

DÉRMAL LEISHMANIASIS IN THE AMAZON REGION OF BRAZIL: *LEISHMANIA (VIANNAIA) LAINSONI* SP.N., A NEW PARASITE FROM THE STATE OF PARÁ⁺

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Until relatively recently dermal leishmaniasis in Brazil was regarded by most as due to a single parasite, *Leishmania braziliensis* Vianna, 1911. It became necessary to modify this conception, however, following epidemiological and parasitological studies which clearly indicated a number of different parasites to be involved as causative agents of the disease (Lainson & Shaw, 1970, *Trans. R. Soc. trop. Med. Hyg.*, 64 :654; 1972, *Brit. Med. Bull.*, 28: 44; 1973, *Bull. PanAm. Hlth Org.*, 7(4) :1). According to biological and biochemical criteria, the parasites were assigned to the *L. mexicana* complex (e.g. *L. mexicana amazonensis* Lainson & Shaw) and the *L. braziliensis* complex (e.g. *L. braziliensis braziliensis* Vianna, and *L. b. guyanensis* Floch). Later, the same authors (Lainson & Shaw, in *Biology of the Kinetoplastida*, Vol. 2, edited by Lumsden and Evans, 1979, Academic Press, London) grouped the leishmanias into two "Sections": the Peripylaria, with a development phase in the hindgut of the sandfly vector (e.g. *L. braziliensis*) and the Suprapylaria, with no such development (e.g. *L. mexicana*). Finally, they have more recently replaced these Sections with the sub-generic names of *Viannia*⁺ Lainson & Shaw and *Leishmania* Safjanova, respectively (Lainson & Shaw, in *The Leishmaniasis in Biology and Medicine*, Vol. 1, edited by Peters & Killick-Kendrick, 1987, Academic Press, London).

During some 22 years of epidemiological studies in the Amazon Region of Brazil, this laboratory has examined a very large number of *Leishmania* isolates from cases of cutaneous and mucocutaneous leishmaniasis of man, by current biological, biochemical and serological methods of identification. A number of parasites have been encountered whose characteristics clearly separate them from the known, major aetiological agents of neotropical leishmaniasis

⁺ Lainson and Shaw (1987) used the name *Viannia* in their original paper. However, the Editors considered this to be incorrect and have replaced it with the name *Viannaia*.

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within the subgenera *Leishmania* and *Viannaia*. It is the object of this communication to describe and name one of these new parasites which, although clearly a member of the subgenus *Viannaia*, differs from the recognized species of this group of leishmanias in morphological, biochemical (isoenzyme profiles) and serological (monoclonal antibodies) characters.

The parasite in question has been isolated from the single, cutaneous lesions of six patients, four from the municipality of Benevides, and two from those of São Domingos do Capim and Vizeu; all in the State of Pará, 25, 120 and 400 km, respectively, from Belém.

Morphological studies have been based on Bouin-fixed smears of amastigotes in smears of skin lesions of infected hamsters, and compared with those of other *Leishmania* species prepared by the same method (Lainson, 1959, *J. Protozool.*, 6 :360). Preparations of promastigotes were from log-phase cultures of the parasite in Difco blood-agar base (B 47) (diphasic medium) (Walton et al., 1977, *J. Parasitol.*, 63 :1118). The flagellates were gently washed by centrifuging in normal saline, and air-dried slide preparations were also fixed in aqueous Bouin solution. All preparations were stained by a modified Giemsa method (Lainson, loc. cit.). Parasites were measured using a Zeiss Morphomat 10.

Isoenzyme profiles were studied by starch-gel electrophoresis; for the preparation of lysates, other methods and enzyme abbreviations, see Miles et al. 1980, *Trans. R. Soc. trop. Med. Hyg.*, 75 :524. We used the enzymes MPI, 6PGDH, GPI, G6PD, PEP, ACON, MDH, PGM, ASAT and ALAT, and compared the profiles with those of the standard marker strains for *L. (V.) braziliensis* (MHOM/BR/75/M2903 from Serra dos Carajás, Pará), *L. (V.) guyanensis* (MHOM/BR/75/M4147 from Monte Dourado, Pará) and *L. (L.) amazonensis* (IFLA/BR/67/PH8 from Utinga, Belém, Pará).

By the methods previously described (Shaw et al., 1987, *Trans. R. Soc. trop. Med. Hyg.*, 81 :69) we examined the parasite with monoclonal antibodies specific to *L. (V.) braziliensis* (B18), *L. (V.) guyanensis* (B19), *L. (V.) panamensis* (B11) and three other *braziliensis* group specific monoclonals (B2, B5, and B12).

