

LEISHMANIASIS IN BOLIVIA. II. THE INVOLVEMENT OF
PSYCHODOPYGUS YUCUMENSIS AND
PSYCHODOPYGUS LLANOSMARTINSI IN THE SELVATIC TRANSMISSION
CYCLE OF *LEISHMANIA BRAZILIENSIS BRAZILIENSIS* IN A
LOWLAND SUBANDEAN REGION

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An epidemiological survey of the vectors of cutaneous leishmaniasis ("espúndia" type) was carried out in the Alto Beni region of Bolivia, an area of Andean foothills at the Eastern limit of the Amazonian lowlands. The climate is typical wet tropical (15°S latitude). Anthropophilic phlebotomine sandfly species were sampled at 20 sites, all forested. The importance of species from the Psychodopygus group, already suspected as a vector in the transmission of Leishmania from the braziliensis complex, was confirmed by: 1) the aggressiveness and diversity of the species encountered (83% of catches, nine species), 2) the discovery of a new anthropophilic species, P. yucumensis and 3) the isolation of a strain of Leishmania braziliensis braziliensis indistinguishable from human strains from the same area, from two species, P. llanosmartinsi and P. yucumensis.

Key words: vector - *Psychodopygus* - cutaneous leishmaniasis - subandean region - Bolivia

Ranging from Venezuela to Argentina, the moist Eastern foothills of the Andes, below 1.800m elevation, are well-known as an endemic area for cutaneous leishmaniasis, and especially for muco-cutaneous leishmaniasis or "espúndia" (Escomel, 1922; Herrero, 1968; Lainson & Shaw, 1979) caused by *Leishmania braziliensis braziliensis*.

The present study was undertaken in the Alto Beni basin of Bolivia, within the tropical zone at about 15° South latitude. These predominantly forested mountainous areas connect the wide Altiplano highlands with the Beni lowlands. Muco-cutaneous leishmaniasis has mainly been described from migrants and natives from these areas (Desjeux, 1974; Desjeux, Quilici & Lapiere, 1974; Walton, Chinel & Eguia, 1973; Walton & Chinel, 1979).

From August 1983 to August 1984, a study among the employees of a petroleum company prospecting in the Alto Beni area highlighted the importance of cutaneous leishmaniasis as the main health problem (Desjeux et al., 1986 submitted for publication). Using the transport facilities of the company, collections of sandflies were made at labor camps and in the surrounding forest, followed by dissections and isolation of *Leishmania* strains. Sandfly captures from humans revealed the aggressiveness of an unrecorded species, recently described under the name of *Psychodopygus yucumensis* (Le Pont et al., 1986). In this paper we present the results of this entomological survey.

MATERIAL AND METHODS

Study area - The study area (Figs. 1 and 2) is located at the junction of the Tuichi, Quiquibey and Hondo Rivers, tributaries of the Alto Beni River. They run diagonally (West to East) across the last two subandean cordilleras (800-1.200m high). These cordilleras are separated by about 40km and run in a Northwest to Southeast direction. Capture sites were in the valley bottoms at 250m as well as on the slopes up to 600m elevation. The mean annual temperature is 24°C, with an annual precipitation of 1.750mm. Night-time relative humidity is 96% throughout the year, while daytime humidity drops sharply at midday during the short dry season.

On well drained slopes grows a dry "terra firme" forest characterized by a continuous canopy and little understory vegetation at ground level. On rocky, steep slopes, the forest is composed of trees having smaller diameter trunks which are often twisted, as well as bamboo clumps and shrubs. The level alluvial zone is dominated by a low forest with palms, epiphytes, scattered, huge buttressed emergent trees and lianas. The discontinuous canopy allows for the development of a dense ground cover of herbs and shrubs.

