Annals of Tropical Medicine & Parasitology, Vol. 95, No. 2, 157–166 (2001)

Trypanosomatidae from wild mammals in the neotropical rainforest of French Guiana

BY J. DEREURE*
Laboratoire d'Écologie médicale et de Pathologie parasitaire, 163 rue Auguste-Broussonet, 34090, Montpellier, France
C. BARNABÉ
Centre d'Études sur le polymorphisme des Microorganismes (CEPM), Unité Mixte de Recherche Centre National de la Recherche Scientifique (CNRS)/Institut de Recherche pour le Développement (IRD) n° 9926, IRD, B.P. 5045, 34032, Montpellier Cedex 1, France

J.-C. VIÉ, F. MADÉLENAT
Programme Faune Sauvage, EDF/CNEH, Savoie Technolac, 73373, Le Bourget-du-Lac, France

AND C. RACCURT
Centre Hospitalier Universitaire d'Amiens, Service de Parasitologie, Mycologie et Médecine des Voyages, Groupe hospitalier Sud, 80054 Amiens Cedex 1, France

Received 1 December 2000, Revised 2 January 2001, Accepted 4 January 2001

The initial filling of the reservoir behind the Petit Saut hydro-electric dam, on the Sinnamary River in French Guiana, threatened the terrestrial and arboreal animals living in the neotropical rainforest being flooded. During a rescue programme between 24 October and 12 November in 1994, many of these animals were checked for infection with trypanosomatids. Overall, 45 blood samples and 54 skin biopsies were collected from 53 mammals (of 13 species representing five orders) and blood samples were also taken from each of nine reptiles (six species from four families). When the skin biopsies and the buffy-coats from the blood samples were cultured in NNN medium, 10 of the cultures, each initiated with mammalian blood, were found to be positive for trypanosomatids. Multilocus enzyme electrophoresis (MLEE) on cellulose acetate plates, with 20 enzyme systems, was then used to investigate each of the positive cultures. The results were analysed by clustering from a genetic distance matrix, using the unweighted pair group method with arithmetic averages (UPGMA), and applying a bootstrap procedure to Wagner parsimony trees.

A stock obtained from *Didelphis marsupialis* was identified as a zymodeme of *Trypanosoma cruzi* (Miles' zymodeme 1) known to cause Chagas disease in French Guiana. Five stocks (one each from *Bradypus tridactylus*, *Tamandua tetradactyla* and *Alouatta seniculus* and two from *Saguinus midas*) were of a single zymodeme close to *Trypanosoma rangeli* reference stock RGB. This is the first confirmation of the presence of *Tr. rangeli* in French Guiana, and the first time that it has been identified, by iso-enzyme analysis, in the neotropical primates *A. seniculus* and *S. midas*. Two other stocks, isolated from *Choloepus didactylus*, were related to *Endotrypanum schaudinni* reference stock LEM 2790. Although the remaining stocks, one from *C. didactylus* and the other from *A. seniculus*, clustered together on UPGMA and in a Wagner tree, they did not appear to be related to any of the reference stocks included in the UPGMA dendrogram.

* E-mail: j-dereure@chu-montpellier.fr; fax: + 33 4 67 63 00 49.

ISSN 0003-4983 (print) ISSN 1364-8594 (online)/01/020157-10
Carfax Publishing

DOI: 1080/00034980120045284

The flagellates belonging to the family Trypanosomatidae are widely represented in Central and South America. Some species, transmitted by blood-sucking arthropods, can infect humans and then cause major public-health problems. In French Guiana, cases of human trypanosomiasis caused by one such species, *Trypanosoma cruzi*, apparently occur only occasionally; there were no more than 10 confirmed cases and five other, proven or probable human infections with trypanosomes between 1940 and 1984 (Raccurt, 1996). The main reservoir host for *Tr. cruzi* in French Guiana is *Didelphis marsupialis* (Dedet *et al.*, 1985*a*). Since 1994, there has been a re-emergence of acute Chagasic cardiopathy observed in young adults in villages along the Maroni river. This is probably related to ecological changes resulting from demographic pressure and/or the increasing frequency of human intrusions into the tropical forest, for tourism, gold prospecting and military training (Raccurt, 1999). There has also been a resurgence of human cases of cutaneous leishmaniasis (CL) since 1978, probably for the same reasons. The mean annual incidence of human CL for the period 1995–2000 is estimated to have been 2.8 cases/1000 (Dedet, 1990). At least three *Leishmania* species are involved in human CL in French Guiana; *Leishmania guyanensis* predominates but three cases have been attributed to *L. amazonensis* and nine to *L. braziliensis* (Courtois *et al.*, 1986; Garin *et al.*, 1989; Dedet *et al.*, 1989*b*, 1994), French Guiana recently being recognized as a new endemic area for the latter parasite (Raccurt *et al.*, 1995). The presence of a fourth species causing human CL (*L. naiffi*) is suspected (Darié *et al.*, 1995). Although the mammalian reservoir hosts of *L. guyanensis* and *L. amazonensis* are well known (Gentile *et al.*, 1981; Lainson *et al.*, 1981; Dedet *et al.*, 1984, 1985*b*, 1989*a*), those of *L. braziliensis* remain unknown.

Among the other trypanosomatids reported from French Guiana, *Endotrypanum schaudinni* was described in 1908 in a sloth (Mesnil and Brimont, 1908), and the presence of *Tr. rangeli* in at least four species of mammal has been suspected, although no infections of this parasite have been reported in humans or reduviid bugs (Floch and Abonnenc, 1949). Given its numerous wild and (peri)domestic hosts (d'Alessandro, 1976), it is fortunate that *Tr. rangeli* appears non-pathogenic for humans.

The present study, in an area of neotropical rainforest in French Guiana, was made possible by an operation to rescue some of the wildlife threatened by the filling of the reservoir behind a newly constructed dam. Ten stocks of trypanosomatid were successfully isolated from some of the rescued mammals and subsequently characterized by iso-enzyme electrophoresis.

ANIMALS AND METHODS

Study Area

The construction of the Petit Saut hydro-electric dam, on the Sinnamary River in French Guiana, eventually led to the flooding of 365 km^2 of primary Amazonian rainforest (Vié, 2001). As the reservoir behind the dam filled for the first time, many of the local animals became trapped on the ever-diminishing islands formed by the rising water. Electricité de France, the company building the dam, then began an operation, involving veterinarians, biologists and technicians, to rescue and study the larger members of the threatened fauna.

Animals

Animals were captured along the Sinnamary River and its tributary streams, between the dam and a point 70 km upstream. Terrestrial vertebrates were caught either in metal cages (Tomahawk Live Trap Co., Tomahawk, WI) baited with fruit or by active trapping with snares or nets placed at the entrances of burrows or at the ends of hollow tree trunks. Arboreal animals were hunted by day or night, depending on their habits, and captured by hand, by felling the trees in which they were sheltering or by using anaesthetic darts. The

location of each animal when caught was recorded as the distance upstream from the dam. Each animal collected was taken to the Petit Saut veterinary facility, were it was identified to species, sexed, weighed, aged, measured and checked for signs of disease. Blood and/or skin samples were collected aseptically from most of the animals (see below) before they were released into a protected area nearby.

Sampling Procedure

BLOOD

Blood samples were collected by aseptic venous puncture, into sterile tubes containing anticoagulant (sodium citrate), and then centrifuged so that buffy coats could be transferred into tubes of NNN culture medium. A small subsample of each buffy coat was used to make a thin smear and this was fixed in methanol and stained with Giemsa's stain (5%). The cultures were incubated at 24–26°C and checked for flagellates after 1 week. If a culture was negative, the liquid phase was placed into a tube of fresh medium each week for 1 month, with weekly checks for parasites.

SKIN

Each animal was anaesthetized before biopsies were taken, with a 2- or 4-mm punch (nose) or a biopsy clipper (ear), from a hairless area of skin which had been cleaned and disinfected with 70% ethanol. Each sample was then placed in a sterile tube containing physiological saline with 100 000 IU sodium benzylpenicillin/ml (PS–SB). After one night at 4°C, each biopsy fragment was crushed and homogenized in PS–SB in a sterile tissue grinder, transferred to a tube of NNN culture medium and then incubated and checked in the same way as the blood samples.

Multilocus Enzyme Electrophoresis

Flagellates from each of the positive NNN cultures were transferred to liquid LIT medium (Camargo, 1964), allowed to multiply and then harvested for electrophoresis and for cryopreservation (Tibayrenc and le Ray, 1984). The electrophoresis was performed using cellulose acetate plates and the methods of Ben Abderrazak *et al.* (1993), with slight modifications. Twenty enzyme systems were assayed: aconitase (ACON; EC4.2.1.3), alanine aminotransferase (ALAT; EC2.6.1.2), diaphorase (DIA; EC1.6.99.2), glyceraldehyde-3-phosphate dehydrogenase (GAPD; EC1.2.1.12), glutamate dehydrogenase NAD + (GDH-NAD +; EC1.4.1.2), glutamate dehydrogenase NADP + (GDH-NADP +; EC1.4.1.4), aspartate amino transferase (GOT; EC2.6.1.1), glucose-6-phosphate dehydrogenase (G6PD; EC1.1.1.49), glucose-6-phosphate isomerase (GPI; EC5.3.1.9), isocitrate dehydrogenase (IDH; EC1.1.1.42), leucine aminopeptidase (cytosol aminopeptidase) (LAP; EC3.4.11.1), malate dehydrogenase (MDH; EC1.1.1.37), (oxaloacetate decarboxylating, NADP +) malate dehydrogenase or malic enzyme (ME; EC1.1.1.40), mannose-phosphate isomerase (MPI; EC5.3.1.8), nucleoside hydrolase with inosine substrate (NHi; EC2.4.2.1); peptidase 1 (Ficin) with leucyl-leucyl-leucine substrate [PEP-1; EC3.4.22.3 (formerly EC3.4.4.12)]; peptidase 2 (Bromelin) with leucyl-L-alanine as substrate [PEP-2; EC3.4.22.4 (formerly E.C.3.4.4.24)]; 6-phosphoglucomutase dehydrogenase (6PGD; EC1.1.1.44); phosphoglucomutase [PGM; EC5.4.2.2 (formerly EC2.7.5.1)]; and superoxide dismutase (SOD; EC1.15.1.1). Eight reference stocks of flagellates were used: six previously characterized isolates of *Tr. cruzi* (Tibayrenc and Ayala, 1988; Brisse, 1998); *E. schaudinni* LEM 2790; and *Tr. rangeli* RGB.

