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DISTINCTIONS BETWEEN PROMASTIGOTES OF *LEISHMANIA* SPECIES DEVELOPING IN THE DIGESTIVE TRACT OF LABORATORY REARED *LUTZOMYIA LONGIPALPIS*

ANA LÚCIA F. F. DA SILVA; PAUL WILLIAMS; MARIA NORMA MELO & WILSON MAYRINK

Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Caixa Postal 2486, 31270 Belo Horizonte, MG, Brasil

Female Lutzomyia longipalpis *were exposed to infection by three different species/strains of* Leishmania. *When the insects were dissected four days after exposure, stained preparations were made of the flagellates contained in the digestive tract. Using traditional morphometric methods,* L. amazonensis, L. guyanensis *and an unnamed species of the* mexicana *complex could be distinguished from one another.*

Key words: *Leishmania* promastigotes – distinctions between species

During studies on the infectivity of different strains of *Leishmania* to laboratory-reared, female *Lutzomyia longipalpis* (Silva et al., 1990), opportunity was taken to make a morphological comparison, by light microscopy of three strains of *Leishmania* that underwent development in the digestive tract of this sand fly.

MATERIALS AND METHODS

Sand flies belonged to the 22nd laboratory generation of material collected in the municipality of Abaetetuba, state of Pará, Brazil. When 4 or 5 days old, females were allowed to take blood meals on hamster lesions, using the methods described by Silva et al. (1990). After blood feeding, the insects were kept individually in polythene tubes, maintained at a temperature of ± 25 °C and in an almost saturated humidity. Four days after the infective meal, the insects were dissected by the method described by Johnson et al. (1963). Observations were made on the position, appearance and numbers of parasites before disrupting the digestive tract to make stained preparations of the flagellates.

The strains of *Leishmania* were: IFLA/BR/67/PH8 – *Leishmania (Leishmania) amazonensis;* MHOM/BR/73/BH121 – *Leishmania (Leishmania) ?* sp *(mexicana complex);* and MHOM/BR/70/M1176 – *Leishmania (Viannia) guyanensis.* Hamsters were inoculated with strains PH8 and BH121 forty days before sand flies were fed on them and, in the case of strain M1176, 60 days before feeding the sand flies.

For morphological studies on the flagellates contained in the digestive tracts of sand flies, the unbroken gut was transferred to a drop of inactivated human serum and dissected to liberate the parasites. The preparation was air dried, fixed in methanol for 3 min, stained with Giemsa for 30 min, washed in running water and air dried. Preparations were permanently slide mounted under Euparol and the slides were dried at 37 °C for 72 h.

Observations were made with an oil immersion objective and camera lucida drawings were prepared at a magnification of 1,000 X. To ensure accuracy of measurements, drawings were further amplified by about 50%. Analysis of variance was used to compare measurements and ratios.

RESULTS

Strain IFLA/BR/67/PH8 (Fig. 1) – In ten freshly dissected sand flies, twenty-five promastigotes were measured, flagellates were seen throughout the mid gut, in both the thoracic and abdominal portions. None were detected in any other part of the digestive tract. They were lying free in the gut lumen or were attached by the flagellum to the wall. Attached and free flagellates were actively moving.

