand flies captured in CDC light-traps around houses in a focus of AVL in Corumbá and Ladário, Mato Grosso do Sul, and found promastigotes in 14 female specimens all with the morphology of *Lu. longipalpis/ Lu. cruzi*. The parasite was identified as *L.* (*L.*) *i. chagasi* by monoclonal antibodies and, in virtue of the apparent absence of males of *Lu. longipalpis* in their captures, these investigators concluded that all of the infected flies were *Lu. cruzi* and that "(...) *Lu. cruzi* is the vector of *Leishmania chagasi* in Mato Grosso do Sul, Brazil." Although the evidence for this supposition is strong, it is not yet conclusive, and Santos et al. (2003) have in fact confirmed the presence of *Lu. longipalpis* in the Corumbá area of study. Until infected females can be conclusively identified as *Lu. cruzi*—by way of biochemical methods (Ryan et al. 1986b), by DNA probes (Ready et al. 1991) or after the production of adults by raising them from the eggs of infected flies (Ryan et al. 1987)—the role of *Lu. cruzi* as a vector of *L.* (*L.*) *i. chagasi* must remain doubtful. Santos et al. (1998) suggested that the epidemiology of AVL in the area of Bolivia bordering Mato Grosso do Sul "(...) certainly should be the same (...)," namely, that *Lu. cruzi* also occurs in Bolivia. The central region of Brazil then presented a differentiated situation regarding the transmission of AVL. As already mentioned, in 1998 it was suggested that *Lu. cruzi* would be involved with the transmission of AVL in Corumbá and Ladário (MS) due to its anthropophily, high density and absence of *Lu. longipalpis* in the transmission areas (Santos et al. 1998). In 2003, the same group reported the finding of natural infection in *Lu. cruzi* (Santos et al. 2003). Subsequently, in 2006, evidence pointed to the participation of *Lu. cruzi* in AVL transmission in the municipality of Jaciara (MT) (Missawa and Lima 2006). Such studies show that in areas where *Lu. longipalpis* is not present, *Lu. cruzi* would be involved in the transmission of AVL.

Among other possible "alternative" vectors, *Lu. intermedia* and *Lu. whitmani* must be included. *Lu. intermedia*, highly suspected as a vector of *L.* (*V.*) *braziliensis* in southeast Brazil, shares a similar habitat to that of *Lu. longipalpis*, is highly anthropophilic and is also known to feed on dogs: In addition, it has been experimentally infected with *L.* (*L.*) *i. chagasi* (Chagas 1940, Paraense & Chagas 1940). In contrast, *Lu. intermedia* has not been recorded further north than Paraíba, in Alagoa Grande and Areia, and part of Pernambuco in Lagoa dos Gatos, Nazaré, Quipapá, Timbaúba and Vitória de Santo Antão (Martins et al. 1978; Young and Duncan 1994). Consequently, it cannot be involved as a secondary vector of AVL in the highly endemic areas in Ceará and Piauí or in the states of Maranhão and Pará. *Lu. whitmani* sensu stricto is a confirmed vector of *L.* (*V.*) *braziliensis* in Northeastern Brazil (Rangel and Lainson 2003) and, as mentioned previously, has been suspected as a vector of AVL in Minas Gerais and Goiás. It is highly anthropophilic and frequently found, together with *Lu. longipalpis*, in chicken houses and human dwelling places. Regarding transmission in the sylvatic habitat in north Brazil, *Lu. whitmani* sensu lato might function as a vector among foxes, but its rarity near houses and its non-anthropophilic habits militates against it being a vector of *L.* (*L.*) *i. chagasi* to humans.

