

TRYPANOSOMA (MEGATRYPANUM) SALOBOENSE N. SP.
(KINETOPLASTIDA: TRYPANOSOMATIDAE)
PARASITE OF MONODELPHIS EMILIAE (MARSUPIALA: DIDELPHIDAE)
FROM AMAZONIAN BRAZIL

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Summary:

Trypanosoma (Megatrypanum) saloboense n. sp., is described in the Brazilian opossum *Monodelphis emiliae* (Thomas, 1912) from primary forest in the Salobo area of the Serra dos Carajás (6° S, 50° 18' W) Pará State, North Brazil. Two morphologically different trypomastigotes were noted. Slender forms, regarded as immature parasites, have a poorly developed undulating membrane adhering closely to the body; large, broad forms with a well developed membrane are considered to be the mature trypomastigotes and have a mean total length of 71.2 µm (62.4-76.2) and a width of 6.1 (5.0-8.0). Infections studied in two opossums were of very low parasitaemia. The large size of *T. (M.) saloboense* readily distinguishes it from the two previously described members of the subgenus *Megatrypanum* of neotropical marsupials, *T. (M.) freitasi* Régo et al., 1957 of *Didelphis azarae* and *D. marsupialis*, and *T. (M.) samueli* Mello, 1977 of *Monodelphis domesticus*, which measure only 49.0-51.5 µm and 42.4 µm respectively. No infections were obtained in hamsters inoculated with triturated liver and spleen from one infected *M. emiliae*, or in laboratory mice inoculated with epimastigotes from a blood-agar culture. No division stages could be detected in the internal organs or the peripheral blood.

KEY WORDS: *Trypanosoma (Megatrypanum) saloboense* n. sp., *Monodelphis emiliae*, Marsupialia, opossum, North Brazil.

Résumé: *TRYPANOSOMA (MEGATRYPANUM) SALOBOENSE* N. SP. (KINETOPLASTIDA: TRYPANOSOMATIDAE) PARASITE DE *MONODELPHIS EMILIAE* (MARSUPIALA: DIDELPHIDAE) DE LA RÉGION AMAZONIENNE DU BRÉSIL.

Trypanosoma (Megatrypanum) saloboense n. sp. est décrit chez *Monodelphis emiliae* (Thomas, 1912), opossum de la forêt primaire de la région de Salobo, Serra dos Carajás (6° S, 50° 18' O) État de Pará, au nord du Brésil. Deux trypomastigotes de morphologies différentes sont observés. Des formes minces, considérées comme immatures, ont une membrane ondulante peu développée qui adhère étroitement au corps du parasite; des formes larges avec une membrane développée sont considérées comme étant des trypomastigotes matures, avec une longueur totale moyenne de 71.2 µm (62.4-76.2) et une largeur de 6.1 (5.0-8.0). Les infections étudiées chez deux opossums étaient de parasitémies très faibles. La grande taille de *T. (M.) saloboense* le distingue assurément de deux membres précédemment décrits du sous-genre *Megatrypanum* chez des marsupiaux néotropicaux, *T. (M.) freitasi* Régo et al., 1957 de *Didelphis azarae* et *D. marsupialis*, et *T. (M.) samueli* Mello, 1977 de *Monodelphis domesticus*, qui mesurent respectivement 49.0-51.5 µm et 42.4 µm. Aucune infection n'a été obtenue chez des hamsters inoculés avec des broyats de foie et de rate de l'un des *M. emiliae* infectés, ni chez des souris de laboratoire inoculées avec des épimastigotes de culture sanguine. Aucun stade en division n'a pu être observé dans les organes internes ou le sang périphérique.

MOTS CLÉS: *Trypanosoma (Megatrypanum) saloboense* n. sp., *Monodelphis emiliae*, Marsupialia, opossum, Brésil.

INTRODUCTION

Wenyon (1926) divided the mammalian trypanosomes into two major biological divisions. Those with development proceeding to the anterior station of the gut (mouthparts or salivary glands) leading to transmission by the bite of the vector, and those in which development in the vector terminates in the faecal medium of the posterior part of the intestine, leading to transmission *via* the faeces. Hoare (1964) gave new descriptive names to these two

groups, namely the Salivaria and the Stercoraria, respectively.

Trypanosomes of the Section Salivaria may or may not possess a free flagellum; the kinetoplast is terminal or subterminal in position, and the posterior end of the parasite is usually rounded. Division of the trypomastigotes takes place in the peripheral blood, and many of these parasites are pathogenic in the mammalian hosts.

Trypanosoma species within the Section Stercoraria were divided by Hoare (1972) into the three subgenera *Megatrypanum* Hoare, 1964, *Herpetosoma* Doflein, 1901 and *Schizotrypanum* Chagas, 1909.

Trypanosomes within the subgenus *Megatrypanum* are large parasites, with the kinetoplast usually situated close to the nucleus and, therefore, far from the posterior end of the organism which is finely pointed. With

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the exception of some members of the subgenus infecting insectivores and ruminants there is a free flagellum. The *Megatrypanum* trypanosomes are considered to be largely non-pathogenic and the majority are seemingly severely host-restricted. Although a few species have been shown to divide as epimastigotes in the blood, or amastigote stages in the internal organs, there has in most instances been a complete failure to detect reproductive stages of the parasites, either in the internal organs or in the peripheral blood. This, and the fact that the blood forms are often divisible into more delicate, slender parasites considered to be developing and immature forms, and large broad forms regarded as the fully developed trypomastigotes, has led to the suggestion that there is, in fact, no division in the vertebrate host and that each trypanosome develops from a single metacyclic form derived from the invertebrate host. Members of the subgenus *Megatrypanum* are found in almost all orders of mammals. They are very similar to many large trypanosomes of amphibians and reptiles and for this reason are considered to be the most primitive of the mammalian trypanosomes.

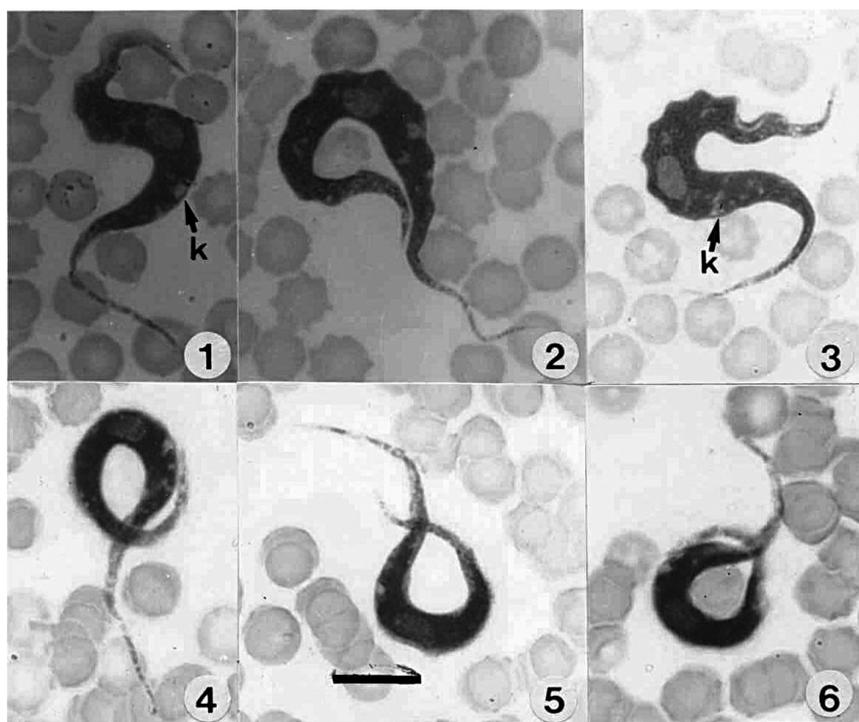
During an examination of sylvatic animals for protozoal parasites in the Salobo area of the Serra dos Carajás, Pará State, north Brazil (6° S, 50° 18' W) in April-May, 2007, a very large trypanosome was encountered in the opossum *Monodelphis emiliae* (Thomas 1912). On size alone, it is readily distinguished from the two species of the subgenus *Megatrypanum* already described in South American marsupials, and the name

Trypanosoma (Megatrypanum) saloboense n. sp. is proposed for the parasite.

MATERIALS AND METHODS

Animals were captured, at ground level in fruit and corn-baited traps. Blood was obtained by cardiac puncture for the preparation of thin blood films which were rapidly air-dried, fixed in absolute methyl alcohol for three minutes and stained by Giemsa's method (30 drops of stain to 15 ml distilled water buffered to pH 7.4). A few drops of blood were cultured in blood-agar slants of BBL-TM culture medium (Becton, Dickinson & Co®) and the liquid phase examined periodically for flagellates. Liver and spleen tissue from one infected opossum was triturated in physiological saline and 0.1 ml of the suspension inoculated intradermally and intraperitoneally into two laboratory hamsters. Epimastigotes from a 3-week-old culture were inoculated intraperitoneally into six young laboratory mice.

All of the trypanosomes encountered in the blood films were photographed, together with the μm scale of a Zeiss slide micrometer, using a Zeiss "Photomicroscope III", $\times 100$ neofluar objective, and $\times 10$ eyepieces. Using the μm scale, total length (including the flagellum) and width were measured for each parasite, together with the distance from the posterior end of the body to the kinetoplast (PK), from the kinetoplast to the centre of the nucleus (KN), centre of the nucleus



Figs 1-6. – *Trypanosoma (Megatrypanum) saloboense* n. sp. of the opossum *Monodelphis emiliae* from Amazonian Brazil. Figs 1-3. Broad, mature trypomastigotes. Figs 4-6. Slender, immature forms. k = kinetoplast in flagellar pocket. Giemsa-stained peripheral blood film. Bar = 10 μm

to the anterior end of the body (NA), posterior end to the centre of the nucleus (PN), length of the free flagellum (FF) and length and width of the nucleus (NL and NW). Measurements (in μm) are given as means, followed by the range in parentheses.

RESULTS

The trypanosome was encountered in the blood films of two of the 20 specimens of *M. emiliae* examined and infections were very light in both cases, with a total of only 25 trypomastigotes detected in four blood films, two from each positive animal. Iso-

lation of the parasite was obtained in culture from one of the infected animals, but growth diminished with each sub-culture and the isolate was finally lost.

As commonly seen among trypanosomes of the sub-genus *Megatrypanum*, there were present the two distinct morphological forms we have previously discussed. The very large, broad parasites possess a well developed undulating membrane, with up to eight undulations (Figs 1-3, 7, 8). The less frequently seen slender forms have a very intensely staining cytoplasm and a poorly developed undulating membrane which is closely applied to the surface of the parasite and frequently undetectable (Figs 4-6, 7, 9). We share the view that these slim forms probably represent immature

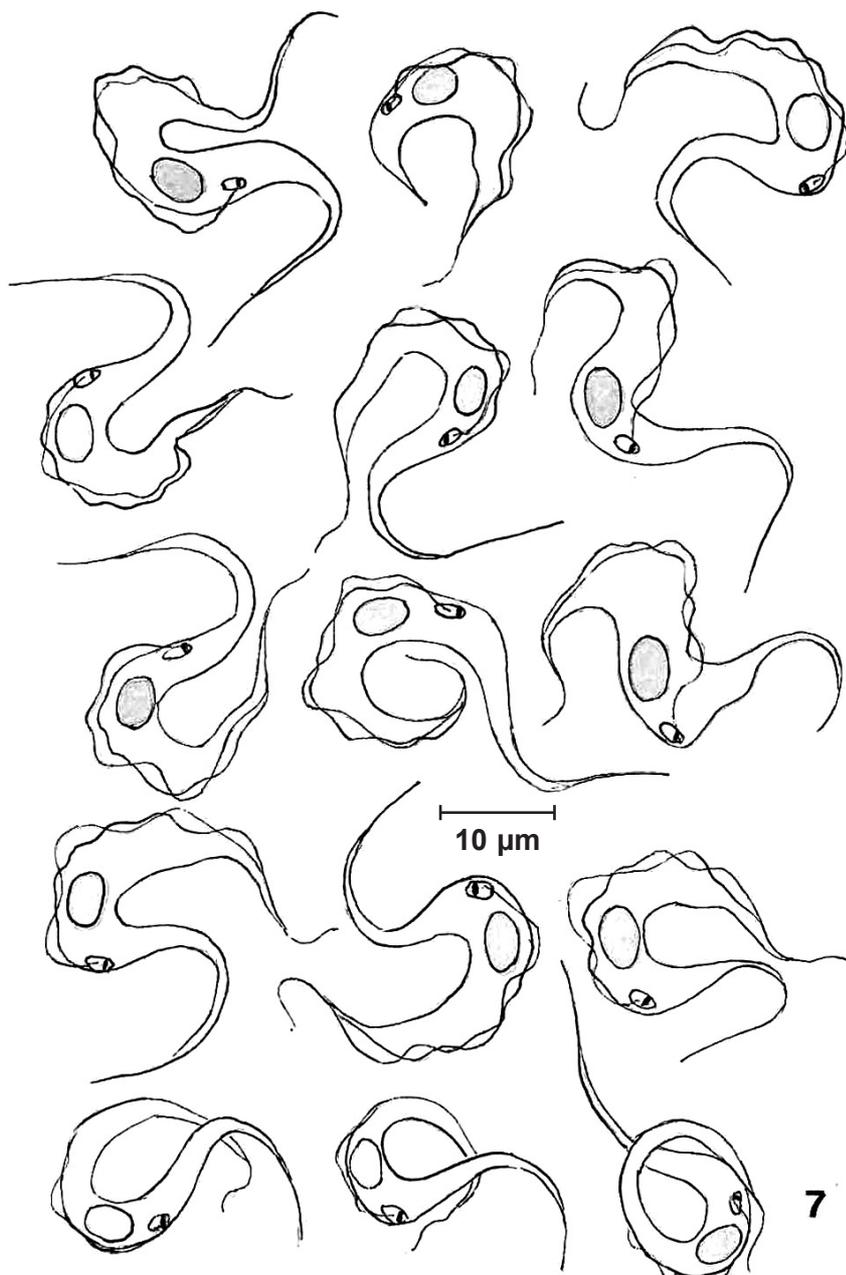
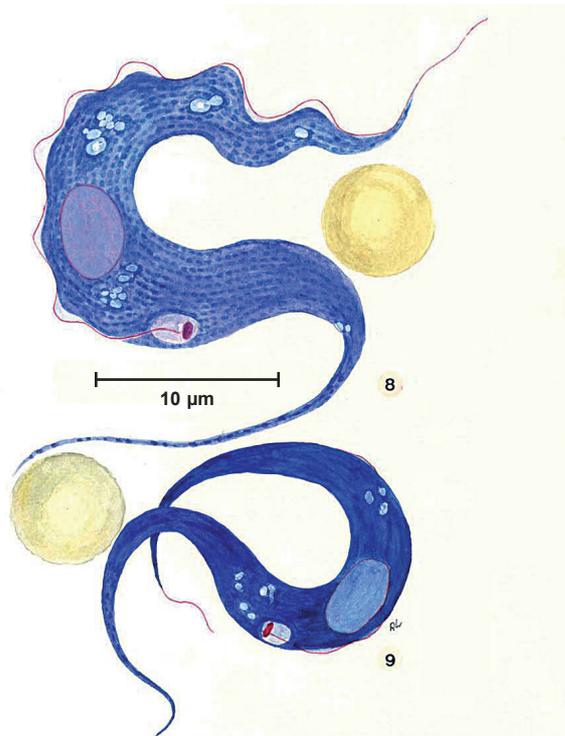


Fig. 7. – Line-drawings of *Trypanosoma (Megatrypanum) saloboense* n. sp. of *Monodelphis emiliae*. The three trypomastigotes at the bottom of the figure are immature, slender forms.



Figs 8, 9. – *Trypanosoma (Megatrypanum) saloboense* n. sp., of *Monodelphis emiliae*, as seen in Giemsa-stained blood films. Fig. 8. Broad, mature trypomastigote. Fig. 9. Slender, immature form.

stages derived from recently acquired metacyclic forms from the invertebrate host, and which will ultimately develop into the broad forms. In Giemsa-stained parasites, the cytoplasm of the broad forms stains a bright blue and has a granular texture due to the presence of innumerable small, darker staining particles: there are occasional vacuoles, particularly noticeable in the posterior part of the body. The oval nucleus is near the middle of the body but slightly more to the anterior part, as shown by the nuclear index (NI) of 1.4 (1.3-1.6), given by the ratio of the distance of the posterior end of the body from the middle of the nucleus to the distance from the middle of the nucleus to the anterior end (PN/NA). It lies longitudinally in the parasite and does not completely occupy the full width of the body. It is virtually colourless or at most stains a very pale pink, and measures 5.4×4.0 (5.0×3.0 - 6.0×4.0). The small, rod-shaped kinetoplast lies in a distinct flagellar pocket and, typical of members of the subgenus *Megatrypanum*, is very close to the nucleus. A major feature of the trypanosome is its very long and finely pointed posterior end: the free flagellum is short, rarely exceeding 6.0 µm.

MEASUREMENTS OF THE LARGE, BROAD FORMS (N = 20)

Total length, including flagellum (L) = 71.2 (62.4-76.2); body length (BL) = 65.2 (56.4-70.2); body width at level of nucleus and excluding the undulating membrane

(BW) = 6.1 (5.0-8.0); posterior end to the kinetoplast (PK) = 30.2 (27.0-33.0); kinetoplast to centre of the nucleus (KN) = 7.2 (5.7-7.7); posterior end to centre of the nucleus (PN) = 37.4 (34.7-39.7); centre of the nucleus to anterior end of the body (NA) = 27.8 (22.7-30.7); free flagellum (FF) = 6.0 (5.0-10.0); nuclear length (NL) = 5.4 (5.0-6.0); nuclear width (NW) = 4.0 (3.0-4.0); nuclear index (NI) = 1.4; kinetoplasmic index (KI) = 5.2; flagellar index (FFI) = 0.1.

MEASUREMENTS OF THE SLENDER FORMS (N = 5)

Total length, including flagellum (L) = 73.8 (67.4-80.4); body length (BL) = 68.0 (61.4-74.4); body width, at level of nucleus (BW) = 4.2 (4.0-5.0); posterior end to kinetoplast (PK) = 31.7 (29.0-33.0); kinetoplast to centre of nucleus (KN) = 6.3 (5.7-7.2); posterior end to centre of nucleus (PN) = 38.0 (34.7-40.2); centre of nucleus to anterior end of body (NA) = 30.0 (26.7-34.7); free flagellum (FF) = 5.8 (5.0-6.0); nuclear length (NL) = 5.0 (5.0-6.0); nuclear width (NW) = 3.1 (3.0-4.0); nuclear index (NI) = 1.3; kinetoplasmic index (KI) = 6.0; flagellar index (FFI) = 0.1.

Type material. Stained blood films (holotypes) to be deposited in the Muséum national d'Histoire naturelle, Depository No. 64/15/4/2007.

DISCUSSION

As far as we are aware, only two species of the subgenus *Megatrypanum* have been described in neotropical marsupials, both in Brazil. *T. (M.) freitasi* Régo, Magalhães & Siqueira, 1957 was described in the opossum *Didelphis azarae* from the State of São Paulo, and re-encountered by Deane (1964) and Rocha e Silva *et al.*, (1976) in *Didelphis marsupialis* from the States of Pará and São Paulo, respectively. The second species, *T. (M.) samueli* Mello, 1977 was described in *Monodelphis domesticus* in the State of Goiás. Both of these species are much smaller than *T. (M.) saloboense*, which may reach up to 76.2 in total length. That of *T. (M.) freitasi* was said to vary from 49.0-51.5 and that of *T. (M.) samueli* was given as only 42.4. Although both of these species have the characteristically sharply pointed posterior end of the body seen in members of the subgenus, that of *T. (M.) saloboense* is particularly long and attenuated (Figs 1-3), with a mean PN of 37.5 compared with only 14.8 and 19.5-20.0 for *T. (M.) samueli* and *T. (M.) freitasi*, respectively. Comparative measurements of the three species from neotropical marsupials are given in Table I.

No infections were detected in the two hamsters inoculated with the liver and spleen suspension and none in the mice inoculated with cultured epimastigotes. Failure to infect mice was also recorded in the descriptions of both *T. (M.) freitasi* and *T. (M.) samueli*, and

Measurements	<i>T. (M.) freitasi*</i>	<i>T. (M.) samueli*</i>	<i>T. (M.) saloboense n. sp.</i>
Total length (L) with flagellum	49.0-51.5	42.4 (± 2.44)	71.2 (62.4-76.2)
Body length (BL)	36.0-40.0	36.3	65.2 (56.4-70.2)
Body width (BW)	7.5-10.0	3.1 (± 0.2)	6.1 (5.0-8.0)
Posterior end to kinetoplast (PK)	9.5-12.5	12.4 (± 0.99)	30.2 (27.0-33.0)
Posterior end to mid-nucleus (PN)	19.5-20.0	14.8	37.4 (34.7-39.7)
Kinetoplast to mid-nucleus (KN)	7.0-9.5	3.0-3.5***	7.2 (5.7-7.7)
Mid-nucleus to anterior end (NA)	16.5-20.5	21.5	27.8 (22.7-30.7)
Free flagellum (FF)	11.0-13.0	7.2	6.0 (5.0-10.0)
Nucleus length and width (NL, NW)	–	–	5.4 × 4.0 (5.0 × 3.0-6.0 × 4.0)
Nuclear index (NI)	1.0-1.2	0.68	1.4
Kinetoplastic index (KI)	2.0	1.2	5.2
Flagellar index (FFI)	0.3	0.2	0.1

* From Régo *et al.*, (1957); ** From Mello (1977); *** calculated from Mello's figures.

Table I. – Comparative measurements (in µm) for *Trypanosoma (Megatrypanum)* species of neotropical marsupials.

it would appear that all three species are considerably host restricted.

The vector of *T. (M.) saloboense* remains to be indicated, but it is of interest that attempts to infect triatomine bugs with *T. (M.) freitasi* and *T. (M.) samueli* have all failed (Régo *et al.*, 1957; Deane, 1964 and Rocha E Silva *et al.*, 1976; Mello, 1977). The same authors recorded failure to detect division of these trypanosomes in the tissues of their hosts, and we found no such stages of *T. (M.) saloboense* in the peripheral blood or impression smears of the viscera of the infected *M. emiliae*. Although infections of *T. (M.) saloboense* and *T. (M.) freitasi* appear to be of low parasitaemia and non-pathogenic, Mello (1977) recorded a high parasitaemia of *T. (M.) samueli* in *M. domesticus* and the animal died after only eight days in captivity. Whether or not death was due to this trypanosome, however, remained uncertain.

As is often the case with trypanosome infections, the number of parasites circulating in the blood of both infected opossums was very low, and we were unable to detect more than 25 examples in four blood films. Although it would clearly have been more satisfactory to have measured a larger number we feel that the consistency of the morphological details of the examples studied, and their marked difference from those of the two previously described *Megatrypanum* species of neotropical marsupials, warrants the use of the new specific name of *T. (M.) saloboense* for the parasite of *M. emiliae*.

Since this paper was prepared, we have also encountered this trypanosome in the closely related opossum *Monodelphis breviceaudata* (Erxleben, 1777) from the same locality. Once again, the parasitaemia was at very low level.

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