Proven and putative vectors of American cutaneous leishmaniasis in Brazil: aspects of their biology and vectorial competence

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The aim of the present review is to give relevant information on aspects of the biology and ecology, including the vectorial competence of Lutzomyia *sand fly species suggested as vectors of American cutaneous leishmaniasis in Brazil. The disease, due to* Leishmania (Viannia) braziliensis, *has been registered in most municipalities in all the Brazilian states and its transmission is associated with more than one sand fly species in each geographical region. A variety of* Leishmania *species can be found in the Amazon basin, where different epidemiological chains have been detected with the participation of different phlebotomine vectors. Finally, a discussion is presented on some sand fly species found naturally infected by* Leishmania*, but for which there is as yet no evidence regarding their epidemiological importance.*

Key words: American cutaneous leishmaniasis - *Lutzomyia* sand fly species - vectorial competence - Brazil

American cutaneous leishmaniasis (ACL) in Brazil is caused by a variety of dermotropic *Leishmania* species and the greatest diversity of these parasites is to be found in the Amazon Region.

Transmission of the causal agents involves different species of phlebotomine sand flies (Diptera: Psychodidae: Phlebotominae) in close relationship with the mammalian reservoir hosts of the parasites, with the consequent formation of a number of different transmission cycles throughout the country. The data from ecological and epidemiological studies on the different species of the subgenera *Viannia* and *Leishmania*, together with those from experimental laboratory transmissions, have either suggested or definitely indicated certain species of sand flies as being responsible for the transmission of ACL. Relatively few, however, have been considered as outstandingly important vectors.

According to the taxonomic criteria proposed by Young and Duncan (1994), three genera of sand flies are recognized in the New World: *Lutzomyia* França & Parrot, 1924; *Brumptomyia* França & Parrot, 1921 and *Warileya* Hertig, 1940. The genus *Psychodopygus* Mangabeira, 1941 has been accepted by some (Fraiha et al. 1971, Fraiha & Ward 1974, Forattini 1973, Lainson et al. 1973) and modifications of the taxonomy have been suggested (Galati 2003). In this review, however, we propose to follow that of Young & Duncan (1994).

Vectorial competence of some neotropical sand flies

Lutzomyia (Nyssomyia) intermedia (Lutz & Neiva, 1912) - *L. intermedia,* described from specimens collected in the Fazenda Ouro Fino, in the state of Minas Gerais (MG), where it was found to be abundant in houses, was one of the first species of sand fly to be described in the Neotropical Region.

In the 1940's, important studies on the biology of *L. intermedia s.l.* were made by Barretto (1943) in the state of São Paulo (SP), together with observations on the behaviour of some other species of sand fly. It is a species found in both primary and secondary forests and particularly in areas where deforestation has gradually modified the ecological profile - clearly seen in those parts of the country that underwent past colonization. As long ago as 1943, Barretto suggested that *L. intermedia s.l.* had, in fact, developed a preference for secondary forest or low, bushy vegetation located in areas suffering from such human impact and according to Tolezano et al. (1980), the environmental changes that took place in SP at the beginning of the 20th century certainly modified the composition of the phlebotomine fauna. While this caused other species of silvatic sand fly to disappear, or survive at low frequency, *L. intermedia s.l.* adapted so well to the peridomestic habitat that it actually has become the predominate species of sand fly in a new epidemiological profile for ACL in this region. These authors further suggested that the small numbers of this sand fly now captured in the remaining forest indicates that transmission of ACL by *L. intermedia* is now taking place solely outside of that habitat.

Like most sand flies, *L. intermedia s.l.* commences its feeding activities at dusk and increases it most markedly during the first half of the night. It has been shown to be abundant inside houses (Lutz & Neiva 1912, Forattini 1953), where it feeds avidly on man. It is also attracted to domestic animals such as dogs and equines, which may have cutaneous lesions due to *Leishmania (V.) braziliensis* and commonly infests their shelters (Forattini 1973, Forattini et al. 1976, Araújo Filho 1979, Rangel et al. 1986, Gomes & Galati 1989).

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Studies in Mesquita, state of Rio de Janeiro (RJ), have resulted in the capture of *L. intermedia* on man and domestic or silvatic animals in a variety of habitats, including the interior of houses, areas approximately 180 m from the houses and in neighbouring forest about 800 m away (Rangel et al. 1999). To the authors, therefore, these findings suggested the existence of genetically heterogenous populations of this sand fly. Meneses et al. (2005), however, used three different molecular markers, MLEE, RAPD and PCR, to study the sand fly populations from three different ecotopes and concluded that they were genetically homogeneous. They suggested that the dispersion of *L. intermedia* to different habitats was merely a reflection of the domiciliation process: a similar variation of habitat has been observed in RJ foci of ACL (Souza et al. 2002). Investigating *L. intermedia* populations from regions with two distinct eco-epidemiologies of *L. (V.) braziliensis* in the state of Espirito Santo (ES), Souza-Rocha et al. (2007) suggested that in addition to the homogeneity estimated by gene flow (F_{st}) this sand fly forms different clusters according to microhabitat: the authors therefore suggested the presence of different transmission cycles of ACL.

With regard to seasonality, Forattini (1973) pointed out an irregular behaviour of the sand fly, which he considered to have a greater population density during the cold months of the year. In RJ, however, Rangel et al. (1990) found it to be present throughout the year, with maximum density in June, August and October, and later studies by Souza et al. (2003) showed that the *L. intermedia* population was at its maximum density in the hottest season of the year.

Just one year after, Sergent et al. (1921) had suggested that ACL in the Old World was transmitted by the bite of phlebotomine sand flies, the young scientist Henrique Aragão (1922) of the Instituto Oswaldo Cruz considered the possibility that these insects were also the vectors of the disease in the Americas. He pointed out the association of sand flies with the presence of human infection in Águas Férreas, near the hillsides of Corcovado and locality of Santa Teresa, city of Rio de Janeiro, and then indicated *L. intermedia* as a putative vector of *L. (V.) braziliensis* by producing an ulcer containing amastigotes on the nose of a dog inoculated with a triturated pool of *L. intermedia* that had fed on the lesions of persons with ACL.

Epidemiological data, accumulated over the years, have steadily increased the suspicion that this sand fly may be an important vector of ACL in many endemic areas of Southeastern Brazil, such as SP and RJ, although in MG and ES its presumed role would be shared with *Lutzomyia (N.) whitmani*, a proven vector (Falqueto 1995, Mayrink et al. 1979). More recently it has been suggested that it participates in the transmission of ACL in the region of Viçosa, MG (Santos et al. 2003) and in the Northeast of Brazil, in the state of Rio Grande do Norte (Ximenes et al. 2000). Souza et al. (2003) also noted the sympatric presence of *L. intermedia* and *L. whitmani* in an area of ACL in RJ and suggested that here, too, both species could be involved in the transmission of the disease: they pointed out that whereas *L. intermedia* predominated in the peridomestic habitat, *L. whitmani* was most frequent in banana plantations. Finally, since the studies of Barretto (1943), the presence of *L. intermedia s.l.* has been indicated in four different municipalities of SP that are considered to be areas of high ACL endemicity.

After the 1970's, the incidence of this disease increased in SP, with an impressive concentration of cases in the Vale do Ribeira, where studies on the sand fly fauna suggested *L. intermedia s.l.* as the vector of the parasite. Changes in the vegetation are presumably the reason for the resulting predominance of *L. intermedia* in this populated environment. Recent investigations in the same area have since shown that this sand fly represented almost 100% of captures made in the domestic environment, indicating the very close contact this insect has with man (de Castro et al. 2005). Souza et al. (2005) have emphasized the tendency of *L. intermedia* to bite man in a variety of habitats throughout RJ.