In the Amazon region, suspicion must fall on *Lu. flaviscutellata* as a conceivable alternative vector of *L.* (*L.*) *i. chagasi*. It is better known as the major sylvatic vector of *L.* (*L.*) *amazonensis* among a variety of rodents and marsupials, but this parasite has been isolated from a fox in Pará (Lainson and Shaw 1987), thus indicating that this sand fly does include foxes among its hosts and that these animals are natural hosts of *L.* (*L.*) *i. chagasi*. *Lu. flaviscutellata* is occasionally found invading the peridomestic habitat in areas where isolated cases of Amazonian AVL have been diagnosed (Lainson et al. 1994). It is not greatly attracted to humans, however, so its role as a secondary vector, if indeed it exists, would be of minor importance. Souza et al. (2003) were unable to find *Lu. longipalpis* in 6 of 18 foci of AVL in the municipality of Rio de Janeiro and suggested the participation of other species of sand flies, such as *Lu. migonei* and *Lu. firmatoi*. When considering the apparent absence of *Lu. longipalpis* in such foci of AVL, however, it must be remembered that with the change of rainy to dry season, the population density of this sand fly may fall to such an extent that no examples can be found until the next wet season.

In studies conducted through multiplex polymerase chain reaction (PCR) assays, it was possible to detect two *Mg. migonei* females from an AVL-transmission area in Pernambuco infected by *L.* (*L.*) *infantum*, the first finding of natural infection of this species, thus suggesting that this could be the vector of *L.* (*L.*) *infantum* in areas of AVL where *Lu. longipalpis* is absent (de Carvalho et al. 2010). Guimarães et al. (2016) observed the production of *L.* (*L.*) *infantum* metacyclic forms and the promastigote-secretory gel in experimentally laboratory-bred flies. The infections were similar those seen in experimentally infected *Lu. longipalpis*, which led the investigators to consider that *Mg. migonei* is a permissive vector. In Argentina, there is also epidemiological evidence (Salomón et al. 2009) of a vectorial role of *Mg. migonei* in AVL transmission.

Table 1 lists infections of *L.* (*L.*) *infantum* detected by different molecular methods. The interpretation of the importance of these infections must be viewed with

**Table 1** List of sand-fly infections of *Leishmania* (*L.*) *infantum* diagnosed by different molecular methods in sand flies

| Genus | Species | Literature source | Country | State |
|---|---|---|---|---|
| *Evandromyia* | *cortelezzii* | Carvalho et al. 2008 | Brazil | MG |
| *Ev.* | *lenti* | Rego et al. 2014 | Brazil | MG |
| *Ev.* | *salesi* | Saraiva et al. 2008 | Brazil | MG |
| *Ev.* | *termitophila* | Saraiva et al. 2010 | Brazil | MG |
| *Expapillata* | *firmatoi* | Donalisio et al. 2017 | Brazil | SP |
| *Lutzomyia* | *almerio* | Savani et al. 2009 | Brazil | MS |
| *Lu.* | *cruzi* | de Pita-Pereira et al. 2008 | Brazil | MS |
| *Lu.* | *forattinii* | de Pita-Pereira et al. 2008 | Brazil | MS |
| *Lu.* | *ischnacantha* | Rego et al. 2014 | Brazil | MG |
| *Micropygomyia* | *peresi* | Rego et al. 2014 | Brazil | MG |
| *Migonemyia* | *migonei* | Moya et al. 2015 | Argentina | MI |
| *Nyssomyia* | *intermedia* | Rego et al. 2014 | Brazil | MG |
| *Ny.* | *neivai* | Saraiva et al. 2008 | Brazil | MG |
| *Ny.* | *whitmani* | Moya et al. 2015 | Argentina | MI |
| *Ny.* | *whitmani* | Saraiva et al. 2010 | Brazil | MG |
| *Pintomyia* | *monticola* | Donalisio et al. 2017 | Brazil | SP |

caution. Under no circumstances can they be considered as vectors but simply as potential vectors that require investigation in greater detail. Savani et al. (2009) observed flagellates in both *Lu. longipalpis* and *Lu. almerio* that were identified by (Small Subunit Ribosomal DNA) SSU rDNA-based PCR as *L.* (*L.*) *infantum*. This raises the intriguing possibility that both were transmitting AVL. Clearly, other sand flies— such as *Lu. cruzi* and *Mg. migonei*—are potential AVL vectors (Santos et al. 1998; Salomón et al. 2010; de Carvalho et al. 2010), but *Lu. longipalpis* is the most important component in AVL transmission and is undoubtedly the principal biological risk factor in the transformation of epidemiological profiles and the increase in the diseases urbanization.

