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LEISHMANIA (VIANNIA) NAIFFI SP. N., A PARASITE OF THE ARMADILLO, *DASYPUS NOVEMCINCTUS* (L.) IN AMAZONIAN BRAZIL

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SUMMARY. A new leishmanial parasite, *Leishmania (Viannia) naiffi* sp. n., is described from the nine-banded armadillo, *Dasypus novemcinctus* (Edentata: Dasypodidae), from Para State, north Brazil. The parasite grows luxuriantly in Difco blood-agar medium (B47), but poorly in the skin of intradermally inoculated hamsters. A comparison of isoenzyme profiles by starch gel electrophoresis separates the parasite from *L. (V.) braziliensis* and *L. (V.) guyanensis* by the enzymes ASAT, ALAT, PGM, GPI, G6PD, PEP, MPI and GD, and from *Leishmania (Leishmania) chagasi*, *L. (L.) amazonensis* and *L. (L.) deanei* by ASAT, ALAT, PGM, GPI, MPI, G6PD, MDH, PEP and ACON. Finally, *L. (V.) naiffi* is serologically differentiated from *L. (V.) braziliensis*, *L. (V.) guyanensis* and *L. (V.) panamensis* on monoclonal antibodies specific for these parasites.

Key-words: *Leishmania naiffi* sp. n. Armadillo. Brazilian Amazonas.

***Leishmania (Viannia) naiffi* n.sp., parasite du tatou, *Dasypus novemcinctus* (L.) en Amazonie brésilienne.**

RÉSUMÉ. *Leishmania (Viannia) naiffi* sp. n. est décrit chez un tatou, *Dasypus novemcinctus* (L.) provenant de l'État du Pará, Brésil. Le parasite se cultive très bien sur le milieu NNN (Difco 47), mais se développe très lentement après inoculation intradermique au Hamster. *L. (V.) naiffi* se sépare de *L. (V.) braziliensis* et *L. (V.) guyanensis* par les profils enzymatiques des enzymes : ASAT, ALAT, PGM, GPI, G6PD, PEP, MPI et GD. Il se sépare de *L. (Leishmania) chagasi*, *L. (L.) amazonensis* et *L. (L.) deanei* par les profils enzymatiques de ASAT, ALAT, PGM, GPI, MPI, G6PD, MDH, PEP et ACON. Enfin, le nouveau parasite se distingue de *L. (V.) braziliensis*, *L. (V.) guyanensis* et *L. (V.) panamensis* grâce aux anticorps monoclonaux.

Mots-clés : *Leishmania naiffi* n. sp. Tatou. Amazonie brésilienne.

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Introduction

Lainson *et al.* (1979) recorded the presence of a *Leishmania* species in the nine-banded armadillo, *Dasypus novemcinctus*, from Mojú, Pará State, north Brazil: the parasite was variously isolated from the spleen, liver and heart-blood of 3 out of 14 animals examined, following the culture of these tissues in NNN blood-agar medium. *In vitro* growth was luxuriant, but the organism grew poorly in hamster skin, in which it either produced an inapparent skin infection or an inconspicuous nodule containing very scanty, small amastigotes. In experimentally infected sandflies, *Lutzomyia longipalpis* (Lutz & Neiva), the parasite showed a typically peripylarian development, attached to the wall of the pylorus and the ileum. On morphology, behaviour in hamster skin and the development in the sandfly, the organism was considered « ... close to members of the *L. braziliensis* group... ».

In a subsequent study, 13 stocks of the parasite, isolated from armadillos in various parts of Pará State, were found to be indistinguishable from each other on the enzyme profiles for ASAT, ALAT, PGM, GPI, G6PD, PEP, MPI, GD, MDH, ACON, DIA, PK, HK and ACP, but distinguishable from *L. (V.) braziliensis* and *L. (V.) guyanensis* on the first 8 of these enzymes. The organism was separated from *L. (Leishmania) chagasi*, *L. (L.) amazonensis* and *L. (L.) deanei* on the profiles for 9 of the 14 enzymes utilized (Lainson *et al.*, 1982).

Arias *et al.* (1985) isolated a *Leishmania* from 4 specimens of the sandfly *Psychodopygus paraensis* (Costa Lima) and 3 *P. ayrozai* (Barretto & Coutinho), all captured in forest in the State of Rondônia, Brazil, and found the parasite to be indistinguishable from that of *D. novemcinctus* on the profiles for 13 different enzymes. It was noted, however, that the promastigotes were restricted to the midgut of « ... sandflies with recent bloodmeals... », and none was seen in the hindgut of any of the infected insects.