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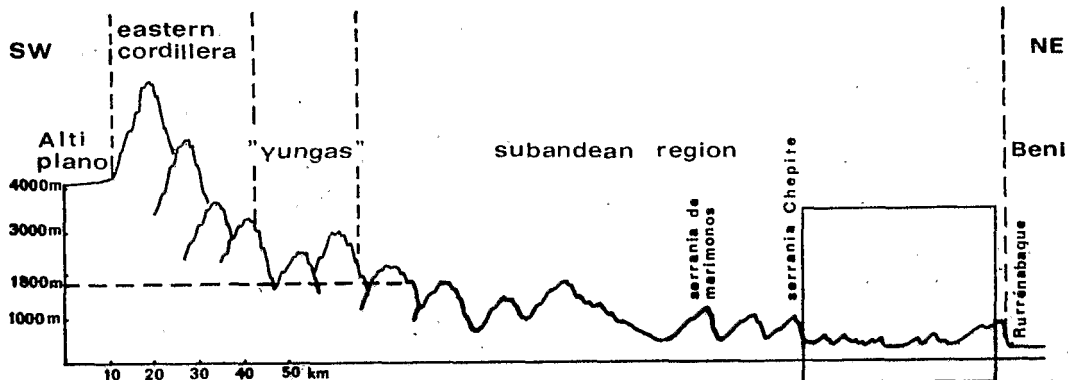


Fig. 1: profile of the andean foothills at 15° South latitude. Shaded portion represents endemic area of cutaneous leishmaniasis (1800 m elevation: altitudinal limit of leishmaniasis sl.) (in the frame, main study area).

Trapping sites and methods – A helicopter, used daily to carry workers and equipment from one campsite to another, allowed the authors to study the anthropophilic sandfly fauna at 19 sites distributed in a 60 by 25 km area (17 sites during August and 2 in May at the end of the rainy season). Another site in a different area on the west side of the Serrania de Marimonos, but still in the Alto Beni region, was also surveyed in August. One catch off Shannon trap was performed in April. Except for the site in the Serrania de Marimonos (1.000m), all other sites were located between 250 and 600m elevation.

Human bait – The major objective of this study was to determine the natural infection rate of *Leishmania* in the anthropophilic sandfly population. Thus, catches from human bait was the main sampling method. Two collectors captured sandflies from their arms, legs and clothing before being bitten. Catches were made into glass vials (6.5 x 1 cm), each containing one fly. With this method, flies were more likely to survive until the following day. At each site one or more capture sessions were conducted.

Shannon trap – Each seismic team from the petroleum company consisted of 50 workers and one technician. Campsites were located 2.500m distant from each other. The blue tents at each campsite were erected in an area roughly cleaned from the low vegetation and used as improvised Shannon traps. Sandflies were attracted both by the light from a lightbulb in each tent and the CO₂ emitted by the people sleeping underneath.

Isolation of strains in the field – From 7 to 10pm., sandflies were collected every half-hour and immediately dissected in a drop of physiological serum. The identification of the flies was based on an examination of the spermatheca and pigmentation. The entire digestive tract was examined for the presence of promastigotes. When an infection was present, the digestive apparatus was disrupted under a binocular microscope and drawn into a 1 cc syringe containing 0.5 cc of physiological serum. The contents of the syringe was then inoculated intradermally in the dorsal portion of the four paws of a golden hamster. The remains of the infected sandflies were kept in vials of 70% alcohol for later slide preparation. The sowing of the suspension into NNN medium culture tubes in the field proved to be inappropriate, since the tubes always exhibited contamination before reaching the laboratory.

***Leishmania* isolate establishment and preservation** – Hamsters were examined every two days for the first week and every week thereafter. Individuals which exhibited negative symptoms were kept for more than 10 months. Once a hamster showed a positive reaction from the inoculation, the granulomas were collected, ground in a mortar and the suspension inoculated into modified biphasic NNN medium (Decker-Jackson & Honiberg, 1978). The isolations were routinely maintained in modified NNN medium, and the isolates preserved in liquid nitrogen at -196°C.

Isoenzyme characterization of the strains – Multiplication of the *Leishmania* strains was made on modified NNN medium. The cultures were centrifuged at 2.500rpm for 10 minutes and the pellets stored without washing at -70°C until use. Just prior to electrophoresis, an equal volume of hypotonic enzyme stabilizer was added. Lysis of the parasites was achieved through freeze-thawing procedures repeated three times.