In 1952, Forattini and Santos worked in an area of ACL transmission in the state of Paraná (PR) and noted promastigotes (referred to as leptomonads) in *L. intermedia s.l.*: they were very like those seen by Aragão (1922). Later on, Forattini et al. (1972) recorded a similar infection, which they attributed to a species of *Leishmania*, in a specimen of this sand fly from SP. Circumstantial evidence indicating the vectorial competence of *L. intermedia s.l.* was obtained when a campaign of DDT application in an area of ACL transmission in RJ resulted in abrupt reduction in the population of *L. intermedia s.l.* and a drop in the number of cases of the disease (Guimarães & Bustamante 1954, Guimarães 1955). In addition, during an epidemic of ACL in the locality of Jacarepaguá, city of Rio de Janeiro, in 1974, 92.7% of 47,761 sand flies captured were *L. intermedia* (Fiocruz Technical Report), while entomological studies during an outbreak of ACL on Ilha Grande, RJ, showed a prevalence of this species of sand fly (Araújo Filho 1979). These observations and the succession of reports on the combined presence of *L. intermedia s.l.* and cases of ACL in several foci of the disease in SP and RJ have all centred attention on this sand fly as the most likely vector (Forattini 1953, Forattini et al. 1976, Araújo Filho 1979, Gomes et al. 1986, Rangel et al. 1986, 1990).

More definite support for the incrimination of *L. intermedia* came when Rangel et al. (1984) captured an infected specimen in the Jacarepaguá focus of ACL. The parasite showed attachment of the promastigotes to the wall of the hindgut (pylorus), characteristic of species of the subgenus *Viannia* and intradermal inoculation of the contents of the gut into the feet of hamsters produced nodular lesions containing amastigotes. Although the parasite was not characterized to species level by the current modern methods, these findings and the fact that the infected san dfly was from an area of active transmission of *L. (V.) braziliensis* makes it highly likely that it was, in fact, this species. Studies on the development of *L. (V.) braziliensis* in experimentally infected *L. intermedia* have added further evidence (Rangel et al. 1992). In addition to the development of the parasite in the pylorus, free promastigotes were shown to migrate to the

foregut and infect the stomodeal valve. A further link in the chain of evidence incriminating *L. intermedia* as a vector of ACL came with the isolation of a parasite from a specimen of this sand fly, captured in the Jacarepaguá focus of the disease and identified as *L. (V.) braziliensis* (Pita-Pereira et al. 2005). This certainly reinforced all the ecological and epidemiological evidence slowly built up over the past years and, in particular, supports the view that the infection in the wild-caught *L. intermedia* found by Rangel et al. (1984) was also this parasite.

During the early studies on *L. intermedia,* there was very little information available regarding the possible mammalian source of infection for this sand fly although from the insect's original habitat in forested areas, it was clearly from silvatic animals and a number of reports on the isolation of *Leishmania* from rodents in localities highly endemic for *L. (V.) braziliensis* put these animals high on the suspect list (see in the discussion on *Lutzomyia wellcomei*).

In more recent times, following the adaption of *L. intermedia* to the peridomestic habitat, the frequent finding of dogs and equines with cutaneous lesions due to *L. (V.) braziliensis* led to the conclusion that the severe deforestation in these areas had resulted in migration of infected wild animals (particularly rodents) to residential areas, the infection of *L. intermedia* in this new habitat and, finally, transmission of *L. (V.) braziliensis* to the domestic animals. These, it has been suggested, may now serve as a further source of infection for the sand flies and the creation of a new, extra-silvatic transmission of human ACL. The observation that sloths (*Bradypus variegatus)* from neighbouring forest frequently visit residential areas in the municipality of Mesquita, RJ, raised the question as to whether this animal might also be a source of infection (Pirmez et al. 1997). In the same area, recent use of the precipitin test on engorged sand flies from the interior of houses and the peridomestic habitat have indicated that the population of *L. intermedia* there feeds on man and on domestic animals such as birds, dogs, equines and rodents (Afonso et al. 2005). Clearly, conclusive evidence is needed to show that domestic animals with cutaneous lesions can serve as a source of infection for the sand fly and, most importantly, to show that amastigotes are available in apparently normal skin, in addition to the very limited area of infected tissue at the border of a lesion.

Lutzomyia (Nyssomyia) neivai (Pinto, 1926) - This sand fly was described from a male specimen collected in a house in the grounds of the Instituto Butantan, city of São Paulo. Later, Pinto placed it in synonymy with *L. intermedia*, a taxonomic position that was accepted by most sand fly specialists. In 1996, however, Marcondes made a study of the morphology of males and females collected in the locality of Fortim Campero, Tarija, Bolivia and compared morphology of the holotype male with other males from this area. He considered that the females of *L. neivai* and *L. intermedia* could be differentiated on morphology of the spermathecae (differences in the common duct), the number of teeth of the ciborium and wing venation. In addition, the males could be separated on wing venation and the morphometry of the ejaculator bomb and genital filament. Still other differences have since been reported (Marcondes et al. 1997, Andrade-Filho et al. 2004).

Regarding the possible participation of *L. neivai* in the transmission of ACL, Marcondes et al. (1997) suggested the possibility of this in the Southeast of Brazil, in the colder and dryer areas.

Studies on the sand fly fauna in 37 municipalities in PR showed that *L. neivai* represented 75.6% of all the specimens collected and distribution of this species in areas of ACL transmission strengthened the suggestion that it may be another vector of the disease (Silva et al. 2008).

Recently, the General Office of Health in Porto Alegre, state of Rio Grande do Sul (RS), registered its first autochthonous case ACL due to *L. (V*) braziliensis and, in the locality of Pitinga, capture of sand flies around the house of an ACL case showed that 94.9% were *L. neivai* and that *L. intermedia* was not present (ACR Azevedo et al., unpublished observations). Recent investigations in Southeastern Brazil suggest that migration of *L. neivai* from forest to residential areas have resulted in the appearance of ACL cases. Studies on the feeding preferences of this sand fly among domestic animals (pig, dog, rabbit and chicken) showed it to be an opportunistic feeder with little host preference (Dias-Sversutti et al. 2007). Stronger evidence supporting the suggestion that it is an important vector of ACL, in certain areas, was provided by a study which reported positive results of tests on sand flies using PCR multiplex coupled to non-isotopic hybridization, suggesting infection with *L. (V.) braziliensis*. The specimens tested were from an endemic area of ACL in Porto Alegre, RS (Pita-Pereira et al. 2009). Additionally, Marcondes et al. (2009, unpublished observations) reported the natural infection of *L. neivai* by a *Leishmania* sp., of the subgenus *Viannia* in a focus of ACL in the state of Santa Catarina. In a study of the biology of this sand fly in an endemic area in SP, however, Casanova et al. (2009) considered that it has low competence as a vector of *Leishmania*.

To complete the incrimination of this sand fly as another vector of this parasite, all that is now needed is its actual isolation and characterization from undoubted *L. neivai* and, if possible, its experimental laboratory transmission by this sand fly.

L. whitmani (Antunes & Coutinho, 1939) - In 1939, Antunes and Coutinho described a new sand fly species which they named *Flebotomus whitmani* in honour of Dr. Whitman, of the Rockefeller Foundation, who was collaborating with the Brazilian government in attempts to control yellow fever. Males and females were described from specimens collected in Ilhéus, state of Bahia (BA). Before this the new species had often been mistaken for *L. intermedia*.

Early information on the biology of *L. whitmani* was given by Barretto (1943) in his entomological studies in SP. It was considered to be a silvatic species, although it could be found inside houses that were situated within or near the forest. At dusk it was found biting man, in addition to feeding on dogs and was shown to be present in large numbers in chicken houses. As the localities

Barretto studied were close to deforested areas, he suggested that the high population density under these circumstances was simply due to the wide variety of blood sources available to this sand fly.

In 1953, Forattini confirmed the presence of *L. whitmani* in both the forest and nearby domestic animal shelters in SP. Later (1960), he commented on the fact that although inicially dependant on primary forest, this sand fly could now be captured, during several months of the year, inside houses, peridomestic pigsties and banana plantations and there was no doubt that it was now thriving in a domestic environment. In the same publication, Forattini (1953) was of the opinion that although there were reports indicating that *L. whitmani* coexists with *L. intermedia* in areas recently occupied by man and may actually outnumber the latter species as the environmental alterations procede, more detailed information was needed to confirm this suggestion.

In RJ in general, the *L. whitmani* population has for some time remained at low level (Rangel et al. 1986, 1990, Oliveira et al. 1995). Souza et al. (2001) registered this sand fly's presence in the Atlantic Forest and, more importantly, its frequency in residential areas close to the forest. The same authors recorded both *L. intermedia* and *L. whitmani* biting man in the peridomestic habitat close to forest and noted that the former prevailed in the residential area while the latter was the most frequent of the two insects in the forest. They observed a higher density of *L. whitmani* in the winter months, while *L. intermedia* was most abundant in the hotter months of the year (Souza et al. 2002).