The establishment of *L.* (*L.*) *infantum* in the Americas was due to the parasite finding a permissive vector, in other words, *Lu. longipalpis*. By encountering other permissive vectors that adapt to the peridomestic habitat, it is likely that AVL could become established in areas that are ecologically inhospitable to *Lu. longipalpis* (Peterson et al. 2017).

## Other Wild Animal Hosts of *Leishmania* (*Leishmania*) *infantum chagasi*

Sherlock et al. (1984, 1988) isolated *L.* (*L.*) *i. chagasi* from two opossums, *Didelphis albiventris*, captured in a focus of AVL in Jacobina, Bahia, but they considered that it was unlikely that this animal represented an important reservoir of the parasite because of the low infection rate (only 2 of 84 examined).

Investigators in Colombia (Corredor et al. 1989a, b; Travi et al. 1994) registered the isolation of the parasite from the common opossum *Didelphis marsupialis* after the *in vitro* culture of spleen, liver and skin in various media and the intraperitoneal inoculation of hamsters. In one focus of AVL, the infection rate of the opossums was as high as 12 of 37 (32%), and it was concluded that this animal is an important reservoir of *L.* (*L.*) *i. chagasi*. Travi et al. (1998a) followed-up these findings by experimentally infecting *D. marsupialis* with both amastigotes and promastigotes of *L.* (*L.*) *i. chagasi* (dog strain). No parasites could be detected by culture of the opossums' blood, and only very few *Lu. longipalpis* were infected when fed on these animals. They nevertheless considered that xenodiagnosis with the sand fly *Lu. longipalpis* was a more sensitive method for detecting infection than PCR. Travi et al. (1998b) then studied a variety of small mammals captured in both undisturbed and degraded dry forest in northern Colombia using PCR and dot-blot hybridization techniques: They made no attempt to isolate the parasite. Positive PCR/hybridization results for *L.* (*L.*) *i. chagasi* DNA were obtained for 3 of 21 (14.3%) *D. marsupialis* caught in undisturbed forest and 13 of 137 (9.5%) specimens of this animal from the degraded forest. Positive results were also recorded for 3 of 34 specimens of the rodent *Proechimys canicollis* from undisturbed forest and in 2 of 4 specimens from degraded forest. The investigators considered these results to indicate active infections of these rodents with *L.* (*L.*) *i. chagasi*. No foxes were examined in these surveys, although the investigators stated that *C. thous* was present in the study areas and "(…) might contribute to the maintenance of *L. chagasi.*" The high percentage of *C. thous* infected in foci of AVL in North Brazil—and the experimental infection of *Lu. longipalpis* fed on an infected fox—together suggest this to be highly likely.

Studies of feeding habits carried out in *Lu. longipalpis* populations from Sobral (CE), Massapê (CE) and Jequié (BA), Brazil and in Colombia (Morrison et al. 1993; Afonso et al. 2012) revealed positivity for opossum blood. These indications, together with reports of natural infection of *D. marsupialis* by *Leishmania* spp., possibly *L.* (*L.*) *infantum chagasi*, add to the discussion about the role of these mammals as potential reservoirs for AVL.