In general, all reproducible bands were numbered for each locus, beginning with 1 for the fastest anodic band. When clearly identified heterozygotes coded for proteins with more than two monomers (e.g. the three-banded pattern seen in GPI for a heterozygote), only the bands corresponding to alleles were numbered, in order to avoid an over-estimation of the genetic distance between the heterozygote and the corresponding homozygotes. As estimates of the genetic divergence between stocks, Jaccard's distances (Dj) were calculated:

$$Dj = 1 - [a/(a + b + c)]$$

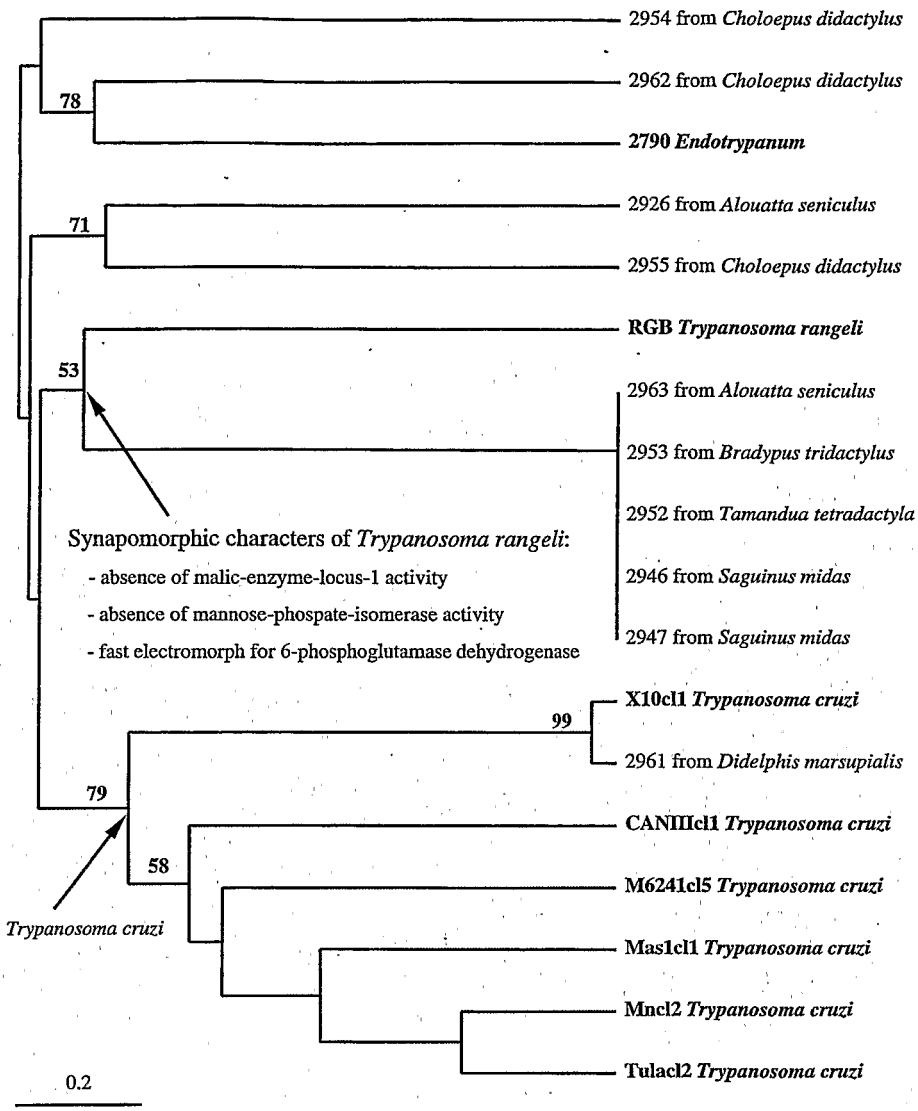


Fig. 1. UPGMA tree derived from Jaccard's genetic distances between the eight reference stocks (shown emboldened) and the 10 new trypanosomatid stocks isolated from wild mammals in French Guiana. The numbers at the forks indicate the highest bootstrap values (out of 100 trees) for trees obtained by Wagner's parsimony method. The new stocks are indicated by their code number (shown here in abbreviated form) and the species of their host mammal.