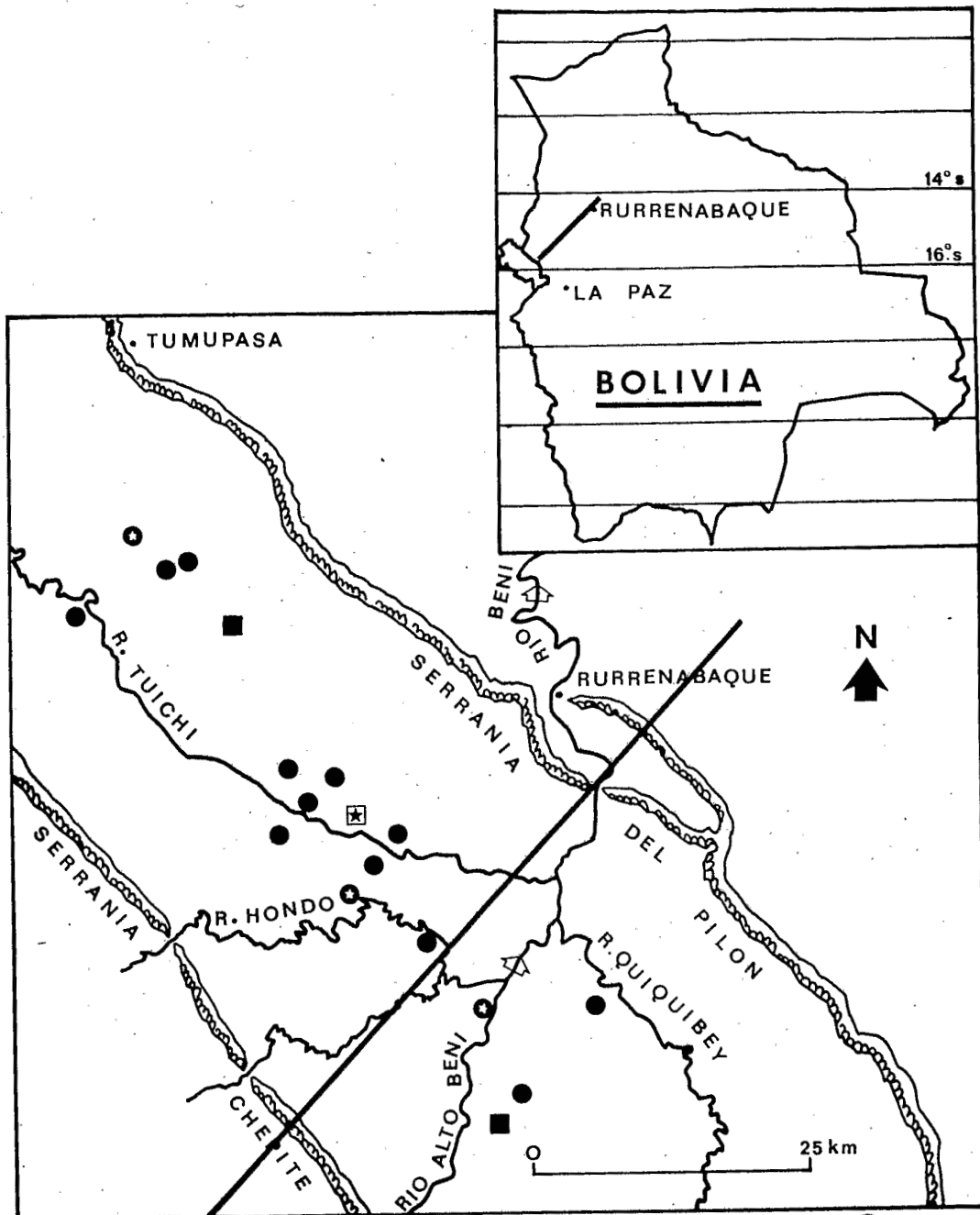


Fig. 2: detailed map of the study sites (with their situation in Bolivia inset) in the Alto Beni basin. ● study site; ★ study site with *Lu. flaviscutellata*; ⊕ study site with *Lu. umbratilis*; ■ study site with infected sandflies (*L. b. braziliensis*).

The following enzymic systems were used for the characterization:

- malate dehydrogenase E.C.1.1.1.37 (MDH)
- malate dehydrogenase (oxaloacetate decarboxylating) Nadp + E.C.1.1.1.40 (ME)
- isocitrate dehydrogenase E.C.1.1.1.42 (ICD)
- 6-phosphogluconate dehydrogenase E.C.1.1.1.44 (6PGDH)
- glucose-6-phosphate dehydrogenase E.C.1.1.1.49 (G6PDH)
- phosphoglucomutase E.C.2.7.5.1. (PGM)
- mannose phosphate isomerase E.C.5.3.1.8. (MPI)

glucose phosphate isomerase E.C.5.3.1.9. (GPI)
 glutamate dehydrogenase E.C.1.4.1.2. (GDH Nad⁺ and GDH Nadp⁺)
 aconitate hydrolase E.C.4.2.1.3. (ACON)
 peptidase (substrate L-leucyl-leucyl-leucine) E.C.3.4.11. (PEP)
 glutamate oxaloacetate transaminase E.C.2.6.1.1. (GOT)

Six reference strains were used:

MHOM/VE/77/L-20	<i>L. mexicana pifanoi</i>
MNYC/BZ/62/M-379	<i>L. mexicana mexicana</i>
IFLA/BR/67/PH-8	<i>L. mexicana amazonensis</i>
MHOM/BR/75/M-2904	<i>L. braziliensis braziliensis</i>
MHOM/BR/75/M-4147	<i>L. braziliensis guyanensis</i>
MHOM/PA/75/N-4037	<i>L. braziliensis panamensis</i>

The electrophoresis was carried out on cellulose acetate plates (Helena Laboratories), running at 200 volts for 30 minutes. The methods used were adapted from Lanham et al. (1981) as previously described (Tibayrenc & Desjeux, 1983).

RESULTS

Sandflies collected from human bait (Table 1) – In identifying the sandflies, the authors have followed the classification given by Theodor (1965), but consider the subgenus *Psychodopygus* as a genus (Forattini, 1971; Ready, 1980).

Detailed analysis showed that *Psychodopygus* were generally abundant throughout the year in well drained forests. Along the sandy riverbanks covered with a more open forest with a dense ground vegetation layer, they could also be found during the dry season (Chaniotis et al., 1971; Ward, Shaw & Lainson, 1973). In sharp contrast collections of *Psychodopygus* from the steep slopes were rare. It should be noted that collections from the slopes consisted principally of numerous dendrophilic species such as those in the *Verrucarum* group and in the series *cruciata*, *Lu. shannoni*, and especially those from the subgenus *Nyssomyia*.

A total of 24 species were from human bait, but 9 species of *Psychodopygus* accounted for 83.6% of the total captures.

TABLE 1

Phlebotomine sandflies captured during April, May and August 1984, using Shannon-traps and human bait at 20 sites of the last subandean valley and one station in the Serrania de Marimonos, Department of La Paz, Bolivia

