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Environmental Changes and the Geographic Spreading of American Cutaneous Leishmaniasis in Brazil

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1. Introduction

Global human population is facing the impacts of centuries of constant changes in natural environments. Impacts in the dynamics of infectious diseases are not only expected, but can already be noticed. Vector-borne diseases are particularly susceptible to environmental changes, since their occurrence depends on the ecological balance between different species in complex transmission cycles [1-3]. Leishmaniasis are among the vector-borne diseases most affected by this *ecological chaos* driven by human actions [4], and one of the expected impacts is the expansion of its geographical distribution [5-7].

Leishmaniasis are among the world's six most neglected diseases, affecting indistinctively men, women and children. Usually they occur among the poorest of the poor, mainly in developing countries, contributing to establishment and maintenance of social inequities [7]. They can be divided in two main clinical forms: visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). Despite this simple classification, a wide clinical spectrum is observed, mostly because of the high diversity of parasites (Trypanosomatidae of *Leishmania* genus), vectors (Phlebotominae sand flies) and reservoir hosts (mammals of several orders) involved in its transmission cycles [7, 8].

The geographical distribution of leishmaniasis includes 98 countries in American, European, Asiatic, African and Australian continents. The World Health Organization estimates the yearly occurrence of about 200,000 to 400,000 VL human cases and 700,000 to 1.2 million CL human cases. More than 90% of global VL cases are recorded in six countries: India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil. Cutaneous leishmaniasis is more widely distributed, with about one-third of cases occurring in tropical regions of the Americas, the

Mediterranean basin, western and central Asia. In the American continent, Brazil is the country with the highest estimated incidences of both visceral and cutaneous leishmaniasis [9].

The distribution of leishmaniasis in the world can be partially explained by its widely distributed vectors. The sand flies are small insects (adults of about 3-5 mm) from order Diptera, family Psychodidae, subfamily Phlebotominae. Although occurring mainly in the tropical, hottest areas of the world (Latin America, South Europe, Africa, South Asia and Australia), their distribution stretches north and south to latitudes of over 40°, such as in Germany [10] and Argentinean Patagonia [11]. Sand flies have primarily crepuscular and nocturnal habits, but adults were captured during the day in dense forests [12], caves [13] and dark, humid animal shelters [14]. Only females are haematophagous and thus are related with *Leishmania* transmission. Their broad feeding habits contribute to the transmission of pathogens between hosts in sylvatic and peridomestic areas [15, 16]. Of approximately 900 described sand fly species, no more than 70 have been implicated in leishmaniasis transmission [17]. All New World vector species belong to *Lutzomyia* genus, while the Old World vectors are grouped in *Phlebotomus* genus [15, 18].

In Brazil, the concept of leishmaniasis as a sylvatic zoonosis is restricted to the Amazon Forest, Atlantic Forest fragments and parts of Cerrado. A new transmission profile has emerged, driven mostly by human-made environmental changes. In past decades, human migration of different origins and purposes resulted in major deforestation and unplanned settlements. These changes favor the dispersion of sylvatic animals (some *Leishmania* reservoir hosts) and sand flies (especially those species with eclectic feeding habits) to peridomestic areas, where new transmission cycles may establish close to human dwellings [19-21].

This new transmission profile is especially evident for American Cutaneous Leishmaniasis (ACL), which is caused by a variety of *Leishmania* parasites. Although some clinical manifestations are more frequently associated with a particular *Leishmania* species or subgenus (*Viannia* or *Leishmania*), none is unique to a species. In addition, a substantial but variable proportion of infections are asymptomatic. Human cases have been occurring with different clinical forms, including localized, disseminated, diffuse and atypical cutaneous and mucosal lesions. Different species of sand flies and reservoirs interact in complex transmission cycles, with particular ecoepidemiological features on each disease focus [22, 23].

According to Brazilian Ministry of Health [23], ACL can be categorized in three epidemiological patterns:

1. Sylvatic: In this case, transmission occurs in primary vegetation areas, where the disease is characterized as a strictly sylvatic zoonosis. Humans get infected occasionally when entering these areas, where the enzootic cycle is maintained;
2. Sylvatic/occupational and impacted areas: This pattern is associated with exploitation of natural environments and deforestation, originated mostly from constructions of roads, hydroelectric power plants, human settlements, wood extraction, agricultural activities, military training and ecotourism. In this case, humans are more intensively exposed to vector contact;

3. Rural/periurban (colonization areas): ACL occurrence is related to human migration, occupation of slopes and aggregation in periurban areas associated with secondary and residual vegetation. Synanthropic and domestic animals such as dogs, horses and rodents are suggested reservoir hosts.

Brazil is currently facing an increasing geographical expansion of ACL, with a shift from the classical predominant epidemiological pattern 1 to frequent observations of pattern 2. All of its states have records of the disease, with a growing number of municipalities affected each year (Figure 1).