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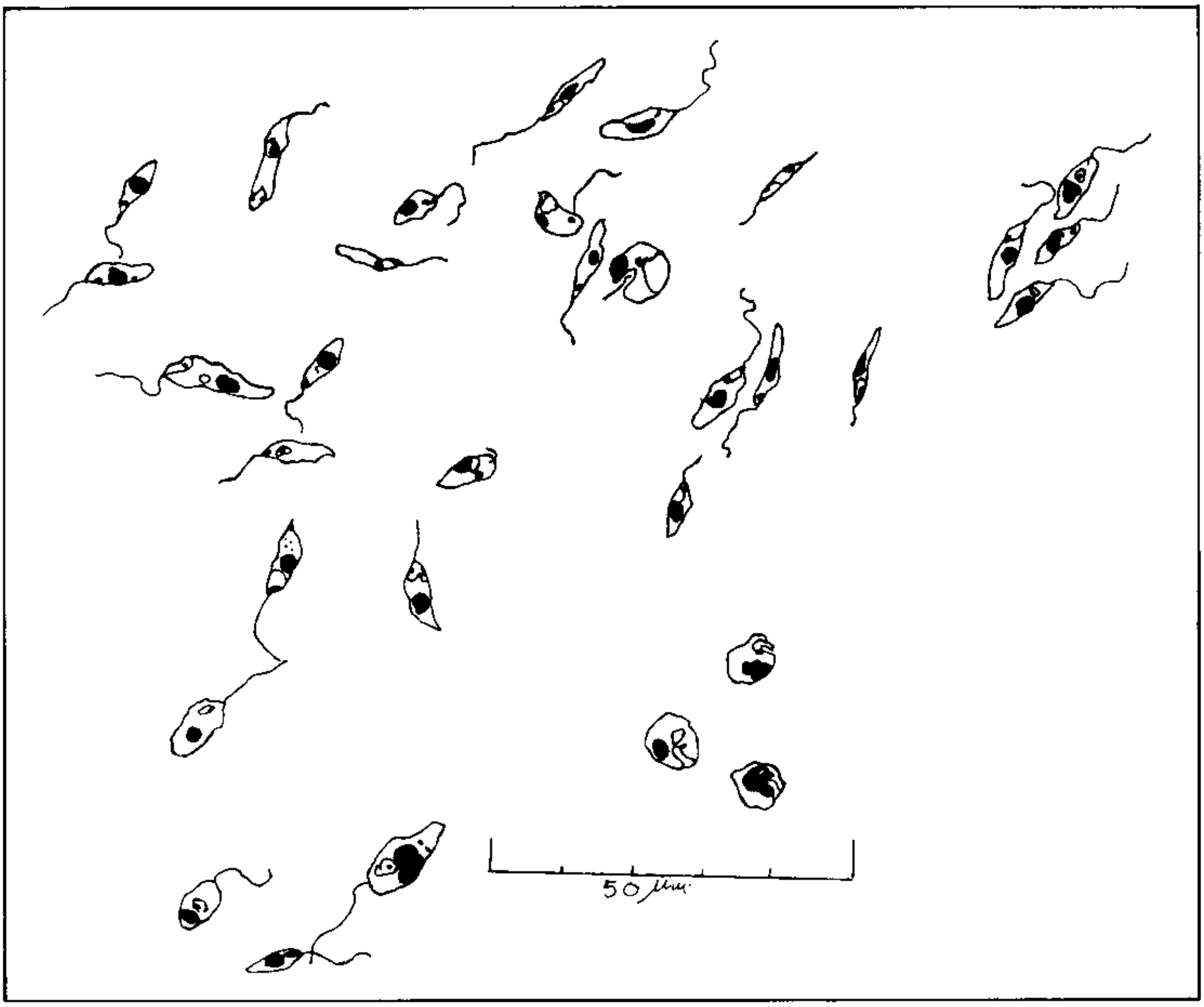

Fig. 1 : promastigotes of strain MHOM/BR/73/BH121 in development in digestive tract of *Lutzomyia longipalpis*.

In stained preparations, some paramastigotes were detected. These were oval, with a short flagellum and the kinetoplast was at the side of the nucleus. Promastigotes varied from long and narrow to short and wide. On average (see Table) the flagellum was short, about two-thirds the length of the body of the promastigotes. The nucleus was in the centre of the body (nuclear index = 1.02 ± 0.50). The kinetoplast was situated about two-thirds of the distance between the anterior tip and the nucleus. In dividing forms, nuclei were in tandem and divided before the kinetoplast.

Strain MHOM/BR/70/M1176 (Figs 2-3) – In the freshly dissected sand flies twenty-five promastigotes were measured, flagellates were observed adhering to the wall of the pylorus and, in fewer numbers, to that of the ileum. Rosettes of promastigotes were seen in the lumen of the pylorus. Attached paramastigotes were almost immobile whereas promastigotes displayed active movements.

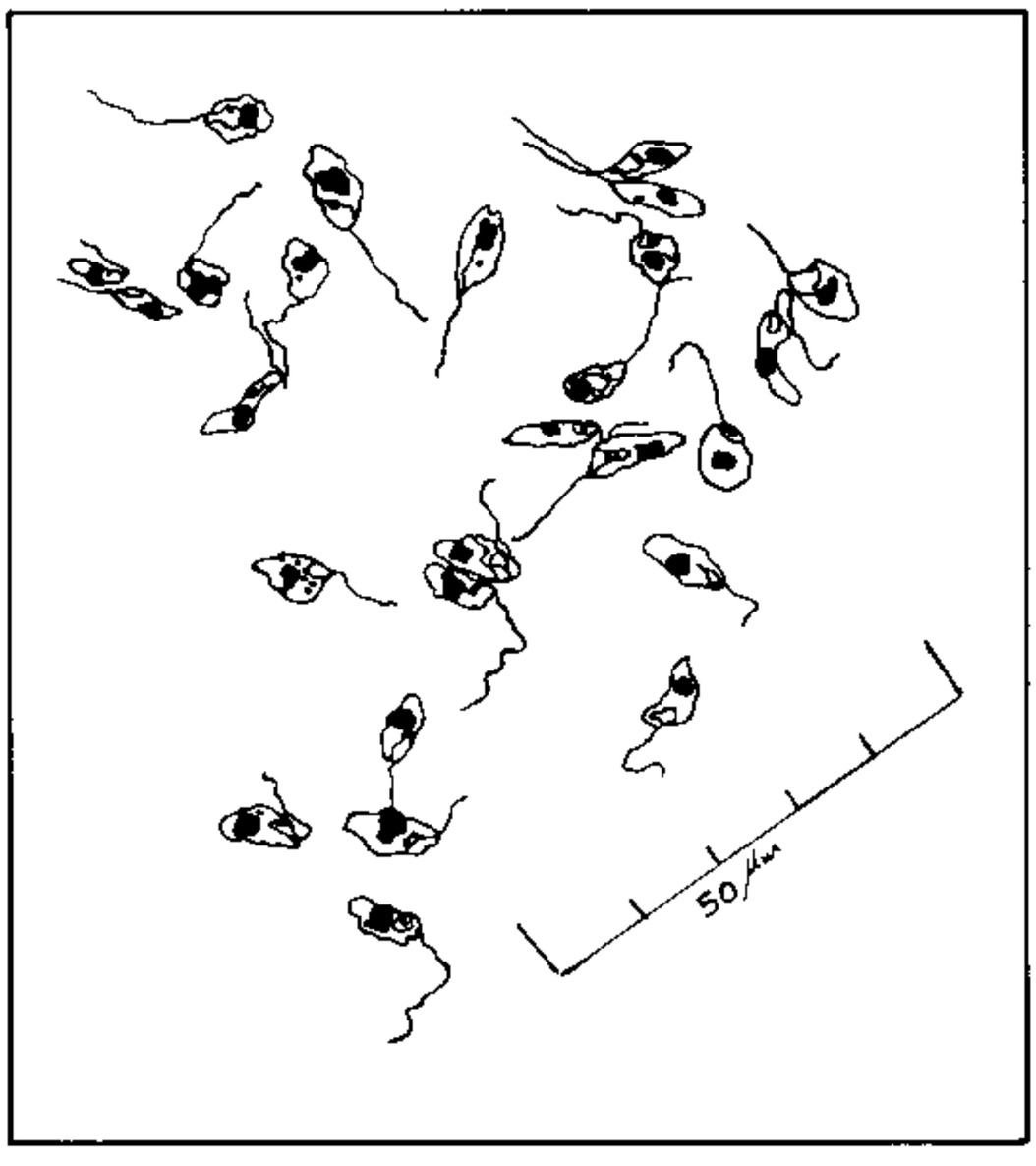

Fig. 2: promastigotes of strain IFLA/BR/67/PH8 in development in digestive tract of *Lutzomyia longipalpis*.

TABLE

Mean measurements and standard deviations of flagellates in the digestive tract of *Lutzomyia longipalpis*, four days after exposure to infection by different species and strains of *Leishmania*

Features measured	*Le. mexicana* complex (MHOM/BR/73/BH121) Promastigotes	*Le. amazonensis* (IFLA/BR/67/PH8) Promastigotes	*Le. guyanensis* (MHOM/BR/70/M1176) Promastigotes	Promastigotes
Total length	21.54 (7.46)[a, b]	17.22 (3.95)[a]	16.88 (3.82)[b]	11.42 (3.13)
Length of flagellum	12.04 (6.47)[a, b]	6.90 (2.88)[a,	9.34 (3.43)[b]	5.73 (3.02)
Body length	8.84 (2.69)	10.30 (2.19)[c]	7.74 (1.51)[c]	5.67 (1.35)
Maximum body width	3.78 (1.28)	3.03 (1.04)	3.29 (0.94)	3.96 (0.49)
Distance between anterior end and nucleus	4.38 (1.47)[a]	5.53 (1.91)[a]	4.54 (1.01)	3.58 (0.80)
Distance between nucleus and posterior end	4.45 (1.80)[b]	5.26 (1.67)[c]	3.20 (0.88)[b, c]	2.09 (0.99)
Nuclear index[d]	0.97 (0.36)[b]	1.02 (0.50)[c]	0.71 (0.20)[b, c]	0.59 (0.26)
Distance between kinetoplast and nucleus	2.54 (0.91)[a]	3.56 (1.42)[a, c]	2.72 (0.89)[c]	2.18 (0.66)

a: statistically significant difference between BH121 and PH8.
b: statistically significant difference between BH121 and promastigotes of M1176.
c: statistically significant difference between PH8 and M1176.
d: ratio, not measurement in μm.

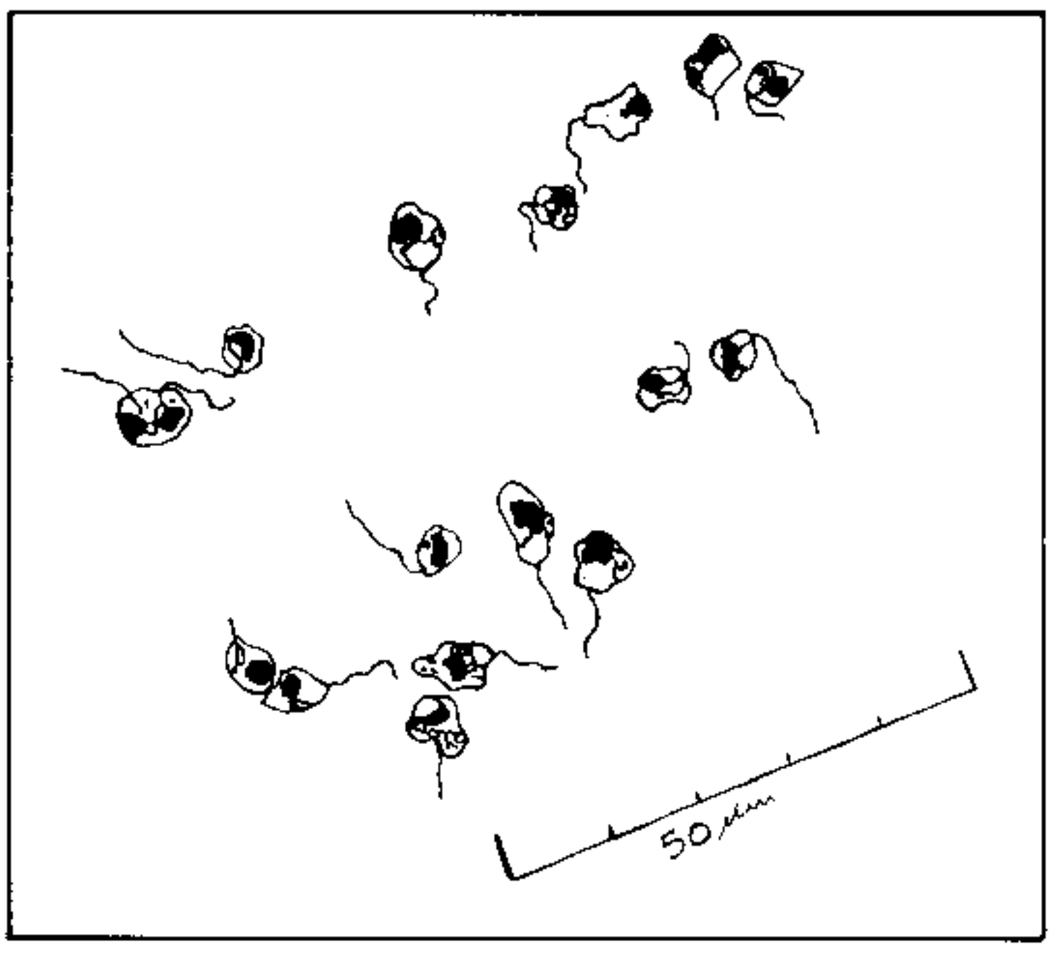

Fig. 3: paramastigotes of strain MHOM/BR/70/M1176 in development in digestive tract of *Lutzomyia longipalpis*.

In stained preparations, paramastigotes were roughly oval in shape, with the average width about two-thirds of the body length, excluding the flagellum. The flagellum was short and about equal to body length (see Table). The nucleus was more intensely stained than the kinetoplast and was situated well within the posterior half (nuclear index = 0.59 ± 0.26). On average, the kinetoplast was situated about two-thirds of the distance between the anterior end and the nucleus. Only one dividing form was observed: it contained two nuclei, two kinetoplasts and had two flagella.

Promastigotes were about 1.5 X the length of paramastigotes. The flagellum, on average, was about 1.2 X body length. The nucleus was in the posterior half of the body (nuclear index = 0.71 ± 0.20). On average, the kinetoplast was situated more than half the distance between the anterior tip and the nucleus. No dividing promastigotes were encountered.

Strain MHOM/BR/73/BH121 (Fig. 4) – In the freshly dissected sand flies twenty-five promastigotes were measured, flagellates were seen only in the mid gut, in both the thoracic and abdominal portions of the stomach. Some were attached to the gut wall, others were free within the lumen. Both attached and free forms were highly mobile.

No paramastigotes were detected in stained preparations. On average (see Table), the flagellum of promastigotes was long, about 1.4 X the length of the promastigotes body. The nucleus was less intensely stained than the kinetoplast, and was situated in a central position (nuclear index = 0.97 ± 0.36). The kinetoplast was situated, on average, at more than one-half the distance between the anterior end and the nucleus. Dividing forms were broad, with two nuclei, two kinetoplast and two flagella, one longer than the other.

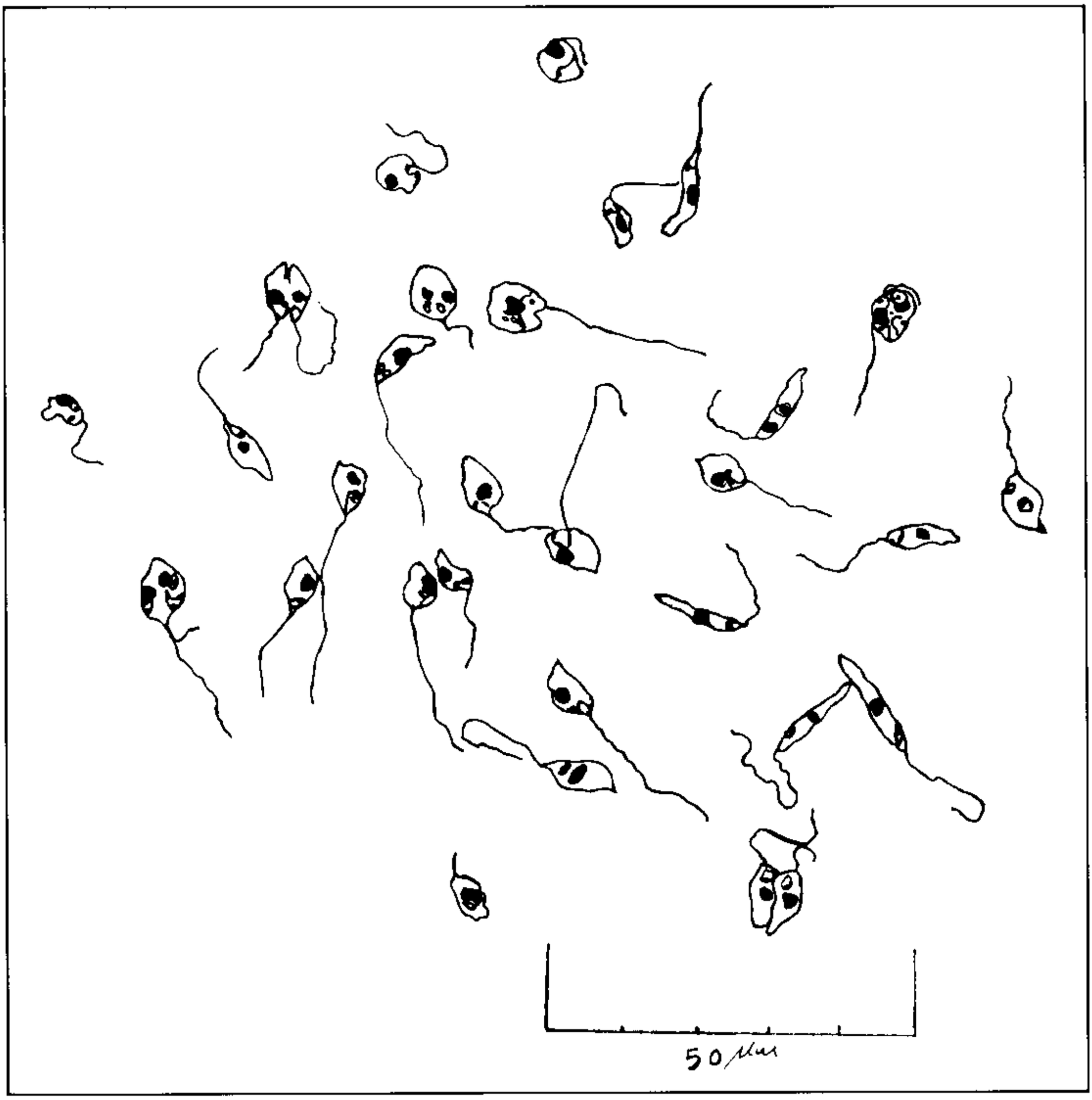

Fig. 4: promastigotes of strain MHOM/BR/70/M1176 in development in digestive tract of *Lutzomyia longipalpis*.

