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Research Note

CUTANEOUS LEISHMANIASIS IN THE AMAZON REGION: NATURAL INFECTION OF THE SANDFLY *LUTZOMYIA UBIQUITALIS* (PSYCHODIDAE: PHLEBOTOMINAE) BY *LEISHMANIA (VIANNIA) LAINSONI* IN PARÁ STATE, BRAZIL

FERNANDO T. SILVEIRA; ADELSON A. A. SOUZA; RALPH LAINSON; JEFFREY J. SHAW; ROSELI R. BRAGA & EDNA E. A. ISHIKAWA

Seção de Parasitologia, Instituto Evandro Chagas, Fundação Nacional de Saúde, Caixa Postal 3, 66001 Belém, PA, Brasil

In Amazonian Brazil the cutaneous leishmaniases are zoonoses, with a variety of wild animal reservoirs among which the various *Leishmania* species are transmitted by different sandfly vectors (Diptera: Psychodidae: Phlebotominae). In terms of human disease the most important parasites of this region are *Leishmania (Viannia) guyanensis* Floch, *L. (V.) braziliensis* Vianna and *Leishmania (Leishmania) amazonensis* Lainson & Shaw. The principal vectors of these organisms are *Lutzomyia umbratilis* Ward & Fraiha, *Psychodopygus wellcomei* Fraiha, Shaw & Lainson and *Lu. flaviscutellata* (Mangabeira) respectively (R. Lainson & J. Shaw, 1968, *Trans. R. Soc. Trop. Med. Hyg., 62:* 385-395; R. Lainson et al., 1973, *loc. cit., 67:* 184-196; R. Lainson et al., 1979, *loc. cit., 73:* 239-242).

Recently F. T. Silveira et al. (1987, *Mem. Inst. Oswaldo Cruz, 82:* 289-292) described another leishmanial parasite, *L. (V.) lainsoni*, which has now been isolated from 20 cases of human cutaneous leishmaniasis, all from the north of Pará State (municipalities of Benevides, Ananindeua, Igarapé-Açu, Ourém, São Domingos do Capim, Acará and Viseu), with exception of one case from Porto Grande, Amapá State. The first of these was from a patient coming from the municipality of Benevides, about 30 km from Belém and, as most of the other cases were from the same region, this prompted us to investigate the sandfly fauna of the area. Although the area was colonized many years ago, there still remains a great deal of natural vegetation, including patches of primary forest interspersed with secondary forest and open agricultural land. The study area chosen was one of "terra firme" primary forest, where one of our patients frequently went hunting. We decided to extend our studies, however, to a second piece of primary forest situated in Utinga (Belém) and extending to the outskirts of the municipality of Ananindeua. The vegetation there is very similar to that of Benevides.

In June, August, September and October, 1988 we made eight sandfly collections (two per month) in each of the two study areas. This period of the year is that with the lowest rainfall. In Benevides, sandflies were captured using both CDC miniature light-traps and a Shannon-trap equipped with a "strip-light" but without animal bait; in Utinga we used only the former. Shannon collections were made between 6.00 and 9.00 p. m., and the sandflies were maintained in a nylon cage (20 x 20 x 20 cm), in plastic bags, with the humidity raised by wet cotton wool, until next day when they were dissected for evidence of flagellate infection and for identification. The CDC traps were placed in the forest overnight, at about 1.0 m above ground level. The sandflies captured were again dissected the next day.

Sandfly guts were dissected out in sterile saline plus antibiotics (200 iu penicillin and 200 μgm streptomycin/ml). Following removal to a fresh drop of this saline, they were examined by phase-contrast microscopy using sterile slides and coverslips. Identification of the sandfly species was largely on spermathecal structure, aided by external characters. After observations on the disposition of flagellates, infected guts were crushed to liberate the parasites and material taken up into a 1.0 ml syringe containing about 0.3 ml of the saline/antibiotic

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TABLE

Isolation and identification of intestinal flagellates found in phlebotomine sandflies captured in Benevides and Utinga, Pará State, Brazil, 1988

Species	Trapping site	No. exam.	No. infec.	Isolation in Hamster-Culture		Parasite
Lu. nordestina	Benevides	22	5	–	+ (2)	*Trypanosoma* sp.
Ps. davisi	Benevides	98	1	–	–	
Ps. paraensis	Benevides	169	2	+ (1)	+ (1)	*Leishmania naiffi*
Lu. gomezi	Benevides	144	1	–	–	
Lu. aragaoi	Utinga	24	1	–	–	
Lu. brachipyga	Utinga	33	1	–	–	
Lu. yuilli yuilli	Utinga	7	1	–	–	
Lu. antunesi	Utinga	21	3	–	+ (1)	*Trypanosoma* sp.
Ps. ayrozai	Utinga	5	1	+	+	*L. naiffi*
Lu. ubiquitalis	Utinga	375	9	+ (8)	+ (8)	*L. lainsoni*

solution. This was used to inoculate two tubes of Difco B45 blood-agar medium (B. C. Walton et al., 1977, *J. Parasitol., 63:* 1118-1119) by passing the needle directly through the rubber caps, which had previously been swabbed with 70% ethyl alcohol. Remaining material was inoculated into the dorsal surface of the back feet of two hamsters.

A total of 1,924 female sandflies were captured in the two areas: 780 (40.5%) from Benevides and 1,144 (59.5%) from Utinga.

In Benevides we identified 27 different species of sandflies, the following being the most prevalent: *Psychodopygus paraensis* (Costa Lima) (21.6%); *Lu. gomezi* (Nitzulescu) (18.4%); *Ps. geniculatus* (Mangabeira) (13.3%); *Ps. davisi* (Root) (12.5%); *Ps. ayrozai* (Barreto & Coutinho) (8.4%) and *Lu. ubiquitalis* (Mangabeira) (6.4%).

In Utinga 29 species were recorded, but only *Lu. dasypodogeton* (Castro) (43.9%) and *Lu. ubiquitalis* (32.7%) were found in any great number.

Results of dissections (Table) – In Benevides the 780 dissected sandflies revealed intestinal flagellates in *Lu. nordestina* (Mangabeira), *Ps. davisi, Ps. paraensis* and *Lu. gomezi.* No infections with *L. (V.) lainsoni* were found, but an unidentified trypanosome was isolated in culture from *Lu. nordestina* and *Leishmania (V.) naiffi* Lainson & Shaw from *Ps. paraensis.*

In Utinga 16 of the 1,144 dissected sandflies showed intestinal flagellates. *L. (V.) lainsoni* was isolated from 8 out of 9 infected specimens of *Lu. ubiquitalis,* both in culture and hamsters.

The total number of *Lu. ubiquitalis* examined from both trapping-sites was 425 (50 from Benevides and 375 from Utinga). The proven infection-rate with *L. (V.) lainsoni* was thus 1.9%. Most of the infections were very heavy, with free elongated promastigotes packing the midgut. Rosettes of short, stumpy flagellates were attached to the pylorus wall (Fig. 1) and extended into the ileum – developmental pattern characteristic of leishmanias of the subgenus *Viannia* Lainson & Shaw (1987, in *The Leishmaniases in Biology and Medicine* eds. W. Peters & R. Killick-Kendrick, Academic Press, London, 120 p).

Growth of the parasites in the blood-agar medium was luxuriant, producing large promastigotes with the excessively elongated flagellum commonly shown by *L. (V.) lainsoni.* Intradermal inoculation of the flagellates from the infected sandflies into the feet of hamsters produced conspicuous nodular lesions two months later, and these contained abundant amastigotes. In Giemsa-stained smears these showed the frequent fusiform shape and voluminous kinetoplast, typical of *L. (V.) lainsoni.* Morphological identification was confirmed by the isoenzyme profiles of the eight isolations, which proved to be indistinguishable from that of our type strain MHOM/BR/81/M6426 (Benevides) (Fig. 2). Finally, all eight isolates failed to react with monoclonal antibodies produced against *L. (V.) guyanensis, L. (V.) braziliensis* and *L. (V.) panamensis.*

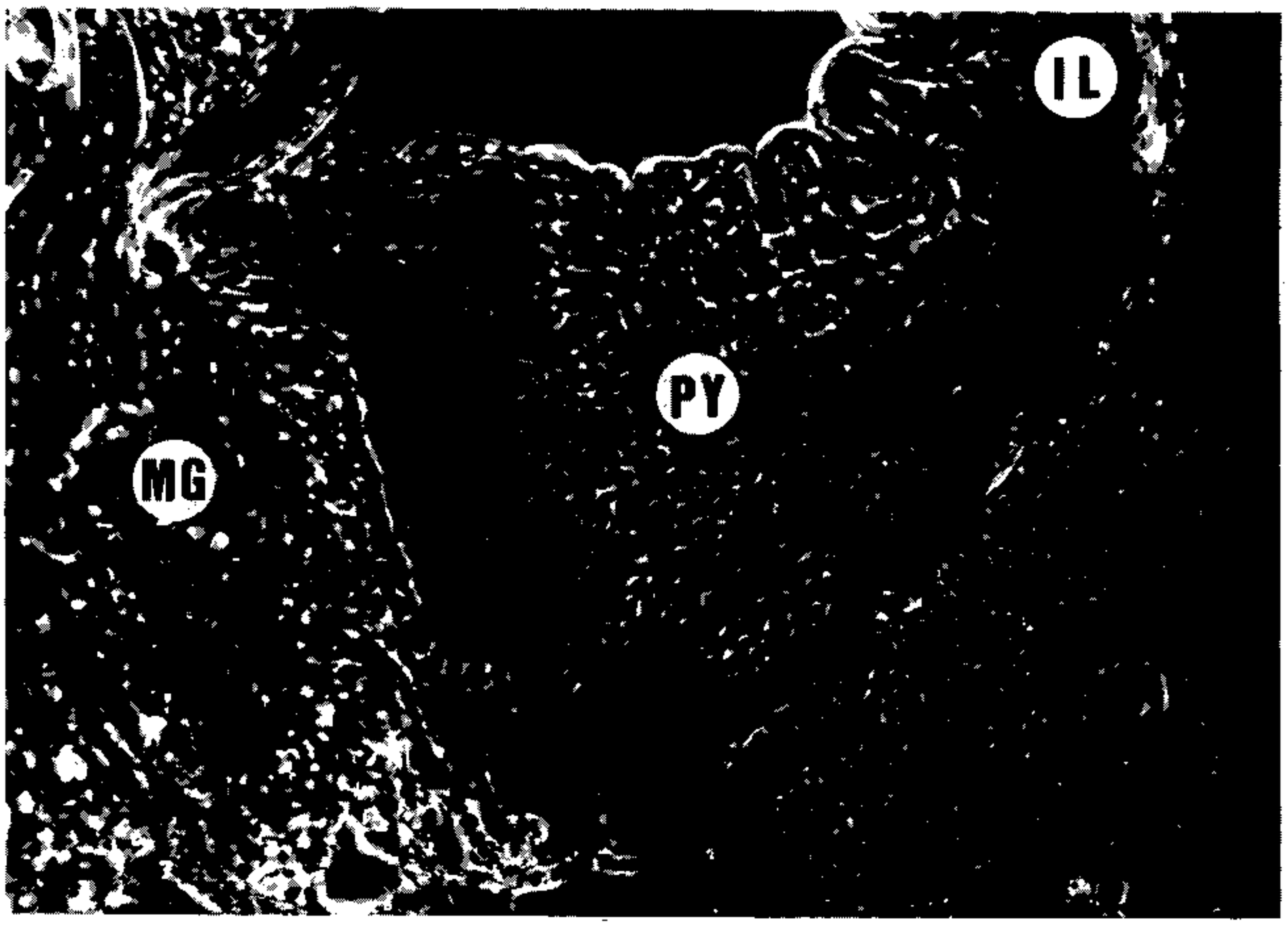

Fig. 1: photomicrograph of the hind gut triangle (pylorus) of a female specimen of *Lutzomyia ubiquitalis* naturally infected with *L. (V.) lainsoni*. Note large numbers of flagellates, often in rosettes, attached to the wall of the hindgut. py = pylorus; il = ileum; mg =midgut.