The tendency of *L. whitmani* to occupy residential areas in MG was discussed by Mayrink et al. (1979) and Passos et al. (1991). It was found feeding on man and domestic animals and thought to possibly be breeding in this habitat. Conversely, it was found in very small numbers in the neighbouring forest.

In Northeast Brazil, in BA and CE, its behaviour has been shown to be similar to that seen in the Southeastern Region: namely, highly attracted to man and well adapted to the domiciliary habitat (Barreto et al. 1982, Vexenat et al. 1986). In BA, it was suggested that *L. whitmani* might be breeding in the cocoa plantations (França et al. 1991).

Regarding seasonality, differences in behaviour have been noted in different regions of Brazil, probably due to differing climatic conditions. In the Southern Region, for example, Barretto (1943) noted its presence at all months of the year, while in studies in Petrópolis, RJ, it was found in greater numbers during the months of low temperature in June, July and August (Souza et al. 2002).

Although the dusk and nocturnal feeding habit of *L. whitmani* follows the usual sand fly pattern (Barretto 1943) it, too, has been shown to be somewhat variable in different regions of Brazil. In the Northeast, Azevedo and Rangel (1991) showed that it can also be captured during the whole of the dawn period, in chicken houses or feeding on equines, with maximum activity from 1-3 am. Souza et al. (2004) noted that in RJ it could be found feeding on man, in the peridomestic habitat, between 4-6 am and such behaviour was previously recorded by Teodoro et al. (1993) in PR; studies on host preferences of *L. whitmani* among domestic animals, made in the same state, showed that this insect is an opportunistic feeder, resulting in a wide choice of hosts in the peridomestic environments of human colonization.

In primary forest, in several different regions of PA, Lainson et al. (1979) noted that *L. whitmani s.l.* has very different habits from those discussed above, in other regions. It was found to be essentially silvatic and was captured principally from the large tree-trunks and in the forest canopy. It was disinclined to bite man and attempts to demonstrate its invasion of houses located very near the forest were completely unsuccessful. Subsequent studies confirmed these observations and led to the conclusion that any divergence from this behaviour is likely to occur only under special conditions (Ready et al. 1986, Lainson 1988, Shaw et al. 1991).

This situation led to the suggestion that *L. whitmani s.l.* might represent a species complex of two or more taxa (Lainson 1988) and has resulted in several comparative studies on populations of this sand fly from widely different areas of Brazil. Some investigations suggested the existence of at least three different lineages of this sand fly, based on biological characters, geographical variations and morphometric features, together with observations on sequences of mitochondrial DNA (Rangel et al. 1996, Ready et al. 1997, 1998). Rangel et al. (1996) made it clear, however, that they did not discard the possibility that the populations they studied, from PA, CE and BA could represent a cline. Ishikawa et al. (1999) studied populations from the North, Northeast, Southeast and the South Regions and indicated the existence of a clade from Rondônia (RO) within the lineage of forested areas, which included haplotypes of the Amazon and Atlantic Forests and Ilhéus (the type locality of *L. whitmani*). They suggested that their findings did not sustain the hypothesis of a cryptic species complex, but rather the occurrence of a recent crossing over of populations in forested areas.

Margonari et al. (2004) studied populations of *L. whitmani* from the Northeast and the Southeast of Brazil. They confirmed observations on the similar morphometry of these, but presented evidence of two biogeographical "clusters". Latterly, however, they suggested the existence of a genetic flow between the two lineages.

Finally, Campbell-Lendrum et al. (1999) maintained that they could observe no significant difference in the anthropophilia of *L. whitmani s.l.* from North Brazil and *L. whitmani sensu stricto* from other areas in Brazil: a finding not supported by some 40 years of entomological observations, in an extensive area of primary forest in PA, by workers in the Instituto Evandro Chagas.

Regarding the incrimination of *L. whitmani* as an important vector of ACL, the first suggestion of this was in 1941, when Pessôa and Coutinho found a specimen from SP infected by flagellates which were considered to possibly be promastigotes of *Leishmania*: as a result, entomological investigations were intensified in areas of ACL transmission in this region and soon showed that although considered as a silvatic species, *L. whitmani* was a highly anthropophilic sand fly with a particularly dense population (Barretto 1943, Forattini 1954).

In Southeastern Brazil, data in the literature suggest the participation of this sand fly in the transmission of ACL in a focus of the disease in Caratinga, MG and in the mountainous area of Afonso Cláudio (Mayrink et al. 1979, Falqueto 1995), and Souza et al. (2002) considered that it might be sharing the role of a vector of *L. (V.) braziliensis*, together with *L. intermedia*, in rural RJ. Recently, the finding of a specimen of *L. whitmani* infected with a *Leishmania* of the subgenus *Viannia* by PCR analysis, in a region very close to Belo Horizonte, MG, has led to the suggestion that this sand fly could be the vector of cutaneous leishmaniasis in that area (Carvalho et al. 2008).

In the South, *L. whitmani* was also considered as a possible vector of ACL in PR, while in the northern part of this state a natural infection with *L. (V.) braziliensis* found in one specimen and the insect's high population density clearly emphasized this sand fly's medical importance (Luz et al. 2000, Teodoro et al. 2003).

In all areas of ACL in Northeast Brazil, this same species of sand fly is considered as an important vector of the disease, based on the the finding of specimens infected by *L. (V.) braziliensis* in the area of Três Bracos, BA (Hoch et al. 1986, Ryan et al. 1990). The sand fly's predominance in houses and the peridomestic habitat in general prompted these authors to suggest the development of a purely domestic cycle of transmission by *L. whitmani*. In Ilhéus, BA, the type locality of this sand fly, its fondnest for human blood and high population density in the domestic habitat led to the same conclusion (Azevedo & Rangel 1991). In the Serra de Baturité, CE, a parasite of the subgenus *Viannia* and others positively identified as *L. (V.) braziliensis* were found in dissected *L. whitmani* (Azevedo et al. 1990, Queiróz et al. 1994) and, once again, these findings together with population density in the peridomestic habitat indicated this fly as the local vector of ACL due to this parasite. Gil et al. (2003) registered *L. whitmani* as the second most prolific sand fly in captures made in the central area of RO, stressed its preferential arboreal habits and recorded the presence of unidentified trypanosomatid parasites in some specimens.

In the municipalities of Rio Branco, Bujari and Xapuri, in the state of Acre (AC), a study of the sand fly fauna and potential vectors of ACL showed that *L. whitmani* was the most abundant species, with its spatial distribution coinciding with proven transmission sites of *L. (V.) braziliensis*: it was therefore suggested that this sand fly was a probable vector of this parasite in that region (Azevedo et al. 2008).

The state of Tocantins (TO) has suffered environmental impacts resulting in ecological changes, due to the construction of hydroelectric plants, agricultural activities and the establishment of new settlement areas, and the increasingly high incidence of ACL in this region has probably been due to these activities. *L. whitmani* is found in most of the endemic municipalities, especially in the areas that have been degraded by man (Vilela et al. 2008). In the Central-Western Region, some studies conducted in areas that suffered environmental changes due to human activities have suggested *L. whitmani* as an important vector of *L. (V.) braziliensis* (Galati et al. 1996, Dorval et al. 2009).

Nowadays, this sand fly is suggested to be the most important vector of ACL in Brazil, having been recorded in a large number of endemic areas (Costa et al. 2007) and in association with wide vegetation diversity. The aforesaid alterations in ecology, together with climatic changes, most probably account for the spread of ACL in Brazil in recent years (Shaw 2007) and, in this respect, *L. whitmani* appears to be a sand fly which readily adapts to new environments, such as degraded areas, in association with domestic animals and man in rural and periurban areas (Costa et al. 2007, Shaw 2008). Using analyses by ecological niche modeling in a study on the ecological distribution of ACL vectors, Peterson and Shaw (2003) predicted that with climatic changes, *L. whitmani* will continue to adapt to new conditions and possibly spread its distribution in Brazil even further.

The very different behaviour of *L. whitmani s.l.* in primary forest in PA, North Brazil has already been discussed and, till now, this sand fly has not been associated with ACL due to *L. (V.) braziliensis* in this region. The suggestion was made, however, that promastigotes of a member of the subgenus *Viannia* found in this sand fly in Monte Dourado, PA an area of ACL due to *Leishmania (Viannia) guyanensis*, were probably those of this parasite and that *L. whitmani s.l.* was participating in its transmission together with the principal vector *Lutzomyia umbratilis* (Lainson et al. 1981b). The parasite was not isolated for characterization, however and in view of the later isolation and identification of *Leishmania (Viannia) shawi* from *L. whitmani s.l.* in another area of primary forest in PA (Lainson et al. 1989) it remains possible that the parasite of *L. whitmani* in Monte Dourado, PA was also *L. (V.) shawi* (Rangel et al. 1996, Lainson & Shaw 1998). Considering the frequency of human infection with *L. (V.) shawi* and the reluctance of *L. whitmani s.l.* to feed on man in the Amazon Region, one is left to the conclusion that there must be a much more anthropophilic sand fly involved in the transmission of *L. (V.) shawi*, with *L. whitmani s.l.* merely maintaining the enzootic in wild animals.

L. (Nyssomyia) umbratilis Ward & Fraiha, 1977 - During a study of the epidemiology of cutaneous leishmaniasis in Surinam, in 1966, Wijers and Linger recorded flagellate infections in a tree-trunk inhabiting sand fly which they referred to as *L. anduzei*. It was thought to be the most likely vector of "bosch yaws", or *pianbois*, due to *L. (V.) guyanensis*, but their attempts to infect hamsters with the flagellates failed and the parasite remained unidentified.

Lainson et al. (1976) worked in the primary forest of Monte Dourado (Jari), PA, Brazil, north of the Amazonas River, where approximately 300 cases of ACL due to *L. (V.) guyanensis* were recorded, in one year, in men working on deforestation. They recorded massive infections with *L. (V.) guyanensis* in four of 55 specimens of a sand fly considered, at the time, to be *Lutzomyia an-*

duzei and isolated the parasite following the intradermal inoculation of hamsters. Suspicions were aroused during these studies, however, that the vector was not in fact *L. anduzei* and subsequent morphological studies showed that it was a closely related and morphologically very similar sand fly which was new to science.

Ward and Fraiha (1977) described the new sand fly as *L. umbratilis* from 10 females collected during the work in Monte Dourado, PA and an intense study of its behaviour was iniciated in the same area (Lainson et al. 1979). It was found that although sand fly species of the subgenus *Psychodopygus* predominated at ground level, *L. umbratilis* was extremely abundant in the forest canopy, but descended to ground level, presumably to oviposit, by way of the tree-trunks, on which it could be collected in great numbers in the early morning. In studies conducted in RO, in the area of Samuel Ecological Station, however, it was noted that *L. umbratilis* predominated in the canopy (Azevedo et al. 1993). In Monte Dourado, PA it was noted that *L. umbratilis* flies off the tree-trunks when disturbed by man's activities and attacks the nearest person. In the same study, *L. (V.) guyanensis* was isolated from 16 more specimens of *L. umbratilis* and, of 77 sand flies attacking two men collecting from the tree-trunks, 72 (92.5%) proved to be *L. umbratilis*. Some idea of the efficiency of this sand fly in the transmission of ACL in the Monte Dourado, PA area may be gained by the fact that the two men developed a total of 13 leishmanial lesions due to *L. (V.) guyanensis* on their arms, probably providing the most conclusive incrimination of a vector of ACL ever obtained!

The explanation of this great number of infected sand flies on the tree-trunks came with the detection of *L. (V.) guyanensis* in 27 of 59 specimens of the sloth *Choloepus didactylus* in the Monte Dourado area, PA (Lainson et al. 1981a, b). This animal spends most of its time in the forest canopy and has thus become the principal mammalian reservoir host of the parasite. As the animal may remain in the same tree for a considerable time there is a gradual build-up of infected *L. umbratilis* on a given tree. This sand fly's similar role as a vector of *L. (V.) guyanensis* and its common presence in the forest canopy and on large tree-trunks at ground level has been recorded in some other areas of the Amazon Region of Brazil (Arias & Freitas 1977a, 1978) and in French Guiana (Le Pont & Pajot 1980). Infection of an undoubted specimen of *L. anduzei* with a parasite having development consistent with that of members of the subgenus *Viannia* has been reported in Manaus, state of Amazonas (AM) (Arias & Freitas 1977b). However, this sand fly can, at most, now be considered only as a possible secondary vector of *L. (V.) guyanensis* and is probably of low importance with regards transmission of ACL to man.

Ready et al. (1986) made a detailed study of the ecology of *L. umbratilis* in the region of Monte Dourado, PA. It is highly anthropophilic and presumably becomes infected after feeding at night, particularly on sloths, but also on other arboreal animals such as the ant-eater *Tamandua tetradactyla*. In Manaus, AM, precipitin tests on blood in naturally fed *L. umbratilis* showed that 66% of them had fed on sloths (Christensen et al. 1982). In addition to its nocturnal feeding habits, however, this sand fly clearly will feed in the early daylight hours if disturbed from its resting place on tree-trunks. It is recorded biting man in the dry season and, particularly, directly after the rainy season.

Areas of high ACL prevalence due to *L. (V.) guyanensis* may be found in communities located in or very close to primary forest and this has led to the erroneous impression that *L. umbratilis* is undergoing the process of adapting to a peridomestic habitat. There are no consistent data that this is so, however and any transmission in this environment is almost certainly due to sand flies that have been attracted to a residential area, from nearby primary forest, by the lights of the houses. Esterre et al. (1986) discussed the acquisition of ACL due to *L. (V.) guyanensis* in persons living in a small village within forest in French Guyana and came to the same conclusion: when the forest was cleared to about 400-500 m around the village, all peridomestic transmission ceased. Guerra et al. (2007) discussed this situation in Manaus, AM, and were clearly of the opinion that the eco-epidemiology of ACL there is the same as that recorded in Monte Dourado, PA. In other forested areas on the outskirts of Manaus, however, *L. umbratilis* was considered to be present in equal numbers in both the forest and in the peridomestic habitat (Barbosa et al. 2008).

There are observations suggesting that *L. umbratilis* is a vector of *L. (V.) guyanensis* in the state of Bolivar, Venezuela (Feliciangeli et al. 1985), possibly indicating an expansion of the Brazilian zoonotic cycle.

Rangel et al. (1998) isolated *L. (V.) braziliensis* from patients with ACL in Peixoto de Azevedo, state of Mato Grosso (MT) and Azevedo et al. (2002) noted that one of the most abundant and highly anthropophilic sand fly in the same area was, morphologically, *L. umbratilis*. In addition, they confirmed observations made by workers in the Instituto Evandro Chagas, Belém, PA (Ward et al., personal communication) that the population of this sand fly south of the Amazonas River, behaved very differently from that studied north of the river (Monte Dourado, PA). Although abundant in the forest canopy, it was not found to accumulate on the tree-trunks at ground level. It was this marked behavioural difference that led Lainson (1988) to suggest that perhaps the populations of *L. umbratilis* north and south of the Amazonas River were not identical and, since that time, the taxonomic status of *L. umbratilis* started to attract special attention.

Azevedo et al. (2002) studied the morphology and the morphometric characters of the head, thorax and abdomen of populations of the insect from Brazil [states of Amapá (AP), PA, AM and MT] and Venezuela (state of Bolivar). They found that analysis of morphological characters could not separate the populations, but that the quantitative characters (morphometry) showed 77% of these separated the Venezuelan population from the Brazilian ones. The analysis did not, however, supply evidence of heterogeneity among the populations from Brazil, but recent studies on *L. umbratilis* populations from Brazil and Venezuela have suggested the existence

of three different populations which are separated by the geographical barriers of the *planalto* of state of Roraima (RR) and the two rivers, Negro and Amazonas; one is in Venezuela and the other two in Brazil (north and south of the Amazonas River (Azevedo 2008).

The same author recorded 52 different species of sand flies in the municipalities of Rio Branco, Xapuri and Bujari, AC (17 being new records for that state); *L. umbratilis* was very abundant in the forest canopy, in close association with the major reservoir of *L. (V.) guyanensis*, the sloth *C. didactylus*. Tojal da Silva et al. (2006) recorded the presence of ACL due to *L. (V.) guyanensis* in the municipality of Rio Branco, leading Azevedo et al. (2005, 2008) to conclude there is, in fact, a transmission cycle of this parasite south of the Amazonas River, involving *L. umbratilis*.

Although the markedly different behaviour of the populations north and south of this river remains to be explained, it does help to account for the very low incidence of ACL due to *L. (V.) guyanensis* throughout PA south of the river compared with the alarmingly high number of cases north of it: the failure of infected *L. umbratilis* of the southern population to concentrate on tree-trunks at ground level will greatly reduce its contact with man. ACL south of Amazonas River is mostly caused by *L. (V.) braziliensis* transmitted by sand flies which are, predominately, not arboreal and it is of particular interest that an isolation of a *Leishmania* from *L. umbratilis s.l.* captured in Peixoto de Azevedo, MT, proved to be *L. (V.) braziliensis* and not *L. (V.) guyanensis* (Azevedo et al. 2002), raising the question as to whether or not some arboreal animals may be hosts of *L. (V.) braziliensis*.

Lutzomyia (Nyssomyia) flaviscutellata (Mangabeira, 1942) - In their various field trips in Brazil, Mangabeira and his collaborators captured approximately 17 thousand specimens of sand flies, representing 57 different species and with 35 of them being new to science. Among the latter was Mangabeira's description of *L. flaviscutellata*, based only on the male insects and collected in the locality of Aurá, close to Belém, PA. Later, Sherlock and Carneiro (1962) described the female of the species following the establishment of a laboratory colony of this sand fly from BA. It must be stressed, however, that the taxonomic status of the material from BA has been questioned (Young & Duncan 1994). The specific name of this sand fly was probably chosen in view of the double colouration of the shield, where the scutelo is clear and the remainder of the structure a dark brown (Latin *flavus* = golden; yellow + *scutu* = shield).

L. flaviscutellata has a wide geographical distribution and can be found in very different habitats, such as primary forest, secondary or copse-like vegetation and lowland *várzea* forest which, during half of the year, is subject to various degrees of flooding. Ready et al. (1983) showed that, together with the various rodents and marsupials on which it feeds, it rapidly adapts to plantations of introduced trees such as *Pinus* and *Gmelina* and it is occasionally captured in the peridomestic habitat of houses located near forest (Lainson et al. 1994). With the continuous environmental and climatic changes, there are modifications in the behaviour of some sand fly vectors of leishmaniasis and in the Brazilian *cerrado* of Central Brazil (extensive, flat areas of low, fire-resistant trees, small palms and thorny bushes) it is possible to note the spread of *L. flaviscutellata*, found in association with domestic animal shelters and the presence of new cases of anergic diffuse cutaneous leishmaniasis (ADCL) (Vilela et al. 2008, Shaw 2008).

L. flaviscutellata is a low-flying sand fly, essentially nocturnal in its biting habits, highly attracted to rodents, but not greatly attracted to man (Lainson & Shaw 1968, Shaw & Lainson 1968, Shaw et al. 1972, Gomes 1994, Vilela et al. 2006, 2007). This is fortunate, as it is the proven vector of *Leishmania (Leishmania) amazonensis* which, in addition to being an agent of single-lesion cutaneous leishmaniasis, is also the cause of ADCL in individuals with a faulty immunological system. ADCL is highly disfiguring and cured with difficulty.

In 1963, Lainson paid a visit to the Instituto Evandro Chagas in Belém, PA and, during a demonstration of the animal trapping programme of the Rockefeller Virus Laboratory, discussed the unique opportunity this held for the examination of these animals for evidence of *Leishmania* infections: ACL was a considerable public health problem in Amazon Region of Brazil. The Director of the programme, the late Dr. Otis Causey, was impressed with the similarity of cutaneous lesions he had seen on the tails of wild rodents and those caused by *Leishmania (Leishmania) mexicana* on the tails of forest rodents in Belize, Central America (Lainson & Strangways-Dixon 1964). He promised to look more closely at the next ones he saw and within a few days presented Lainson with a stained smear of a lesion on the tail of the rodent *Oryzomys capito*, rich in amastigotes. At first it was thought that the parasite was *L. (V.) braziliensis* (Guimarães & Costa 1966), but following subsequent study of the parasite it was given the name of *Leishmania mexicana amazonensis* (Lainson & Shaw 1972) later amended to *Leishmania (Leishmania) amazonensis* (Lainson & Shaw 1987).

With the knowledge that rodents were important reservoir hosts of the parasite, rodent-baited Disney traps were used to capture sand flies attracted to them. By far the greatest number trapped were *L. flaviscutellata* and dissection of these revealed eight out of 2,706 to be heavily infected with promastigotes, proved to be those of *L. (L.) amazonensis* (Lainson & Shaw 1968): during this and continuing studies a total of 45 heavily infected *L. flaviscutellata* were recorded in 7,498 females dissected and on no occasion was the parasite encountered in other species of sand flies from the same area.

Finally, *L. (L.) amazonensis* was experimentally transmitted from hamster to hamster by the bite of *L. flaviscutellata* (Ward et al. 1977).

Lutzomyia (Nyssomyia) antunesi (Coutinho, 1939) - This sand fly was first described from male specimens from Manacapuru, AM and examples were deposited in the University of São Paulo. The female was described by Floch and Abonnenc (1944), under the synonym of

Phlebotomus machicouensis, from material collected in Machicou, French Guiana. The sand fly has its major concentration in the Amazon Region: the females are highly anthropophilic, low-flying and, like most sand fly species, feed at night.

While working in an area of visceral leishmaniasis on the island of Marajó, PA, Ryan et al. (1984) captured a small number of *L. antunesi* together with the catch of *Lutzomyia longipalpis* and three of them had promastigote infections. Intradermal inoculation of these into hamsters did not produce cutaneous lesions and the authors wondered if perhaps the parasites were *Leishmania (Leishmania) infantum chagasi* and that *L. antunesi* was functioning as a secondary vector of this parasite. However, no supportive evidence that this might be so has been forthcoming.

Silveira et al. (2002) described a small outbreak of ACL among soldiers carrying out manoeuvres in degraded primary forest in Belém, PA and a woman living very close to the same forest. The parasite differed from all other known *Leishmania* species in the Amazon Region of Brazil and was given the name of *Leishmania (Viannia) lindenbergi* in honour of Adolfo Lindenberg who first registered the existence of human ACL in Brazil (*úlcera de Bauru*), in SP in 1909. Nightime capture of sand flies attacking man was made and 50.7% of the captured specimens proved to be *L. antunesi*, which were taken principally when biting legs and feet. Apart from the large number attacking man, suspicion that the vector might be *L. antunesi* was increased by information that the soldiers had spent most of the time in trenches. This resulted in their arms and head being at ground level and it was precisely on these parts of the body that the lesions occurred. It remains to be seen, however, if *L. antunesi* is, in fact, the vector.

This species has been recorded in forest in TO, particularly in dense forest in the municipality of Guaraí, where the population density was very high (Vilela et al. 2006, 2007).

Lutzomyia migonei (França, 1920) - Data in the literature suggests that *L. migonei* should be regarded as a silvatic species, as it is found in both primary forest and, in lesser abundance, in secondary growth. It is, however, also commonly found inside houses and domestic animal shelters (Barretto 1943, Forattini 1973, Araújo Filho 1979, Rangel et al. 1986). The studies of Araújo Filho (1979) indicated that the highest population density of this sand fly was, in fact, in the peridomestic and domestic habitats.

Like *L. intermedia*, *L. migonei* is one of those primordially silvatic sand flies that has managed to survive the drastic ecological upheavels following deforestation for agriculture or other necessities. Whilst adapting to the new peridomestic and domestic habitats, it probably still helps to maintain the enzootic cycle of *Leishmania* in surviving secondary vegetation. It is notably anthropophilic, can also be found feeding on domestic animals such as dogs, chicken and equines and has retained its attraction to wild animals, as shown by its collection using armadillos as bait (Barretto 1943, Forattini 1973, Araújo Filho 1979, Rangel et al. 1986, 1994). Its feeding habits follow those of most other sand flies, commencing at dusk and increasing during the night: using humans as bait it has been possible to show that feeding activity slowly decreases in the more advanced hours, although feeding on a dog increased in the later hours of the night (Barretto 1943, Forattini 1973, Araújo Filho 1979, Azevedo & Rangel 1991). Regarding its seasonality, *L. migonei* is not found at every month of the year, being absent in the dry and colder months (Barretto 1943, Forattini 1973).

The role of this sand fly in the transmission of ACL due to *L. (V.) braziliensis* was initially associated with foci of the disease in the Southeastern Region of Brazil, but now seems to have extended to the Northeastern Region: it is, however, only considered as a secondary vector. The first evidence suggesting the insect's medical importance was in the 1940's, when a specimen of *L. migonei* was found naturally infected by leptomonad flagellates (probably promastigotes of *Leishmania*) in SP (Pessôa & Coutinho 1941); and in 1943 Barretto recorded this sand fly in zones of both high and low ACL endemicity in the same region. Recent studies by Camargo-Neves et al. (2002) (again in SP) renewed interest in *L. migonei* as a vector of ACL by recording an increase in the number of ACL cases which coincided with an increase in the population of this insect. The authors also commented on this sand fly's zoophilic and anthropophilic biting habits.

On Ilha Grande, RJ, Araújo Filho (1979) found a coincidental distribution of *L. migonei* and houses where human cases of ACL had been recorded or where individuals had positive Montenegro skin tests. It was suggested that this sand fly might be sharing the role of vector of *L. (V.) braziliensis* with *L. intermedia*. In another area of ACL, in Jacarepaguá, where *L. migonei* occurs in high density and is considered as a predominant species, this sand fly has shown a preference to feed on dogs, suggesting that this sand fly might simply be maintaining canine cutaneous leishmaniasis due to this parasite (Rangel et al. 1986). This, it was argued, might also have been the case on Ilha Grande. In contrast, *L. migonei* was found to represent only 15.8% of the local sand fly fauna captured in an area of ACL in Mesquita, RJ and presumably plays a minor role in transmission (Rangel et al. 1990). It was found in an agricultural area, close to Petrópolis, RJ, where human cases of ACL and canine infections have been recorded (Souza et al. 2002) and the same authors (2001) recorded its presence in the Biological Reservation Forest of Poço das Antas, RJ.

In Southeastern Brazil, Ferreira et al. (2001) indicated the high population density and anthropophilic habit of *L. migonei* in the town of Afonso Claudio, an area of ACL transmission in ES, where the insect was suggested to be a secondary vector. Alexander et al. (2002) have registered the presence of the sand fly in coffee plantations in MG, where cases of ACL were appearing.

In the Northeast Region, *L. migonei* has been shown to invade human dwelling places, especially in the rainy season; it was found feeding on equines and also biting man, although with less voracity (Azevedo & Rangel 1991, Queiroz et al. 1994). In some localities it represent-

ed 50% or more of captured sand flies. In a focus of ACL in Baturite, state of Ceará (CE), it was found infected with flagellates with a pyloric development typical of a *Leishmania* of the subgenus *Viannia* (Azevedo & Rangel 1991). On this occasion the parasite was not isolated and identified, but a further infection of *L. migonei* was found and this time the organism was isolated and characterized as *L. (V.) braziliensis* (Queiroz et al. 1994): due to the low attraction of *L. migonei* to man recorded by the authors in that area, however, it was suggested to be only a secondary vector of ACL.

In the town of Campo Grande, state of Mato Grosso do Sul (MS), *L. migonei* was recorded in an urban area by Oliveira et al. (2003) and more recently a specimen infected with *L. (V.) braziliensis* was captured in Jacarepaguá, RJ, where human and canine case of ACL have been reported (Pita-Pereira et al. 2005).

Lutzomyia (Pintomyia) fischeri (Pinto, 1926) - This species was described by Pinto following a study of sand flies captured around the city of São Paulo and Barretto (1943) registered its occurrence in 41 different municipalities of SP, in secondary forest. As it was most frequent where houses and animal shelters were localized nearby, it was suggested that this sand fly was in the process of adapting to the domiciliary environment. Forattini (1953) also noted this occurrence of *L. fischeri* in the peridomestic habitat of ACL transmission in SP and recorded it in domestic animal shelters, particularly the pigsties. Studies in the same areas showed it to be present throughout the year: the highest population density was in the hotter and more humid months, but it could be captured in reasonable numbers during colder and dryer weather. Main feeding activity is from dusk onwards, but it was shown also to feed in daylight. More recent studies in this Region of Brazil showed that during the years 1986-1995, this sand fly was present in the domiciliary habitat in 53.6% of the municipial districts of SP with cases of ACL (Camargo-Neves et al. 2002).

In MG, *L. fischeri* was shown to be among the four species of sand flies with the highest population density in coffee plantations that were in areas of ACL transmission (Alexander et al. 2002), while its presence was also recorded in areas of ACL in SC (Marcondes et al. 2005).

L. fischeri is considerably anthropophilic, although also feeding on dogs and birds. Recently, in the locality of Posse, district of Petrópolis, RJ, it was shown to have the same feeding pattern in the peridomestic habitat and was the third most frequent species of sand fly captured (Souza et al. 2002). It was also recorded in the Atlantic Forest, during a comparative study with the sand fly species encountered in the *caatinga* of BA (Dias-Lima et al. 2003).

In considering the low frequency of the sand fly in the forest, Forattini (1973) felt that *L. fischeri* would have no participation in the transmission of ACL, although it was known to be present in endemic areas in SP (Barretto 1943). Although it has never been found harbouring *Leishmania*, Coutinho and Barretto (1941) admitted that it could possibly be acting as a secondary vector, based on its high attraction to man and abundance in deforested areas where there were cases of ACL, a conclusion shared by Lainson (1983), who suggested that it might help maintain *L. (V.) braziliensis* among wild animals in the surviving pockets of forest.

Lutzomyia (Pintomyia) pessoai (Coutinho & Barretto, 1940) - Interestingly, this sand fly was, up to 1940, confused with *L. fischeri* and, according to Barretto (1943), some of the observations regarding the biology of *L. fischeri* made in the 1930's possibly referred to *L. pessoai*.

In SP, Barretto (1943) indicated the silvatic origin of *L. pessoai* and showed that the greatest population density of this sand fly was close to areas of recent deforestation, or in forest which was frequently invaded by man and where he had constructed houses and introduced domestic animals. Forattini (1954) recorded it in houses, in SP, which were as far as 300 m from forest where, he suggested, this sand fly probably bred in the forest soil.

L. pessoai was shown to be present throughout the year and its capture during the coldest months suggests that like *L. whitmani* it does not hibernate (Barretto 1943). Complementary studies on seasonality indicated its population to be most dense in the hotter and rainier months (Forattini 1973). Its peak feeding activity occurs at dusk and during the first hours of nightfall (Barretto 1943, Forattini 1954, 1960). It may, however, be collected biting man or animals in the forest during the daylight hours (Barretto 1943).

There is circumstantial evidence that *L. pessoai* may participate in the transmission of ACL in the Southeast of Brazil, such as its high population density, considerable attraction to man and its presence inside houses in endemic areas in SP. In this latter region it has been found with flagellates considered to probably be those of a *Leishmania* species (Pessôa & Coutinho 1940, 1941). In RS, amplification of DNA has indicated the presence of a parasite of the subgenus *Viannia* in two specimens of *L. pessoai* (Silva & Grunewald 1999). To date however, undoubted *L. (V.) braziliensis* infection of this sand fly has not been demonstrated.

L. (Psychodopygus) wellcomei (Fraiha, Shaw & Lainson, 1971) - In 1968, the Meridional Mining Company, undertaking mineral exploration in PA, requested the Instituto Evandro Chagas to investigate an alarming number of men acquiring ACL due to *L. (V.) braziliensis* whilst working on road construction through primary forest in the Serra dos Carajás. It required only a few days for one particular sand fly to become highly suspected as the vector, due to its avid feeding on man.

It proved to be a previously undescribed sand fly, which was named *L. (Psychodopygus) wellcomei* Fraiha, Shaw e Lainson, 1971, in honour of Sir Henry Wellcome, founder of the Wellcome Trust, London, who was to sponser the Institute's leishmaniasis programme for nearly 40 further years.

Ward et al. (1973) made a study of sand flies captured during a two month period (December and January) using human bait, rodent-baited Disney traps and aspiration from tree-trunks, all at ground level and captures

with CDC light traps on platforms built in the trees at five and 11 m above the forest floor. A total of 23 different species were caught and approximately 65% of all the sand flies captured while biting man were *L. wellcomei*. Heavy promastigote infections were encountered in three specimens of this sand fly and the parasite was isolated in culture and the skin of hamsters; subsequent studies showed it to be *L. (V.) braziliensis*. Finally, Ryan et al. (1987) obtained experimental transmission of the parasite to hamsters by placing the animals in cages with large numbers of newly caught sand flies. All fed flies were separately maintained in glass vials until they had oviposited, when they were dissected to detect promastigotes and the eggs of all infected specimens maintained in order to rear males for positive identification. This was necessary as the females of *L. wellcomei* are morphologically indistinguishable from those of a sympatric species, *L. complexus,* while the males have distinctly different morphology.

L. wellcomei is an essentially silvatic and highly anthropophilic species (Ward et al. 1973, Wilkes et al. 1984). In addition, Ward et al. (1973b) found that 25.5% of all sand flies attracted to rodent-baited traps were of this species: this and the fact that this sand fly has a vertical flight-range of only 1-2 m above ground level, led to their suggestion that the silvatic reservoirs of *L. wellcomei* are terrestrial animals, the most highly suspected being rodents and marsupials (Lainson et al. 1973). The isolation of parasites with the biological characters of *L. (V.) braziliensis* from the rodents *Oryzomys concolor*, *O. capito*, *Oryzomys nigripes*, *Akodon arviculoides*, *Proechimys* spp, *Rattus rattus* and *Rhipidomys leucodactylus* and the opossum *Didelphis marsupialis* in Brazil, tended to support this view (Lainson & Shaw 1970, 1979, Forattini et al. 1972, Forattini 1973, Lainson et al. 1981b, Rocha et al. 1988). Finally, a more definitive identification of this parasite from the Brazilian rodents *Bolomys lasiurus* and *R. rattus* was obtained by multilocus enzyme electrophoresis (Brandão-Filho et al. 2003).

Regarding its behaviour and seasonality, *L. wellcomei* is most abundant in the rainy season (November-April) and enters into diapause during the dryer months, when it is very rarely encountered. Limiting forest work to the dryer months can, therefore, greatly reduce the risk of acquiring ACL in areas where this sand fly is found. The great importance of *L. wellcomei* as a vector of *L. (V.) braziliensis* is due to its tendency to not only feed at night but also during broad daylight, particularly in cloudy weather. The number of infected females captured during the day was, in fact, found to be greater than that obtained during the night, suggesting that transmission is actually most frequent during the day (Wilkes et al. 1984).

The presence of *L. wellcomei* has been recorded in areas out of the Amazon region, such as in forest of the Serra de Baturité, CE (Ready et al. 1983, Azevedo & Rangel 1991). The former authors suggested that sand flies recorded as being *Lutzomyia squamiventris* by Lucena (1953) in the area of Guaramiranga, CE, were possibly *L. wellcomei*, as the females of the two species, are morphologically very similar.

In the Serra de Baturité area, CE (Queiroz et al. 1994) detected flagellates in *L. wellcomei* (infection-rate 0.05%). Unfortunately, the parasites were not identified and the importance of *L. wellcomei* as a vector of ACL in Northeast Brazil has still to be assessed.

Finally, although *L. wellcomei* has been found in the Atlantic Rainforest region of state of Pernambuco there has, as yet, been no proven association of this species with local ACL in that region (Andrade et al. 2005, Silva & Vasconcelos 2005).

Lutzomyia (Psychodopygus) complexa (Mangabeira, 1941) - This sand fly was described from a single male, captured in the municipality of Abaeté, PA, in 1938, by members of the Commission of Studies of American Visceral Leishmaniasis. Like *L. wellcomei*, the females are highly anthropophilic, although they seem not to share that sand fly's daytime biting habits.

The females of *L. complexa* and *L. wellcomei* are morphologically indistinguishable, although the males are easily identified by the structure of the external genitalia. Ready et al. (1991), however, used DNA probes to distinguish the two species and showed that a fragment of DNA highly repetitive for *L. wellcomei* was not detected in either sex of *L. complexa*.

In Serra dos Carajás, PA, the two species share the same forest habitat, which at first created difficulties in pin-pointing the principal vector of *L. (V.) braziliensis* in that area and necessitated the rearing of males from the eggs of infected females to obtain the all-important males. In a transect running from high up on the range of hills down to the lowland forest, Ready et al. (1984) showed that the predominant species at the higher altitude (700 m, or more, above sea level) was *L. wellcomei* and that this predominance was slowly reversed with decreasing altitude until *L. complexus* predominated, in large numbers, in the forest at the foot of the hills (200 m) and *L. wellcomei* was completely absent at 150 m and lower. As ACL due to *L. (V.) braziliensis* is commonly found in the latter lowland forest, in various regions of PA, this is a clear indication that vectors other than *L. wellcomei* are involved (Shaw et al. 1987). In later studies in Paragominus, where *L. wellcomei* is uncommon, several females of the *squamiventris* series were found infected and, as all of the males captured proved to be *L. complexa*, it was considered sufficient evidence to incriminate this sand fly as the vector of *L. (V.) braziliensis* in that region (Souza et al. 1996).

Azevedo et al. (2002) showed that *L. complexa* represented 8.2% of all captured sand flies in an area of ACL transmission in Peixoto de Azevedo, MT, although the participation of this species as a vector in this region has yet to be established.

Lutzomyia (Psychodopygus) ayrozai (Barretto & Coutinho, 1940) - *L. ayrozai* is recognized as a highly anthropophilic sand fly species in the more mountainous area of Southeast Brazil (Aguiar & Soucasaux 1984), where it is more frequently found in the hot and humid months: the population suffers reduction during the colder and dryer part of the year. Studies in the Serra dos Órgãos, RJ, showed its feeding activity to start at dusk, extending from approximately 5-12 pm: it feeds, pref-

erentially, at ground level (Aguiar & Soucasaux 1984). In the Atlantic Forest, PR, it has been indicated as one of the predominant species; with its population density controlled by such factors as mean monthly temperature and rainfall index (Marcondes et al. 2001).

Leishmania (Viannia) naiffi, a relatively uncommon cause of human ACL in the Amazon Region of Brazil, has been isolated and characterized from *L. ayrozai* (Arias et al. 1985, Lainson et al. 1990). This sand fly is not so highly anthropophilic in this region, however, as it is in the Serra dos Órgãos, RJ.

Lutzomyia (Psychodopygus) paraensis (Costa Lima, 1941) - This sand fly was first described from a male specimen captured near Belém, PA. Observations on its biology were made in Monte Claro, north PA (Lainson et al. 1979), who suggested that the insect's nocturnal feeding habits would give it little opportunity to feed on man, whose activities in the forest are usually during the daylight hours.

Following studies in primary forest of the Serra dos Carajás, PA, an area of high transmission of *L. (V.) braziliensis*, Lainson et al. (1973) discussed the importance of sand flies of the subgenus *Psychodopygus* (given generic ranking by the authors). In addition to infections in *L. wellcomei*, heavy promastigote infections were also found in two out of 175 *L. paraensis* (1.14%) and one out of 127 *L. amazonensis* (0.79%). No proven infection resulted following intradermal inoculation of hamsters with the parasites from the latter two species of sand flies, although a small but persistent swelling was noted at the site of inoculation, some two or three months later. Amastigotes were not demonstrable in the lesions, although intradermal subinoculation of the triturated skin into further hamsters produced a similar small and reddened swelling. The lesions in hamster skin due to *L. (V.) braziliensis* isolated from man and *L. wellcomei* in this area are known to be slow in development, inconspicuous and to contain very scanty amastigotes: in addition, *L. (V.) naiffi* may behave in a similar fashion, or even produce a completely occult infection which can be detected only by subsequent culture of skin from the area of inoculation. Further isolation and identification of these parasites from *L. paraensis* and *L. amazonensis* are clearly needed in the Serra dos Carajás, PA, but as isolates of *L. (V.) naiffi* have been made from both *L. ayrozai* and *L. paraensis*, elsewhere in Amazon Region of Brazil (Arias et al. 1985, Lainson et al. 1990), it remains likely that they were this parasite.

Lutzomyia (Psychodopygus) amazonensis (Root, 1934) - We have mentioned the finding of heavy promastigote infection in this anthropophilic sand fly, in the discussion on *L. paraensis*. It was captured in primary forest in the Serra dos Carajás, PA, an area of high incidence of ACL due to *L. (V.) braziliensis* and further attempts are needed to isolate and characterize its parasite.

L. amazonensis is widely distributed in primary forest throughout the Amazon Region. In Brazil it has been reported in PA, AM, AC, RO, AP and RR. In neighbouring countries it has been recorded in Bolivia, Colombia, Ecuador, French Guiana, Peru, Suriname and Venezuela.

Lutzomyia (Psychodopygus) hirsuta hirsuta (Mangabeira, 1942) - This species is commonly encountered in captures of man-biting sand flies and the females can easily be confused with those of some other anthropophilic species. It has been shown to host a *Leishmania* species of the subgenus *Viannia* (Ryan et al. 1987, R Lainson & EF Rangel, unpublished observations).

The former authors encountered three infected specimens in Tucuruí, PA and a single infected female was found by the latter authors in forest near Além Paraíba, MG, the type locality of *L. (V.) braziliensis*.

Lutzomyia hirsuta hirsuta is widely distributed in the Amazon Basin and, in addition to Brazil, has been recorded in Colombia, Ecuador, Peru, Bolivia, French Guiana and Suriname.

Lutzomyia (Trichophoromyia) ubiquitalis (Mangabeira, 1942) - This species was first described by Mangabeira, following a study of males only, taken from the burrows of the armadillo *Dasypus novemcinctus* (Xenarthra) and the *paca Agouti paca* (Rodentia) and from specimens attracted to a horse, in the locality of Aurá, near Belém, PA. It is the first species of the subgenus *Trichophoromyia* to be incriminated as a vector of a species of *Leishmania* (Lainson & Shaw 1998).

In 1983, in the Serra dos Carajás, PA, a *Leishmania* was isolated from a single female specimen of this sand fly and identified only as a member of the subgenus *Viannia* by its pyloric development in the sand fly. Later comparison of the morphology and isoenzyme profiles of this parasite and another unidentified *Leishmania* species from a case of ACL acquired on the outskirts of Belém, PA in 1981, showed the two isolates to clearly be the same parasite: it differed from all other species of *Leishmania* known in the Amazon Region and was named *Leishmania (Viannia) lainsoni* (Silveira et al. 1987). Later, the reservoir host of the parasite was shown to be the large rodent *Agouti paca* (Silveira et al. 1991a) in the burrows of which Mangabeira had first found *L. ubiquitalis*.

During further eco-epidemiological studies in the same area near Belém, the parasite was re-encountered in eight more specimens of *L. ubiquitalis* and further isolates were made from cases of ACL (Silveira et al. 1991b).

Surprisingly, *L. ubiquitalis* was found disinclined to bite man in the field. It was noted, however, that when retained in the laboratory for some time it fed on him readily and when entomological studies were resumed in the area where the infected sand flies were caught, a single female was finally observed to feed and engorge on a man's arm. It was suggested that, in nature, certain factors or changing conditions stimulate *L. ubiquitalis* to attack man and could be induced in the laboratory (Lainson et al. 1992).

Tojal da Silva et al. (2006) characterized isolates of *Leishmania* isolated from man in an area of intense

ACL transmission in Rio Branco, AC, as *L. (V.) guyanensis*, *L. (V.) braziliensis* and *L. (V.) lainsoni* and research on potential vectors by Azevedo et al. (2008) led to the suggestion that in that area, too, the vector of the latter parasite is *L. ubiquitalis*.

Lutzomyia (Lutzomyia) gomezi (Nitzulescu, 1931) - Most first descriptions of sand fly species have been based on male specimens, but that of *L. gomezi* was made with the female; it was captured in the area of San Cristobal, state of Tachira, Venezuela.

Up till now, information on the role of *L. gomezi* as a vector of ACL is scanty and comes mainly from Panamá, where the major vector of *L. (V.) panamensis* is considered to be *Lutzomyia trapidoi*, but with *L. gomezi* acting as a secondary vector due to its considerable attraction to man (Johnson et al. 1963, Christensen et al. 1969).

In Brazil, this sand fly has been recorded in the states of Maranhão, Goiás, AC, AP, AM, RO, RR, PA, MT and BA and in other countries, in Peru, French Guiana, Venezuela, Colombia, Ecuador, Costa Rica, Honduras, El Salvador, Panama, Nicaragua and Trinidad (Young & Duncan 1994, Aguiar & Medeiros 2003).

Workers in the Instituto Evandro Chagas, Belém, PA have, on several occasions, encountered specimens of *L. gomezi* heavily infected with promastigotes, the development of which was the same as that of *Leishmania* species in the subgenus *Viannia*. They were, however, unable to isolate the parasite for characterization. Its failure to produce a detectable infection after intradermal inoculation into the skin of hamsters is notably similar to the frequent production of occult infection by *L. (V.) naiffi* in the same animal. *L. gomezi* feeds on a variety of wild animals and possibly they include the armadillo *D. novemcinctus*, the only known host of this parasite.

Lutzomyia (*Viannamyia) tuberculata* (Mangabeira, 1941) - In 1979 a *Leishmania* species of the subgenus *Viannia* was isolated by workers of the Instituto Evandro Chagas from a single specimen of this sand fly taken from a large tree-trunk in the Utinga forest, near Belém, PA. The parasite, maintained in liquid nitrogen, remained uncharacterized until Braga et al. (2003) showed it to differ from all other known *Leishmania* species recorded in Amazon Region of Brazil and named it *Leishmania (Viannia) utingensis*.

Till now, the wild mammalian host of the parasite is unknown and it has not been found infecting man. As *L. tuberculata* shows no inclination to bite man it is perhaps unlikely that human ACL due to *L. (V.) utingensis* exists. If, however, there is some other sand fly host which is sometimes attracted to man, cases may well be found in the future.

Lutzomyia tuberculata is commonly found on the trunks of large trees in primary or secondary forest and the mammalian host of *L. (V.) utingensis* is, therefore, likely to be an arboreal animal. In Brazil, *L. tuberculata* is widely distributed throughout the Amazon Basin and in neighbouring countries such as French Guiana, Suriname, Colombia and Venezuela. In Central America it has been recorded in Panama.

The general opinion is that the genus *Leishmania* evolved from a monogenetic insect flagellate and, this being so, the phlebotomine sand fly is best regarded as the primary host of this parasite (Lainson 1997). During the sand fly's blood meal, inoculated flagellates must have eventually adapted to spend part of the parasite's life-cycle in the secondary, vertebrate host, in the amastigote form, thus forming a reservoir of infection for further sand flies.

We have little knowledge regarding the degree of specificity of the *Leishmania* species for their sand fly hosts in nature. In many instances the results of experiments, using different colonized sand fly species in the laboratory, may be misleading due to the excessive number of amastigotes (or promastigotes from in vitro cultures) fed to these insects: this may overpower a sand fly's resistance to infection by a given *Leishmania* species. Ecological studies, however, strongly suggest natural barriers which limit the life-cycle of leishmanial parasites to certain sand fly species, or others that are closely related.

The steadily increasing number of neotropical *Leishmania* species being described is largely due to isolation of the parasites from cases of human leishmaniasis. There are, however, several species which are not yet known to infect man, most probably because their sand fly vectors are not attracted to him: notable examples are *Leishmania (Leishmania) hertigi* and *Leishmania (Leishmania) deanei* of porcupines, *Leishmania (Leishmania) enriettii* of guinea-pigs and *L. (V.) utingensis* of the sand fly *L. tuberculata*. Just how many more species of *Leishmania* there are to be discovered in other non-anthropophilic sand flies remains to be seen and some idea will only be gained by the continued dissection of large numbers of these and the isolation and characterization of any parasites detected. A daunting task, considering that almost 400 species of sand flies of the genus *Lutzomyia* have been recorded in the Americas (Young & Duncan 1994) and that the list will doubtless continue to grow!

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