

## A NEW TRYPANOSOME IN *SAIMIRI* MONKEYS FROM COLOMBIA

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### SUMMARY

In a squirrel monkey, *Saimiri sciureus*, from Colombia the authors found a new trypanosome resembling *T. cruzi* which they named *Trypanosoma sanmartini*. It easily cultivates, is infective to mice and develops in the intestinal tract of *Rhodnius prolixus*, but differs from *T. cruzi* in morphological details and by being confined to the blood of the vertebrate host, producing no leishmania forms in the viscera. It occasionally invades the haemolymph of the bug and could be transmitted through its bite. Cross immunity experiments confirmed the distinction between *T. sanmartini* and *T. cruzi*.

*Editor's summary*

### INTRODUCTION

Trypanosomes have been reported on many occasions from the monkeys of the New World, and before identifying a fresh isolation as a new species, it is necessary to verify both that it possesses different characters from any other flagellate, and that it is not merely an old parasite in a new guise as the result of inhabiting an abnormal host. When, therefore, we encountered what we thought was a new trypanosome in the blood of a *Saimiri* monkey in Colombia in 1959, we realized that we should have to study it for a sufficiently long time to be sure of its nature.

The infected *Saimiri* (S 12) was captured in October 1959 near Leticia on the upper reaches of the Amazon, and the *cruzi*-like trypanosome in its blood has been examined by us in Cali and London, in subinoculated animals, in culture and in reduviid bugs. The account which follows is based on our findings. The organism is named *Trypanosoma sanmartini* sp. nov. in honour of Dr. Carlos Sanmartin Barberi at whose invitation the senior author visited Colombia in 1959, and under whose auspices the work was carried out.

### STAGES IN THE VERTEBRATE HOST

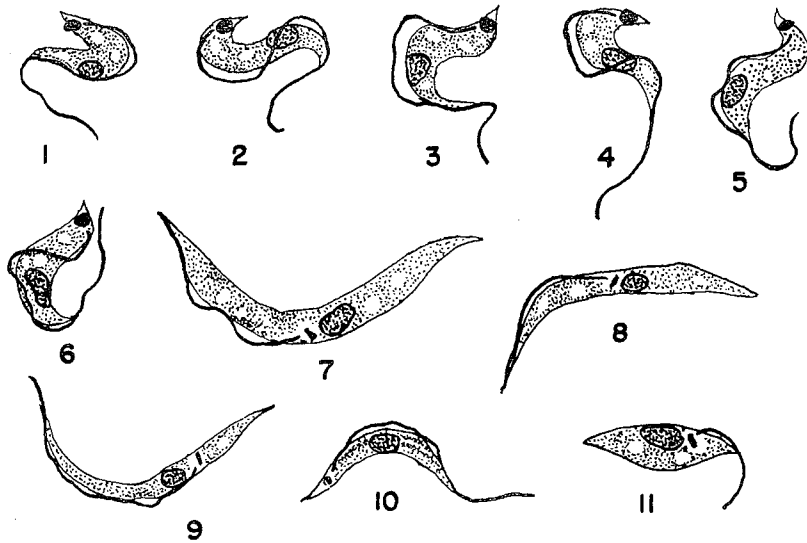
The natural host of *T. sanmartini* is the squirrel monkey *Saimiri sciureus* and apparently the parasite is confined to the blood. The trypanosome resembles *T. cruzi* in general appearance, but it possesses some clear differentiating features.

It is a curved trypanosome (Figs. 1-6) often in the form of the letter S, with an average length of 19  $\mu$  including the free flagellum (usually extending about 7  $\mu$  beyond the anterior tip of the body). The flagellum is attached to the trypanosome by a moderately developed undulating membrane. A large egg-shaped kinetoplast occupies the posterior extremity, and the actual tip is often hidden by this structure. The nucleus is oval and sometimes lies anteriorly to the mid-point of the body. One or two vacuoles may be present in the cytoplasm.

The organism preserves its form in dried smears, unlike *T. cruzi*, which easily disintegrates into "shadow" forms. The new trypanosome apparently possesses tougher structure.

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Figs. 1-6 — Blood forms of *Trypanosoma sanmartini* in *Saimiri* monkeys from Colombia. Figs. 7-10 — Culture forms of *T. sanmartini*. Fig. 11 — Crithidial form of *T. sanmartini* in dejecta of bug.

The dimensions of measured specimens (from 20 trypanosomes) are given in Table I.

TABLE I

Measurements (in micra) of blood forms of *T. sanmartini*.

	Minimum	Maximum	Mean
Total length	17	24	19
Kinetoplast-Nucleus	4	9	6
Length of nucleus	2	3.5	3
Nucleus-Anterior tip	3	6.5	4
Free flagellum	4	9	6
Greatest width	2	3	2.5

The examination of smears and sections of organs of both naturally infected monkeys, and other animals infected with a) metacyclic trypanosomes, b) blood trypanosomes, and c) cultures, revealed no leishmanial forms.