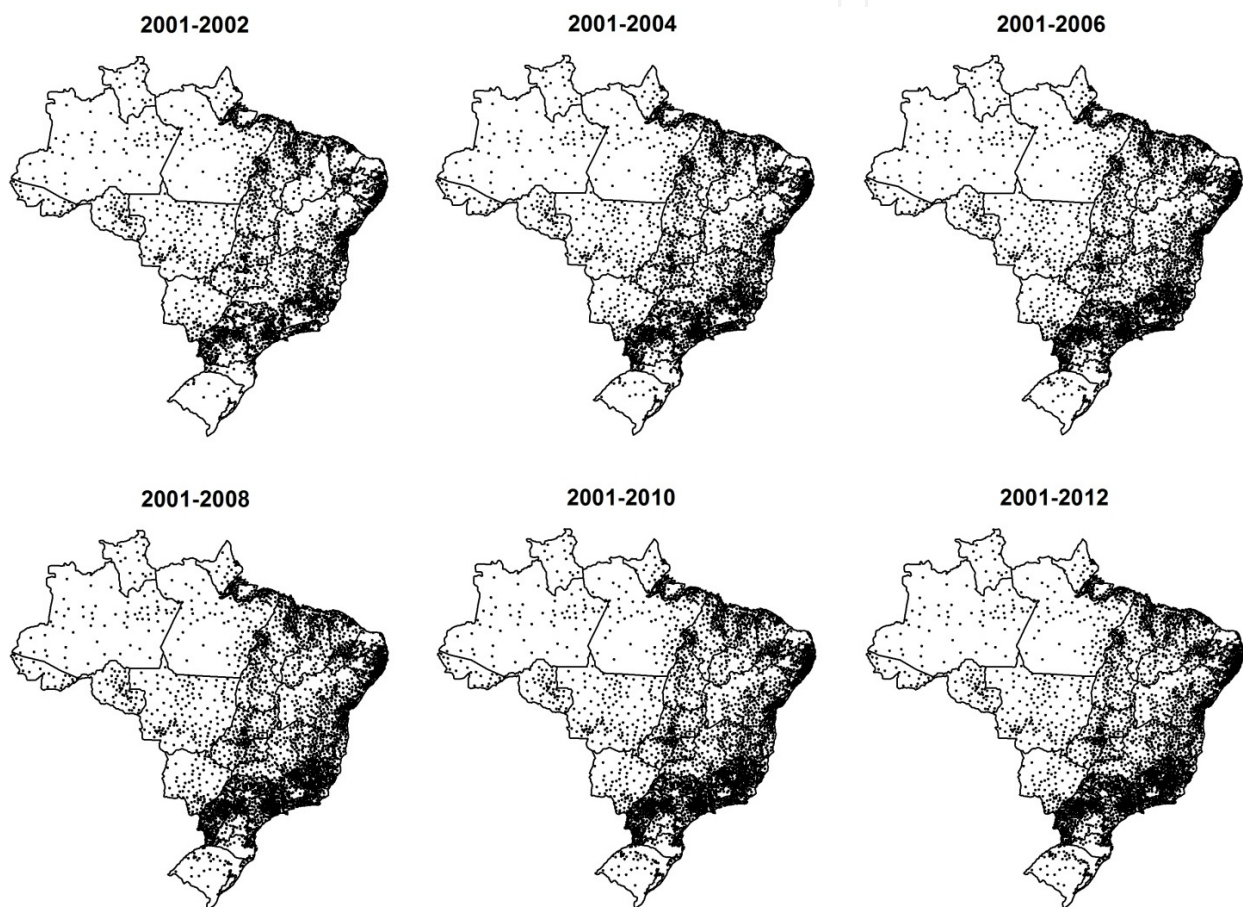


Figure 1. Brazilian municipalities with records of American Cutaneous Leishmaniasis, 2001 to 2012. Each point on the map represents one municipality with ACL human case records

This expansion can probably be explained by the growing environmental changes, which in turn affect vector behavior. Some ACL vector species have been showing evidences of adaptation to man-modified environments, establishing in peridomestic areas, even in outskirts of large cities [22, 23]. In this case, two sand fly species are particularly good examples, in different ecoepidemiological situations: *Lutzomyia* (*Nyssomyia*) *whitmani* and *Lutzomyia* (*Nyssomyia*) *flaviscutellata*. On the following sections the geographical distribution in Brazil and relation with ACL transmission of these species are presented.

2. *Lutzomyia (Nyssomyia) whitmani* (Antunes & Coutinho 1939)

Lutzomyia (N.) *whitmani* was described by Antunes & Coutinho in 1939 [24] as *Flebotomus whitmani* in honor of Dr. Whitman, from Rockefeller Foundation, an institute that collaborated with the Brazilian government at the time in the Yellow Fever Service. The new species was described based on male and female specimens captured in Ilhéus municipality, Bahia state. This species can be observed in all five regions of Brazil and, in the American continent it is also present in Argentina, French Guiana, Paraguay and Peru [7, 18].

The role of *L. (N.) whitmani* as ACL vector is evident throughout the Brazilian territory. The first observation of its importance in ACL transmission cycle was made in São Paulo state, where females were caught naturally infected by flagellates, possibly *Leishmania* [25]. In the same state, the biology of some sand fly species was studied, and *L. (N.) whitmani* was frequently found in deforested areas [26]. According to Pessoa & Coutinho [25], this species is considered highly anthropophilic, constantly invading houses for biting humans.

Between decades of 1930 and 1940, during the human colonization of South and Southeast Brazilian regions, ACL transmission was related with *L. (N.) whitmani*, with its occurrence mainly in sylvatic areas [27]. At this time, this sand fly species used to inhabit mainly forests. Man and domestic animals were bitten when they entered these areas or when houses were built near or inside forests [26]. Other studies on the ecology of *L. (N.) whitmani* showed aspects of its natural breeding places, monthly variation, high density and adaptation to domestic areas [28].

In Brazil, *L. (N.) whitmani* was already detected in 634 of its 5566 municipalities, occurring in all 27 federative units (Figure 2). The states with the higher spatial aggregation of municipalities with the vector occurrence are Pernambuco, Minas Gerais, São Paulo and Paraná, which are also areas of high concentration of ACL human cases [29] (see Figure 1).

Lutzomyia (N.) whitmani is widely distributed across Brazilian biomes. Its presence was recorded in Amazon, Cerrado, Caatinga, Atlantic Forest and Pantanal (Figure 3), occurring mainly in Cerrado and Atlantic Forest [30]. When observing its occurrence in different Brazilian vegetation types, the vector occurs in municipalities with predominance of dense ombrophilous forest, deciduous ombrophilous forest, semideciduous ombrophilous forest, savannah and steppe (Figure 4). The species was not observed in municipalities predominantly covered by marshes and sandbanks [29].

In São Paulo state, *L. (N.) intermedia* and *L. (N.) whitmani* were the predominant species during deforestation of primary forests [28]. However, as deforestation continued to expand, *L. (N.) whitmani* showed lower abundances, suggesting that this species would be more dependent of primary forest than *L. (N.) intermedia*. On the other hand, *L. (N.) whitmani* was found frequently inside houses built near the forest. In Southeast Region, this species can be found during all months of the year [22, 26]. In São Roque municipality, São Paulo state, *L. (N.) whitmani* was the predominant sand fly species among *Leishmania (V.) braziliensis* transmission areas [31], showing higher abundances in the hotter months of the year [32].