Method of catches	Human Bait														Shannon Trap							
	August 1984														May 1984		Total					
	Tuichi River											Hondo River		Marimonos	Alto Beni River			Human Bait				
Period of catches																						
Place of catches																						
Identification of the station	B24- H11	B20- H11	B20- H12	B20- H14	B22- H11	B22- H12	B21- H10	B21- H13	B21- H10	B23- H11	B23- H13	B23- H14	unde- term.	B24- H10	B25- H11	unde- term.	B63- H16	B26- H10	B12- H12	B12- H13	B12- H13	B12- H16
Altitude (m)	383 369						391 286 267			233		982		396 413								
<i>Gr. Psychodopygus</i>																						
<i>P. ayrozoai</i>	14	1	29	165	4		52	453	56	62	58	1	1	118					32		33	
<i>P. yucumensis</i>																						
<i>P. c. carrerae</i>	333	59	27	191	29	1	11	132	98	260	202	126	6	142	4	2	60	28			1711	12
<i>P. llanosmartinsi</i>	7	58	3	6					1	19		49	11	15	1		15	186	34		407	87
<i>P. h. hirsuta</i>	4	1	1	11			3	2				10		4			2	1	1		40	
<i>P. nocticola</i>	1	1		6				1				2		13							25	
<i>P. amazonensis</i>		3		7								3	3	9	1	8					38	
<i>P. davisi</i>	1	28				1	1	3	2	4	6	12	2	16							76	
<i>gr. guyanensis</i>																						
<i>Sg. Nyssomyia</i>																						
near <i>Lu. shawi</i>	41	29	7	15		1	61	9		1	18	1	61	1	2	5	2	50	53	225	582	99
<i>Lu. yuilli</i>		9			1															2	12	
<i>Lu. flaviscutellata</i>																						
<i>Lu. umbratilis</i>																						
<i>Gr. Verrucarum</i>																						
<i>Lu. serrana</i>																						
<i>Lu. nevesi</i>			1	1										1			5	1		1	11	
Divers																						
<i>Lu. campbelli</i>	1																					
<i>Lu. aclydifera</i>		1																				
<i>Lu. shannoni</i>			1																			
<i>gr. cruciata</i> (2 sp.)							2	1	1					1							6	
<i>gr. vexator</i>							7														8	
<i>Brumptomyia</i> sp.		5																				
<i>Pressatia</i> sp.																						
<i>Trichophoromyia</i> sp.																						
Totals	402	220	42	268	195	7	77	200	563	324	313	71	257	25	319	12	18	158	301	263	4035	203

Psychodopygus ayrozai and one species of the *P. guyanensis* complex are rare and seem to have a patchy distribution. Individuals of *Psychodopygus carrerai carrerai* are morphologically similar to the Colombian *P.c. carrerai* (Young, 1979). This is the most abundant (42% of total captures) in the study area, followed by *P. yucumensis*, also well distributed and *P. llanosmartinsi*. Less frequent were *P. hirsuta hirsuta*, *P. davisi*, *P. amazonensis* and *P. nocticola*.

The following important sandfly group includes the species of the subgenus *Nyssomyia*. One species very close to *Lu. shawi* was the main representative. This species presents infuscation of the low part of the pleura as in *P. llanosmartinsi*. *Lu. flaviscutellata* and *Lu. umbratilis* were both found in the study area. *Lu. anduzei* described by Llanos (1973) from Peru is most likely conspecific with *Lu. umbratilis*. The presence of *Lu. umbratilis* in the Andean foothills of Bolivia extends its distribution to the Southeast when compared with the collections by Llanos in Peru and Lainson from the Mato Grosso region (1976). Capture of *Lu. umbratilis* at only two sites is not surprising since dry season populations of this species are mainly found in the canopy (Le Pont & Pajot, 1980). *Lu. yuilli* is rare, but this could be due to seasonal variation. One species close to *Lu. trapidoi* was mentioned for the first time in Bolivia. It was not collected off human bait, but from a miniature CDC light trap (a few specimens).

TABLE II

Results of dissections of anthropophilic sandflies carried out at four sites in a subandean valley adjacent to regions Alto Beni-Beni, Bolivia, endemic for cutaneous leishmaniasis. In brackets, number of phlebotomine sandflies with a peripyloric infection by promastigotes

Identification of the station	May 1984, end of rainy season				August 1984, dry season			
	B12 H13		B12 H12		B20 H12		B20 H14	
Biotope	range flank		flat		range flank	flat	flat	
	Number dissected	Number infected by <i>L.b.b.</i>	Number dissected	Number infected by <i>L.b.b.</i>	Number dissected	Number dissected	Number dissected	Number infected by <i>L.b.b.</i>
<i>P. ayrozai</i>			32(1)	0				
<i>P. yucumensis</i>			1				29(1)	1
<i>P. c. carrerai</i>			28		1	26	191(3)	0
<i>P. llanosmartinsi</i>	34		186(3)	1	2	1	6	
<i>P. h. hirsuta</i>	1		1			1	11	
<i>P. nocticola</i>							6	
<i>P. amazonensis</i>							7	
near <i>Lu. shawi</i>	225(4)	0	53		2	5	15(1)	0
<i>Lu. yuilli</i>	2							
<i>Lu. serrana</i>					1		1	
<i>Lu. nevesi</i>	1				1		2	
<i>Lu. shannoni</i>					1			
gr. <i>cruciata</i> (2 sp.)					1			
Total	263		301		9	33	268	

The *Verrucarum* group is represented by only two species, although collections made off platform outside of the campsites would probably have shown a greater number of species from the canopy. A small number of rare or occasionally anthropophilic species were recorded, with the two species in the *cruciata* group being *Lu. evangelistai* and *Lu. sherlocki*.

Sandflies collected from Shannon traps — The species close to *Lu. shawi* as well as *P. llanosmartinsi* were highly attracted to the lighted campsites, with *P. yucumensis* attracted to a lesser extent.

Sandfly dissection and *Leishmania* isolates (Table II) — In total, 874 anthropophilic sandflies were dissected. 13 specimens exhibited natural infections by promastigotes (peripyloric type): five near *Lu. shawi*, three *P.c. carrerai*, one *P. ayrozai*, one *P. yucumensis* and three *P. llanosmartinsi*. Strains of *Leishmania* could be isolated in hamsters from only one *P. llanosmartinsi* (out of 220 dissections during the rainy season from two sites), one *P. yucumensis* (out of 29 dissections in the dry season at two sites) and from one specimen of a species close to *Lu. shawi* (out of 278 dissections during the rainy season at two sites). In all infected flies the infection was heavy, filling

the stomach and the pylorus, the latter being swollen and covered with stumpy attached forms. The cardia was filled with free, mobile, elongated flagellates and the proboscis was also infected.

Two infected flies (one *P. llanosmartinsi* and one *P. yucumensis*) had residues of a recent blood meal in the midgut. These sandflies were dissected and inoculated to hamsters (four paws). The area of inoculation rapidly became swollen (7 to 10 days) and indurated. In the hamster inoculated from *P. llanosmartinsi* three paws became positive, while in the one inoculated from *P. yucumensis* only two paws developed a lesser positive response. The re-isolation of *Leishmania* strains from the swollen parts of the two hamsters did not raise any particular problem.

In the case of the hamster inoculated from the species close to *Lu. shawi*, swelling and induration followed the same time course as the others.

Isoenzyme characterization – The electrophoretic results showed that the two *Leishmania* strains isolated from the sandflies exhibited the same enzymatic profile for 11 of 13 enzymes and were similar to a *Leishmania braziliensis braziliensis* reference strain (M-2904). Variants were observed in MDH and ICD, the same as for the 41 human strains isolated from the same general area (Alto Beni 32, Yungas 9).

DISCUSSION

Two species *Psychodopygus llanosmartinsi* and *Psychodopygus yucumensis* were found infected with *Leishmania braziliensis braziliensis*. One of them *P. yucumensis* is a species recently described (Le Pont et al., 1986). Identification of the parasite was supported by isoenzyme characterization showing that isolated strains were indistinguishable from a *L. b. braziliensis* reference stock (M-2904) for 11 of 13 enzymes. As these isoenzyme profiles were also the same as the 41 strains isolated from human cases in the same area (Desjeux et al., submitted for publication), it is evident that *P. llanosmartinsi* and *P. yucumensis*, considering their aggressive anthrophilic behaviour, are involved in the transmission of cutaneous leishmaniasis caused by *L. b. braziliensis* in the lowland subandean region of Bolivia.

Two other *Psychodopygus* species (*P. c. carrerai* and *P. ayrozai*) were found infected, but not by *L. b. braziliensis*. Other species belonging to the subgenus *Nyssomyia* accounted for only 15% of man biting sandflies. Only the most abundant representative of this group, a species close to *Lu. shawi*, was infected by an unidentified *Leishmania*.

From our study of 20 collections of anthrophilic sandflies, it has become evident that the genus *Psychodopygus* is predominant in the study area (nine species, 83% of captures). This is similar to the results obtained by Ward et al. (1973) in the Serra dos Carajás in the Eastern Amazon basin (eight species, 95% of captures) as well as those met by Marins de Aguiar & Soucasaux (1984) in the selvatic hilly region of Southeast Brazil (four species, 93% of captures). Lainson et al. (1973) also emphasized the importance of *Psychodopygus* in the transmission of *L. b. braziliensis* in Northern Brazil, since a strain of the parasite could be isolated from *P. wellcomei*, and two other species, *P. amazonensis* and *P. paraensis* were highly suspected as potential vectors.

