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Sand Fly Fauna Associated With Dwellings and Forest Habitats Along the BR-319 Highway, Amazonas, Brazil

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Abstract

Roads and highways can affect the spread of insect-borne diseases by limiting or amplifying the spatiotemporal distribution of vectors, pathogens, and hosts, which can, in turn, lead to the creation of a *nidus* of infection. The aim of this study was to compare the diversity (richness and abundance) of phlebotomine sand flies in household and forest edge environments found along two different segments of an Amazonian highway. Sampling was conducted along the northern and southern portions of highway BR-319, in Amazonas State, Brazil. At each sampling point, Hoover Pugedo traps were set in indoor and outdoor habitats, and at forests edges, and captures were made between 06:00 pm and 06:00 am. A total of 1,189 sand flies were captured and 48 species were identified. As expected, a greater number of species and individuals were captured in forest edge environments. Permutational Multivariate Analyses of Variance (PERMANOVA) and Permutational Analyses of Multivariate Dispersions (PERMDISP) analyses showed that sand fly fauna differed significantly among habitats, but no variance in species composition was observed between the two road segments. Some of the captured species were species that have been implicated as vectors of *Leishmania* spp. Ross, 1903 (Kinetoplastida:Trypanosomatidae).

Key words: HP trap, leishmaniasis, Phlebotominae, road, vector ecology

Human activity and land-use changes are considered to be the main instigators of disease transmission scenarios (Coosemans and Mouchet 1990, Pongsiri et al. 2009). Disease transmission via dipteran vectors can be exacerbated by biophysical alterations to the environment such as deforestation, habitat fragmentation, extractive and agricultural activities, and global and regional emigration and immigration, including the rural–urban movement of human populations (Knudsen and Slooff 1992, Patz et al. 2004, Wilcox and Ellis 2006).

At present, the increased volume and assortment of global traffic has shaped patterns of disease transmission by favoring the dispersion of some etiologic agents and their vectors (Tatem et al. 2006a,b). In the past, this kind of dispersion was limited by the relative sparsity of terrestrial and maritime routes; for example, paleoparasitological studies have indicated that only transpacific migrations of Asiatic populations could have brought hookworm and ancylostomiasis to the Americas (Araújo et al. 1988).

The fact is that terrestrial traffic routes can and do affect the transmission dynamics of insect-borne diseases by limiting or expanding the spatio-temporal distribution of vectors and their hosts (Reisen 2010). In the Brazilian Amazon during the early 1970's, thousands of malaria cases were triggered by the construction and opening of the Madeira-Mamoré Railway (1907-1912), and the BR-364 and Trans-Amazon highways (Katsuragawa et al. 2008). Colonization along the Trans-Amazon highways increased human contact with forested areas and led to several cases of arboviruses, including cases of Mayaro, Guaroa, Itaporanga, and Oropouche (Pinheiro et al. 1977). In these localities, studies on leishmaniases have revealed a high incidence of infection among wild animals trapped at various sites along the highway, and skin tests performed on colonists have revealed a high incidence of positive reactions to leishmanin (up to 60%); however, up until now, active cases of cutaneous leishmaniasis have been uncommon. It is anticipated that the incidence of the disease will rise sharply in the succeeding, nonimmune generation and that cutaneous leishmaniasis will then become a serious problem. Several authors have established that dense populations of highly anthropophilic sand fly species are present at every point studied along the new roads, and that sylvatic reservoirs such as rodents and opossums are also present. Some of these fly species will undoubtedly act as vectors (Lainson and Shaw 1973, Pinheiro et al. 1977, Fraiha et al. 1978).

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During the Brazilian military-dictatorial government (1964-1985), a large-scale road-network project was undertaken in the Amazon with the aim of fostering human colonization and structural and economic development. This network comprised the Trans-Amazon highways (BR-230, linking the eastern and western Amazon), BR-364 (Cuiabá-Porto Velho), BR-163 (Santarém-Cuiabá), and BR-319 (Manaus-Porto Velho) (Fearnside and de Alencastro Graça 2006, Katsuragawa et al. 2008, Fearnside 2009). Between 1972 and 1973, BR-319 was built to connect the state capitals of Manaus in Amazonas and Porto Velho in Rondônia, via an 877 km long route (Fearnside and de Alencastro Graça 2006). At present, only the northern and southern extremities of the highway experience significant traffic. The middle segment (340 km of road) is inaccessible for much of year, mainly due to the rainy season (Fearnside and de Alencastro Graça 2006). Our study focused on the occurrence and diversity of sand fly fauna in domiciliary and forest habitats along highway BR-319. This study compared the abundance, richness and species composition of phlebotomine sand flies 1) in indoor, outdoor, and forest edge habitats, and (2) along two road segments (northern segment and southern segment) of highway BR-319, in the Brazilian Amazon.

