Phlebotomine sand flies (Diptera: Psychodidae) associated with changing patterns in the transmission of the human cutaneous leishmaniasis in French Guiana

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Between March 2000 and December 2001 a survey of the sand flies (Diptera: Phlebotominae) of French Guiana was carried out during 14 nights of captures with CDC light-traps and Malaise traps, and resulted in the collection of 2245 individuals of 38 species. The most abundant species were Lutzomyia (Trichophoromyia) ininii *Floch & Abonnenc,* Lu. (Psychodopygus) squamiventris maripaensis *Floch & Abonnenc, and* Lu. (Nyssomyia) flaviscutellata *Mangabeira. Half of the collected sand flies females were dissected under field conditions and five species were found harboring* Leishmania-*like parasites*. *The* Leishmania *(Kinetoplastidae: Trypanosomatidae) species were identified by molecular typing, and for the first time* Lu. (Nys.) flaviscutellata *was found harboring* Leishmania (Viannia) guyanensis *and* Lu. (Tri.) ininii *harboring unknown* Leishmania*. The first record for French Guiana of* Lu. (Psy.) squamiventris maripaensis *harboring* L. (V.) naiffi*, was also reported. The patterns of diversification of the human cutaneous leishmaniasis transmission in French Guiana are discussed.*

Key words: sand flies - *Lutzomyia* - *Leishmania* - human cutaneous leishmaniasis - French Guiana

With about 300 cases of human cutaneous leishmaniasis (HCL) reported annually (Carme 2001, Lightburn et al. 2001) and some extreme situations (Couppié et al. 2004), this disease still represents a major public health problem in French Guiana. The first HCL cases were confirmed in French Guiana in 1943 (Floch 1943) and past studies showed that *Leishmania (Viannia) guyanensis* is the predominant species of *Leishmania* (Floch 1957, Dedet et al. 1989, Raccurt 1996) but *L. (L.) amazonensis*, *L. (V.) braziliensis*, *L. (V.) lainsoni,* and *L. (V.) naiffi* have also been diagnosed (Dedet et al. 1985, Raccurt et al. 1995, Basset et al. 2001, Pratlong et al. 2002). The sand flies of French Guiana have been extensively studied until the 1990s and 77 sand flies species were reported (Floch & Abonnenc 1952, Léger et al. 1977). The entomological studies have shown that the sand fly *Lutzomyia (Nyssomyia) umbratilis* Ward & Fraiha was the major vector of *L. (V.) guyanensis* (Le Pont & Pajot 1980, Pajot et al. 1982). The other sand flies species found infected with *Leishmania* parasites were *Lu. (Nys.) flaviscutellata* Mangabeira, harboring *L. (L.) amazonensis* (Dedet et al. 1989) and *Lu. (Lut.) gomezi* Nitzulescu and *Lu. (Psy.) yullii pajoti* Abonnenc, Léger & Fauran harboring unidentified *Leishmania* species (Killick-Kendrick 1990, Young & Duncan 1994). The sand flies species transmitting *L. (V.) braziliensis*, *L. (V.) lainsoni*, and *L. (V.) naiffi* are partly known in other South-American countries (Naiff et al. 1991, Young & Duncan 1994, Feliciangeli 2004), but have not been so far identified in French Guiana. Furthermore, in the last ten years, the geographical distribution of the HCL transmission in French Guiana has changed, previous foci have disappeared and new foci are reported. The people at risk for HCL contamination have also changed and now the military personnel represent 25 to 85% of the annual number of cases (Lightburn et al. 2001). Finally, the environment of French Guiana is continuously changing due to human pressure on the forested area for road construction, housing development, and gold mining.

For these reasons, a research program to update our knowledge on the HCL transmission in French Guiana was started in 2000. The objectives of the program were to identify the species of sand flies acting as vector of HCL in the probable contamination places, to identify the *Leishmania* parasites when present in the collected sand flies, to characterize the foci and finally to collect information on the biology and ecology of the species.

MATERIALS AND METHODS

The study sites - From March 2000 to December 2001, 14 collections of sand flies were carried out in different areas of French Guiana. The prospected locations were chosen in relation with the report of HCL cases during the past weeks or the month before the cap-

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tures. The report of the HCL cases remained anonymous and only geographical locations were given by the Direction Départementale des Affaires Sanitaires et Sociales (DDASS) reports and the military headquarters for the study. The procedures of the report of human cases were in accordance with the ethical standards and the research program was approved by the DDASS of French Guiana representing the French Ministry of Health.

Different ecological conditions of French Guiana were prospected for sand flies collection at different periods of the year. The forested areas along the littoral at Regina were prospected in March, April, October, and November 2000, and at Nancibo in December 2001 (Fig. 1). The forested areas in the interior of the country at Maripasoula and the Upper Maroni River were prospected in October 2000 and at Saül in December 2000. The savannas areas at Montsinery were prospected in February and March 2001 and at Sinnamary in September 2001. Finally, some agricultural areas were also prospected at Cacao in June 2001, Montsinery in July 2001 and Saül in October 2001 (Fig.1, Table).

The sand flies collections and species identification - Each collection of sand flies included two consecutive nights of capture with three types of traps, one CDC light-trap (Bioquip model), one modified CDC light-trap (with a green plastic hat and a more powerful light-bulb, Gantier model) and one Malaise trap. One or two traps of each type were left between 6 p.m. and 7 a.m. in different places (close to trees, bushes, armadillos' hole, etc.) and at different heights, varying from 0.30 to 10 m. The sand flies were collected in the traps at regular intervals during the nights. The intervals were in most cases, each 3 h, which is at 9 p.m., 12 p.m., 3 a.m., and 6 a.m.

The collected sand fly females were dissected under field conditions just after the empting of the trap, to search for natural infection to *Leishmania* spp. The infected sand flies were kept in Dulbecco's Modified Eagle's Medium (ref D5523, Sigma Chemicals Co.) supplemented with fetal bovine serum (ref F2442, Sigma) and brought to the Laboratory of Immunology of the Pasteur Institute of French Guiana for a tentative culture of the *Leishmania* parasite. Some of the positive samples

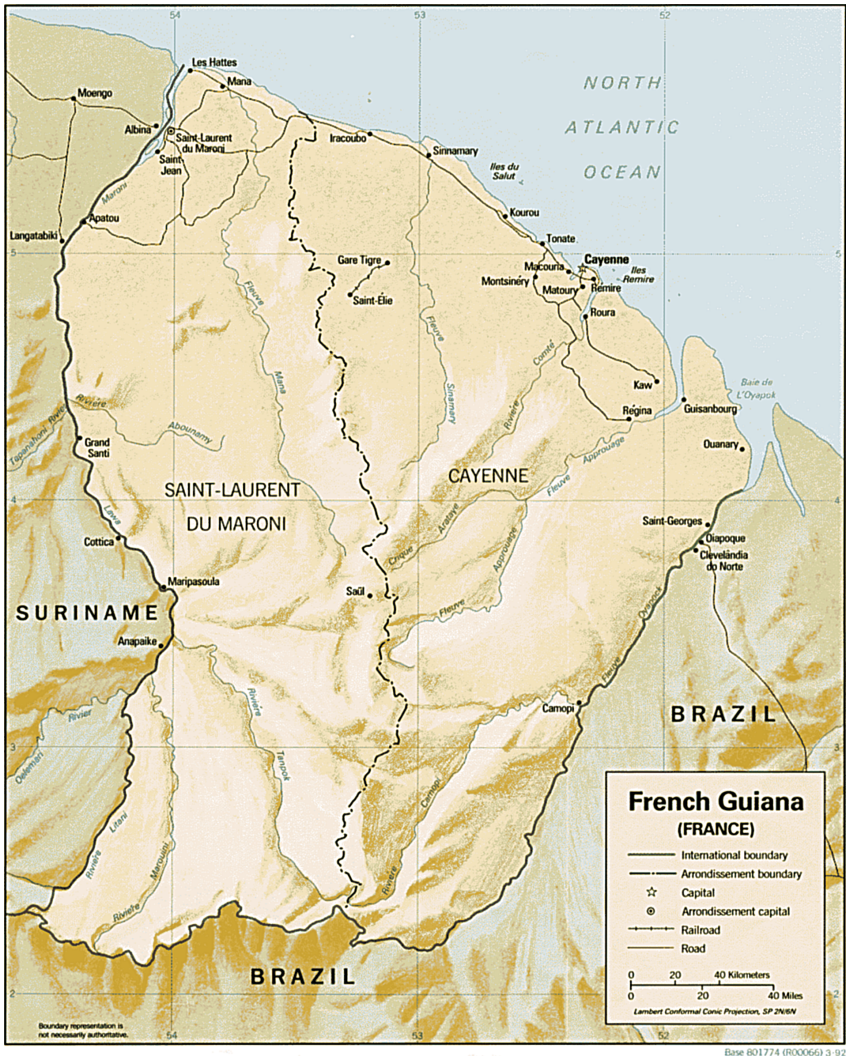

Fig. 1: map of French Guiana with the areas prospected (underlined) during the sand flies survey from March 2000 to December 2001.

TABLE

List of the sand flies species collected between March 2000 and December 2001 in French Guiana. Among the 14 collections, in only one collection the individuals could not be identified. The numbers in parenthesis are the number of dissected females for the species found positive for *Leishmania* parasites. The report of vector species is based on Young and Duncan (1994)

	Place and date of collection																										Total	
	Régina								Maroni River		Saül		Montsinery								Cacao		Sinnamary		Nancibo			
	March 2000		April 2000		October 2000		November 2000		October 2000		December 2000		October 2001		February 2001		March 2001		July 2001		June 2001		September 2001		December 2001			
List of species	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f
1 *Brumptomyia (Brumptomyia) pintoi* (Costa Lima 1932)																	1										1	0
2 *Brumptomyia (Brumptomyia) travassosi* (Mangabeira 1942)							1	1																			1	1
3 *Lutzomyia (Ara.) aragaoi* (Costa Lima 1932)	2			2			1	2				3															3	7
4 *Lutzomyia (Ara.) brasiliensis* (Costa Lima 1932)																		1									0	1
5 *Lutzomyia (Dre.) dreisbachi* (Causey & Damasceno 1945)							3			1		1	21	19	3	2							8	4		2	35	29
6 *Lutzomyia (Eva.) brachyphalla* (Mangabeira 1941)	1						4	3					1	1				4	1							1	7	9
7 *Lutzomyia (Eva.) infraspinosa* (Mangabeira 1941)			2	3			4	3	4	13			5	10	3	4	3		2	5	8	18		1		2	31	59
8 *Lutzomyia (Eva.) monstruosa* (Floch & Abonnenc 1944)			1											1								1		2			1	4
9 *Lutzomyia (Mic.) micropyga* (Mangabeira 1942)														4													0	4
10 *Lutzomyia (Mig.) pacae* (Floch & Abonnenc 1943)																									1	1	1	1
11 *Lutzomyia (Nys.) anduzei* (Rozeboom 1942) [a]															1												1	0
12 *Lutzomyia (Nys.) flaviscutellata* (Mangabeira 1942) [b]		1				1	1	22 (7)	3	15		3			2	5		5		6	1		6	7	25	104	38	169 (43)
13 *Lutzomyia (Nys.) umbratilis* (Ward & Fraiha 1977) [a]				8				1	1	1	1	17	27	4	1			2		9	7	18	1	2		2	38	64
14 *Lutzomyia (Nys.) yuilli pajoti* (Abonnenc, Léger & Fauran 1979)											7			24	35												24	42
15 *Lutzomyia (Osw.) rorotaensis* (Floch & Abonnenc 1944)									1	6			8			1				1							9	8
16 *Lutzomyia Pressatia* sp.										1																	0	1
17 *Lutzomyia (Pre.) choti* (Floch & Abonnenc 1941)										2			1	1								1					1	4
18 *Lutzomyia Psathyromyia* sp.																								1			0	1
19 *Lutzomyia (Psa.) punctigeniculata* (Floch & Abonnenc 1944)																										1	0	1
20 *Lutzomyia (Psa.) shannoni* (Dyar 1929)																					1				1		2	0
21 *Lutzomyia (Psy.) amazonensis* (Root 1934)																										1	0	1
22 *Lutzomyia (Psy.) ayrozai* (Baretto & Coutinho 1940)								2				1				1				1					10		10	5
23 *Lutzomyia (Psy.) claustrei* (Abonnenc, Léger & Fauran 1979)								6		4					10	3	2	9		5	3		2	1	21	44	38	72
24 *Lutzomyia (Psy.) corrossoniensis* (Le Pont & Pajot 1978)				1																							0	1
25 *Lutzomyia (Psy.) davisi* (Root 1934)															1											1	1	1
26 *Lutzomyia (Psy.) guyanensis* (Floch & Abonnenc 1941)		2		1																							0	3
27 *Lutzomyia (Psy.) hirsuta* (Mangabeira 1942)	6			1				1						2	15	2			1	1					16	20	38	27
28 *Lutzomyia (Psy.) nocticola* (Young 1973)								3											4							1	4	4
29 *Lutzomyia (Psy.) panamensis* (Shannon 1926) [c]		1																			2					5	2	6
30 *Lutzomyia (Psy.) paraensis* (Costa Lima 1941)				4				3									2	1	1						53	74	56	82
31 *Lutzomyia (Psy.) squamiventris maripaensis* (Floch & Abonnenc 1946) [d]							1	8	1					14	7	14	1		2	13		2	1	3 (3)	64	153	77	207 (56)
32 *Lutzomyia (Sci.) fluviatilis* (Floch & Abonnenc 1944)														1										1	2		2	2
33 *Lutzomyia (Sci.) sordelli* (Shannon & Del Ponte 1927)		1		1			6	7	1	1		1			1	3			2	2	5	4	1	3	1	1	17	24
34 *Lutzomyia (Tri.) brachypiga* (Mangabeira 1942)															1												1	0
35 *Lutzomyia (Tri.) flochi* (Abonnenc & Chassignet 1948)					1																						1	0
36 *Lutzomyia (Tri.) ininii* (Floch & Abonnenc 1943)	15	20	34	52	13	5	71	68	2				3	8	39	51 (33)	23	14	3	10 (10)	3			1	2	3	208	232(136)
37 *Lutzomyia (Tri.) ubiquitalis* (Mangabeira 1942) [e]	22	10	8	6	5	1	56	2	2				3	2	3				2				4		1	3	106	24
38 *Lutzomyia (Tri.) trychopyga* (Floch & Abonnenc 1945)			2												4	1	1	1					3	1	2	1	12	4
39 Total	46	35	47	79	19	7	148	132	15	51	1	26	93	102	91	87	32	37	18	53	30	44	26	27	199	420	765	1100

a: vector of *L. (V.) guyanensis; b*: vector of *L. (L.) amazonensis; c*: vector of *L. (V.) braziliensis; d*: vector of *L. (V.) naiffi.*

were also sent to the Laboratory of Parasitology of the Faculty of Medicine of Montpellier for identification by molecular typing, which was carried out by systematic sequencing of a part of the RNA pol II large sub-unit gene.

All sand flies collected were brought to the Laboratory of Entomology and processed for identification. Processing and mounting of the sand flies males and females was made according to the following protocol (Tran-Kiem 2000): the individuals were rinsed in distilled water until flotation, then left 10 min in the warmed up Marc-André Liquid (distilled water 300 g, chloral hydrate 400 g, acetic acid 300 g) for lightening, then rinsed twice with alcohol 90°, then mounted in a drop of Creosote, the exceeding of Creosote was removed and the Euparal mounting liquid was added. The species were then identified firstly with the informatics' key (Lebbe et al. 1987) and confirmed with the available descriptions (Floch & Abonnenc 1952, Young & Duncan 1994). The Laboratory of Mycology of the Faculty of Pharmacy of Châtenay-Malabry (Paris IX) further confirmed the species identifications. The molecular confirmation of the *Lutzomyia* species was not performed due to the absence of reliable methods for the sand flies species of French Guiana.