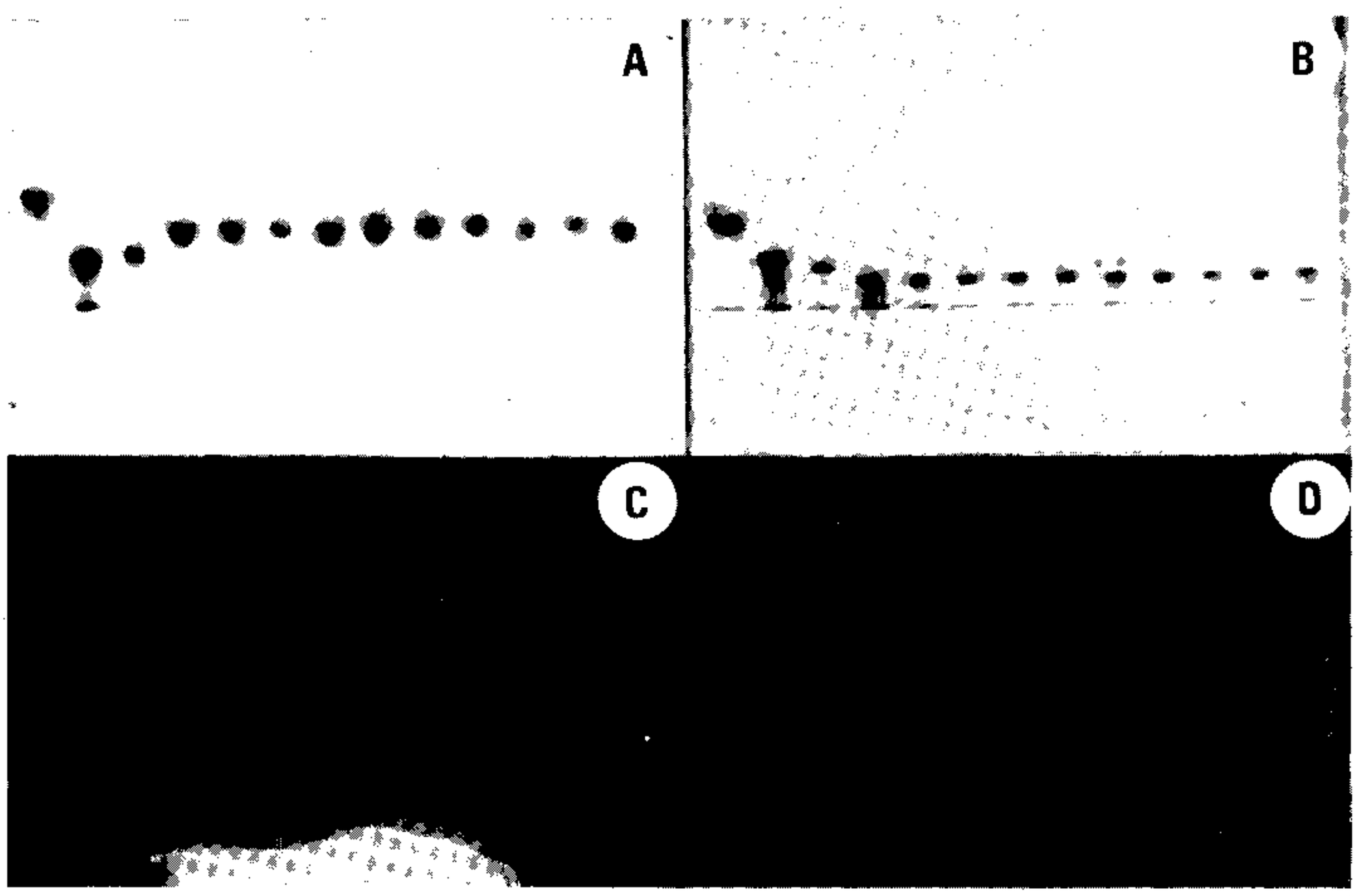

Fig. 2: polaroid photograph of the enzyme profiles (enzyme electrophoresis) of the eight isolates of *Leishmania* from *Lutzomyia ubiquitalis*, all indistinguishable from that of the type strain of *L. (V.) lainsoni* MHOM/BR/81/M6426, Benevides, Pará. Four of 10 enzymes used are shown: (A) MPI, (B) 6PGDH, (C) GPI and (D) G6PD. Order of parasites from left to right are: (1) *L. (L.) amazonensis*, (2) *L. (V.) guyanensis*, (3) *L. (V.) braziliensis*, (4 and 13) *L. (V.) lainsoni*, type strain and (from 5 to 12) *L. (V.) lainsoni* isolated from *Lu. ubiquitalis*.
Scale: distance between the points of origin of each parasite = aproximately 1.0 cm.

A parasite designated as "an unnamed parasite of the sub-genus *Viannia* (IUBI/BR/83/M7556)" was isolated by Lainson et al., from a specimen of *Lu. ubiquitalis* from the River Paranapanema area, in the foothills of the Carajás, Pará, in 1983. This has since been characterized as *L. (V.) lainsoni.*

Under normal forest conditions we have not found *Lu. ubiquitalis* to be an anthropophilic sandfly. The fact that human infections with *L. (V.) lainsoni* were registered in the same region as that from which the infected flies came, however, leads us to assume that under certain conditions it will bite man, and transmit the parasite to him. This is in keeping with the relatively rare ocurrence of human infection, and our hypothesis is amply supported by the results of a recent experiment in which 71 out of 83 (85%) *Lu. ubiquitalis,* caught in Utinga, were successfully fed on man in the laboratory, 48 h after capture. There is, too, a coincidence in the distribution of our human cases of infection with *L. (V.) lainsoni* and the geographical distribution of *Lu. ubiquitalis* in Pará, as given by A. V. Martins et al., (1978, in *American Sand Flies,* Academia Brasileira de Ciências, Rio de Janeiro, RJ). It is hoped that the recent establishment of a laboratory colony of *Lu. ubiquitalis* will permit an experimental evaluation of the vectorial capacity of this sandfly.

In conclusion, our additional isolation of the parasite *L. (V.) naiffi* from one specimen each of the sandflies *Ps. paraensis* and *Ps. ayrozai* tends to support the view of J. Arias et al., (1985, *Am. J. Trop. Med. Hyg., 34:* 1098-1108) that these species may be natural vectors of that parasite. Furthers studies in this connection are in progress.

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