Finally, Lainson & Shaw (1987) referred to the parasite of *D. novemcinctus* as an unnamed member of the subgenus *Viannia* Lainson & Shaw (= the Section Peripylaria of Lainson & Shaw, 1979).

It is the purpose of this communication to describe this organism in greater detail and to propose for it the name of *Leishmania (Viannia) naiffi*, in honour of our old friend Sr. Roberto Daibes Naiff, to whose technical skills we owe much in our first isolation of the parasite.

Leishmania (Viannia) naiffi sp. n.

SPECIFIC DIAGNOSIS

Type Host: the nine-banded armadillo, *Dasypus novemcinctus* (L.).

Locality in Host: spleen and liver; more rarely isolated from heart-blood; not encountered in the skin.

Type Locality: Monte Dourado, Pará State, north Brazil (0°47' S; 52°40' W). Other isolates from armadillos captured in Mojú, Barcarena, Serra dos Carajás and Salvaterra (Island of Marajó), all in the State of Pará.

Strain Designation: MDAS/BR/79/M5533.

Amastigotes (Fig. 1): all measurements* in μm . Length 2.70 ± 0.43 (1.47-4.81); width 2.07 ± 0.42 (1.13-3.03); nucleus length 1.30 ± 0.20 (1.00-1.75); nucleus width 0.93 ± 0.15 (0.75-1.25); kinetoplast length 0.79 ± 0.10 (0.60-1.03); kinetoplast width 0.55 ± 0.10 (0.41-0.81).

Promastigotes (Fig. 2): total body length 10.11 ± 3.02 (4.60-19.93); width 2.60 ± 0.56 (1.40-4.14); nucleus length 1.60 ± 0.18 (1.25-2.00); nucleus width 1.27 ± 0.17 (1.00-1.50); nucleus centre to anterior tip 5.11 ± 1.39 (1.60-10.30); nucleus centre to mid-kinetoplast 3.36 ± 1.31 (1.10-8.44); nucleus centre to posterior tip 6.04 ± 2.00 (1.60-16.71); mid-kinetoplast to anterior tip 2.35 ± 0.40 (1.67-3.33); free flagellum 15.57 ± 3.38 (10.26-27.90).

Development in the Sandfly Host: peripylarian, with the characters of the genus and subgenus.

Sandfly Vector: isolations have been made from *Psychodopygus paraensis* and *P. ayrozai*, but the nature of the infections leaves doubt as to the role of these sandfly species as vectors.

Isoenzyme Profiles: distinguished from *L. (V.) braziliensis* and *L. (V.) guyanensis* on profiles for ASAT, ALAT, PGM, GPI, G6PD, PEP, MPI and GD. From *L. (L.) chagasi*, *L. (L.) amazonensis* and *L. (L.) deanei* by those of ASAT, ALAT, PGM, GPI, MPI, G6PD, MDH, PEP and ACON.

Monoclonal Antibodies: separated from *L. (V.) braziliensis*, *L. (V.) guyanensis* and *L. (V.) panamensis* on monoclonal antibodies specific for these parasites (Shaw *et al.*, 1986).

Kinetoplast DNA Buoyant Density (Table I): 1.696 g ml^{-1} .

Behaviour in Hamster: producing either an inapparent skin infection or an inconspicuous nodule at the side of intradermal inoculation. Amastigotes very scanty and usually only detected by culture.

Behavior in in vitro Culture: grows luxuriantly in Difco blood-agar medium (B47; Walton *et al.*, 1977).

Host Specificity: known, so far, only from *D. novemcinctus*.

Type Material: hapantotype slides (amastigotes and promastigotes) held in the Department of Parasitology, Instituto Evandro Chagas, Fundação SESP, Belém, Pará, Brazil. Cultures held in cryobanks at the Instituto Evandro Chagas, the London School of Hygiene and Tropical Medicine, and the Department of Epidemiology, Yale University, U. S. A.

* Methods in Silveira *et al.* (1987).

FIG. 1. — Amastigotes of *Leishmania (Viannia) naiifi* sp. n. as seen in impression smears of hamster skin.

FIG. 2. — Promastigotes of *L. (V.) naiifi*, from 4 day-old culture in Difco NNN blood-agar medium. Both smears fixed in aqueous Bouin's fluid and stained by Giemsa's method.

Discussion

Table I gives the kinetoplast DNA buoyant densities for a number of leishmanial parasites within the subgenus *Viannia*, as listed in Lainson & Shaw (1987). That of *L. (V.) naiffi*, kDNA b. d. 1.696, together with its very different isoenzyme profiles, supports our view that the parasite should not be included in the *braziliensis* complex (kDNA b. d. 1.692-4).