#### CULTURAL FORMS

The trypanosome grows easily in NNN, Geiman's and Noguchi-Wenyon media, in which, at 28°C, it quickly assumes a crithidial form; it may be maintained at this temperature or better at the lower temperature of the laboratory.

Polymorphic forms are to be found in the older cultures, in which leishmanial as well as short or long crithidial stages occur (Figs. 7, 8). The latter measure about 12-15  $\mu$  in length, though sometimes they may be longer. The kinetoplast varies from a small dot to a rod-shaped body and the egg-like shape of the adult is rarely seen. Even in the metacyclic trypanosomes, which eventually appear, the kinetoplast maintains the rod-shape (Figs. 9, 10), i.e., while it is still only just behind the nucleus and before it has taken up its position at the posterior extremity.

#### BEHAVIOUR IN REDUVIID BUGS

The trypanosome develops readily in the intestinal tract of *Rhodnius prolixus*, after the bugs have taken a blood meal from an

infected animal. In the same circumstances, *Triatoma infestans* failed to become infected, and a slight infection only was obtained in *T. phyllosomae*.

Infection of *Rhodnius* occurred after the bugs had fed either on monkeys or on sub-inoculated mice. *Rhodnius* were fed on the original *Saimiri* monkey, and four weeks later, this batch of bugs was still heavily positive. In another experiment, in which a second *Saimiri* monkey (S 13) was used, *Rhodnius* bugs were again successfully infected.

*Rhodnius prolixus* were also fed on infected baby mice, and showed numerous crithidia in the mid gut some weeks later; the infection persisted for at least two months. This species of bug became infected even after feeding on baby mice inoculated with cultures of the parasite which had been maintained in the laboratory for nearly 2 years, though the infection rate was much lower in the bug, and was occult in the mice.

Crithidial stages and metacyclic trypanosomes are found in the mid gut, and these forms resemble the cultural flagellates (Fig. 11); they are of relatively small size.

The great point of interest in the infection of *Rhodnius prolixus* is the occasional invasion of its haemolymph by the developing parasite. Thus, bugs which had fed on infected mice in October 1959, first showed crithidia in the haemolymph on December 21<sup>st</sup>, while a bug which had fed on the original infected *Saimiri*, on October 21<sup>st</sup>, 1959, showed crithidia in the haemolymph on January 5<sup>th</sup>, 1960. The infection in the haemolymph was inconstant, and only a few specimens were invaded in this way; yet, as will be noted below, transmission by the bite of these bugs occurred on several occasions.

#### BEHAVIOUR OF *T. SANMARTINI* IN ANIMALS

*In monkeys:* A clean *Saimiri* monkey (S 13) was inoculated with 0.1 ml of dejecta of an infected *Rhodnius* on 22-12-59, and

*T. sanmartini* was first detected in its blood 3 weeks later. The parasitaemia increased, and continued in a varying degree until the death of the animal on 21-6-61. The trypanosomes increased greatly in number after a splenectomy, performed on 31-3-60.

*In mice:* Mice about 4 to 8 days old are easily infected with the blood forms of the organism, which take a week to become detectable in the peripheral blood. Intraperitoneal inoculation of cultural forms also proved infective to baby mice in which the parasitaemia persisted for at least a month.

Two *Rhodnius prolixus* with flagellates in the haemolymph were allowed to feed on batches of mice (4-10 days old); one bug transmitted the infection to 2 mice, with an incubation period of 4 days, the second bug successfully infected 3 mice with an incubation period again of 4 days. The parasitaemia lasted in several of these animals for a month.

*In guinea pigs:* Guinea pigs, 6 weeks old, were inoculated with cultural forms and infected dejecta, but no infection was detected either by direct examination of blood or by xenodiagnosis.

#### DISCUSSION

It is necessary first to compare this trypanosome of the *Saimiri* monkey with other trypanosomes reported from New World monkeys. Table II summarizes the relevant information, and a useful comparative account has recently been given by DEANE & DAMASCENO<sup>5</sup>. In addition, an unnamed trypanosome of *Saimiri* monkeys (*S. boliviensis*) has been described from Peru (MORALES<sup>12</sup>), which in all respects appears to be identical with *T. cruzi*.

*T. saimirii* has been found in *Saimiri* monkeys by RODHAIN<sup>13</sup>, by DEANE & DAMASCENO<sup>5</sup> and by others, the dimensions of their respective organisms differing slightly, but they were all found to possess a kinetoplast far from the posterior tip, which clearly differentiates this species from ours.

TABLE II  
Trypanosomes of New World monkeys.

Name	Author	Host	Locality	Morphology
<i>Minasense</i>	Chagas	<i>Callithrix</i>	Brazil	30 $\mu$ in length; + 10 $\mu$ flagellum
<i>Saimirii</i>	Rodhain	<i>Saimiri</i>	Brazil	31 $\mu$ total length; kinetoplast 5 $\mu$ from tip
Unnamed (? <i>minasense</i> )	Renjifo	<i>Callicebus</i>	Colombia	Large and wide
<i>Advieri</i>	Floch	<i>Ateles</i>	French Guiana	21 $\mu$ in length + 5 $\mu$ flagellum ends drawn out
<i>Lesourdi</i>	Leger	<i>Ateles</i>	French Guiana	14 $\mu$ in length; 5 $\mu$ broad
<i>Diasi</i>	Deane & Martins	<i>Cebus</i>	Brazil	34 $\mu$ in length
<i>Cebus</i>	Floch & Abon-nenc	<i>Cebus</i>	French Guiana	40 $\mu$ in length; kinetoplast 7 $\mu$ from tip
<i>Devei</i>	Leger	<i>Midas</i>	French Guiana	44 $\mu$ in length
	Deane	<i>Leontocebus</i>	Brazil	Kinetoplast near nucleus
<i>Forestali</i>	Romaña	<i>Alouatta</i>	Argentina	Close to <i>T. mycetæ</i>
<i>Manguinhense</i>	Arantes & Fonseca	<i>Alouatta</i>	Brazil	
<i>Brimonti</i>	Giaquinto	<i>Alouatta</i>	Guatemala	
<i>Mycetæ</i>	Brumpt	<i>Alouatta</i>	Brazil & French Guiana	22 $\mu$ in length; kinetoplast 5 $\mu$ from tip
<i>Prowazeki</i>	Berenberg-Gosler	<i>Cacajao</i>	Brazil	14 $\mu$ excluding flagellum; kinetoplast near tip

All other species in Table II again exhibit marked differences except for *T. prowazeki*, about which little information is available. The host — cacajao ou uakari — is well removed from the *Saimiri* or squirrel monkey, and because the South American trypanosomes in general seem to have considerable host-specificity, we think we are justified in giving a specific identity to the Colombian trypanosome. CHAGAS<sup>3</sup> regarded *T. prowazeki* as distinct from *T. cruzi*.

However, a greater problem is the question of its differentiation from *T. cruzi*, particularly as CHAGAS<sup>3</sup> himself, identified as *T. cruzi*, a trypanosome which he found

in *Saimiri* monkeys coming from Pará in Brazil. (This record has been excluded from Table II, as being a human trypanosome). *T. cruzi* however differs in the following respects from *T. sanmartini*:—

1. *T. cruzi* is longer — 25  $\mu$  instead of 19  $\mu$ .
2. The kinetoplast of *T. cruzi* is placed near the posterior extremity, instead of practically occupying the tip.
3. *T. cruzi* produces leishmanial forms in the tissues, which are absent in *T. sanmartini*.

4. *T. cruzi* is infective to *Triatoma infestans*; *T. sanmartini* is normally non-infective. Moreover the latter occasionally invades the haemolymph of *Rhodnius*, while *T. cruzi* is confined to the gut.

Confirmation of the distinction between these two species was provided by cross-immunity tests. Six baby mice were inoculated with a culture of *T. sanmartini*, and half were given a second dose after 3 weeks. The animals were then challenged with the Sonya strain (GARNHAM<sup>9</sup>) of *T. cruzi* and all responded with normal infections of the latter; this experiment thus indicated the absence of cross immunity between the two organisms.

In this investigations we encountered a number of other trypanosomes in Colombian monkeys. We found a *T. rangeli*-like organism in 3 out of 20 *Lagothrix lagothrix* (Leticia), in 1 out of 3 *Aotus trivirgatus* (Villavicencio), and in 2 out of 5 *Saimiri* (Villavicencio). But a detailed study of these organisms has not yet been undertaken.

#### DIAGNOSIS

*Trypanosoma sanmartini* sp. n. is a small curved trypanosome with an egg-shaped kinetoplast occupying the posterior tip, with no leishmanial stages in the tissues, but with a developmental cycle in the gut and haemolymph of *Rhodnius prolixus*. The vertebrate host is *Saimiri sciureus*. Type locality: Leticia, Colombia.

#### RESUMO

Nôvo tripanosoma do macaco "Saimiri", da Colômbia.

No sangue de um "macaco-de-cheiro", *Saimiri sciureus*, da Colômbia, os autores encontraram um nôvo tripanosoma, semelhan-

te ao *T. cruzi*, e que designaram como *Trypanosoma sanmartini*.

Cultiva fãcilmente, infeta camundongos e evolui no intestino do *Rhodnius prolixus*, mas difere do *T. cruzi* por detalhes morfológicos e porque no vertebrado limita-se ao sangue, não produzindo formas leishmãnicas nas vísceras. Ocasionalmente invade a hemolinfa do triatomíneo e pode ser transmitido por sua picada. Experiências de imunidade cruzada confirmaram ser o nôvo tripanosoma distinto do *T. cruzi*.

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