SHORT COMMUNICATION

Minicircle kDNA Microheterogeneity in *Endotrypanum* Indicate Diversity within this Genus

Antonia MR Franco, Gérzia MC Machado, Célia FS Moreira, Gabriel Grimaldi Jr+

Departamento de Imunologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

A comparison of kDNA restriction-endonuclease fragment patterns from strains representing selected Endotrypanum *zymodemes was done by schizodeme analysis. As the degree of heterogeneity within mini-circles varied among species or strains of* Endotrypanum*, the fingerprint obtained with each of the restriction enzymes was unique for each of these parasites. The data have revealed that this trypanosomatid genus is much more complex than it was originally thought to be.*

Key words: *Endotrypanum* - Trypanosomatidae - minicircle kDNA analysis

Parasitic protozoa of the genus *Endotrypanum* (Kinetoplastida: Trypanosomatidae) are biologically diverse group of microorganisms in that infection appears to be restricted to edentates. These parasites have three distinct motile flagellated stages in their life cycle: promastigote (that lives within the alimentary tract of the sand fly vector), epimastigote and trypomastigote (the later stage residing within erythrocytes of their vertebrate hosts, sloths of the genera *Choloepus* and *Bradypus*) forms (Shaw 1992).

In nature, *Endotrypanum* spp. are probably transmitted by the bite of infected phlebotomine sand flies (Diptera: Psychodidae) (Shaw 1964). Arias et al. (1985) identified *E. schaudinni* infection in sand flies and sloths captured in the Amazon region of Brazil. Studies using kinetoplast DNA probe for detecting the parasite in sand flies also demonstrated *Endotrypanum* in *Lutzomyia shannoni*, *L. umbratilis* and *L. anduzei* (Rogers et al. 1988). However, infections with other biologically distinct trypanosomatid protozoa, such as *Leishmania* and *Trypanosoma* are also found in sloths and in sand flies in Neotropical forests (Shaw 1992).

Taxonomic studies of *Endotrypanum* isolates from the Americas indicate genetic diversity among these microorganisms (Franco & Grimaldi Jr 1999). Since the description of the genus, the number of strain variants of this parasite has increased, although only two species have been described. As a result of using numerical zymotaxonomy for classifying these organisms (Franco et al. 1996), the taxonomic horizon of *Endotrypanum* spp. has been widened.

Here we have studied minicircle kinetoplast DNA (kDNA) polymorphism (population heterogeneity) among references strains and *Endotrypanum* isolates from the Brazilian Amazon region, by analysis of restriction-endonuclease fragment patterns of kDNA (Grimaldi Jr et al. 1992). Minicircle sequences have been used as biochemical markers in classification of *Leishmania* using kDNA fingerprinting (Grimaldi Jr et al. 1992) or hybridization (Rogers et al. 1988).

The genus *Endotrypanum*, analyzed in this study (identification of the strains, their geographic origin and the source of stocks used are given in the Table), may represent an heterogeneous complex of parasite species or strain variants, as classified by zymotaxonomy (Franco et al. 1996). A comparison of kDNA fragment patterns from strains representing selected *Endotrypanum* zymodemes and *Leishmania* species (that are also frequently found in sloths) was done by schizodeme analysis of endonuclease *Alu* I, *Hinf* I, *Msp* I, and *Taq* I, digests of kDNAs, fractioned by gradient acrylamid gel electrophoresis, using the method described previously (Grimaldi Jr et al. 1992). With these digests, sequence microheterogeneity in mini-circle DNA was revealed and could be compared between different parasites.

As the degree of heterogeneity within minicircles varied among species or strains of *Endotrypanum*, the fingerprint obtained with each

+Corresponding author: Fax: +55-21- 280.1589. E-mail: grimaldi@ioc.fiocruz.br
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TABLE

Origin and identification of the *Endotrypanum* stocks and other reference strains employed in this study