from a female common opossum (*Didelphis marsupialis*; caught 22 km from the dam) and appeared very similar to the *Tr. cruzi* reference stock X10 cl1 Z1, which is representative of Miles' zymodeme 1. Another five of the new isolates—MBRA/GF/94/LEM 2953 from a male three-toed sloth (*Bradypus tridactylus*; 16 km), MTAM/GF/94/LEM 2952

TABLE

Trypanosomatid infections detected, by culture of blood and skin samples, in the mammals captured in the area of Petit Saut hydro-electric dam, in French Guiana

		No. of cultures prepared and (no. found positive)		*Trypanosomatid stock isolated*	
Order	*Species*	*Blood*	*Skin*	*Code(s)*	*Species*
Rodentia	*Proechimys* sp.	2 (0)	3 (0)		
	Myoprocta acouchy	1 (0)	6 (0)		
	Dasyprocta agouti	4 (0)	4 (0)		
	Coendou prehensilis	8 (0)	9 (0)		
Carnivora	*Potos flavus*	2 (0)	2 (0)		
Marsupiala	*Didelphis marsupialis*	2 (1)	2 (0)	MDID/GF/94/LEM2961	*Trypanosoma cruzi*
Edentata	*Bradypus tridactylus*	12 (1)	12 (0)	MBRA/GF/94/LEM 2953	*Trypanosoma rangeli*
	Choloepus didactylus	4 (3)	4 (0)	MCHO/GF/94/LEM2954	*Endotrypanum schaudinni*
				MCHO/GF/94/LEM2962	*Endotrypanum schaudinni*
				MCHO/GF/94/LEM2955	Undetermined
	Tamandua tetradactyla	1 (1)	1 (0)	MTAM/GF/94/LEM2952	*Trypanosoma rangeli*
	Dasypus novemcinctus	2 (0)	3 (0)		
	Dasypus kappleri	2 (0)	2 (0)		
Primates	*Alouatta seniculus*	3 (2)	4 (0)	MALO/GF/94/LEM2963	*Trypanosoma rangeli*
				MALO/GF/94/LEM2926	Undetermined
	Saguinus midas	2 (2)	2 (0)	MSAG/GF/94/LEM2946	*Trypanosoma rangeli*
				MSAG/GF/94/LEM2947	*Trypanosoma rangeli*

where *a* is the number of bands common to both stocks, *b* is the number found only in one stock, and *c* is the number found only in the other stock (Jaccard, 1908). The unweighted pair group method with arithmetic averages, better known as UPGMA (Sokal and Sneath, 1963), was used to cluster the stocks from the genetic distance matrix. The software package PHYLIP was used for a cladistic analysis (Felsenstein, 1993). A bootstrap procedure (Felsenstein, 1985) was applied to Wagner parsimony trees (Kluge and Farris, 1969).

RESULTS

From 24 October to 12 November 1994, 53 mammals (from five orders and 13 families) and nine reptiles (belonging to six species in four families) were captured. None of the animals had lesions indicative of leishmaniasis. No parasites were detected in the cultures of the nine (blood) samples from the reptiles. Overall, 45 blood samples and 54 skin biopsies were taken from the mammals, with some animals supplying several samples each (see Table). Although all of the cultures of the skin biopsies remained negative, trypanosomatid stocks were isolated from cultures of blood collected from 10 animals representing six species of mammal (see Table). All the positive animals were adult. As two of the 20 enzymes investigated (DIA and ME) showed activity at two independent loci, a total of 22 loci could be read. Six off the 14 different zymodemes observed were found within the stocks from French Guiana (Fig. 1).