Material and Methods

Study Area

The BR-319 highway is about 885 km long. The longer portion of the highway runs through Amazonas State (859.5 km), while the remainder runs through Rondônia State, Brazil. This study was conducted along two different road segments: the northern segment (165 km) and the southern segment (180 km) (Fleck 2009). The middle segment (≈500 km) is generally impassable due to damaged drains and bridges, and sections that remain unpaved; as a result, there is very little human habitation along this segment of the highway (Fleck 2009). Highway BR-319 is located in the Amazon rainforest; however, subtle differences between vegetation physiognomies can be observed along the highway's northern and southern segments. The northern segment is covered by a dense rainforest (closed canopy) and it passes through lowland terrains that experience flooding. In contrast, the southern segment belongs to the region of open rainforest with palm trees, and deforestation in the area has been intense. There are also slight climatic differences between the two segments. Both segments are located in the equatorial zone, but the northern region has a warm and highly humid climate with no marked dry season, while the southern segment passes through areas that have a warm and humid climate but experience one or two dry months (IBGE 1997).

Data Sampling

Nine sample points were surveyed overall. Points 1 to 5 were established in the northern segment, and points 6 to 9 were established in the southern segment (Fig. 1). Sand flies were captured at each of the nine points during one night in four sampling periods (January, March, April, and May/June 2011). Hoover Pugedo (HP) light traps (HP trap, Brazil) were used to capture the insects (06:00 pm–06:00 am) inside and outside domiciles at all sampling points, and at forest edges where forest was present. Total sampling effort was 48 h *per* habitat/point (four events × 12 h).

Sand Fly Identification

The captured sand flies were slide mounted in Berlese fluid and identified using the identification keys of Young and Duncan (1994) and Galati (2003). The nomenclature adopted for sand fly taxonomy follows the proposals of Galati (2003), since this classification have widely been adopted by American taxonomists, due to the frequency



Fig. 1. Sampling points (n = 9) of phlebotomine fauna surveyed along highway BR-319, State of Amazonas, Brazil. **Northern Segment**: Pt 1 – Careiro da Várzea village; Pt 2 – Tescon Company Office; Pt 3 – Tupana village; Pt 4 – Sr. Paulista household; Pt 5 –6°BEC/AM detachment. **Southern Segment**: Pt 6 –7°BEC/AM Base; Pt 7 – Canteiro Delta; Pt 8 – Cristolândia village; Pt 9 – 5°BEC/RO detachment.

of updating and accessibility (www.fsp.usp.br/~egalati) (Galati et al. 2017).

Data Analyses

Differences in sand fly abundance and richness between habitats and road segments were compared using Kruskal–Wallis tests and Dunn post-hoc tests, which were based on median values. The *P*-values of the Dunn test were corrected to control for the false discovery rate using the Benjamini–Hochberg '*P*' adjustment method ('bh' option). Permutational Multivariate Analyses of Variance (PERMANOVA) and Permutational Analyses of Multivariate Dispersions (PERMDISP) were used to evaluate differences in phlebotomine species composition with respect to different road segments and/or habitats. These analyses were based on sand fly abundance data and its Bray–Curtis dissimilarity distances, taking into account species composition across habitats and road segments. All graphs and analysis were done in the R Platform, version 3.4.2 (R Core Team 2017).

Results

A total of 1,189 sand fly specimens were captured and 48 species were identified in 1,296 HP-hours sampling effort (9 points \times 3 habitats \times 4 samplings \times 12 h). *Trichophoromyia ubiquitalis* (Mangabeira) was the most frequent and abundant species across all sampling points and capture events (49% of total individuals recorded) (Table 1). Nearly half of the identified species were recorded very infrequently in the samples: sixteen species were represented by only one

3

specimen(singletons) and eight were represented by two specimens (doubletons) (Table 1). Two sampling points—Pt 2 and Pt 7—were not included in the statistical analyses because in the Pt 2 named TESCON, Northern segment, a unique specimen of *Psychodopygus amazonensis* (Root) was captured, and in the Pt 7, named 'Canteiro Delta', Southern Segment, no phlebotomine were captured.

Outdoor, Indoor, and Forest Habitats

As expected, sand fly abundance (H = 14.46, df = 2, P = 0.0007) and richness (H = 11.30, df = 2, P = 0.0000) were significantly higher in forest edges than in outdoor/indoor habitats (Fig. 2a and b). Domiciliary habitats exhibited no variation in number of sand fly individuals and species (Dunn-test, respectively, P = 0.4910 and P = 0.3701). Abundance and richness differed statistically in the Edge-Indoor pairwise comparison (Dunn-test, respectively, P = 0.0022 and P = 0.0115) and the Edge-Outdoor comparison (Dunn-test, respectively, P = 0.0008 and P = 0.0022).

Only 23% of the species obtained were captured in all three habitats, and the greatest number of species was found consistently in edge habitats (50%). Interestingly, no sand fly species found in both indoor and outdoor habitats were absent from edge habitats (Fig. 3a, Table 1). These dissimilarities in species composition were

 Table 1.
 Number of individuals and sand fly species in forest edge, indoor, and outdoor habitats along highway BR-319, State of Amazonas, Brazil, in 2011

Sand fly species	Forest edge	Indoor	Outdoor	Total
Trichophoromyia ubiquitalis*	472	13	98	583
Trichopygomyia rondonienses (Martins, Falcão & Silva)	73	1	13	87
Psychodopygus chagasi	72	1	-	73
Psychodopygus davisi*	43	12	5	60
Psychodopygus amazonensis	39	2	6	47
Nyssomyia antunesi*	40	2	2	44
Bichromomyia flaviscutellata*	28	10	2	40
Nyssomyia umbratilis*	29	8	1	38
Trichophoromyia flochi (Abonnenc & Chassignet)	30	-	-	30
Nyssomyia fraihai (Young & Porter)	4	-	20	24
Psychodopygus corossoniensis (Le Pont & Pajot)	18	3	1	22
Psychodopygus carrerai	20	1	-	21
Trichopygomyia trichopyga (Floch & Abonnenc)	19	-	-	19
Psathvromvia aragaoi (Costa Lima)	14	1	3	18
Psychodopygus geniculatus (Mangabeira)	11	-	1	12
Nyssomyia richardwardi* (Ready & Fraiha)	2	4	1	7
Micropygonyja rorotaensis	-	3	1	5
Psathyromyja scaffi (Damasceno & Arouck)	5	-	-	5
Trichothoromyja eurytyvga (Martins Falcão & Silva)	5	-	_	5
Psathuromyja lutziana (Costa Lima)	3	1		4
Trychophoromyja dunhami (Causey & Damasceno)	4	-		4
Fuandromyia intrai (Young & Arias)	3			3
Psathuromyia abomanci (Floch & Chassignet)	3	_	_	3
Trichotygomyia wagleyi (Causey & Damasceno)	3			3
Microtygomyja wicrotyga (Mangabeira)	2			2
Micropygomyju micropygu (Wangabena)	- 1	- 1	-	2
Nucropygomyta phosa (Damasceno & Causey)	1	1	-	2
Ryssomyta winimani (Antanes & Coutinno)	2	2	-	2
Posthyromyta drashela (Mangahaira)	2	-	-	2
Posthemounia bizanizulata* (Duar)	2	-	-	2
Producto de transmonte de transmonte de la companya	2	-	-	2
<i>Viscondopygus claustrei</i> (Abonnenc, Leger & Fauran)	2	-	-	2
<i>Viannamyia tuberculata</i> (Mangabelra)	<u>∠</u>	-	-	۲ ۲
Bichromomyla reaucta (Feliciangeli, Kamirez Perez & Kamirez)	1	-	-	1
Evanaromyla infraspinosa (Mangabeira)	1	-	-	1
Evandromyta saulensis (Floch & Abonnenc)	-	1	-	1
Evanaromyla sericea (Floch & Abonnenc)	-	-	1	1
Lutzomyla evangelistai (Martins & Fraiha)	1	-	-	1
Micropygomyia trinidadensis (Newstead)	1	-	-	1
Migonemyia gorbitzi (Blancas)	1	-	-	1
Pressatia choti (Floch&Abonnenc)	-	1	-	1
Psathyromyia punctigeniculata (Floch & Abonnenc)	1	-	-	1
Psychodopygus ayrozat [*]	1	-	-	1
Psychodopygus hirsutus* (Mangabeira)	-	-	1	1
Psychodopygus paraensis*	-	1	-	1
Psychodopygus squamiventris squamiventris * (Lutz & Neiva)	1	-	-	1
Irichophoromyia castanheirai (Damasceno, Causey & Arouck)	1	-	-	1
Trichophoromyia gibba (Young & Aryas)	1	-	-	1
Viannamyia furcata (Mangabeira)	-	1	-	1
Total	964	69	156	1,189

*Sand fly species involved in Leishmania spp. Ross, 1903 (Kinetoplastida: Trypanosomatidae) infection, according to Lainson (2010) and Ready (2013).



Fig. 2. Sand fly abundance (A) and richness (B) in forest edge, indoor, and outdoor habitats along highway BR-319, State of Amazonas, Brazil.



Fig. 3. Number of sand fly species exclusive to or shared by (A) indoor (In), outdoor (Out) and forest edge (Fe) habitats; and (B) northern (Ns) and southern (Ss) segments along highway BR-319, State of Amazonas, Brazil.

corroborated by the PERMANOVA results. Sand fly fauna differed significantly between habitats (pseudo- $F_{2,31} = 0.1237$; P = 0.002) and these variations in composition can be attributed to habitat differences because multivariate dispersion was homogeneous among groups (similar distances of dispersion to the centroids; PERMDISP test; pseudo- $F_{2,31} = 1.8581$; P = 0.191; Table 2).

Road Segments

No difference was observed between northern and southern road segments with respect to sand fly abundance (H = 0.0877, df = 1, P = 0.77, Fig. 4a) and richness (H = 0.0203, df = 1, P = 0.89, Fig. 4b). Nearly 65% of the species captured were unique to either the northern or southern segment of highway BR-319 (Table 3, Fig.3b), but multivariate variance and beta-dispersion analyses showed a significant overlap of phlebotomine species across both road segments (PERMANOVA and PERMDISP, P > 0.05, Table 2).

Discussion

At the global level, the spread of vectors has been attributed to international traffic volume and climatic similarities between source-sink localities, which, combined, can determine the success of vector establishment in a new area (Tatem et al. 2006a). Nevertheless, other factors such as vegetation play an important role in determining vector colonization (Reisen 2010). Along highway BR-319, in the Brazilian Amazon, we verified that Phlebotominae vector abundance, richness, and species composition are strongly affected by habitat type, and the presence of hosts and humans. Due to their relative isolation from one another, the northern and southern segments of highway BR-319 exhibit quite characteristic vector fauna. Although 65% of species were exclusive to one of the two segments, permutational analyses based on relative abundance revealed a fair level of phlebotomine fauna similarity between both segments. Furthermore, a general pattern of large number of species with few specimens (singletons and doubletons) and few dominant species was also observed (Pessoa et al. 2007, Silva et al. 2010, Rosário et al. 2017).

Disregarding geographical extension, differential deforestation rates along highway BR-319 could be responsible for the current spatial pattern in sand fly species composition. In southeastern Brazil, the replacement of natural forests with agriculture has impacted Leishmania spp. transmission by altering vector habits, domiciliation levels among sand fly species, and mammal reservoir movements (among peridomiciliar areas, plantations, and residual forest) (Gomes 1994). In the Amazon region, peak abundance of Nyssomyia umbratilis (Ward & Fraiha), a vector of Leishmania guyanensis (Floch), was observed immediately after selective timber extraction; nevertheless, sand fly fauna in this region returned to its previous, lower level of abundance 1-2 mo after timber extraction was complete (Freitas et al. 2003). Pessoa et al. (2007) demonstrated that selective logging has greatly impacted sand fly fauna in the Brazilian Amazon-the rate of trypanosomatids infection among females significantly declined after the selective timber harvest. However, medically important sand fly species can be apparently tolerant to the modified environments. At instance, behavioral changes in the species Bichromomyia flaviscutellata (Mangabeira) and N. umbratilis have been associated with dam waterlogging in the hydroelectric system at the Amazonian Jari basin (Furtado et al. 2016). In the current study, large tracts of deforestation were not observed in the sampled areas, yet a significant number of sand flies (69) were captured inside domiciles. This situation seems to be common in the Amazon region (Machado et al. 2012, Chagas et al. 2018), and deserves more investigation. Two-hundred and forty-six phlebotomine individuals were obtained in the intradomicile (mainly Sciopemvia sordellii [Shannon & Del Ponte] and Micropygomvia rorotaensis [Floch & Abonnenc]) during 9,216 h of sampling in a rural settlement in the central Amazon, Brazil (Chagas et al. 2018). Rural settlements and road building have been identified as the landscape changes that are primarily responsible for forest depletion and management (Fearnside 2017).

From 1978 to 1981, sand fly fauna was surveyed at eight localities along highway BR-319, by Castellón et al. (1994). Captures were

Table 2. Permutational analyses of variance (PERMANOVA) and dispersion (PERMDISP) results, based on Bray–Curtis dissimilarities for sand fly species composition and their relative abundance in habitats and road segments along highway BR-319, State of Amazonas, Brazil

	Analyses	Source	df	SS	MS	pseudo-F	R^2	Р
Н	PERMANOVA	Habitat	2	1.6084	0.8042	2.1886	0.1237	0.002
А	Multivariate	Residuals	31	11.3906	0.3674		0.8763	
В	Variance	Total	33	12.9990	NA		1.0000	
Ι								
Т	PERMDISP	Habitat	2	0.0650	0.0325	1.8581		0.191
Α	Multivariate	Residuals	31	0.5425	0.0175			
Т	Beta-dispersion							
	PERMANOVA	Road Segment	1	0.2679	0.2679	0.6735	0.0206	0.836
R	Multivariate	Residuals	32	12.7310	0.3978		0.9794	
0	Variance	Total	33	12.9990			1.0000	
А								
D	PERMDISP	Road Segment	1	0.0011	0.0011	0.1041		0.73
	Multivariate	Residuals	32	0.3401	0.0106			
	Beta-dispersion							

df = degrees of freedom; MS = mean sum of squares; pseudo-F = F value obtained by 999 permutations; SS = sum of squares.



Fig. 4. Sand fly abundance (A) and richness (B) along the northern and southern segments of highway BR-319, State of Amazonas, Brazil.

made using center for disease control (CDC) light traps, tree trunk swamp sampling, and human-bait captures, and 57 phlebotomine species were recorded. Of these, 52 species were obtained with CDC traps installed 1 m and 6 m above the forest floor. This was the first survey conducted along highway BR-319. Despite the temporal gap (1994–2018) and methodological differences, that study and the current one found 28 species in common, and both studies found that the most frequent and abundant species were *T. ubiquitalis*, *Psychodopygus chagasi* (Costa Lima), *Psychodopygus davisi* (Root), and *Nyssomyia antunesi* (Coutinho) (Castellón et al. 1994, based on Table 3).

The physical road itself may have shaped the spatial distribution of sand flies along highway BR-319. This was found to be the case in north-eastern Venezuela where a higher number of malaria cases occur in rural villages along main roads than along secondary roads; however, this variable (main road) was not a good predictor in models that evaluated malaria persistence in the region (Grillet et al. 2010). A clear spatio-temporal pattern was observed in the dissemination of visceral leishmaniasis in the State of Mato Grosso do Sul, Brazil. Historically, the implementation of three traffic routes was determinant: a railroad (1909–1952), the federal BR-262 highway (≈1980), and the Brazil-Bolivia gas pipeline (1998–2004) (Correa Antonialli et al. 2007). In this context, our habitat sampling data highlighted the indoor occurrence of *P. davisi, B. flaviscutellata, T. ubiquitalis,* and *N. umbratilis*—all of which are either implicated or relevant vectors of *Leishmania* spp (Ready 2013). Although there was no significant variation in species composition between road segments, the primary vector species were different: *N. antunesi* was more abundant in the southern segment, while *N. umbratilis* and *T. ubiquitalis* were captured in similar numbers along both road segments. Fraiha et al. (1978) made a survey of anthropophilic sand flies along Transamazonic highway BR-230, where it intersects BR-319. Human bait was used to capture specimens of *Psychodopygus ayrozai* (Barretto & Coutinho) (formerly referred as *P. titinabulus*), *Psychodopygus carrerai* (Barretto), *P. chagasi, P. davisi, Psychodopygus paraensis* (Costa Lima), and two undescribed species at that moment. The current study captured all of these species in indoor habitats and some of these species are involved in *Leishmania* infection (Table 1).