Stock	Designation[a]	Species[b] (zymodeme/group)[c]	Geographic origin
Endotrypanum strains			
E01	MCHO/BR/89/RO9627[a]	*Endotrypanum* sp. (EZ01/A)	Brazil, Rondônia
E02	MCHO/BR/89/RO1635[a]	*Endotrypanum* sp. (EZ01/A)	Brazil, Rondônia
E03	MCHO/BR/89/RO1634[a]	*Endotrypanum* sp. (EZ01/A)	Brazil, Rondônia
E05	MCHO/BR/89/RO1602[a]	*Endotrypanum* sp. (EZ01/A)	Brazil, Rondônia
E06	MCHO/BR/89/RO1471[a]	*Endotrypanum* sp. (EZ01/A)	Brazil, Rondônia
E18	MCHO/BR/85/IM2384[a]	*Endotrypanum* sp. (EZ01/A)	Brazil, Rondônia
E11	MCHO/CR/62/A-9[b]	*E. monterogeii* (EZ01/A)	Costa Rica
E09	MBRA/PA/00/415P01	*Endotrypanum* sp. (EZ01/A)	Panama
E31	MCHO/BR/85/IM2259[c]	*Endotrypanum* sp. (EZ02/A)	Brazil, Pará
E22	MCHO/BR/89/IM3606[c]	*Endotrypanum* sp. (EZ03/A)	Brazil, Rondônia
E36	MCHO/BR/89/IM3603[c]	*Endotrypanum* sp. (EZ03/A)	Brazil, Rondônia
E14	MCHO/BR/80/M6159[c]	*E. schaudinni* (EZ06/B)	Brazil, Pará
E32	MCHO/BR/85/IM2380[a]	*Endotrypanum* sp. (EZ08/B)	Brazil, Rondônia
E17	MCHO/BR/85/IM2382[a]	*Endotrypanum* sp. (EZ09/B)	Brazil, Rondônia
E33	MCHO/BR/85/IM2393[a]	*Endotrypanum* sp. (EZ11/B)	Brazil, Rondônia
E12	MCHO/BR/88/M11602[c]	*E. schaudinni* (EZ12/C)	Brazil, Pará
Leishmania strains			
L565	MHOM/BR/75/M4147	*Leishmania guyanensis*	Brazil, Pará
L894	MHOM/EC/87/G-07	*L. panamensis*	Ecuador, Pichincha
L888	MCHO/EC/82/LSP1[b]	*L. equatorensis*	Ecuador, Guayas

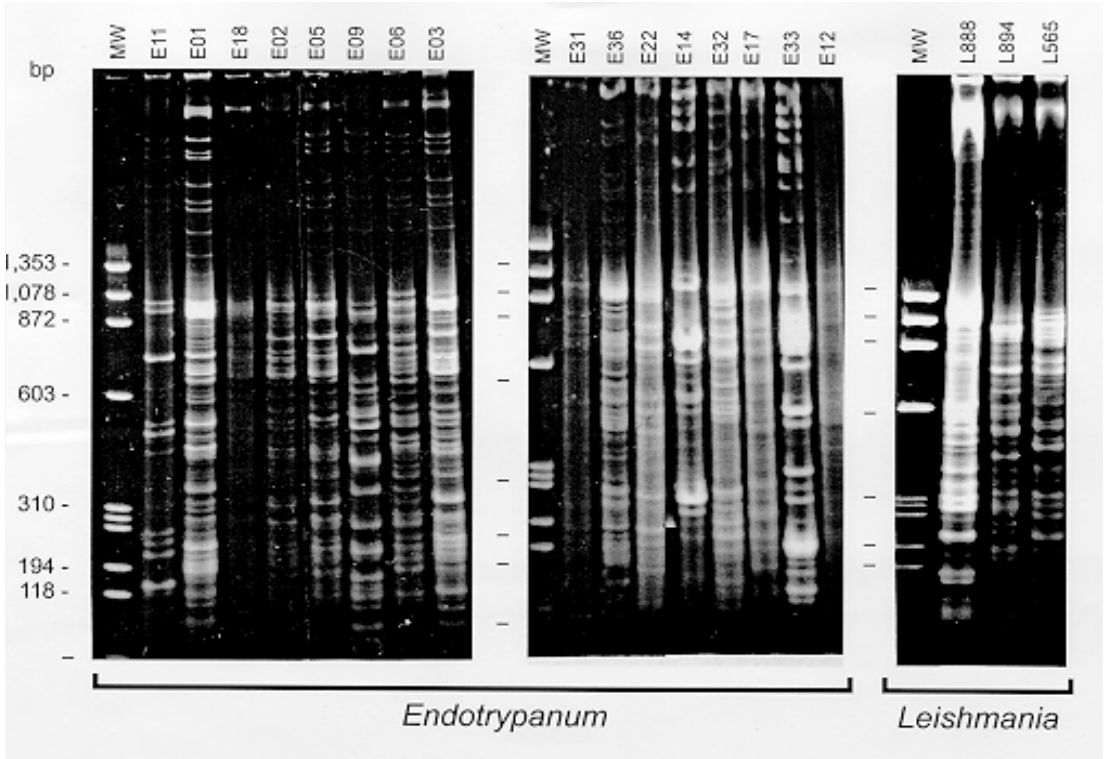
a: designations: host [M= Mammalia: BRA: *Bradypus infuscatus*; CHO: *Choloepus* sp. ([a]*C. juruanus* Lönnberg 1942; [b]*C. hoffmani*; [c]*C. didactylus*)/country of origin/year of isolation/original code]; *b:* stock identification was established by enzyme activities (Medina-Acosta et al. 1994) and monoclonal antibodies (Franco et al. 1997) analyses; *c*: *Endotrypanum* zymodeme/phenetic group classified by enzyme electrophoresis according to their enzyme patterns and numerical analyses (Franco et al. 1996).

of the restriction enzymes was unique for each of these parasites. Overall, the stocks representing different zymodemes could be discriminated into distinct parasites, according to the major sequence classes (minicircle fragments or digests) released by the restriction enzymes tested. Moreover, sequence microheterogeneity in minicircle DNA was detected among isolates of *Endotrypanum* sp. from *C. juruanus* collected at the same locality in the State of Rondônia, Brazil, with *Taq* I (Figure) and other enzymes (data not shown). These strains (E01, E02, E03, E05, E06 and E18) revealed schizodeme profiles that were distinguishable from those seen with other parasite strains (E11 and E09) clustered into the same zymodeme (Franco et al. 1996).

The taxonomy of *Endotrypanum* spp. is still controversial. Since the original description, only two species have been named as *E. schaudinni* Mesnil and Brimont, 1908 and *E. monterogeii* Shaw, 1969. However, *E. monterogeii* is phenotypically related to *E. schaudinni* (which was isolated from *C. didactylus* in Brazil), since ultrastructural and biochemical parameters were similar (Croft et al. 1980), in contrast to the description by Shaw (1969), who reported serological differences between these species. Lopes et al. (1990), also grouped both species in the same cluster by comparison of nuclear DNA restriction fragment patterns.

As a result of using numerical zymotaxonomy for classifying these organisms, the data indicated that (i) *E. schaudinni* represents an heterogeneous complex of parasite strain variants (EZ05, EZ06, EZ12) and (ii) the *E. monterogeii* reference strain, which was isolated from *C. hoffmanni* in Costa Rica, is phenetically closely related to other parasites from Brazil and Panama (Franco et al. 1996). In this study, the minicircle kDNA analysis was able to confirm the polymorphism in *E. schaudinni*, as well as to differentiate the heterogenous population of parasites that were clustered into the same zymodeme (EZ01).

In conclusion, minicircle kinetoplast DNA polymorphism analysis appears to be a typing system useful for epidemiologic and taxonomic studies of *Endotrypanum*, being sensitive to differentiate distinct zymodemes belonging to this genus while simultaneously revealing considerable molecular diversity.



Acrylamide gradient (5-12%) gel electrophoresis comparison of kDNA fragment patterns, generated with the restriction enzyme *Taq* I, among representative strains complexes of *Endotrypanum* and selected *Leishmania* species. The stock codes of the strains studied by kDNA analysis are indicated above the lanes and information on their origin is given in the Table. The apparent sizes of kDNA fragments are estimated relative to fragments from a *Hae* III digest of 0 x 174 RF DNA and their molecular weights are indicate in base pairs (bp) beside the figures.The linearized minicircles migrate at about 0.8 kb and, in some lanes, the minicircle digests appear as bands (ladders) between 0.05 and 0.07 kb.

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