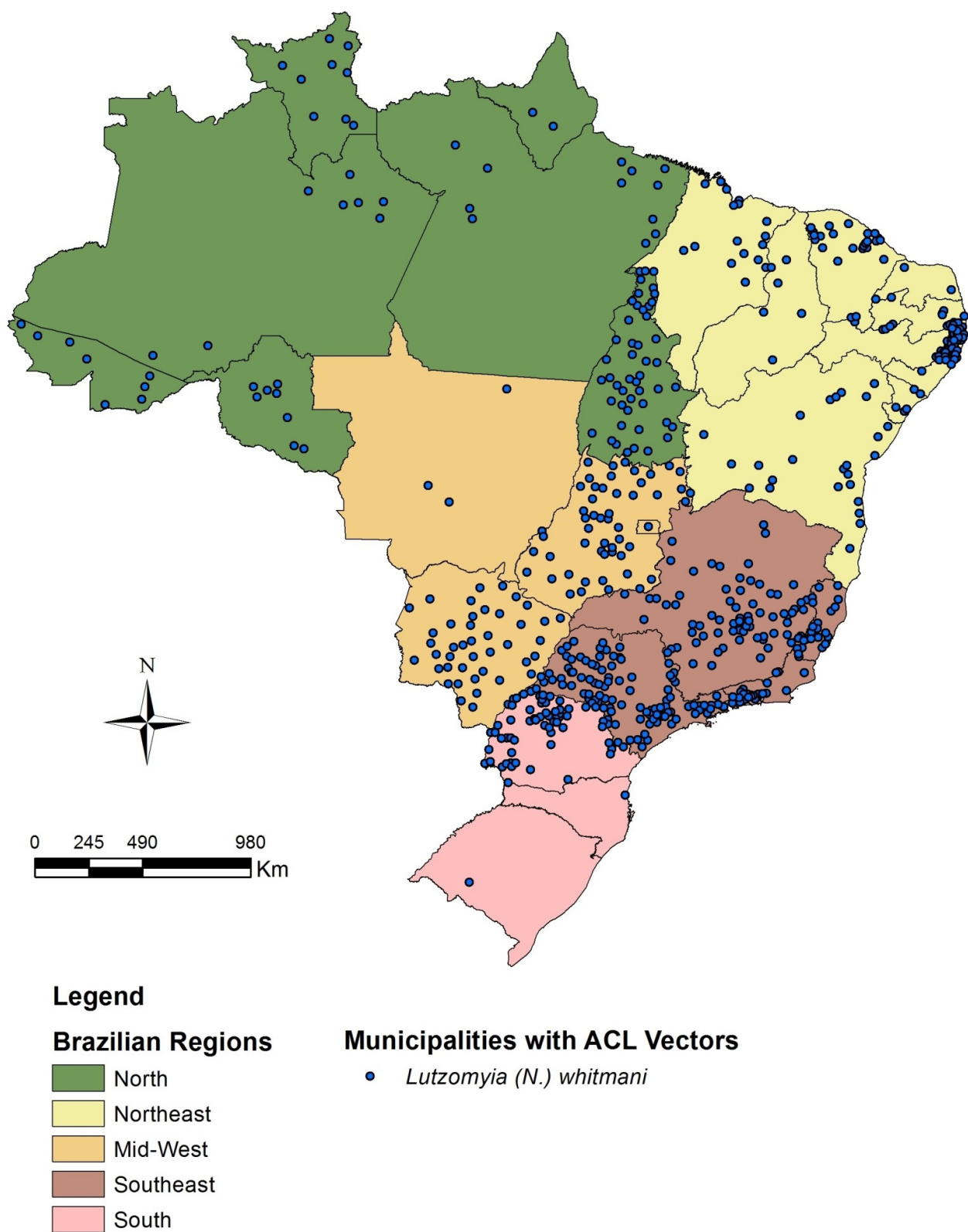


Figure 2. Brazilian municipalities with *Lutzomyia (Nyssomyia) whitmani* occurrence