Another recently discovered member of the subgenus, *L. (V.) lainsoni*, is readily differentiated from *L. (V.) naiffi* and the other listed species by its unusual kDNA b. d. of 1.707 (Dr. D. C. Barker, pers. comm.) and morphological peculiarities of both its amastigotes and its promastigotes (Silveira *et al.*, 1987).

The frequency of *L. (V.) naiffi* in *D. novemcinctus* (17 out of 113 (15.4 %) examined), and our failure to find the parasite in any other mammalian host, suggested to us a very restricted host/sandfly vector association—possibly within the armadillo burrows. It came as somewhat of a surprise, therefore, when Arias *et al.* (1985) isolated the parasite from the sandflies *Psychodopygus paraensis* and *P. ayrozai*, both of which are caught biting man but seem unattracted to armadillos (Arias *et al.*, 1985; Ready *et al.*, in Lainson & Shaw, 1987). Ready & Lainson (unpublished data) failed to trap these species in Disney-traps baited with armadillos, and did not encounter them in the phlebotomine fauna of many armadillo burrows examined in the type locality of *L. (V.) naiffi*.

The infected specimens of *P. paraensis* and *P. ayrozai* examined by Arias *et al.*, were all caught on the same night, in the same trapping-site. This, and the restriction of the parasite to the midgut of sandflies which had taken a recent

TABLE I. — Kinetoplast DNA buoyant densities for some leishmanial parasites within the subgenus *Viannia* Lainson & Shaw.

Species	kDNA b. d.
<i>Leishmania (Viannia) braziliensis</i>	1.692 g ml ⁻¹ *
<i>L. (V.) guyanensis</i>	1.692*
<i>Leishmania (V.)</i> sp. (MHOM/BZ/82/BEL1 of Lainson & Shaw, 1987), Belize C. Am.	1.692***
<i>Leishmania (V.)</i> sp. (MCHO/BR/71/M1411 of Lainson & Shaw, 1987), Pará, Brazil.	1.692*
<i>L. (V.) peruviana</i>	1.693*
<i>L. (V.) panamensis</i>	1.694*
<i>Leishmania (V.)</i> sp. (MDID/BR/71/M1597 of Lainson & Shaw, 1987), Pará, Brazil.	1.694*
<i>L. (V.) naiffi</i>	1.696**
<i>L. (V.) lainsoni</i>	1.707**

* Chance 1976, 1979 ; Chance *et al.*, 1974, 1977.

** Dr. D. C. Barker, personal communication.

*** Barker & Butcher, 1983.

blood-meal, suggests that the infections may have been the result of opportunistic feeding on the reservoir-host. A similar situation has been recorded for *L. (L.) deanei* of porcupines, which has been isolated from *Lutzomyia furcata* (Mangabeira) trapped in a hollow tree housing an infected animal (Miles *et al.*, 1980; Lainson & Shaw, 1987). The parasite was only seen in those sandflies with partially digested blood-meals, and seemed incapable of surviving after digestion of the blood, as confirmed by observations on experimentally infected *Lu. furcata*.

Table II shows the isolation records following the *in vitro* culture of viscera (spleen and liver), heart-blood and skin (from the ears and nose) of the 17 infected armadillos examined to date. *L. (V.) naiffi* is essentially a visceralizing parasite, and found most readily in the spleen: only on 2 occasions was it isolated from the blood, and skin cultures were all negative.

L. (V.) guyanensis (a major cause of human cutaneous leishmaniasis in north Brazil and the Guyanas) is similarly a predominantly visceral parasite in the wild, reservoir-hosts (Lainson *et al.*, 1981). Sandfly transmission of both *L. (V.) naiffi* and *L. (V.) guyanensis* among such animals is presumably dependant on the periodic invasion of the peripheral blood by infected macrophages from the internal organs.

TABLE II. — Isolation of *Leishmania (Viannia) naiffi* from the armadillo, *Dasypus novemcinctus*, following culture of viscera, blood and skin in Difco blood-agar medium.

Animal Number	Results of Cultures				
	Spleen only	Liver only	Pooled Spleen Liver	Heart Blood	Skin
M5169	+	+		+	cont.
M5210	—	+		+	—
M5271	+	—		—	—
M5310	+	—		—	N/E
M5533	+	—		—	—
M5534	+	cont.		—	cont.
M5647	+	—		—	—
M5648	+	—		—	—
M5932	+	+		—	—
M5956	+	+		—	—
M6067			+	—	—
M6084			+	cont.	cont.
M6309			+	—	—
M6330			+	—	—
M6934			+	—	—
M8516			+H	N/E	N/E
M11712			+	—	—

cont. = contaminated cultures; N/E = not examined; +H = cultures contaminated, but parasite isolated from the skin of a hamster inoculated intradermally with pooled spleen and liver.

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