The amastigote:

In Bouin-fixed smears, these are readily distinguished from all recognized species of the subgenus *Viannaia* by their typically elongated nature and voluminous kinetoplast. The lesion they produce in hamster skin is similar to that caused by parasites of the *braziliensis* complex – namely, a small self-limiting nodule or ulcer. It differs, however, in the very abundant amastigotes it contains.

The promastigote:

Experimental infection of laboratory-bred sandflies (*Lutzomyia longipalpis*) confirmed the inclusion of the parasite within the subgenus *Viannaia*, by its prolific growth as paramastigotes and promastigotes attached to the wall of the hindgut (pylorus).

As observed in Difco culture medium the promastigotes differ strikingly from those of *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis* and *L. (V.) peruviana* in their large size, which even exceeds that of *L. (L.) amazonensis*. They are also distinguished by an extraordinarily long flagellum, which may attain the length of 36.42 μm . Unlike *L. (V.) braziliensis*, and some other species within the subgenus *Viannaia*, *L. (V.) lainsoni* grows luxuriantly not only in Difco blood-agar base (B 47) but also in simple NNN medium, in which it produces vast numbers of flagellates in colonies which can be seen with the naked eye, growing on the liquid and solid surfaces of the medium.

Isoenzyme profiles:

L. (V.) lainsoni is distinguished from *L. (V.) braziliensis*, *L. (V.) guyanensis* and *L. (L.) amazonensis* on the profiles of at least six enzymes (MPI, 6PGDH, GPI, G6PD, PEP and ACON) of the ten utilized (Fig. 1).

Monoclonal antibodies:

No positive reactions were recorded with any of the monoclonal antibodies used, in an indirect immunofluorescent antibody test.

From all the above observations, the parasite described here is clearly distinct from all other recognized neotropical leishmanias, and we propose the name *Leishmania (Viannaia) lainsoni* in honour of Professor Ralph Lainson whose dedication to the leishmanias during the past 30 years has undoubtedly played a great part in the advances seen in our understanding of New World leishmaniasis.

Studies on the DNA will be published in due course.

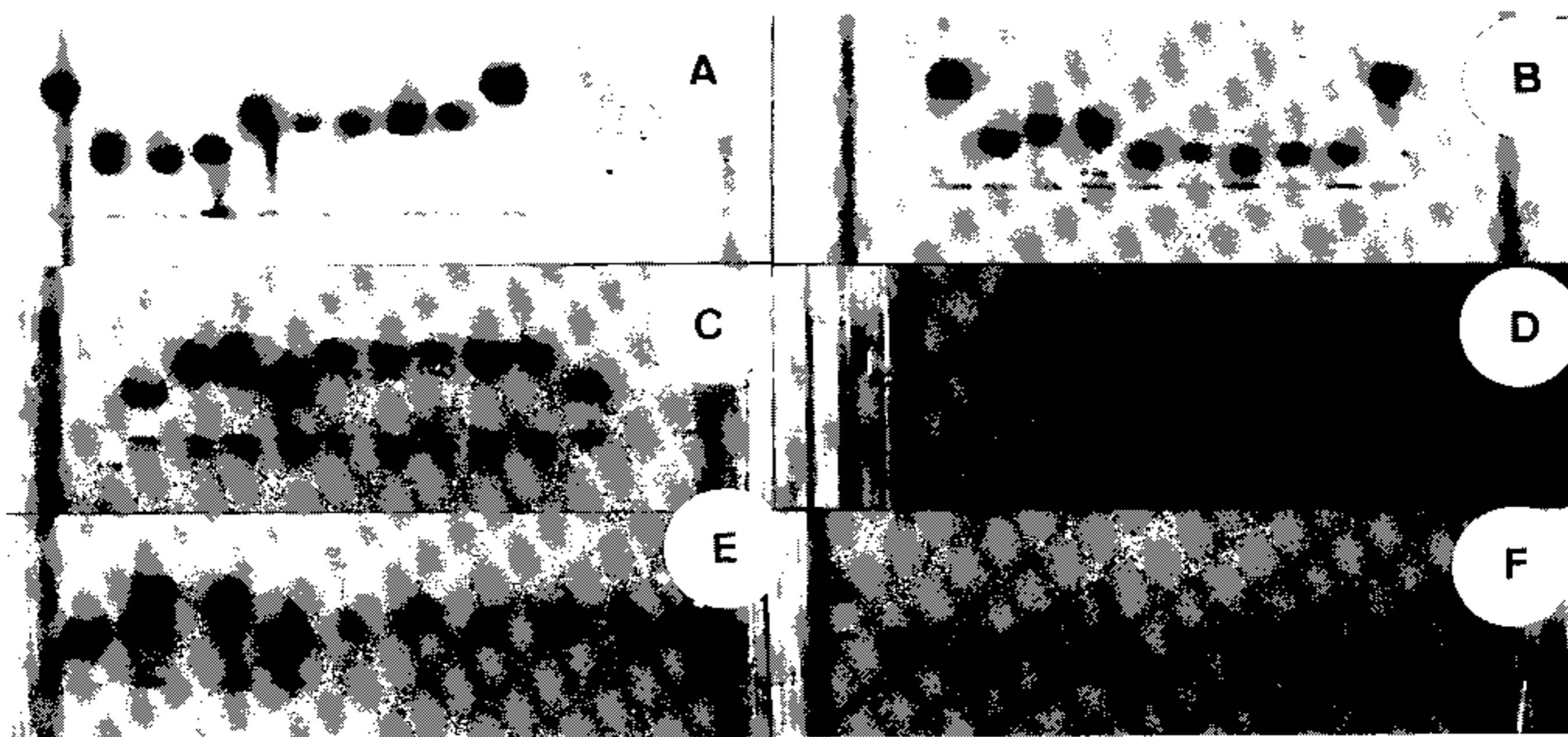
Leishmania (Viannaia) lainsoni sp.n.