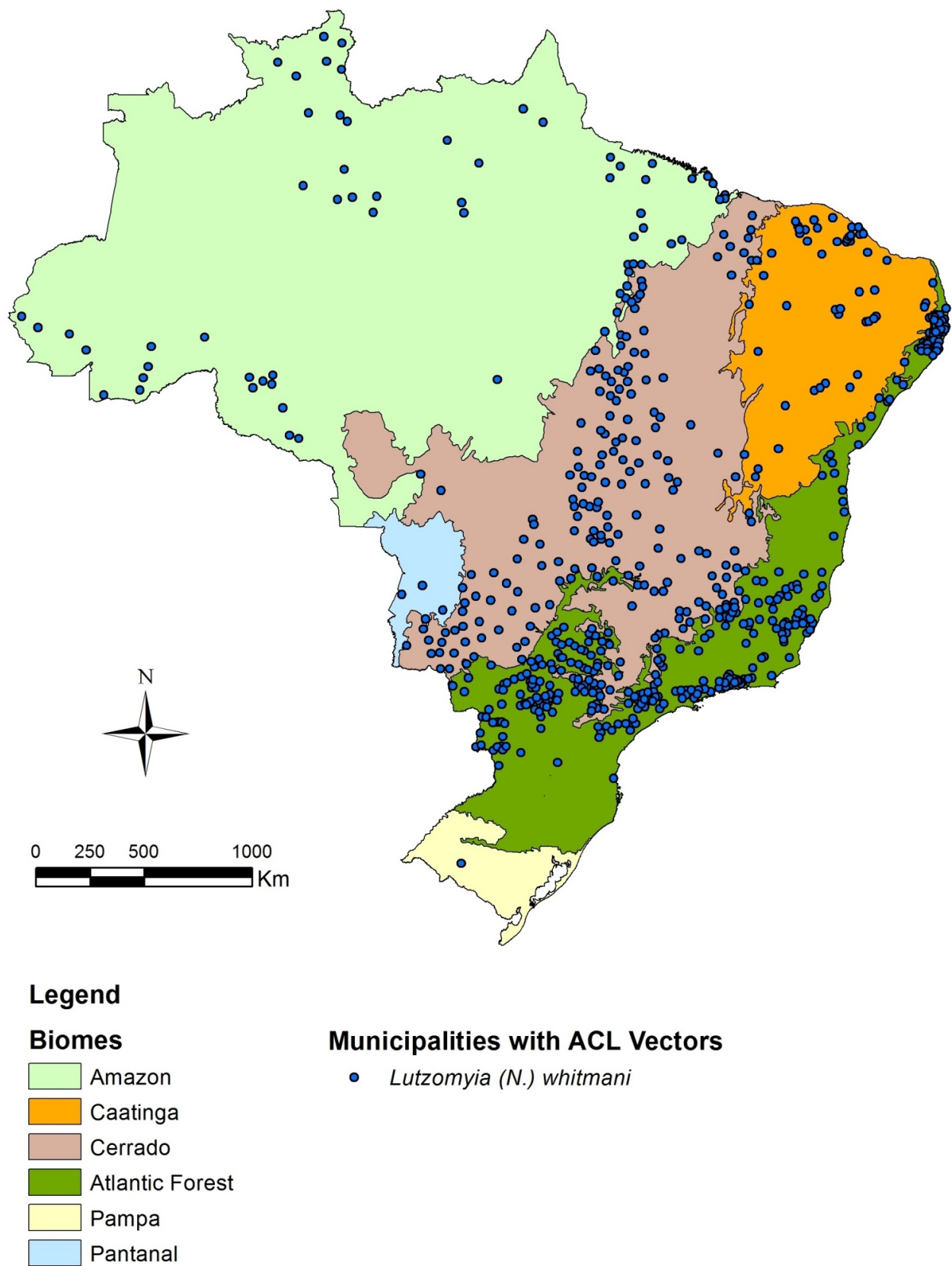
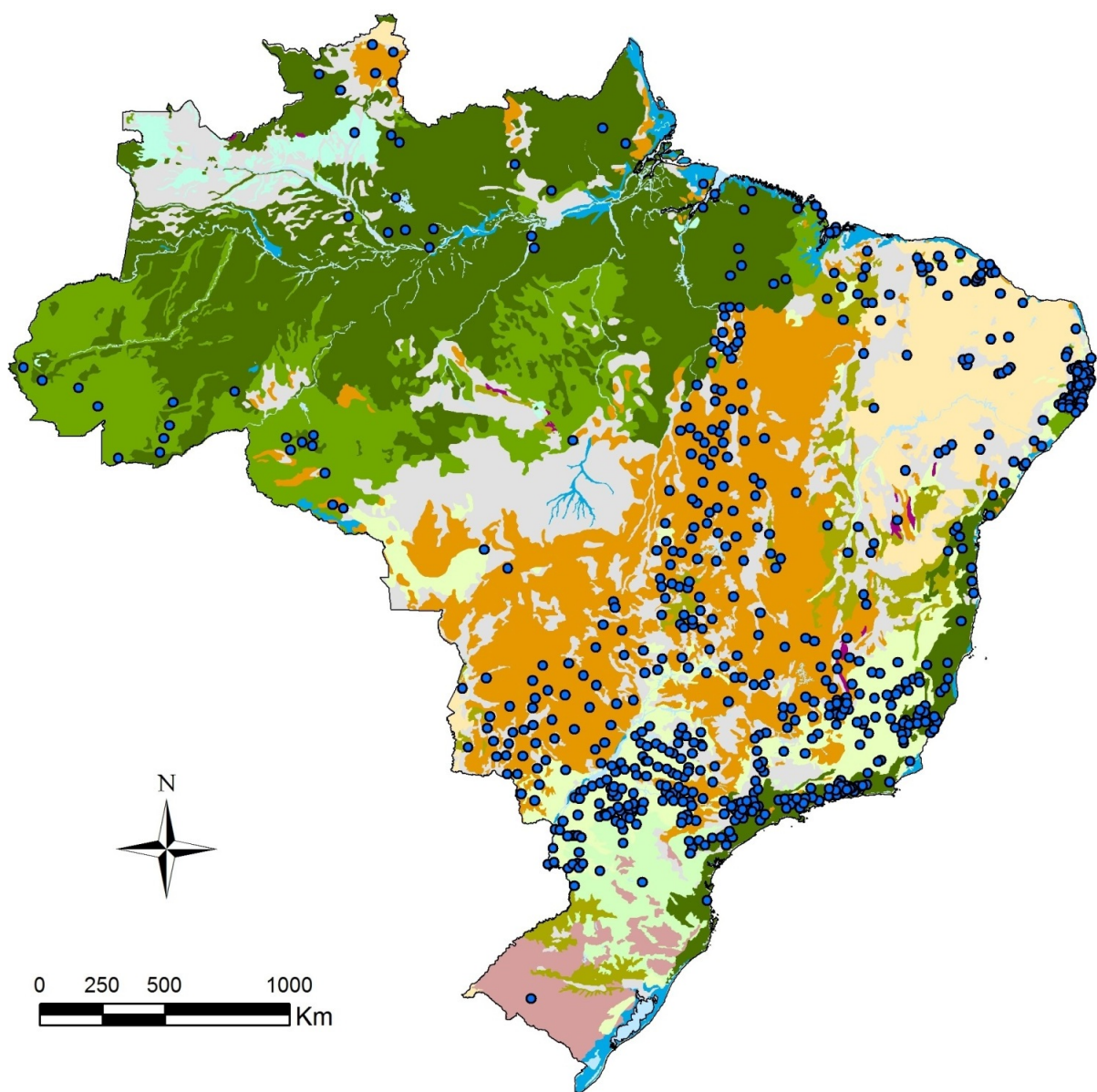


Figure 3. Brazilian municipalities with *Lutzomyia (Nyssomyia) whitmani* occurrence and biomes



Legend

Municipalities with ACL Vectors

• *Lutzomyia (N.) whitmani*

Vegetation Type

Dense Ombrophilous Forest	Savannah
Open Ombrophilous Forest	Savannah/Steppe
Mixed Ombrophilous Forest	Steppe
Semideciduous Ombrophilous Forest	Pioneer Vegetation Areas
Deciduous Ombrophilous Forest	Ecological Tension Areas
Campinarana	Water

Figure 4. Brazilian municipalities with *Lutzomyia (Nyssomyia) whitmani* occurrence and vegetation types

This species was also observed in Atlantic Forest protected areas and inside houses near the forest in Rio de Janeiro state [33]. In the same state, studies performed in rural areas of ACL transmission showed the co-occurrence of *L. (N.) intermedia* and *L. (N.) whitmani* biting humans. In peridomestic areas, *L. (N.) intermedia* was predominant, while *L. (N.) whitmani* was more frequent in the nearest forest. With this spatial separation, the authors suggested that both species would be sharing *Leishmania (V.) braziliensis* transmission on the same focus, throughout the year. *Lutzomyia (N.) whitmani* was captured during all year, but was more frequent in months with lower temperatures [34].

Also in Southeast region, besides São Paulo and Rio de Janeiro states, *L. (N.) whitmani* was associated with *Leishmania (Viannia) braziliensis* transmission in Caratinga (Minas Gerais state) and in a mountainous region of Afonso Cláudio (Espírito Santo state) [35, 36].

In South Brazil, *L. (N.) whitmani* is probably associated to ACL transmission in Paraná state. Studies performed in the north of this state detected it as predominant sand fly species and naturally infected by *Leishmania (V.) braziliensis* parasites [37].

Leishmania (V.) braziliensis in Northeast region is also probably transmitted by *L. (N.) whitmani*. In Bahia and Ceará states this vector shows similar habits to the Southeast region populations: high anthropophily and presence in domestic areas [38-40]. In Ceará state, *L. (N.) whitmani* was found naturally infected by *Leishmania* of *Viannia* subgenus [41]. Afterwards, new infections were detected and the parasite characterization confirmed to be *Leishmania (V.) braziliensis* [42]. Other evidences of this vector's role in ACL transmission in the region were its high abundance and anthropophily [40, 42].

In Bahia state, *L. (N.) whitmani* was found naturally infected by *Leishmania (V.) braziliensis* in Três Braços [43]. This finding, associated with the high frequency of this sand fly in peridomestic and domestic areas allowed the hypothesis of occurrence of a domestic transmission cycle in this area [44]. In Ilhéus municipality, *L. (N.) whitmani* was suggested as ACL vector, considering its almost absolute predominance over other sand fly species (99.7%), its high anthropophily and its occurrence on every sand fly capture point, most of them coincident with areas of ACL human cases [40].

In the Mid-West Region, in Corguinho municipality (Mato Grosso do Sul state), *Leishmania (V.) braziliensis* was isolated from every tested ACL patient by monoclonal antibodies. *Lutzomyia (N.) whitmani* was suggested as vector because it was observed in high abundances and anthropophilic [45]. Furthermore, its predominance over other sand flies was observed in eight of ten ecotopes studied in the locality. *Lutzomyia (N.) whitmani* was present both in ground level and in the forest canopy, suggesting its eclectic feeding habits on mammals and birds. Although in this locality the species is not very common in peridomestic areas, its high abundance and anthropophily are strong evidences of its role in ACL transmission [46].

The behavior of *L. (N.) whitmani* in North region seems to be different from other regions. In these areas, the species was considered mainly sylvatic, being captured on tree trunks and canopies, besides showing low attractiveness for humans [47]. Afterwards, novel studies confirmed such observations and suggested that, if the species were to be anthropophilic, it would be only in some situations [48, 49]. In 1989, in Pará state, a parasite was isolated from

L. (N.) whitmani, and after its characterization as *Leishmania (V.) shawi*, the sand fly species was suggested as its vector [50].

3. *Lutzomyia (Nyssomyia) flaviscutellata* (Mangabeira 1942)

Lutzomyia (N.) flaviscutellata was described by Mangabeira [51] as *Flebotomus flaviscutellatus*, based on two male specimens captured in Belém (Pará state). Later, Sherlock & Carneiro [52] described a female collected in Salvador (Bahia state), although its identification has been questioned by several authors [18, 27, 53]. At the same time, the species *Phlebotomus apicalis* was described by Floch & Abonnenc [54] in French Guiana. Three years later, after a review of the specimens, *P. apicalis* was considered synonym of *L. (N.) flaviscutellata* [55].

In the following years, descriptions of *L. (N.) olmeca* [56], *L. (N.) olmeca bicolor* [53] and *L. (N.) olmeca nociva* [57], all of them morphologically similar to *L. (N.) flaviscutellata*, led some authors to consider these four species as the “*L. flaviscutellata* complex” [58]. However, they are all currently considered valid species, with more recent taxonomic reviews supporting their status [18, 59].

Lutzomyia (N.) flaviscutellata is currently widely distributed across Latin America, occurring in Bolivia, Brazil, Colombia, Ecuador, French Guiana, Peru, Suriname, Trinidad and Venezuela [7, 18].

This sand fly species is associated with *Leishmania (Leishmania) amazonensis* transmission in Brazil. This parasite, when infecting humans, can cause localized cutaneous lesions and eventually develop a more severe clinical form, diffuse cutaneous leishmaniasis (DCL). This clinical form is rare, with chronic development, where the immunodepressed patient shows frequent relapses and insufficient responses to available therapies [60].

The first observation of this sand fly's role in ACL transmission cycle was from a study in the Utinga forest, an Amazon area in Belém municipality (Pará state) [61]. In this area, wild rodents of *Proechimys* and *Oryzomys* genus were captured with cutaneous lesions on tails and feet, from where *Leishmania* parasites were isolated. These rodents were then used as baits and 98% of captured sand flies were *L. (N.) flaviscutellata*. Captured sand flies were dissected and flagellates were isolated from eight females.

Studies of the feeding habits of *L. (N.) flaviscutellata* showed higher preference for small sylvatic rodents (*Proechimys* sp., *Oryzomys* sp.), agoutis (*Dasyprocta* sp.) and porcupines (*Coendou* sp.), having the species also fed on opossums (*Philander* sp.), monkeys (*Saimiri* sp.) and chickens (*Gallus gallus*). Few females fed on humans, so the authors considered the species as having low anthropophily [62]. This preference for biting small rodents indicates that captures of this species tend to be more efficient when using animal baited traps, such as the Disney trap [63].

Despite its strong zoophilic habits and low anthropophily, *Lutzomyia (N.) flaviscutellata* has recently been captured in peridomestic areas, suggesting its dispersion to human dwellings [64-67]. This hypothesis is plausible, since the species also occurs in secondary forests in the

Amazon. In a study performed in the late 1980s in Pará state, *L. (N.) flaviscutellata* was the predominant sand fly species in an area where the primary forest was replaced with exotic trees (*Pinus* and *Gmelina*), with occasional captures in peridomestic areas of houses near the forest [68]. In a review of the Amazonian ACL transmission cycles, *L. (N.) flaviscutellata* was considered one of the few vector species that could adapt to deforestation and become peridomestic [69].

In Brazil, *L. (N.) flaviscutellata* was detected in 131 municipalities, mostly in North and Mid-West regions, with occurrences also in Southwest and Northeast regions (Figure 5).

Lutzomyia (N.) flaviscutellata is considered mainly an Amazonian species, although it can also be found in Cerrado and some few occurrences were recorded in Atlantic Forest, Caatinga and Pantanal (Figure 6).

In the Amazon, *L. (N.) flaviscutellata* is more commonly found in seasonally flooded areas of “igapó forests”, when compared with non-flooded areas of “terra-firme forests” [70]. Its vertical distribution was also studied in the Amazon. The species has a very low flight, with 26 times more specimens captured 0.2 meters above ground than at 1.2 meters. This observation reinforces its association with small rodents and the fact that human cutaneous lesions caused by *Leishmania (L.) amazonensis* are mainly located in the lowest parts of the body [71].

The species was also captured in peridomestic areas of Manaus (Amazonas state) [72], Ilha de Marajó (Pará state) [73] and Santarém (Pará state) [74]. Other examples of surveyed Amazon forest areas of the North region with records of *L. (N.) flaviscutellata* include the states: Acre [75, 76], Amazonas [13, 76-79], Amapá [80], Pará [61, 62, 68, 70, 81], Rondônia [82, 83] and Roraima [84, 85].

Also in the North region, Tocantins state has most of its area covered by Cerrado. It was in this biome that *L. (N.) flaviscutellata* was captured during a four-year sand fly fauna monitoring in the ACL endemic areas of Porto Nacional and Guaraí municipalities. This vector species was found in peridomestic captures in rural settlements and periurban areas [66, 67] and was suggested as *Leishmania (L.) amazonensis* vector in Porto Nacional [66]. In municipalities of the south of the same state, *L. (N.) flaviscutellata* was captured near houses in areas directly and indirectly impacted by a hydroelectric power plant construction in Tocantins River [86].

In Bela Vista municipality (Mato Grosso do Sul State, Mid-West region), an ACL outbreak associated with *Leishmania (L.) amazonensis* in a military training unit led to a sand fly fauna monitoring during years 2004 to 2006. Using light traps, few specimens of *L. (N.) flaviscutellata* were caught [87]. When a modified Disney trap baited with hamsters (*Mesocricetus auratus*) was used, *L. (N.) flaviscutellata* was the species with the highest female abundance [88]. Despite its capture with these methodologies, some females were also captured in white and black Shannon traps [89], suggesting that the species can also feed on humans, and therefore be a possible *Leishmania (L.) amazonensis* vector in this locality [87].

The sand fly fauna of an ecotourism area in Bonito (Mato Grosso do Sul state) was studied. In Cerrado areas, *L. (N.) flaviscutellata* was caught with light traps mainly inside the forest, but it was also found in yards and kennels of houses [64].

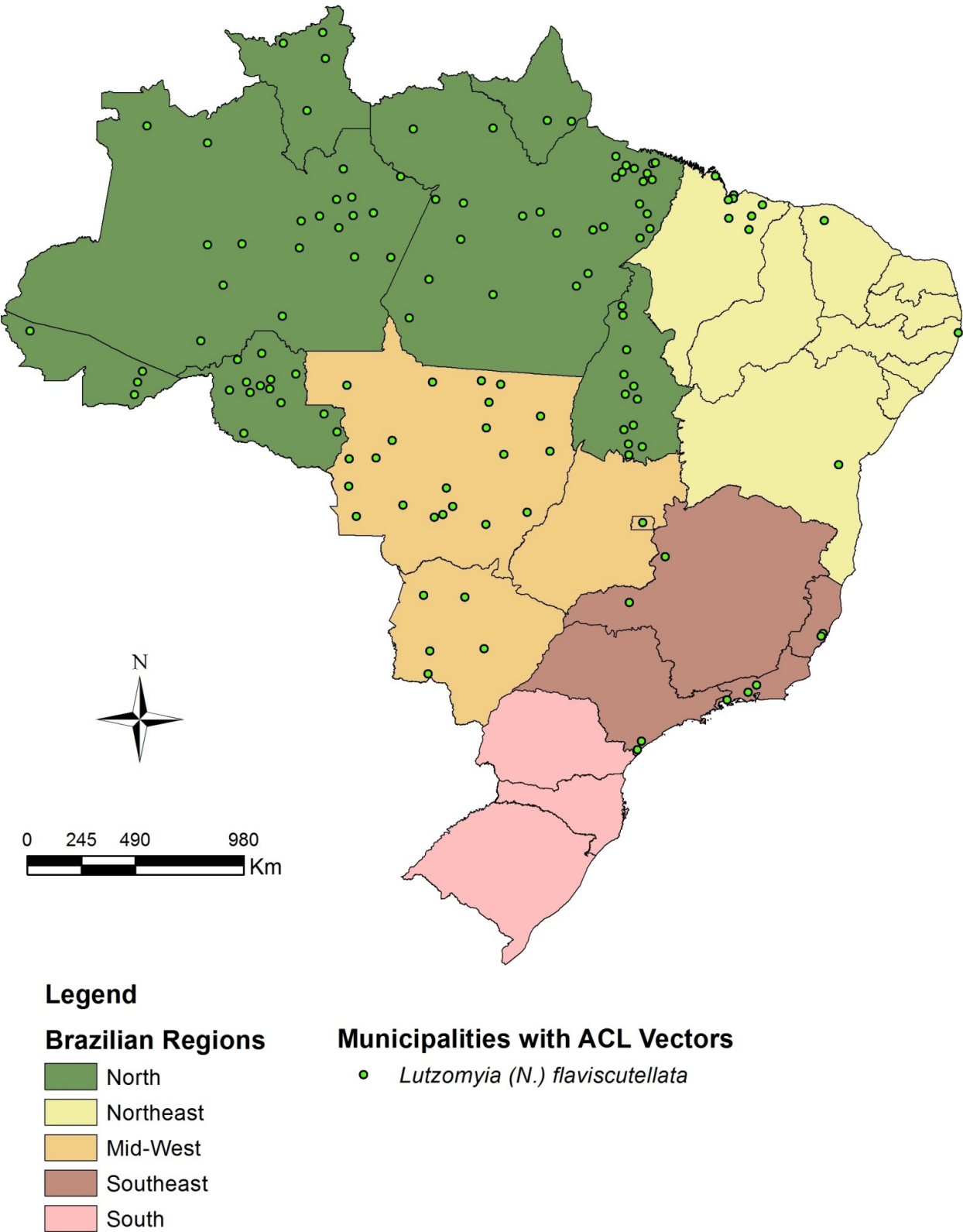


Figure 5. Brazilian municipalities with *Lutzomyia (Nyssomyia) flaviscutellata* occurrence

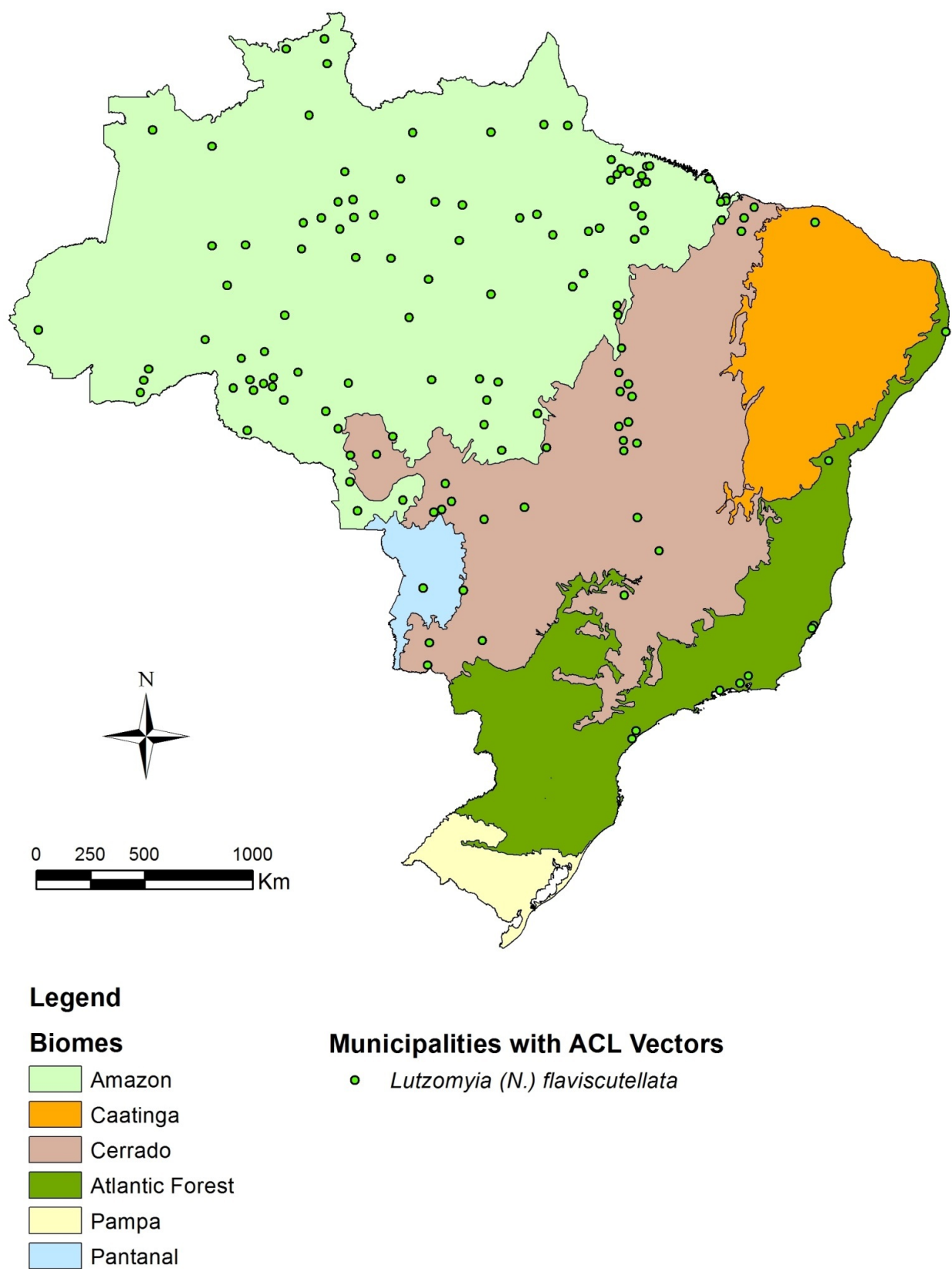


Figure 6. Brazilian municipalities with *Lutzomyia (Nyssomyia) flaviscutellata* occurrence and biomes

In Southeast region, another ecotourism area was surveyed, in Rio de Janeiro state, Atlantic Forest biome. In Angra dos Reis municipality, the state's biggest continental island - Ilha Grande - has records of sporadic ACL cases since the first outbreak in the decade of 1970. At the time of the ACL outbreak, the sand fly fauna was monitored and *L. (N.) flaviscutellata* was captured inside the forest with Disney traps, baited with *Proechimys* rodents [90]. Over three decades later, the same localities were surveyed, and *L. (N.) flaviscutellata* was captured inside the forest and in peridomestic areas of several fisherman villages in Ilha Grande [65]. Even though there are no recorded human cases of *Leishmania (L.) amazonensis* infection in Ilha Grande, one DCL case was recorded in 2007 in Paraty, a municipality neighbor to Angra dos Reis [91].

4. Conclusion: Two American cutaneous leishmaniasis vectors as drivers of its geographical expansion in Brazil

Both *Lutzomyia (N.) whitmani* and *L. (N.) flaviscutellata* are widely spread in Brazilian territory. Each one with its particular epidemiological importance, their geographical distributions overlap areas of ACL occurrence in Brazil (Figure 7).

Since it has a wide geographical distribution and it is associated with two ACL parasites (*Leishmania (V.) braziliensis* and *Leishmania (V.) shawi*), currently, *Lutzomyia (N.) whitmani* is considered the most important ACL vector in Brazil. Its importance is due mainly to its role in transmission cycles related with ACL epidemiological pattern 2 (sylvatic/occupational and impacted areas). This sand fly species was found in several localities associated with areas of environmental changes of different origins, such as deforestation, road constructions, human settlements and agricultural activities. This epidemiological pattern is frequently observed in Brazil, and constitutes the main evidence of the disease's geographical spreading.

Lutzomyia (N.) flaviscutellata, with evidences of dispersion to peridomestic areas especially in the Cerrado biome, confirms the ruralization process of the previously considered strictly sylvatic cycle of *Leishmania (L.) amazonensis*. The possibility of this enzootic cycle to be maintained in secondary forests and even become peridomestic was previously discussed [69]. This could be happening, in part, because of the adaptation process of the vector to man-modified environments. At first, it would be logical to think that a strictly sylvatic cycle would disappear with deforestation of primary forests [92], but the *Leishmania (L.) amazonensis* cycle shows evidences of occurrence in secondary forests and peridomestic areas, where the vector could be dispersing to domestic animal shelters [22].

Considering the great challenge that is controlling ACL, a disease with complex epidemiology directly associated with environmental changes, studies that aim to characterize and monitor its spatial and temporal trends can support the Epidemiological and Entomological Surveillance actions of Health Departments. These studies can help to identify receptive areas for new ACL outbreaks and population groups at higher risk of infection, so that control actions can be better planned and more effective.

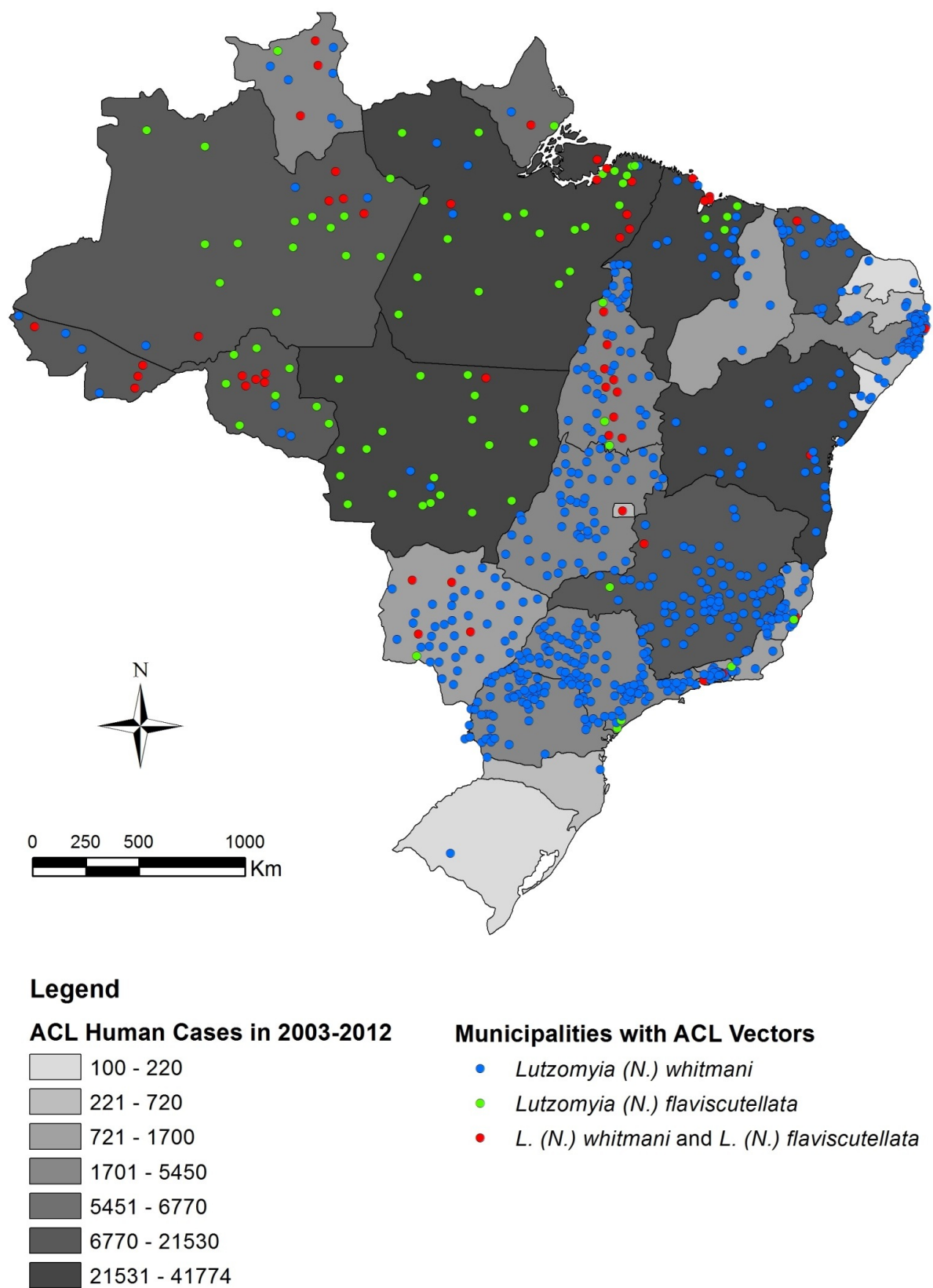


Figure 7. Brazilian municipalities with *Lutzomyia (Nyssomyia) whitmani* and *Lutzomyia (Nyssomyia) flaviscutellata* occurrence and American Cutaneous Leishaniasis human cases recorded by state in the past ten years (2003-2012)

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