One of the stocks isolated from a rescued mammal (MDID/GF/94/LEM 2961) came

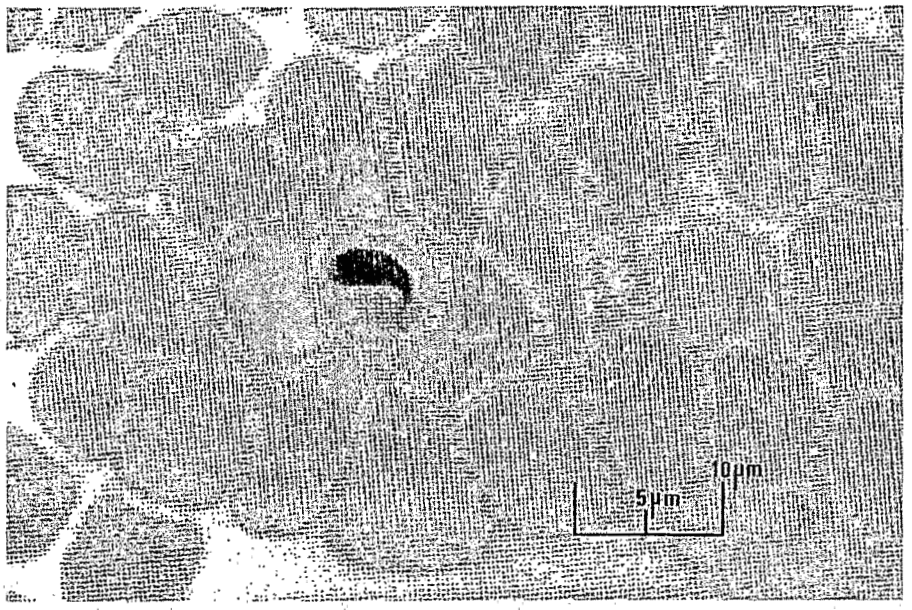


Fig. 2. An intra-erythrocytic form of *Endotrypanum schaudinni* in a bloodsmear of a two-toed sloth (*Choloepus didactylus*) captured in the area of Petit Saut hydro-electric dam in French Guiana.

from a male southern tamandua (*Tamandua tetradactyla*; 20 km), MALO/GF/94/LEM 2963 from a female red-howler monkey (*Alouatta seniculus*; 20 km), and MSAG/GF/94/LEM 2946 and MSAG/GF/94/LEM 2947 from two female golden-handed tamarins (*Saguinus midas*; both15 km)—shared the same zymodeme which was close to that of the *Tr. rangeli* RGB reference stock. Two of the other new isolates—MCHO/GF/94/LEM 2954 and MCHO/GF/94/LEM 2962, from two male two-toed sloths (*Choloepus didactylus*; 16 and 25 km, respectively)—appeared to be similar to the *Endotrypanum schaudinni* reference stock (LEM 2790). An intra-erythrocytic form was found on the stained smear of the buffy-coat from the sloth from which MCHO/GF/94/LEM 2954 was isolated (Fig. 2). The other two new stocks from the rescued mammals—MCHO/GF/94/LEM 2955 from a male two-toed sloth (30 km) and MALO/GF/94/LEM 2926 from a female red-howler monkey (24 km)—could not be related to any other stock in the UPGMA dendrogram. Curiously, although these two isolates clustered together on the dendrogram, LEM 2955 grew as promastigotes in culture whereas LEM 2926 developed as trypomastigotes.

Using Wagner's parsimony procedure, only one bootstrap value (99) was found to be significant, at the node clustering the *Tr. cruzi* reference stock X10 cl 1 and the LEM2961 stock. However, some other values which were high enough to be relevant are indicated on the UPGMA tree (Fig. 1).

DISCUSSION

Prior to the MLEE described here, the 10 new isolates from French Guiana had been compared with, and found to differ markedly from, several reference strains of *Leishmania* species known to occur in South America (unpubl. obs.). Although none of the parasites isolated from the rescued animals therefore appeared to belong to the genus *Leishmania*, this was not surprising, since very few cases of human cutaneous leishmaniasis have been ob-

served among the individuals working on the Petit Saut dam (unpubl. obs.).

One of the new isolates, LEM 2961, not only appeared to be *Tr. cruzi* of Miles' zymodeme 1, the only zymodeme previously identified in French Guiana (Dedet *et al.*, 1985*a*; Lewicka *et al.*, 1995), but also came from *D. marsupialis*, the 'classical' reservoir host of this zymodeme (Miles *et al.*, 1978).

Five (50%) of the new stocks appeared to be of *Tr. rangeli*, a species known to occur in Edentata (*Ch. hoffmanni*, *Ta. tetradactyla*), Marsupiala (*D. marsupialis*, *Philander opossum*), Carnivora (*Procyon lotor*), non-human primates (*Cebus fatuelles*, *Ce. capucinus*) and, more rarely, in humans and domestic animals (*Felis catus*, *Canis familiaris*) in South America (Hoare, 1972; Pessoa and Martins, 1988; Brenière *et al.*, 1993). In French Guiana, a trypanosome resembling *Tr. rangeli* in morphology was found in a spider monkey (*Ateles paniscus*) by Floch and Abonnenc (1949) and, more recently, in smears of blood from *Al. seniculus* and *S. midas* (B. de Thoisy, J. C. Michel, I. Vogel and J.-C. Vié, unpubl. obs.). All five of the stocks isolated in the present study, from four species of mammalian host in the same biotope, were of the same electrophoretic type as RGB, the Venezuelan stock of *Tr. rangeli* used for reference, and clustered with it on the UPGMA dendrogram as well as on the Wagner tree. These results, the absence of the ME1 locus in all five stocks, and other characters specific to *Tr. rangeli* (Fig. 1) clearly show that these five stocks are of *Tr. rangeli*. However, the genetic distance between these stocks and the RGB reference stock ($Dj = 0.83$) is large enough to show that *Tr. rangeli* is highly polymorphic in terms of MLEE markers, as previously reported (Brenière *et al.*, 1993). The results of further studies on these stocks, using slow 'molecular-clock' markers such as the gene sequences coding for ribosomal RNA, should be interesting. Recently, five isolates of *Tr. rangeli* and 59 of other *Trypanosoma* spp. were studied by phylogenetic analysis of their ssrRNA sequences and comparison of their miniexon sequences (Stevens *et al.*, 1999). The results indicate that *Tr. rangeli* can be grouped with some species within the subgenera *Schizotrypanum* (*Tr. cruzi*, *Tr. dionisii*, *Tr. vespertilionis*), *Herpetosoma* (*Tr. leeuwenhoeki*) or *Megatrypanum* (*Tr. minasense*, *Tr. conorhini*) but not with other members of the same subgenera. The taxonomic position of *Tr. rangeli* therefore remains controversial. Hoare (1972) set *Tr. rangeli* in the Stercoraria section. Although Añez (1982) suggested that it be moved to the Salivaria, in a new subgenus (*Tejeraia*), this proposal was criticised by d'Alessandro and Saravia (1992).