RESULTS

A total of 14 collections were carried out and 2245 sand flies were collected including 1265 females, and 980 males, among which 1865 individuals (83%) were identified (1100 females and 765 males) (Table). A lot of individuals (17%) could not be identified because they were either damaged during the transportation between the field and the laboratory, damaged during the laboratory process of lightening and mounting, or because the specimens could not be attributed to a species description. A total of 38 species were collected, which is close to the 43 species reported during the last sand flies survey in French Guiana (Léger et al. 1977), and about half of the 78 species previously described from French Guiana (Floch & Abonnenc 1952, Oliveira et al. 2001). The most abundant species in our survey were *Lu. (Tri.) ininii, Lu. (Psy.) squamiventris maripaensis* and *Lu. (Nys.) flaviscutellata* and the 3 species were found harboring *Leishmania-like* flagellates parasites (Table). The most abundant species reported during the 1977 survey were *Lu. (Tri.) ubiquitalis* Mangabeira followed by *Lu. (Tri.) ininii*, and *Lu. (Psy.) squamiventris maripaensis*. Thus, the overall results do not show any significant changes in the species diversity and densities, except for the high density of *Lu. (Tri.) ubiquitalis* collected in Maripasoula in 1977 (Léger et al. 1977). The collection made in Maripasoula during our survey resulted in 32 individuals that could not be identified.

About half (534 females, *i.e.* 48.5%) of the sand flies females collected were dissected in the field, examined from the presence of *Leishmania* parasites and placed on culture when found positive. Five sand flies females were found harboring *Leishmania*-like flagellates parasites, with an overall infection rate of 0.84%. Four positive sand flies could be identified. No *Leishmania* culture was successful, but molecular typing on four of these *Leishmania* field samples allowed the identification of 2 *Leishmania* species. One female of *Lu. (Nys.) flaviscutellata* was found harboring *L. (V.) guyanensis* and one female of *Lu. (Psy.) squamiventris maripaensis* was found harboring *L. (V.) naiffi.* The other two molecular typing were significant of a *Leishmania* genus, but the species were not identified.

The highest sand flies density and species diversity were found during high and medium rainfalls periods, in natural and forested areas (December 2001 at Nancibo). In the contrary, the lowest sand flies density were found during the dry season, in forested and savanna areas, submitted to important ecological changes due to military activities (Regina in August and October 2000), agricul-

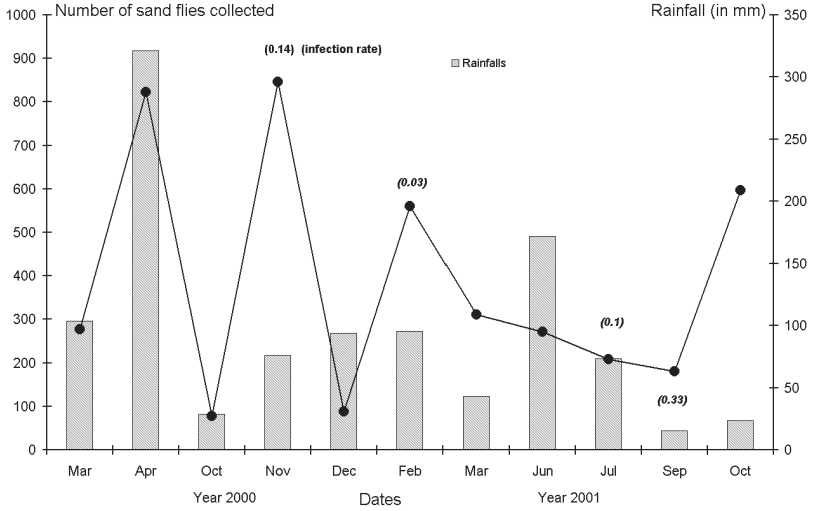

Fig. 2: fluctuations in the densities of the sand flies collected in French Guiana between March 2000 and December 2001, in the disturbed forested areas of Regina, Saul, and Cacao (-●-) and in the savannah areas submitted to agricultural pressure of Montsinery and Sinnamary (-◇-) with the monthly rainfall fluctuations in the location of the collection. The numbers in parenthesis represent the infection rates of the infected species.

tural practices (Sinnamary in September 2001), and gold mining activities (Saül in December 2000) (Fig. 2). The infected sand flies females were collected in October and November 2000 at Regina, in February and July 2001 at Montsinery and in September 2001 at Sinnamary.