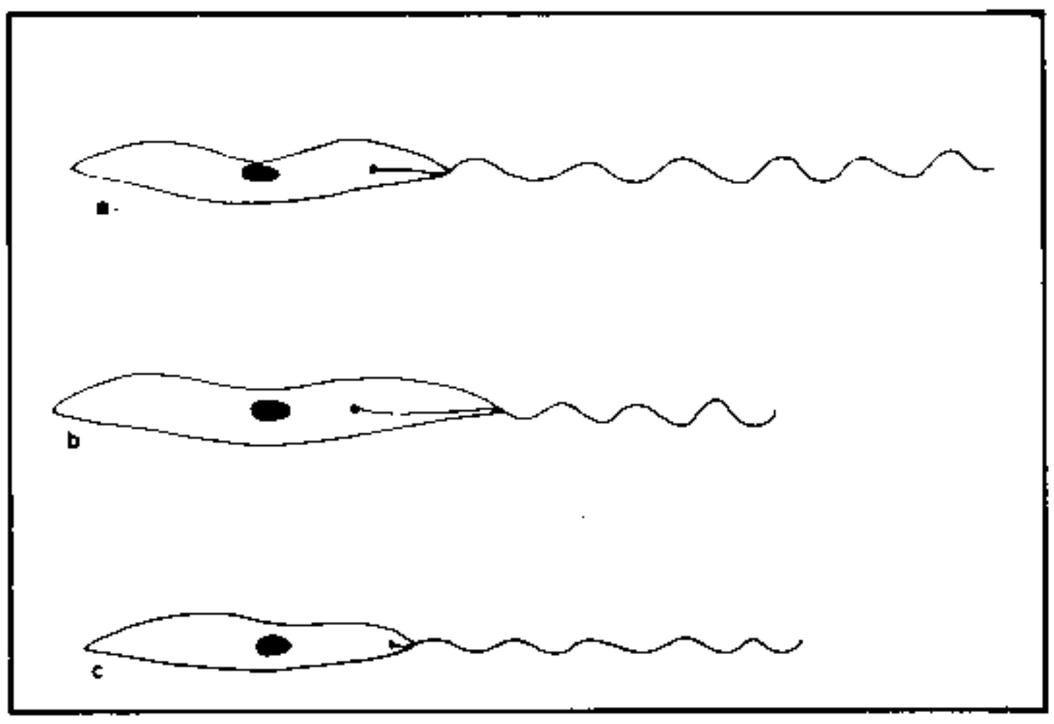

Fig. 5: schematic diagrams of promastigotes: a – strain MHOM/BR/73/BH121; b – strain IFLA/BR/67/PH8; c – strain MHOM/BR/70/M1176. (Based on mean measurement shown in the Table).

DISCUSSION

The purpose of the present study was to compare the flagellate forms of strain MHOM/BR/73/BH121 with those of *Le. amazonensis* and *Le. guyanensis* after the three had undergone development in *Lu. longipalpis* for a fixed period of time (four days).

Strain MHOM/BR/73/BH121 is a component of the antileishmaniasis vaccine developed by Mayrink et al. (1979) but has not yet been defined to specific level. Some biological and biochemical characteristics of the strain were recorded by Melo et al. (1987). It belongs to the subgenus *Leishmania* and can be included

in the *L. mexicana* complex; it differs from *Le. amazonensis*, however in the electrophoretic mobility patterns of five isoenzymes. Silva et al. (1990) showed that it has a low infectivity rate (9%) towards *Lu. longipalpis* in comparison to *Le. amazonensis* (37%) and *Le. guyanensis* (100%). In comparing promastigotes of strain MHOM/BR/73/BH121 after four days of development in *Lu. longipalpis* with those of *Le. amazonensis* and *Le. guyanensis*, several morphometric differences were detected.

Lainson & Shaw (1987) remarked that both the amastigotes and promastigotes of species of the subgenus *Viannia* are smaller than those of the *mexicana* complex. Promastigotes of the strains of *Le. amazonensis* and *Le. guyanensis* used in the present study were of similar size when comparing the total body length, including the flagellum. However, there was a statistically significant difference in the lengths of the bodies of the promastigotes when flagellum length was excluded. Promastigotes of the two species also differed in the distance between the kinetoplast and nucleus, the distance between the nucleus and the posterior end, and in their nuclear indices. The promastigotes of strain MHOM/BR/73/BH121 differed from those of *Le. guyanensis* in four respects: total body length, including the flagellum; length of the flagellum; distance between the nucleus and the posterior end; and in nuclear indices.

Strain MHOM/BR/73/BH121 was found to differ from *Le. amazonensis* in four parameters: total body length, including the flagellum; length of the flagellum; distance between the kinetoplast and the nucleus; and the distance between the nucleus and the anterior end.

Within the limits of this study, it is clear that after four days of development in *Lu. longipalpis*, the promastigotes of strain MHOM/BR/73/BH121, *Le. amazonensis* and *Le. guyanensis*, can be distinguished from one another by using a modified morphometric method first introduced by Bruce et al. (1909) for the specific identification of African trypanosomes. The methods used herein could be a useful adjunct in field studies, even when specific monoclonal antibodies, DNA probes and facilities for isoenzyme analyses are available.

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