Drastic alterations of the environment modify the ecology of vector/reservoir/parasite and their interactions, which potentially changes the epidemiology of leishmaniases. In the new epidemiological situation created by deforestation, some wild mammalian hosts may invade areas inhabited by humans and thus come in contact with sand flies that are either in the process of domiciliation or totally adapted to the new environment. Eclectic feeding habits would allow such sand flies to transmit the parasite between humans and domestic mammals (da Costa et al. 2007, Ramos et al. 2014). Although the abundance

Sand fly species	Ns	Ss	Total
Trichophoromyia ubiquitalis	302	281	583
Trichopygomyia rondoniensis	65	22	87
Psychodopygus chagasi	56	17	73
Psychodopygus davisi	9	51	60
Psychodopygus amazonensis	20	27	47
Nyssomyia antunesi	12	32	44
Bichromomyia flaviscutellata	22	18	40
Nyssomyia umbratilis	23	15	38
Trichophoromyia flochi	30	-	30
Nyssomyia fraihai	20	4	24
Psychodopygus corossoniensis	10	12	22
Psychodopygus carrerai	7	14	21
Trichopygomyia trichopyga	12	7	19
Psathyromyia aragaoi	18	-	18
Psychodopygus geniculatus	-	12	12
Nyssomyia richardwardi	-	7	7
Micropygomvia rorotaensis	3	2	5
Psathvromvia scaffi	5	-	5
Trichophoromyia eurypyga	5	-	5
Psathyromyia lutziana	1	3	4
Trychophoromvia dunhami	4	_	4
Evandromvia inpai	3	-	3
Psathvromvia abonnenci	3	-	3
Trichopygomvia waglevi	3	-	3
Micropygomvia micropya	2	-	2
Micropygomvia pilosa	1	1	2
Nyssomyia whitmani	- 1	1	2
Psathyromyja brasiliensis	2	-	2
Psathyromyja dendrothyla		2	2
Psathyromyja higeniculata	2	-	- 2
Psychodotygus claustrei	- 1	1	- 2
Viannamvia tuberculata	2	-	- 2
Richromomyja reducta	-	1	- 1
Evandromvia infrastinosa	1	-	1
Fuandromvia saulensis	-	1	1
Fuandromnja sericea		1	1
I utzomvia evangelistai	-	1	1
Microtwgomvia trinidadensis	1	-	1
Migonemuja gorbitzi	1		1
Pressatia choti	1		1
Psathyromyja punctigeniculata	1	1	1
Psychodotway awardi	- 1	1	1
Psychodotways hirsytys	1		1
Psychodopygus hirsuius Psychodopygus paraansis	1		1
Pencho dotrogue cauaminantrie cauaminantrie	1	- 1	1
r syenouopygus synumivenins synumivenins Trichothoromvia castanhairai	- 1	-	1
Trichothoromyja gibba	1	-	1
Viamamia furcata	-	1	1
Total		1 527	1 1 100
10(a)	0.02	537	1,107

Table 3. Number of individuals and sand fly species captured with light traps along the Northern (Ns) and Southern (Ss) segments of highway BR-319, State of Amazonas, Brazil, in 2011

and richness of sand fly fauna was found to be significantly different between BR-319 highway habitats, vector species that were present in both indoor and outdoor habitats were also present in forest edge habitats (*P. davisi*, *B. flaviscutellata*, *T. ubiquitalis*, and *N. umbratilis*). The presence of these species in all habitats may increase the risk of human *Leishmania* transmission to man.

The literature shows that the construction of roads and highways alters sand fly composition and behavior and thereby changing the transmission dynamics of leishmaniases (e.g., Fraiha et al. 1978, Lainson 1988, Confalonieri et al. 2014). This problem has been also widely discussed in relation to other vectors and associated etiological agents (Pinheiro et al. 1977, Katsuragawa et al. 2008). Interestingly, there is a convergence between these previous studies and the present one, despite their considerable spatiotemporal distance. Ecological changes observed in the Amazon region can include the appearance of Leishmaniasis triad (i.e., vector/reservoir/ parasite) and subsequently human disease arises in areas where they were not previously documented.

The construction of roads and highways places humans in direct contact with sand fly vectors and wild reservoirs. It is therefore extremely important to conduct further studies that examine the impact that this type of anthropic action has on the spread of leishmaniases, considering sampling point distances, extent of forest loss, and sand fly fauna composition before and after landscape changes.

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