Specific diagnosis:

Type host: Man. Silvatic, mammalian host as yet unknown.

Type locality: Municipality of Benevides, Pará State, north Brazil.

Strain designation: MHOM/BR/81/M6426



Comparative enzyme profiles that distinguish *L. (V.) lainsoni* from other *Leishmania* species. Enzymes are: (A) MPI, (B) 6 PGDH, (C) GPI, (D) G6PD, (E) PEP and (F) ACON. Reading from left to right, the parasites are: (1) *L. (L.) amazonensis*, (2) *L. (V.) braziliensis*, (3) *L. (V.) guyanensis*, (4) *Leishmania* sp. (in process of characterization), (5, 6, 7, 8 and 9) *L. (V.) lainsoni* and (10) *L. (L.) amazonensis*. Scale: the cotton threads at the origin measure approximately 1.0 cm.

Promastigotes: Total body length: 17.43 ± 2.91 (10.94 - 22.17)

Flagellum length: 25.70 ± 5.00 (10.54-36.42)

Development in the sandfly host with the characters of the genus and subgenus. Cultivation in simple blood-agar medium (NNN) very luxuriant.

Amastigotes: In air-dried, aqueous Bouin-fixed smears the amastigotes are elongate and with a bulky kinetoplast placed anterior to the nucleus.

Length 3.49 ± 0.37 (2.01 - 4.30); width 1.40 ± 0.18 (1.08 - 1.91); kinetoplast length 0.96 ± 0.16 (0.62 - 1.34); kinetoplast width 0.48 ± 0.09 (0.31 - 0.70); nuclear length 1.21 ± 0.13 (0.94 - 1.50); nuclear width 0.89 ± 0.12 (0.63 - 1.14); P-K (posterior tip to kinetoplast) 1.98 ± 0.24 (1.18 - 2.47); P-N (posterior tip to nucleus) 1.11 ± 0.21 (0.77 - 1.76); all measurements in μm .

Sandfly vector: as yet unknown.

Isoenzyme profiles: Distinguished from *L. (V.) braziliensis*, *L. (V.) guyanensis* and *L. (L.) amazonensis* on profiles for MPI, 6PGDH, GPI, G6PD, PEP, and ACON.

Monoclonal antibodies: Separated from other named members of the subgenus *Viannaia* by monoclonal antibodies.

Behaviour in hamster: Producing a small, self-limiting nodule containing very abundant amastigotes.

Pathology in man: producing single, simple cutaneous ulcers. No evidence of naso-pharyngeal involvement. Infection readily cured by antimonial treatment.

Type material: Hapantotype slides (amastigotes & promastigotes) Department of Parasitology, Instituto Evandro Chagas, FSESP, Belém, Pará, Brazil. Cultures held in the following cryobanks: Instituto Evandro Chagas; London School of Hygiene & Tropical Medicine; Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro.