Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *86* (1): 73-79 jan./mar. 1991

TRYPANOSOMA RANGELI (TEJERA, 1920) ISOLATED FROM A SYLVATIC RODENT (*ECHIMYS DASYTHRIX*) IN SANTA CATARINA ISLAND, SANTA CATARINA STATE: FIRST REPORT OF THIS TRYPANOSOME IN SOUTHERN BRAZIL

M. STEINDEL; J. C. CARVALHO PINTO; H. K. TOMA; R. H. R. MANGIA*;
R. RIBEIRO-RODRIGUES**& A. J. ROMANHA**

Universidade Federal de Santa Catarina, Departamento de Microbiologia e Parasitologia, Caixa Postal 476, 88049 Florianópolis, SC, Brasil *Instituto Oswaldo Cruz, Departamento de Protozoologia, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brasil **Centro de Pesquisas René Rachou, Laboratório de Imunologia e Biologia Molecular, Caixa Postal 1743, 30190 Belo Horizonte, MG, Brasil

*A trypanosome strain isolated from a sylvatic rodent (*Echimys dasythrix*) from Santa Catarina Island (Santa Catarina State, Brazil) was characterized by the following methods: experimental transmission and development in invertebrate and vertebrate hosts, morphometry, cross protection, complement sensitivity, lectin agglutination and isoenzyme profiles. Comparisons were made with standard* Trypanosoma cruzi *and* T. rangeli *strains. All methods except isoenzyme analysis led to the identification of the isolate as* T. rangeli. *The isoenzyme differences found could be explained on the basis of polymorphism. Therefore this is the first report of* T. rangeli *in southern Brazil, increasing the geographical distribution of this parasite.*

Key words: *Trypanosoma rangeli* – rodent – *Echimys dasythrix* – experimental development – complement lysis – isoenzyme patterns – epidemiology

Trypanosoma rangeli is found in a wide range of mammals and triatomine bugs, often occuring together with *T. cruzi* in the same vertebrate and invertebrate hosts. The geographical distribution of *T. rangeli* comprises Central, Northern and Western South America (D'Alessandro, 1976).

The classical test for recognizing mammalian trypanosomes as *T. rangeli* is finding parasites in the haemolymph and salivary glands of infected triatomine bugs and inducing transmission to a vertebrate host by their bite (Hoare, 1972; D'Alessandro, 1976). However, additional methods such as complement lysis of cultured epimastigotes (Schottelius, 1982; Salgado, 1988), isoenzyme patterns (Kreutzer & Sousa, 1981; Miles et al., 1983; Ebert, 1986; Salgado, 1988), lectin binding (Schottelius & Muller, 1984; Miranda-Santos & Pereira, 1984; Salgado, 1988) and monoclonal antibodies (De-Simone et al., 1987; Hudson et al., 1987) have been used to differentiate *T. cruzi* from *T. rangeli* and other morphologically similar trypanosomes.

Received 22 August 1990.
Accepted 23 October 1990.

In Brazil, parasites similar to *T. rangeli* were observed in faeces of *Panstrongylus megistus* used in human xenodiagnosis in a patient from Alagoas State (Lucena & Marques, 1954), in the haemolymph of a sylvatic nymph of *Rhodnius domesticus* in Bahia State (Barrett & Oliveira, 1977) and in the blood of *Metachirops opossum opossum* and *Didelphis marsupialis*, from the Amazon region (Deane, 1958a, b). Miles et al. (1983) characterized 46 strains of trypanosomes isolated from different wild mammals, triatomine bugs and a sandfly from the Amazon Basin, which were identified as *T. rangeli* by biological and isoenzyme methods.

In Santa Catarina Island, Santa Catarina State, *T. cruzi* has been the only human infective trypanosome described. It was detected in sylvatic vectors and reservoirs (Leal et al., 1961; Schlemper Jr et al., 1985). Recently, we have isolated in the same island, a trypanosome distinct from *T. cruzi* from the rodent *Echimys dasythrix*, described previously as *T. cruzi* sylvatic reservoir. In the present work, we characterize for the first time in southern Brazil this parasite, as *T. rangeli*, on the basis of: 1) experimental transmission and development in invertebrate and vertebrate hosts, 2)

morphometry of blood trypomastigotes, culture and salivary gland forms, 3) cross protection, 4) human and guinea pig complement susceptibility, 5) lectin agglutination and 6) isoenzyme profiles.

MATERIALS AND METHODS

During a survey of the sylvatic cycle of *T. cruzi* at Ribeirão da Ilha, Santa Catarina Island, we isolated from the rodent *E. dasythrix* by haemoculture in LIT medium (Liver Infusion Tryptose) and by xenodiagnosis with nymphs of *P. megistus* and *Triatoma infestans* a different trypanosome strain coded as SC-58.

Experimental transmission and development in invertebrate and vertebrate hosts – Outbred 1-month old male albino mice, were inoculated i.p. either with 15-day-old culture parasites grown in LIT or flagellates from infected bug faeces. Searches for blood forms were made daily after the 3rd day from inoculation in fresh and Giemsa stained smears; haemoculture in biphasic medium (blood agar + LIT) on the 6th day of infection and xenodiagnosis with 30 fourth instar nymphs of *R. prolixus* and *R. domesticus* on the 10th day of infection.

The search for parasites was made in bugs after 30 days of xenodiagnosis by fresh and Giemsa stained smears of faeces, haemolymph and salivary glands. The nymphs presenting parasites in the haemolymph were fed on uninfected mice that were immobilized in narrow tubes, to prevent the eventual contact of bug faeces with the animals. Fresh and stained blood smears and haemoculture were made further to confirm the infection of mice by triatomine bite. To check any cross protection, groups of 5 mice infected with SC-58 strain were challenged 60 days after infection with 10^5 *T. cruzi* Y strain blood trypomastigotes (Silva & Nussenzweig, 1953). Parasitaemia and mortality were followed, having as the control mice infected with Y strain only.

Morphometry – The morphometry was made as described by Hoare (1972).

Complement lysis – Epimastigotes from seven-day-old cultures were harvested and washed 3 times in PBS + 0.5% Foetal Bovine Serum (FBS) at 1,000 g for 10 min at 4 °C and resuspended in PBS at the concentration of 10^8 cells/ml and maintained on ice. *T. cruzi* Y and SC-28 strains and *T. rangeli* strain H-14, were used as controls. SC-28 is a typical sylvatic *T. cruzi* strain, isolated on the same island as SC-58, from the opossum (*D. marsupialis*) and *T. rangeli* strain H-14 was isolated from a human case in Honduras by haemoculture (Salgado, 1988). The complement was obtained from fresh human and guinea pig sera. Serum inactivated at 56 °C for 30 min was used as a negative control. Fifty μl of the parasite suspension were incubated with 100 μl of undiluted serum in U bottom microtitration plates for 30 min at 25 °C and the lysis confirmed microscopically.

Lectin agglutination – Epimastigotes from seven-day-old cultures were harvested and washed in PBS at 1,000 g for 10 min at 4 °C and resupended in PBS at the concentration of 10^8 cells/ml. WGA lectin from *Triticum vulgaris* (SIGMA Chemical Company) was used at final concentrations of 25, 125 and 625 μg/ml. As inhibition control we used WGA (125 μg/ml) preincubated with an equal volume of 0.2M N-acetyl-D-glucosamine for 10 min. For agglutination tests 25 μl of parasite suspension and 25 μl of WGA solutions at different concentrations were incubated at 25 °C for 30 min in microtitration plates. As a control of autoagglutination, some flagellates were also incubated in PBS without lectin. The presence or absence of agglutination was observed at microscope.

Isoenzyme profiles – Culture forms were harvested and washed 3 times in KRT buffer (Krebs-Ringer-Tris) pH 7.3 at 1,000 g for 15 min at 4 °C. The extract was prepared as described by Romanha (1982). Enzyme electrophoresis was performed in thin layer starch gels refrigerated at 4 °C. Six enzymes were analyzed: alanine aminotransferase (ALAT) (E.C.2.6.1.2); aspartate aminotransferase (ASAT) (E.C.2.6.1.1); glucose phosphate isomarase (GPI) (E.C.5.3.1.9); phosphoglucomutase (PGM) (E.C.2.7.5.1); malic enzyme (ME) (E.C. 1.1.1.40) and glucose 6 phosphate dehydrogenase (G6PD) (E.C. 1.1.1.49). The running and development conditions were those described by Romanha (1982). *T. rangeli* H-14 strain and three standard zymodemes of *T. cruzi* (Z1, Z2 and ZC) were used as controls.

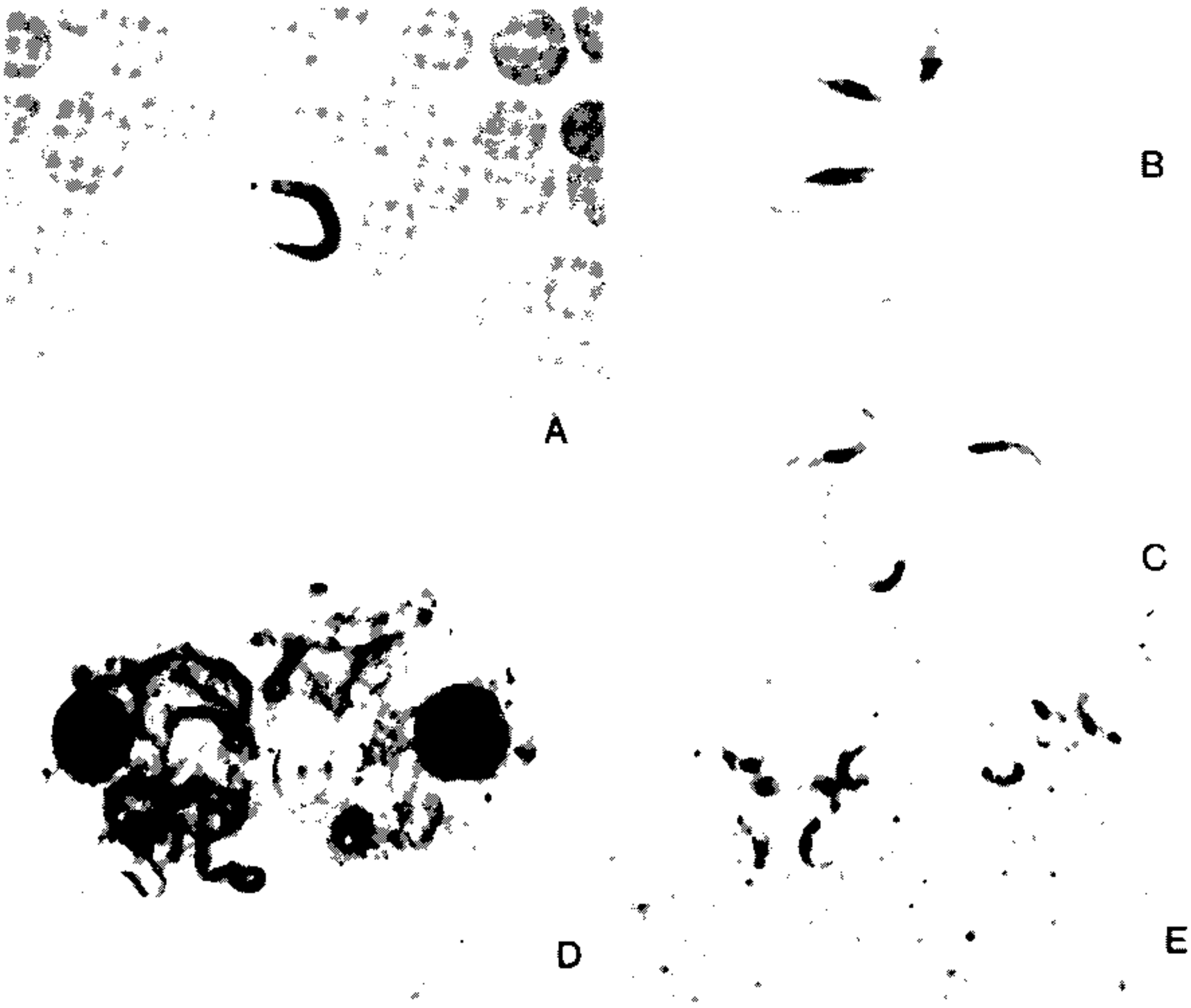

Fig. 1: morphology of parasites from SC-58 strain. A – Blood trypomastigote from experimentally infected mice. B – Culture forms in LIT medium: short epimastigotes with small free flagellum. C – Large epimastigotes and trypomastigote forms. D – Parasites inside *Rhodnius domesticus* hemocytes. E – Metatrypanosomes from salivary glands of experimentally infected *R. domesticus*. X 1,000.

TABLE I

Morphological comparisons between strain SC-58 blood trypomastigotes and previously published data on *Trypanosoma rangeli*

Strain code (host)	Measured parasites	Measures (μm^a)				
		L	PK	KN	NA	F
SC-58 (*E. dasythrix*)	25	25,5-34,0 (30,7)	3,5-5,0 (4,0)	5,5-11,0 (9,2)	7,0-10,0 (9,0)	7,0-11,0 (9,5)
IM-144 (*D. marsupialis*)[b]	20	28,3-36,7 (32,7)	2,7-4,3 (3,4)	7,0-11,7 (9,4)	7,0-12,0 (10,4)	7,0-11,0 (9,6)
BUG 1798 (*R. robustus*)[b]	25	27,0-37,0 (29,1)	1,7-5,7 (3,3)	7,0-12,7 (9,2)	6,6-11,7 (9,1)	5,0-10,3 (7,5)
R 1625 (man)[b]	23	28,0-36,0 (32,1)	2,7-4,0 (3,3)	9,3-12,7 (10,7)	6,3-11,7 (8,8)	7,0-10,7 (9,4)
Various[c]	–	25,0-37,0	1,8-7,0	8,2-10,0	5,0-12,0	5,0-11,0

a: after Hoare, 1972; L = total length; PK = distance from posterior end to kinetoplast; KN = distance from kinetoplast to nucleus; NA = distance from nucleus to anterior end; F = free flagellum. Ranges given with mean in parentheses.
b: from Miles et al., 1983.
c: from D' Alessandro, 1976.

TABLE II

Morphological data from strain SC-58: culture and salivary gland forms

Forms[b]		Measures (μm[a])				
		L	PK	KN	NA	F
Culture epimastigote	large	35,8 ± 7,7[c]	11,2 ± 1,4	1,9 ± 1,9	13,2 ± 2,9	10,9 ± 4,5
	short	20,6 ± 3,5	8,7 ± 1,4	1,2 ± 0,3	5,5 ± 1,0	5,5 ± 0,8
Culture trypomastigotes	large	39,5 ± 5,5	14,2 ± 3,3	2,3 ± 0,6	11,9 ± 8,0	11,0 ± 2,1
	short	16,7 ± 2,2	2,1 ± 0,9	3,6 ± 1,0	5,4 ± 1,2	5,7 ± 0,1
Salivary gland trypomastigotes		9,3 ± 0,9	0,9 ± 0,3	2,3 ± 0,4	3,0 ± 0,4	3,1 ± 0,4

a: see Table I.
b: fifty randomly chosen parasites were measured.
c: mean value plus or minus standard deviation.

RESULTS

Experimental development – Only mice inoculated with cultures of SC-58 strain were positive by haemoculture and xenodiagnosis. *R. domesticus* and *R. prolixus* fed on infected mice showed parasites in their faeces after 30 days of infection, and 3 (10%) *R. domesticus* and 1 (3.3%) *R. prolixus* presented parasites in the haemolymph and salivary glands after 38 days of infection (Fig. 1). *R. domesticus* and *R. prolixus* nymphs with parasites in the haemolymph were capable of infecting mice by successive bites. All SC-58 strain infected mice challenged with *T. cruzi* Y strain presented the same parasitaemia and mortality as the controls.

Morphometry – The morphometric data of 25 blood trypomastigotes from strain SC-58 compared with data from other authors are shown in Table I. The SC-58 strain was highly polymorphic in culture and the parasites from salivary glands were the typical metatrypanosomes (Table II; Fig. 1).

Complement lysis – While neither SC-58 or H-14 strain epimastigotes were lysed by human and guinea pig sera complement, 95% of the Y and SC-28 strain epimastigotes of *T. cruzi* were lysed. No lysis was observed when Y and SC-28 strain epimastigotes were incubated with inactivated human and guinea pig sera (data not shown).

Lectin agglutination – Agglutination of Y strain epimastigotes increased with the WGA concentration. The epimastigotes of SC-58 and H-14 showed only a weak agglutination at the highest concentration of lectin. The preincubation of 0.1M N-acetyl D-glucosamine with WGA lectin inhibited completely the agglutination of Y strain epimastigotes. Autoagglutination was not observed for any of the parasite strains (Table III).

TABLE III

Lectin agglutination of epimastigote culture forms of *Trypanosoma cruzi* Y, *T. rangeli* H-14 and SC-58 strains

Trypanosome	WGA concentration (μg/ml)				
strain	25	125	625	NAcGlu[a]	PBS
Y	+	++	+++	–	–
H-14	–	–	+	–	–
SC-58	–	–	+	–	–

a: 0,1 M N-acetyl-D-glucosamine was pre-incubated with 125 μg/ml WGA and then the parasites were added.
Agglutination intensity: (+++) high, (++) medium, (+) low and (–) absence.

Isoenzyme patterns – The comparative diagram of the isoenzyme patterns presented in Fig. 2 shows strain SC-58 as closer to *T. cruzi* than to *T. rangeli*. Three (ASAT, ALAT and G6PD) out of six enzymes showed the same pattern for SC-58 and *T. cruzi* Z1. SC-58 showed the same pattern as *T. rangeli* only for ME.

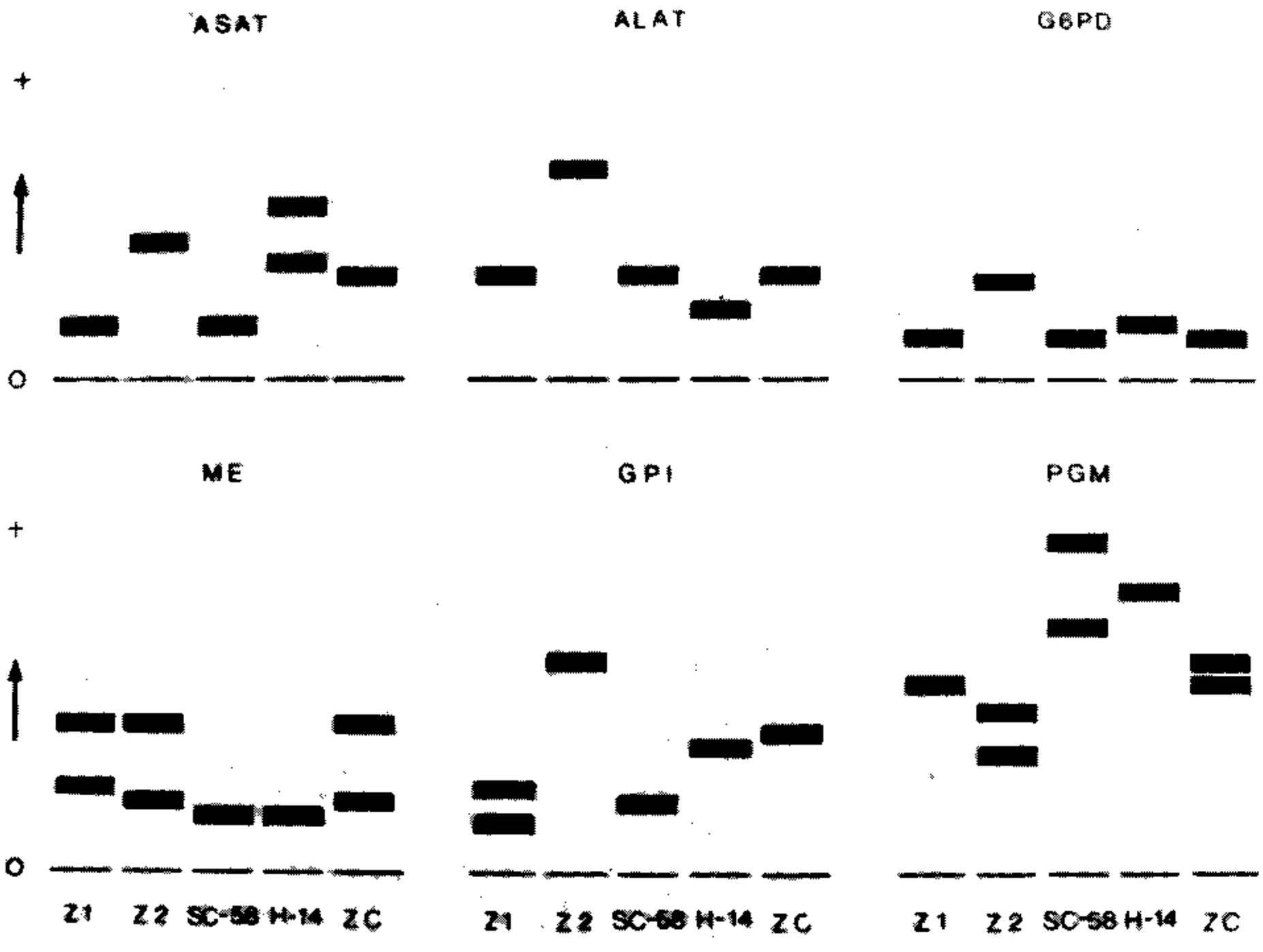

Fig. 2: diagram of the isoenzyme patterns presented by SC-58, *Trypanosoma rangeli* H-14 and *T. cruzi* Z1, Z2 and ZC standard zymodemes.

DISCUSSION

The presence of *T. rangeli* in southern South America, which is based only on finding *T. rangeli*-like organisms in triatomine faeces or in mammalian blood, is controversial (D'Alessandro & Prado, 1977). In Brazil, Miles et al. (1983) were the first to characterize *T. rangeli* by biological methods and isoenzyme patterns in the Amazon basin. Our strain SC-58 fulfilled the main biological characteristics of *T. rangeli.*

Tobie (1961) showed that some strains of *T. rangeli* had different development patterns in *R. prolixus.* The infection rate of strain SC-58 was greater in the haemolymph and salivary glands of *R. domesticus* than *R. prolixus.* The experimental transmission of *T. rangeli* by infected bug faeces to mice was shown by Coutinho & Nussenzweig (1952) and Grewal (1956), but was not confirmed by Tobie (1964) or Cuba Cuba (1973) and ourselves. Faeces of *P. megistus, T. infestans, R. domesticus* and *R. prolixus* infected with our strain SC-58 were not infective to mice.

The culture forms of SC-58 showed a high polymorphism during maintenance in LIT medium and the blood trypomastigotes and salivary gland metatrypanosomes presented morphological data in accordance with previous published reports for *T. rangeli* (Tobie, 1964; Hoare, 1972). Mice infected with SC-58 were not protected against a *T. cruzi* Y strain challenge. Absence of cross protection was also reported by D'Alessandro (1976).

Isoenzyme patterns have been used to differentiate *T. cruzi* from *R. rangeli* (Kreutzer & Sousa, 1981; Miles et al., 1983; Ebert, 1986; Salgado, 1988). In contrast to the other markers used in our investigation isoenzyme patterns showed SC-58 closer to *T. cruzi* Z1 strain than to *T. rangeli* H-14. The isoenzyme polymorphism in *T. cruzi* is well reported but in *T. rangeli* and other trypanosomatids little is known. Miles et al. (1983), Holguin et al. (1987) and Salgado (1988) observed very little or no polymorphism in the isoenzyme patterns among *T. rangeli* strains from the Brazilian Amazon basin, Colombia and Honduras. On the other hand,

Kreutzer & Sousa (1981) and Ebert (1986) found a high polymorphism among *T. rangeli* strains from Colombia and Venezuela. Therefore the isoenzyme polymorphism could explain why SC-58 was not so similar to *T. rangeli* H-14 from Honduras.

The possibility of mixed populations (*T. cruzi/T. rangeli*) in strain SC-58 was discarded because in 5 out of 6 enzymes, the isoenzyme patterns consisted of single bands and the only enzyme presenting two bands showed identity neither with standard *T. cruzi* or *T. rangeli.* Another piece of evidence against mixed populations is that the isoenzyme patterns of SC-58 isolated after 6 cyclic passages (mice-*R. domesticus*-mice) were the same as the initial patterns.

The characteristics of the SC-58 strain, namely, invasion of the haemolymph and salivary glands of *R. domesticus* and *R. prolixus,* transmission to mice by the bite of infected triatomine bugs, resistance to human and guinea pig complement lysis and lack of agglutination with WGA lectin, demonstrate that SC-58 strain is *T. rangeli,* being therefore reported for the first time in southern Brazil. Among all the characters we used, only isoenzyme patterns were not indicative of *T. rangeli.* Should we have used isoenzyme patterns alone, we could have reached the wrong conclusion, that is the description of a new *T. cruzi* zymodeme. Therefore we emphasize the importance of using several characters when identifying new trypanosomes.

The geographical distribution of the rodent *E. dasythrix* comprises an area between Rio Grande do Sul and Bahia States (Cabrera, 1961). According to Lent & Wygodzinsky (1979), *R. domesticus* is found in Santa Catarina, Paraná, São Paulo, Rio de Janeiro, Espírito Santo and Bahia States. It is clear that in these states *R. domesticus* and *E. dasythrix* are sympatric, and the presence of *T. rangeli* in *R. domesticus* at Bahia State (Barret & Oliveira, 1977) may explain the spreading of *T. rangeli* southward. Therefore the occurrence of *T. rangeli* in other southern and southeastern states of Brazil could be expected. Mammals infected with *T. rangeli* often present subpatent parasitaemia, and the parasite can only be detected by haemoculture and/or xenodiagnosis. In Santa Catarina Island, *E. dasythrix* is very common in secondary forests, living in tree holes, bromeliad clumps and making nests in the tree tops. Leal et al. (1961) observed a close relation between *R. domesticus* and *E. dasythrix* nests in epiphytic bromeliads. In spite of searching thoroughly in the sylvatic environment, we did not find *R. domesticus* naturally infected by *T. rangeli.* Nevertheless, our experimental evidence indicates that this triatomine might be the natural vector of *T. rangeli* parasites in Santa Catarina Island.

The finding of *T. rangeli* in southern Brazil has epidemiological significance, because single or mixed infections could be expected in vertebrate reservoirs, triatomine vectors and man, making difficult the diagnosis of Chagas' disease there.

ACKNOWLEDGEMENT

To Dr Zigman Brener for revising and criticizing the manuscript.

REFERENCES

BARRETT, T. V. & OLIVEIRA, T. S., 1977. A trypanosome indistiguishable from *Trypanosoma rangeli* in the haemolymph of *Rhodnius domesticus* from Brazil. *Trans. R. Soc. Trop. Med. Hyg., 71:* 445-446.

CABRERA, A., 1961. Catalogo de los mamiferos de America del Sur. *Rev. Mus. Arg. Cienc. Nat., 4:* 309-732.

COUTINHO, J. D. & NUSSENZWEIG, V., 1952. Infecção experimental de triatomíneos pelo *Trypanosoma rangeli* Tejera, 1920. *Folia Clin. Biol., 18:* 181-188.

CUBA CUBA, C., 1973. *Evolução de uma cepa peruana de* Trypanosoma rangeli *em* Rhodnius ecuadoriensis *e* Panstrongylus herreri. Master Thesis, Universidade Federal de Minas Gerais, Belo Horizonte, 84 p.

DEANE, L. M., 1958a. Encontro de tripanossomo do tipo rangeli em gâmbas da espécie *Didelphis marsupialis marsupialis* no Estado do Pará. *Rev. Brasil. Malariol. Doenças Trop., 10:* 451-458.

DEANE, L. M., 1958b. Novo hospedeiro de tripanossomos dos tipos cruzi e rangeli no Estado do Pará. O marsupial *Metachirops opossum opossum. Rev. Brasil. Malariol. Doenças Trop., 10:* 531-541.

D'ALESSANDRO, A., 1976. Biology of *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920. p. 328-403. In W. H. R. Lumsden & D. A. Evans (eds). *Biology of Kinetoplastida.* Vol. 1. Academic Press, London.

D'ALESSANDRO, A. & PRADO, C. E. del., 1977. Search for *Trypanosoma rangeli* in endemic areas of *Trypanosoma cruzi* in Argentina and Brazil. *Am. J. Trop. Med. Hyg., 26:* 623-627.

DE-SIMONE, S. G.; CARVALHO, P.; OLIVA, O. F. P.; ANDRADE, S. G.; GALVÃO-CASTRO, B., 1987. *Trypanosoma cruzi* strain specific monoclonal antibodies: identification of Colombian

strain flagellates in the insect vector. *Trans. R. Soc. Trop. Med. Hyg., 81:* 750-754.

EBERT, F., 1986. Isoenzymes of *Trypanosoma rangeli* stocks and their relation to other trypanosomes transmitted by triatomine bugs. *Trop. Med. Parasit., 37:* 251-254.

GREWAL, M. S., 1956. *Trypanosoma rangeli* Tejera, 1920 in its vertebrate and invertebrate hosts. *Trans. R. Soc. Trop. Med Hyg., 50:* 301-302.

HOARE, C. A., 1972. *Herpetosoma* from man and other mammals: *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920. p. 288-313. In *The trypanosomes of mammals: a zoological monograph*. Blackwell Scientific Publications, Oxford.

HUDSON, L.; GUHL, F.; MARINKELLE, C. J.; RODRIGUES, J., 1987. Use of monoclonal antibodies for the differential detection of *Trypanosoma cruzi* and *Trypanosoma rangeli* in epidemiological studies and xenodiagnosis. *Acta Trop., 44:* 387-392.

HOLGUIN, A. F.; SARAVIA, N. G.; D'ALESSANDRO, A., 1987. Lack of enzyme polymorphism in *Trypanosoma rangeli* stocks from sylvatic and domiciliary transmission cycles in Colombia. *Am. J. Trop. Med. Hyg., 36:* 56-58.

KREUTZER, R. D. & SOUSA, O. E., 1981. Biochemical characterization of *Trypanosoma* spp. by isoenzyme eletrophoresis. *Am. J. Trop. Med. Hyg., 30:* 308-317.

LEAL, H.; FERREIRA NETO, J. A.; MARTINS, C. M., 1961. Dados ecológicos sobre triatomíneos silvestres da Ilha de Santa Catarina, Brasil. *Rev. Inst. Med. Trop. São Paulo, 3:* 213-220.

LENT, H. & WYGODZINSKY, P., 1979. Revision of the Triatomine (Hemiptera, Reduviidae) and their significance as vectors of Chagas' Disease. *Bull. Am. Mus. Nat. Hist., 163:* 520 p.

LUCENA, D. T. & MARQUES, R. J., 1954. Primeiro caso de infecção humana por *Trypanosoma rangeli* Tejera, 1920 no Brasil. *Rev. Brasil. Med., 11:* 535-540.

MILES, M. A.; ARIAS, J. E.; VALENTE, S. A. S.; NAIFF, R. D.; SOUZA, A. A.; POVOA, M. M.; LIMA, J. A. N.; CEDILLOS, R. A., 1983. Vertebrate hosts and vectors of *Trypanosoma rangeli* in the Amazon Basin of Brazil. *Am. J. Trop. Med. Hyg., 32:* 1251-1258.

MIRANDA-SANTOS, I. K. F. & PEREIRA, M. E. A., 1984. Lectins discriminate between pathogenic and non-pathogenic South American trypanosomes. *Am. J. Trop. Med. Hyg., 33:* 839-844.

ROMANHA, A. J., 1982. *Heterogeneidade isoenzimática em* Trypanosoma cruzi. PhD. Thesis, Universidade Federal de Minas Gerais, Belo Horizonte, 110 p.

SALGADO, L. S. A., 1988. *Comparação de diferentes metologias na identificação de* Trypanosoma cruzi *e* Trypanosoma rangeli *em amostras de tripanosomatídeos de Honduras*. Master Thesis, Universidade Federal de Minas Gerais, Belo Horizonte, 108 p.

SCHLEMPER Jr., B. R.; STEINDEL, M.; GARGIONI, R.; FARIAS, C. J. M.; OLIVEIRA, R.; XIMENEZ, J. A., 1985. Reservatórios e vetores silvestres do *Trypanosoma cruzi* e suas relações com o domicílio humano na Ilha de Santa Catarina. *Arq. Cat. Med., 14:* 91-96.

SCHOTTELIUS, J., 1982. Differentiation between *Trypanosoma cruzi* and *T. rangeli* by their different complement sensitivity. *Tropenmed. Parasit., 33:* 147-150.

SCHOTTELIUS, J. & MULLER, V., 1984. Interspecific differentiation of *Trypanosoma cruzi; Trypanosoma conorhini* and *Trypanosoma rangeli* by lectins in the combination with complement lysis. *Acta Trop., 41:* 29-38.

SILVA, L. H. P. & NUSSENZWEIG, V., 1953. Sobre uma cepa de *Trypanosoma cruzi* altamente virulenta para o camundongo branco. *Folia Clin. Biol., 20:* 191-203.

TOBIE, E. J., 1961. Experimental transmission and biological comparision of strains of *Trypanosoma rangeli. Exp. Parasitol., 11:* 1-9.

TOBIE, E. J., 1964. Increased infectivity of a cyclically maintained strain of *Trypanosoma rangeli* to *Rhodnius prolixus* and mode of transmission by invertebrate host. *J. Parasitol., 50:* 593-598.