Our isolation of *L. b. braziliensis* from *P. llanosmartinsi* and *P. yucumensis* reinforces the results of the Brazilian studies, and emphasizes the lack of bioecological data on this important group of disease vectors.

Few data are available on the seasonal transmission of *L. b. braziliensis* in the wild, although it should be mentioned that *P. wellcomei*, *P. amazonensis* and *P. paraensis* are found to be infected in December and January at the beginning of the rainy season. In our study area, *P. llanosmartinsi* was found infected at the end of the rainy season and *P. yucumensis* during the dry season.

Further similarities exist with the results obtained in the Serra dos Carajás. In 1973, Lainson et al. reported the difficulty in isolating strains of *L. b. braziliensis* from humans as well as from infected flies. In our studies we have noted that *Leishmania* strains from *P. llanosmartinsi* and *P. yucumensis* rapidly infected hamsters, whereas strains from two other individuals of *P. llanosmartinsi*, exhibiting equally massive peripyloric infections, were not infective. It is possible to suggest two explanations which account for this phenomenon:

- 1) In the Andean foothills of Bolivia, it can be noted from clinical observation and parasitological data (experimental inoculation) that *Leishmania* strains (*L. b. braziliensis*) from the low elevations seem more virulent than strains from the Yungas area at higher elevations (Desjeux et al., submitted for publication). The reason for the virulence is not clear, but it is worth noting that in the subandean zone the transmission cycle is strictly wild with sporadic transmission. In the Yungas region the cycle affecting man appears to be mainly peridomestic, with epidemic trans-

mission and the involvement of other species of sandflies, although two species of *Psychodopygus* could still be found between 1.000 and 1.800m elevation.

2) The lack of infectivity of extracts from sandflies which, at first sight, appeared to have the same degree and type of infection, and from which verified *L. b. braziliensis* strains were later cultured may be accounted for by the findings of Sacks and Perkins in a recent study (1984; 1985). They demonstrated that there exists a sequential development of *Leishmania* from a non-infective to an infective stage in culture and in the sandfly vector and that this change is growth cycle dependant. According to these authors, the infective stage in the sandfly appears late, at the stationary stage of promastigote growth and in response to adverse environmental conditions, such as when the sandfly is in a state of fasting at the end of the digestion of a bloodmeal. They note that this infective stage is short and coincides with the search for another bloodmeal by the fly (the infective stage occurring between two gonotrophic cycles). In the case of the *Leishmania* strains from *P. llanosmartinsi* and *P. yucumensis* which immediately infected the inoculated hamsters, we noticed that the flies had black blood residues in the midgut which clearly indicates the terminal phase of a gonotrophic cycle. If the hypothesis of Sacks and Perkins is accepted, it means that the parasites were at their optimum infectivity. On the other hand, the infected *P. llanosmartinsi* which did not infect the hamster had no residual blood in the gut, thus it is possible that the parasites had not yet reached the same stage of infectivity.

RESUMO

Uma pesquisa epidemiológica sobre os vetores da leishmaniose tegumentar (do tipo espúndia) foi empreendida na região do Alto Beni, na Bolívia, uma zona de contrafortes andinos no limite Este das baixas planícies amazônicas. O clima é tipicamente tropical úmido (15° de latitude Sul). As espécies de flebotomos antropofílicos foram amostradas em vinte estações, todas na floresta. A importância das espécies do grupo *Psychodopygus*, já suspeitas como vetores na transmissão da *Leishmania* do complexo *braziliensis*, foi comprovada por: 1) a agressividade e a variedade das espécies encontradas (83% das capturas e nove espécies); 2) a descoberta de uma nova espécie antropofílica, *P. yucumensis* e 3) o isolamento de uma amostra de *Leishmania braziliensis braziliensis* indistinguível das amostras humanas da mesma região, de duas espécies, *P. llanosmartinsi* e *P. yucumensis*.

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