Because of positive PCR hybridization tests for *L.* (*L.*) *i. chagasi* in wild-caught *Pr. canicollis*, the Colombian workers investigated the susceptibility of another spiny rat, *P. semispinosus*, to experimental infection with *L.* (*L.*) *i. chagasi* by the intracardial and intradermal inoculation of promastigotes (Travi et al. 2002). No parasites could be isolated from these spiny rats on periodic culture of liver aspirates, but at autopsy they were isolated in cultures of splenic material from 5 of 10 of the animals. No parasites could be found in stained spleen smears, and repeated xenodiagnosis (*Lu. longipalpis*) failed to reveal parasites. Finally, results of PCR hybridization examination of skin (ears) were all negative.

The investigators concluded that "The inability to infect *P. semispinosus* experimentally with *L. chagasi* indicates that it is not highly susceptible to this *Leishmania* species (…)"; that "(…) *L. chagasi* infection in *Proechimys semispinosus* is contained and compartmentalized"; and that "*Proechimys canicollis*, which is naturally infected with *L. chagasi* in Northern Colombia, may be a more capable reservoir host than *P. Semispinosus*."

During studies on leishmaniasis in the Amazon region of north Brazil by investigators from the Instituto Evandro Chagas, a total of 2637 wild animals— including rodents, marsupials, procyonids, canids and edentates—were examined for leishmanial infection (Lainson et al. 1987): This list included large numbers of the opossum *D. marsupialis* and the spiny rat *P. guyannensis*, many of which were captured near the houses of patients with AVL and—as in the case of opossums—frequently in the backyards of such houses. No infections with *L.* (*L.*) *i. chagasi* were detected in any animal other than the fox *C. thous* after culture of spleen and liver tissue and inoculation of this material intraperitoneally into hamsters. At the time of these studies, the PCR/hybridization technique had not been developed and, in view of the finding of Travi et al. (1998b) (i.e. that tissues of wild-caught *P. canicollis* gave positive PCR results for *L.* (*L.*) *i. chagasi* DNA in Colombia), Lainson et al. (2002) examined the susceptibility of laboratory-bred *P. guyannensis* to experimental infection with a canine strain of *L.* (*L.*) *i. chagasi* from north Brazil. The animal proved to be totally resistant to infection by way of promastigotes and amastigotes after massive intraperitoneal inoculation of the parasite, and the results of subsequent PCR/hybridization tests made on liver and spleen tissue were negative. This failure in attempts to feed laboratory-bred *Lu. longipalpis* on *P. Guyannensis*, or to capture this sand fly in traps baited with the rodent and placed in or near houses infested by *Lu. longipalpis*, led to the conclusion that this species of spiny rat plays no part in the eco-epidemiology of AVL in north Brazil.

At the 3rd World Congress on Leishmaniasis in April 2005, investigators at the Adolfo Lutz Institute, São Paulo, Brazil, presented the results of an examination of wild animals for evidence of *Leishmania* infections in two localities of endemic cutaneous leishmaniasis in the state of São Paulo. Each animal was examined by "(…) one or more of the following methods: detection of rK39 antibody in whole blood; intradermal inoculation of hamsters with skin biopsies from lesions and/or hipocromic spots or culture and/or DNA extraction for PCR and RFLP tests." Among the positive results were "*L.* (*L.*) *chagasi* in 1 *Akodon* sp. and 2 *D. Marsupialis*." Unfortunately, the published abstract of the presentation (Tolezano et al. 2005) does not indicate by which method these results were obtained or, more importantly, if the parasite was isolated from these three animals.

The finding of a benign *L.* (*L.*) *i. chagasi* infection in marsupials and rodents suggests their possible role as source of infection to sand flies. Of greater importance, however, is the fact that it raises the question as to what extent these animals may act as reservoirs of infection for the sand-fly vector and thus play a role in the epidemiology of human AVL.