DISCUSSION

This is the first report of *Lu. (Nys.) flaviscutellata* harboring *L. (V.) guyanensis*. This species is also considered a proven vector of *L. (L.) amazonensis* (Killick-Kendrick 1990). This positive sand fly was collected in Regina in November 2000, and the overall infection rate for this species was 2.32%. During the same capture, only one female of *Lu. (Nys.) umbratilis*, the proven vector of *L. (V.) guyanensis* in French Guiana (Le Pont & Pajot 1980) was collected (Table). The second species of *Leishmania* identified, *L. (V.) naiffi* was found in a female of *Lu. (Psy.) squamiventris maripaensis*. This sand fly species was reported as a vector of *L. (V.) naiffi* in Brazil (Naiff et al. 1991), but this is the first time that *Lu. (Psy.) squamiventris maripaensis* can be considered a vector of *L. (V.) naiffi* in French Guiana, where the HCL species has already been reported in human cases (Basset et al. 2001, Pratlong et al. 2002). The positive female of *Lu. (Psy.) squamiventris maripaensis* was collected in September 2001 in the savanna area of Sinnamary and the overall infection rate for this species was 1.78%. Two females of *Lu. (Tri.) ininii* were found harboring unidentified *Leishmania* flagellates with an overall infection rate of 1.47%, and this could be the first report of *Lu. (Tri.) ininii* as probable vector of HCL.

The results are showing a natural *Leishmania* circulation in French Guiana expanding all year long and widespread in the country, in particular in areas submitted to important ecological changes due to military activities (Regina) and agricultural practices (Montsinery and Sinnamary). The infected sand flies were collected during periods with low rainfalls (October 2000 and September 2001) and medium rainfalls (November 2000 and February 2001). However, the highest infection rates were found in September 2001 at Sinnamary (0.33) and November 2000 at Regina (0.14). Furthermore, when the densities of the potential vectors are observed, *Lu. (Nys.) flaviscutellata* was most abundant in November 2000 (at Regina) and December 2001 (at Nancibo), *Lu. (Tri.) ininii* was must abundant in November 2000 (at Regina) and *Lu. (Psy.) squamiventris maripaensis* was most abundant in December 2001 (at Nancibo), confirming a transmission season mostly at the end of the year when the dry season is also ending and the rainfalls start to increase (Fig. 2). Some HCL foci could be detected at Regina, Montsinery and Sinnamary, with not only report of clinical cases but also presence of infected sand flies.

Although, the annual HCL incidence do not show a recent increase in French Guiana because 2.3 cases per 1000 inhabitant were reported in 1989 (Dedet 1990) against 1.5 to 2 cases per 1000 inhabitant reported between 1986 and 2000 (Carme et al. 2001), the transmission patterns are changing with an increase in the number of *Leishmania* species diagnosed (Basset et al. 2001) and the report of new sand flies vector. HCL is probably endemic in the Amazonian region since the arrival of the first human beings (Andrade Fiho & Brazil 2003) and this is demonstrated by the great diversity of HCL transmission found in this region. French Guiana is included in the Amazon geographic area and the small number of *Leishmania* parasites and sand flies vectors previously found in this country may be due to a report of cases mostly for the population living along the littoral area. Actually, French Guiana is submitted to an important human pressure of deforestation for gold mining, road construction and agricultural practices. Consequently, the population exposed to the natural leishmaniasis forested cycles is increasing and the HCL transmission is gaining in diversity. The first report of three sand flies species found infected with *Leishmania* parasites in French Guiana, *Lu. (Nys.) flaviscutellata* for *L. (V.) guyanensis*, *Lu. (Psy.) squamiventris maripaensis* for *L. (V.) naiffi*, and *Lu. (Tri.) ininii* for unknown *Leishmania* species is the major finding of this survey, and suggests that more extensive studies should be undertaken for a better knowledge of HCL transmission patterns and prevention in the Amazonian ecosystem of French Guiana.

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