The first description of *E. schaudinni* was made by Mesnil and Brimond (1908), using a parasite collected in French Guiana from *Ch. didactylus*, which is also the local reservoir host of *L. guyanensis* (Gentille *et al.*, 1981). Noyes *et al.* (1996) considered *E. schaudinni* to be very close to *L. herreri*. In the present study, while the UPGMA tree clustered two of the new stocks, LEM 2954 and LEM 2962, with LEM 2790, the *E. schaudinni* reference stock, it is worth noting that the genetic distances between these three stocks are very high and that only one of the new stocks (LEM 2922) was close to the reference stock in the Wagner's consensus tree. In contrast to the group of new stocks of *Tr. rangeli*, which shared the same genetic pattern for malic enzyme, there was no synapomorphic pattern apparent for the stocks suspected to be *E. schaudinni*. The identity of the LEM 2954 stock must therefore remain in doubt, despite the intra-erythrocytic form seen in a sample from the sloth from which this stock was isolated (Fig. 2). Schizodeme analysis of kDNA restriction-endonuclease fragment patterns has demonstrated genetic diversity among the genus *Endotrypanum* (Franco and Grimaldi, 1999). In a revised classification, based on several molecular criteria (sialidase activity, G6PDH enzyme structure and malic-enzyme loci), Cupolillo *et al.* (2000) recently proposed that *Endotrypanum* be placed in the Paraleishmania section of the genus *Leishmania*.

The two remaining stocks among those isolated in the present study (MCHO/GF/94/LEM 2955 and MALO/GF/94/LEM 2926) were found to clustered together, but not

closely, on both the UPGMA and Wagner trees. However, as the genetic distances between these two stocks and all of the others investigated were very large, they could not be identified. It is possible that these stocks could be of *Endotrypanum*, given the high level of enzymatic polymorphism observed in this genus. It is remarkable that one of these stocks developed as trypomastigotes in culture and the other as promastigotes. Although the small number of reference stocks employed in the present study clearly limited the probability of accurate identification of the rarer genotypes, there have been previous problems in identifying some trypanosomatids from French Guiana (Lewicka *et al.*, 1995).

The present results confirm the extreme genetic diversity of the parasites of the family Trypanosomatidae that infect the wild mammals of French Guiana. Although iso-enzyme analysis permits clusters within *Tr. cruzi* to be identified and *Tr. cruzi* to be distinguished from *Tr. rangeli*, it does not permit the upper levels of the branching within this large family to be resolved clearly. It would be well worth studying the trypanosomatids of French Guiana (and elsewhere) using other genetic markers.

ACKNOWLEDGEMENTS. We are grateful to Professor R. W. Ashford for his critical reading of the manuscript. We thank B. de Thoisy, C. Fournier, P. Fournier, C. Genty, T. Grouex, J. Kéravec, D. Pélissier, C. Richard-Hansen, C. Sissakian and all the administrative, technical and scientific staff at Petit Saut who participated in the 'Faune-Sauvage' operation. We are also grateful to Y. Balard, H. Corbières, Y. Garrel, P. Lami, M. Lefebvre, C. Martinez, C. Rouquairol and G. Serres of the Laboratoire d'Écologie médicale et de Pathologie parasitaire of the Medical School and the Universitary Hospital of Montpellier (France) for their technical participation. This work was one of a series of environmental studies funded by Électricité de France and the Centre National d'Équipement Hydraulique.

REFERENCES

AÑEZ, N. (1982). Studies on *T. rangeli* Tejera 1920. IV. A reconsideration of its systematic position. *Memórias do Instituto Oswaldo Cruz*, 77, 405–415.

BEN ABDERREZAK, S., GUERRINI, F., MATHIEU-DAUDÉ, F., TRUC, P., NAEUBAUER, K., LEWICKA, K., BARNABÉ, C. & TIBAYRENC, M. (1993). Isoenzyme electrophoresis for parasite characterization. *Methods in Molecular Biology*, 21, 361–382.

BRENIÈRE, S. F., BOSSENO, M. F., BARNABÉ, C., URDANETA-MORALES, S. & TIBAYRENC, M. (1993). Population genetics of *Trypanosoma cruzi* and *Trypanosoma rangeli*: taxonomical and epidemiological purpose. *Biological Research*, 26, 27–33.

BRISSE, S. (1998). *Phylogénie moléculaire des clones naturels de* Trypanosoma cruzi, *agent de la maladie de Chagas*. Thesis, Université Montpellier II, France.

CAMARGO, E. P. (1964). Growth and differentiation in *Trypanosoma cruzi*. I. Origin of metacyclic trypanosomes in liquid media. *Revista do Instituto de Medicina Tropical de São Paulo*, 6, 93–100.

COURTOIS, D., RIOUX, J. A., PRATLONG, F., MORENO, G., LANOTTE, G. & COURRIER, P. L. (1986). *Leishmania braziliensis* Vianna, 1911 s. st. en Guyane Française. *Annales de Parasitologie Humaine et Comparée*, 61, 593–594.

CUPOLILLO, E., DEDINA-ACOSTA, E., NOYES H., MOMEN, H. & GRIMALDI JR, G. (2000). A revised classification for *Leishmania* and *Endotrypanum*. *Parasitology Today*, 16, 142–144.

D'ALESSANDRO, A. (1976). Biology of *Trypanosoma* (*Herpetosoma*) *rangeli* Tejera, 1920. In *Biology of the Kinetoplastida*, Vol. 1, eds Lumsden, W. H. R. & Evans, D. A. pp. 328–403. New York: Academic Press.

D'ALESSANDRO, A. & SARAVIA, N. (1992). *Trypanosoma rangeli*. In *Parasitic Protozoa*, Vol. 2, eds Kreier, J. P. & Baker, J. R. pp. 1–54. San Diego: Academic Press.

DARIÉ, H., DENIAU, M., PRATLONG, F., LANOTTE, G., TALARMIN, A., MILLET, P., HOUIN, R. & DEDET, J. P. (1995). Cutaneous leishmaniasis of humans due to *Leishmania* (*Viannia*) *naiffi* outside Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 89, 476–477.

DEDET, J. P. (1990). Cutaneous leishmaniasis in French Guiana: a review. *American Journal of Tropical Medicine and Hygiene*, 43, 25–28.

DEDET, J. P., DESJEUX, P., GOYOT, P. & GOSSELIN, H. (1984). Infestation naturelle de *Proehimys cuvieri* Petter 1978 (Rongeur, Echimyidés) par *Leishmania mexicana amazonensis* Lainson et Shaw 1972 (Kinetoplastida, Trypanosomatidé) en Guyane Française. *Comptes Rendus de l'Académie des Sciences, Paris*, 298, 85–87.

DEDET, J. P., CHIPPEAUX, J. P., GOYOT, P., PAYOT, F. X., TIBAYRENC, M., GEOFFROY, B., GOSSELIN, H. & JACQUET-VIALET, P. (1985*a*). Les hôtes naturels de *Trypanosoma cruzi* en Guyane Française. Endémicité élevée du zymodème 1 chez les marsupiaux sauvages. *Annales de Parasitologie Humaine et Comparée*, 60, 111–117.

DEDET, J. P., PAJOT, F. X., DESJEUX, P., GOYOT, P., CHIPPEAUX, J. P. & GEOFFROY, B. (1985*b*). Natural host of *Leishmania mexicana amazonensis* Lainson et Shaw 1972 (Kinetoplastida, Trypanosomatidae) in French Guiana. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 79, 302–305.

DEDET, J. P., GAY, F. & CHATENEY, G. (1989*a*). Isolation of *Leishmania* species from wild mammals in French Guiana. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 83, 113–115.

DEDET, J. P., PRADINAUD, R. & GAY, F. (1989*b*). Epidemiological aspects of human cutaneous leishmaniasis in French Guiana. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 83, 616–620.

DEDET, J. P., PRATLONG, F., MARTINI, A., LEFEBVRE, M. & DEREURE, O. (1994). *Leishmania* (*Viannia*) *braziliensis* en Guyane Française: une réalité à prendre en considération. *Nouvelles de Dermatologie*, 13, 188–189.

FELSENSTEIN, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783–791.

FELSENSTEIN, J. (1993). *PHYLIP (Phylogeny Inference Package) 3.5c edit.* Seattle: Department of Genetics, University of Washington.

FLOCH, H. & ABONNENC, E. (1949). Sur une nouvelle trypanosomiase humaine américaine. *Bull*

FLOCH, H. & ABONNENC, E. (1949). Sur une nouvelle trypanosomiase humaine américaine. *Bulletin de la Société de Pathologie Exotique*, 42, 394–402.

FRANCO, A. M. & GRIMALDI JR, G. (1999). Characterization of *Endotrypanum* (Kinetoplastida: Trypanosomatidae), a unique parasite infecting the neotropical tree sloth (Edentata). *Memórias do Instituto Oswaldo Cruz*, 94, 261–268.

GARIN, J. P., PIENS, M. A., PRATLONG, F. & RIOUX, J. A. (1989). *Leishmania braziliensis* Vianna, 1911 en Guyane Française. *Bulletin de la Société de Pathologie Exotique*, 82, 233–235.

GENTILE, B., LE PONT, F., PAJOT, F. X. & BERNARD, R. (1981). Dermal leishmaniasis in French Guiana: the sloth (*Choloepus didactylus*) as a reservoir host. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 75, 612–613.

HOARE, C. A. (1972). *The Trypanosomes of Mammals.* Oxford: Blackwell Scientific.

JACCARD, P. (1908). Nouvelles recherches sur la distribution florale. *Bulletin de la Société Vaudoise de Sciences Naturelles*, 44, 223–270.

KLUGE, A. G. & FARRIS, J. S. (1969). Quantitative phyletics and the evolution of anurans. *Systematic Zoology*, 18, 1–32.

LAINSON, R., SHAW, J. J. & POVOA, M. (1981). The importance of edentates (sloths and anteaters) as primary reservoirs of *Leishmania braziliensis guyanensis*, causative agent of 'pian bois' in North Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 75, 611–612.

LEWICKA, K., BRENIÈRE-CAMPANA, S. F., BARNABÉ, C., DEDET, J. P. & TIBAYRENC, M. (1995). An isoenzyme survey of *Trypanosoma cruzi* genetic variability in sylvatic cycles from French Guiana. *Experimental Parasitology*, 81, 20–28.

MESNIL, F. & BRIMONT, E. (1908). Sur un hématozoaire nouveau (*Endotrypanum* n. gen.) d'un édenté de Guyane. *Comptes Rendus de la Société de Biologie*, 65, 581.

MILES, M. A., SOUZA, A., POVOA, M., LAINSON, R. & TOYÉ, P. J. (1978). Isozymic heterogeneity of *Trypanosoma cruzi* in the first autochtonous patients with Chagas' disease in Amazonian Brazil. *Nature*, 272, 819–821.

NOYES, H. A., CAMPS, A. P. & CHANCE, M. L. (1996). *Leishmania herreri* (Kinetoplastida: Trypanosomatidae) is more clodely related to *Endotrypanum* (Kinetoplastida: Trypanosomatidae) than to *Leishmania*. *Molecular and Biochemical Parasitology*, 80, 119–123.

PESSOA, S. B. & MARTINS, A. V. (1988). *Parasitologia Médica.* Rio de Janeiro: Guanabara Koogan.

RACCURT, C. (1996). *Trypanosoma cruzi* en Guyane Française: revue des données accumulées depuis 1940. *Médecine Tropicale*, **56**, 79–87.

RACCURT, C. P. (1999). Acute Chagasic cardiopathy in French Guiana: a re-emergent hazard linked to ecological change? *Annals of Tropical Medicine and Parasitology*, **93**, 183–184.

RACCURT, C. P., PRATLONG, F., MOREAU, B., PRADINAUD, R. & DEDET, J. P. (1995). French Guiana must be recognized as an endemic area of *Leishmania* (*Viannia*) *braziliensis* in South America. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **89**, 372.

SOKAL, R. R. & SNEATH, P. H. A. (1963). *Principles of Numerical Taxonomy*. San Francisco: Freeman.

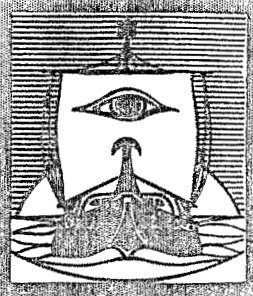
STEVENS, J. R., TEIXEIRA, M. M., BINGLE, L. E. & GIBSON, W. C. (1999). The taxonomic position and evolutionary relationships of *Trypanosoma rangeli*. *International Journal for Parasitology*, **29**, 749–757.

TIBAYRENC, M. & AYALA, F. J. (1988). Isozyme variability in *Trypanosoma cruzi*, the agent of Chagas' disease: genetical, taxonomical and epidemiological signifiance. *Evolution*, **42**, 277–292.

TIBAYRENC, M. & LE RAY, D. (1984). General classification of the isoenzymic strains of *Trypanosoma* (*Schizotrypanum*) *cruzi* and comparison with *T.* (*S.*) *c. marinkelli* and *T.* (*Herpetosoma*) *rangeli*. *Annales de la Société Belge de Médecine Tropicale*, **64**, 239–248.

VIÉ, J. C. (2001). Wildlife rescues—the case of the Petit Saut hydroelectric dam in French Guiana. *Oryx*, in press.

VOLUME 95 NUMBER 2 MARCH 2001



ANNALS OF TROPICAL MEDICINE & PARASITOLOGY

Published for the **Liverpool School of Tropical Medicine**

ISSN 0003-4983