An effective reservoir host of any parasite is one that can participate in the maintenance and dissemination of that parasite in nature. When parasites are dependent on haematophagous vectors for their transmission, it is clearly necessary to show that these can be infected when fed on the host in question. Until this is done, the infected animal is best referred to as a "potential reservoir." The isolation of *L.* (*L.*) *i chagasi* from numerous specimens of the fox *C. thous* and the opossum *D. marsu-*

*pialis*—and the experimental infection of *Lu. longipalpis* fed on these animals—places them firmly in the category of natural reservoirs of *L.* (*L.*) *i. chagasi* (Lainson et al. 1990; Travi et al. 1998a). In contrast, although positive results of PCR tests on the tissues of some wild rodents do suggest that these may also represent reservoirs, isolation of the parasite and experimental infection of *Lu. longipalpis* fed on the infected animals is needed to confirm this.

In Minas Gerais, the species *Rattus norvegicus* was found infected by *L. infantum* [= *L.* (*L.*) *i. chagasi*] using the *Leishmania* nested-PCR technique, thus suggesting the possibility of this rodent's participation in the AVL zoonotic cycle (Lara-Silva et al. 2014).

## Other Parasites Associated with Visceral Leishmaniasis

*L.* (*L.*) *amazonensis* has been isolated from AVL patients from Bahia, Brazil (Barral et al. 1991) and *Endotrypanum colombiensis* from cases of AVL in Venezuela (Rodriguez-Bonfante et al. 2003). In 2007, *L.* (*L.*) *amazonensis* was identified in two dogs from Araçatuba, São Paulo state, diagnosed clinically as having canine visceral leishmaniasis (Tolezano et al. 2007). This same parasite has also been identified in dogs from two CVL-endemic locations in Minas Gerais state (Dias et al. 2011; Valdivia et al. 2017).

In the above-mentioned areas, *Lu. longipalpis* is the CVL vector, and Sherlock (1996) transmitted *L.* (*L.*) *amazonensis* with flies of this species from Jacobina, Bahia. In addition, this natural infection of this same parasite has been found using molecular methods in *Lu. longipalpis* captured in an endemic AVL region of Mato Grosso do Sul (Paiva et al. 2006; Savani et al. 2009).

The natural vector of *L.* (*L.*) *amazonensis* is *Bi. flaviscutellata*, which can be found in both primary and secondary forest but not in peri-domestic habitats. Evidence is accumulating that it is being transmitted in this habitat, amongst dogs, by *Lu. longipalpis*. It is possible that this cycle starts by a dog becoming infected by an infected *Bi. flaviscutellata* when it goes into forested areas, and on its return to the peri-domestic area it becomes a source of infection of *Lu. longipalpis*. However, given this fly's presence in forests, it cannot be ruled out that it also takes part in the sylvatic cycle of *L.* (*L.*) *amazonensis*.

## Amazonian American Visceral Leishmaniasis: Indigenous or Introduced?

Although there remains little doubt that peri-domestic/intra-domestic infestations by *Lu. longipalpis* and/or *Pi. evansi* originate(d) from sylvatic populations, the origin of *L.* (*L.*) *i. chagasi* in Amazonia has been controversial, particularly in the more remote forested areas. This led Lainson and Shaw (1998) to consider that the parasite was indigenous (i.e., present before humans' presence in the Americas) and not imported by immigrants from the northeast foci. This hypothesis was further motivated by finding infections in healthy wild foxes (Silveira et al. 1982; Lainson et al. 1969, 1987) and records of sporadic, widely separated cases in Pará state that were not significantly associated with immigrant families from the northeastern VL foci. A similar occult infection in *C. thous* was recorded in a focus of AVL in Corumbá, Mato Grosso do Sul, Brazil (Mello et al. 1988). Furthermore, infections registered in men sleeping in lumber camps in or near forest, far from fixed habitations, suggested a feral source of the parasite (Lainson, Shaw, Silveira and Souza, unpublished observations).

If the parasite was indigenous in Amazonian foxes, then where did it come from? Wild canids have been present in South America since the Pleistocene era some 2–3 million years ago (Perini et al. 2010) and are considered to have originated from North America. This was around the same period that *donovani* and *infantum* split from the ancestral visceral line (Barratt et al. 2017). It also marked the beginning of the ice age. which lasted until about 12,000 year ago. Given these scenarios, it seems very unlikely that an African parasite of canids could have reached American canids before the European colonization of the American continent.

Only now are we beginning to appreciate the speed at which vector-borne pathogens can spread. Its introduction into an area where there is a suitable vector results in its rapid expansion as has been witnessed recently with the Zika virus (Bogoch et al. 2016). A more plausible explanation of fox infections in Amazonia is that they became infected from dogs habouring *L .*(*L.*) *infantum* that were imported with immigrants from the Iberian Peninsula. Settlers from this region began to arrive in western Amazonia at the end of the seventeenth century. It is inconceivable that during a period of >200 years that infected dogs were not inadvertently imported. Local foxes would have found the farmsteads a source of food and could have become infected while visiting them. In these early settlements, there is also strong evidence that wild *Lu. longipalpis* adapted quickly to a peri-domestic environment. This assumption exists because in the municipality of Igarape Miri, Pará, *Lu. longipalpis* was found in the chicken houses of numerous widely separated houses located along a stretch of road that had only been opened 18 months earlier (Lainson, Shaw, Silveira and Souza, unpublished observations). On returning to their feral habitats, an enzootic was set up with the help of local forest sand flies that probably included *Lu. longipalpis*. A similar situation could have occurred in other regions where foxes are found infected. It is important to remember that the occult nature of *L.* (*L.*) *infantum* in wild canids makes them excellent maintenance reservoirs. In the Old World, it has been suggested that the origin of human visceral leishmaniasis, due to parasites of the *L.* (*L.*) *donovani* complex, was a rural enzootic of wild canids, such as foxes, jackals and wolves, that later spread to dogs (Lysenko 1971).

## Final Comments

We can never know the exact origin of the *L.* (*L.*) *infantum* infections found in American wild animals, but current evidence suggests that they are from infected dogs imported by immigrants during a period of hundreds of years. The presence of sylvatic sand flies, such as *Lu. longipalpis* and *Pi. evansi*, most probably resulted in the maintenance and spread of wild enzootic cycles of *L.* (*L.*) *infantum* in such animals as foxes, which are closely related to Old World foxes (Perini et al. 2010). A similar situation occurred with the yellow fever virus, which was imported from west Africa in the 1600s and spread throughout the Americas where it is now an enzootic of wild animals, such as monkeys, in Amazonia (Bryant et al. 2007).

Within this scenario there are two possible ways in which peri-domestic transmission cycles can be established. One is from peri-domestic flies being infected from a roaming wild animal, such as a fox, or from imported infected dogs. Both situations require established populations of peri-domestic phlebotominae, such as *Lu. longipalpis*. Another feasible source of infection for either a human or a dog is going into a wooded area where there are flies that have become infected from a wild animal. This situation is probably rare.

Transmission of the parasite to dogs or humans in situations where peri-domestic fly populations have become infected is then only a question of time when a single infected dog, often with a vast supply of amastigotes for these sand flies in its skin, sets the scene for a small focus of canine or human visceral leishmaniasis. When human habitations become overcrowded, with conditions of poor hygiene and an abundance of domestic animals, particularly dogs, the concentration of the vector *Lu. longipalpis* (and/or *Pi. evansi* in some parts of Latin America) may reach very high levels, which present the risk of a serious outbreak of canine and human disease. At this stage of events, the dog becomes the major reservoir of infection for humans, and the wild-animal host may in fact no longer be present in the immediate area. Control measures in such foci of infection will certainly decrease the number of human cases of AVL, but—unfortunately—total elimination of the disease is unlikely due to a persistent source of *L.* (*L.*) *i. chagasi* in the wild-animal enzootic, a problem equally difficult to resolve in the matter of controlling cutaneous leishmaniases.

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