Some Lessons from the South: Molecular and Virulence Studies on *T. evansi* and *T. equiperdum Venezuelan strains*

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Pérez,Y, Perrone, T †, Recchimuzzi,G, Sánchez, E , Biteau,N. , Mijares,A. , Baltz, T and Aso, PM.

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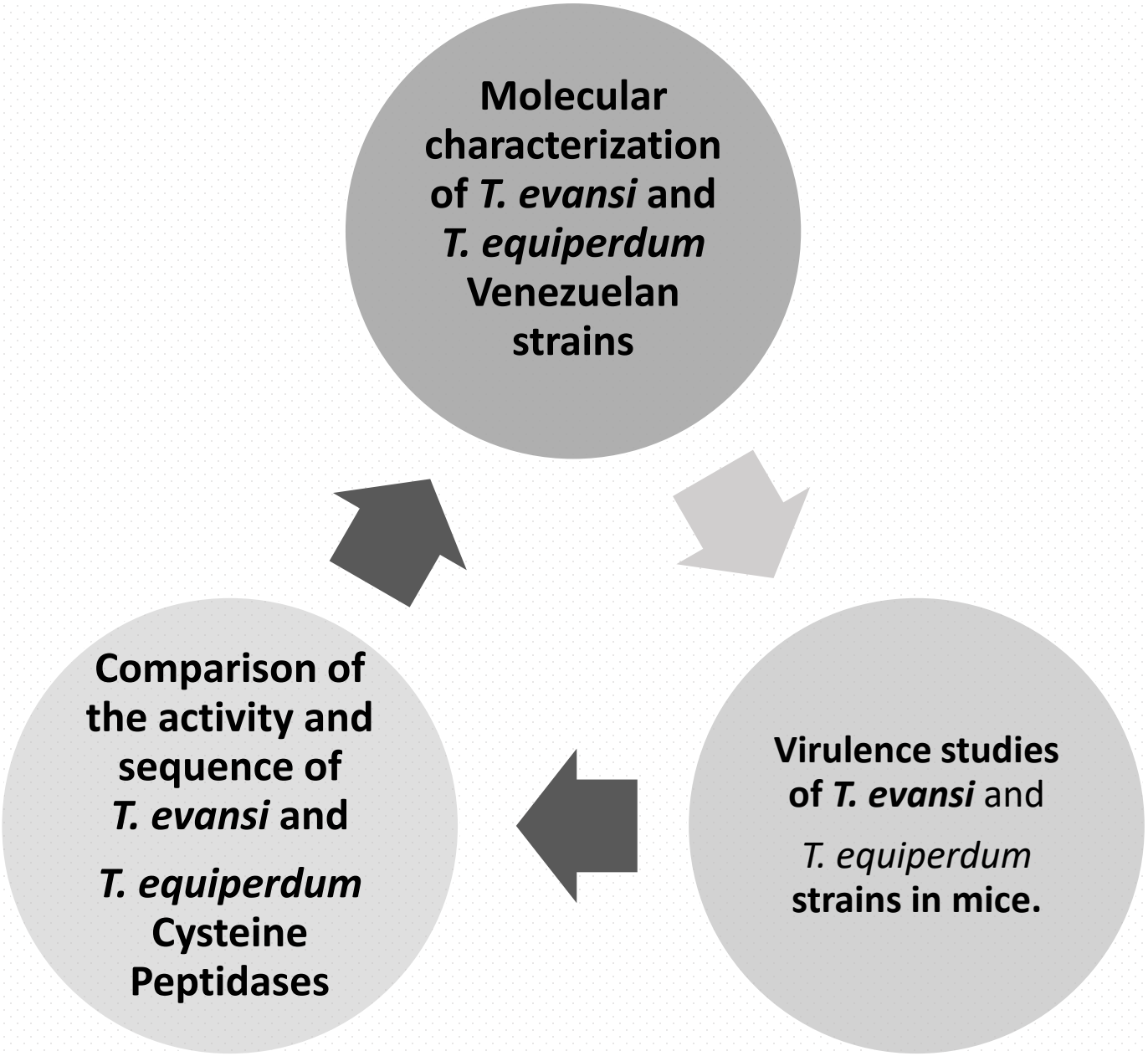
Molecular characterization of *T. evansi* and *T. equiperdum* Venezuelan strains
Virulence studies of *T. evansi* and *T. equiperdum* strains in mice.
Comparison of the activity and sequence of *T. evansi* and *T. equiperdum* Cysteine Peptidases

Trypanosoma spp. RAPDs

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Molecular profiles of Venezuelan isolates of *Trypanosoma* sp. by random amplified polymorphic DNA method

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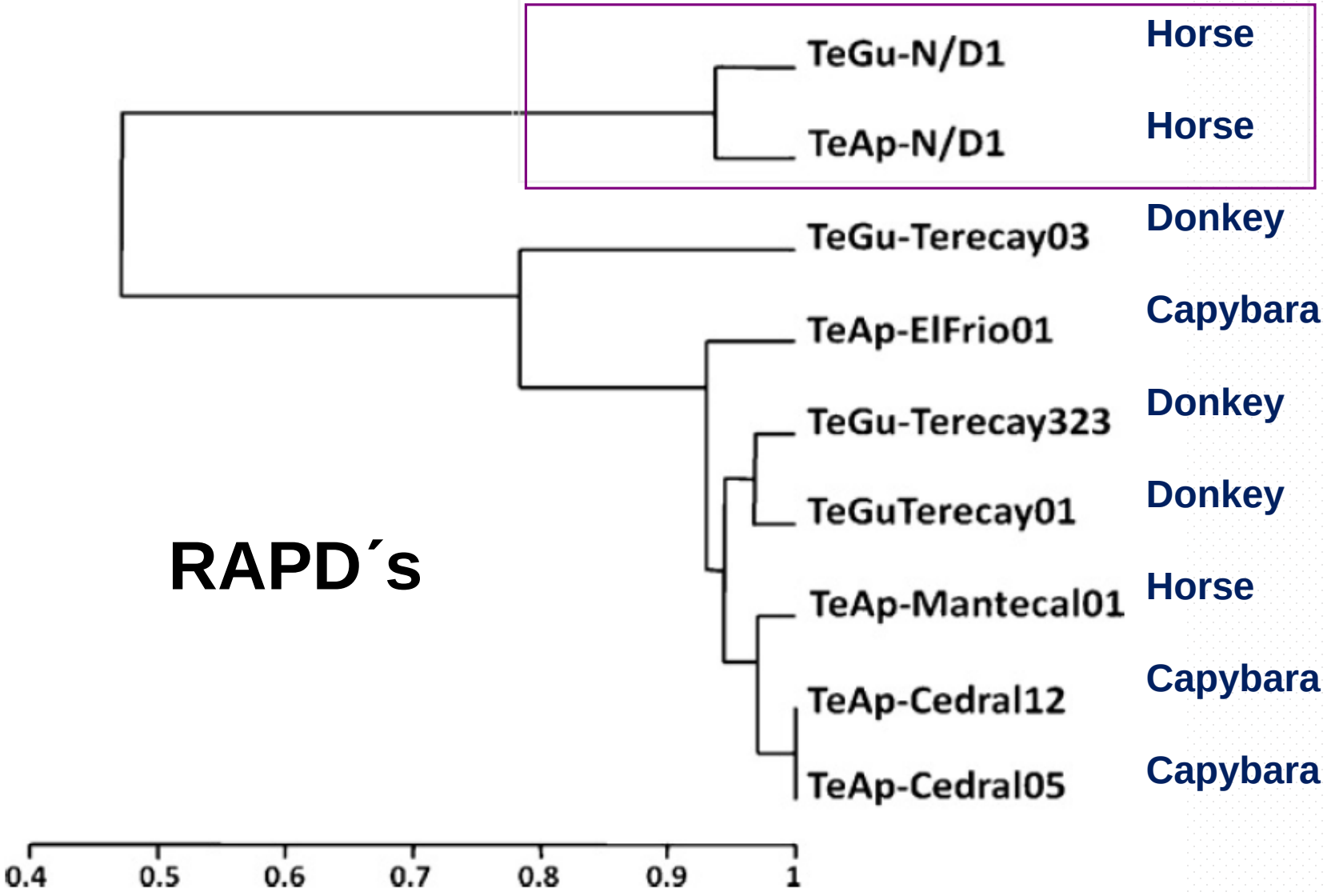

Fig. 5. UPGMA Cluster Analysis Jaccard's Coefficient based on randomly amplified polymorphic DNA profiles between nine Venezuelan isolates of *Trypanosoma* sp.

Sánchez *et al. Parasites & Vectors* (2015) 8:536
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Parasites & Vectors

RESEARCH

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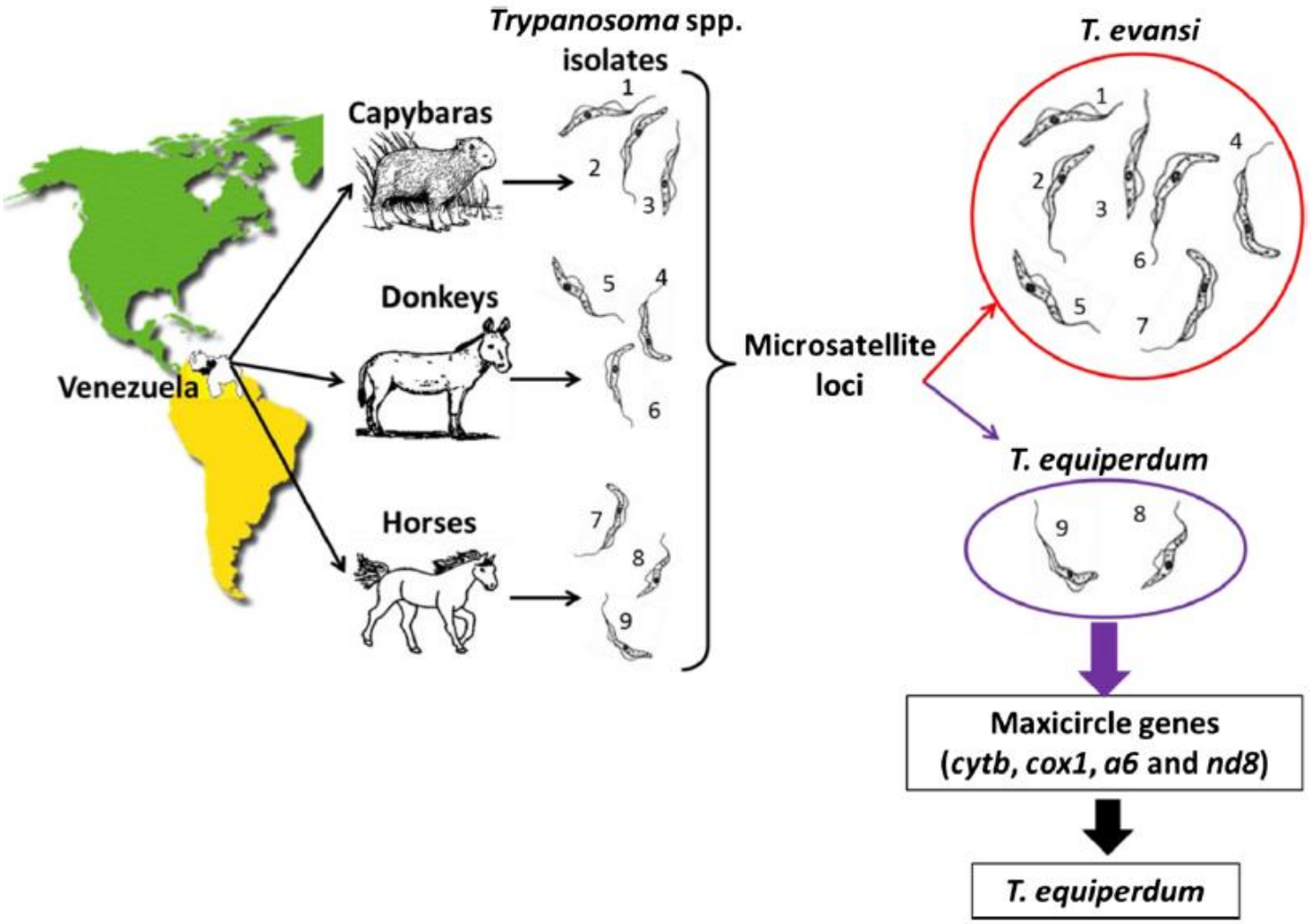
Molecular characterization and classification of *Trypanosoma* spp. Venezuelan isolates based on microsatellite markers and kinetoplast maxicircle genes

E. Sánchez, T. Perrone, G. Recchimuzzi, I. Cardozo, N. Biteau, PM Aso, A. Mijares, T. Baltz, D. Berthier, L. Balzano-Nogueira and MI Gonzatti



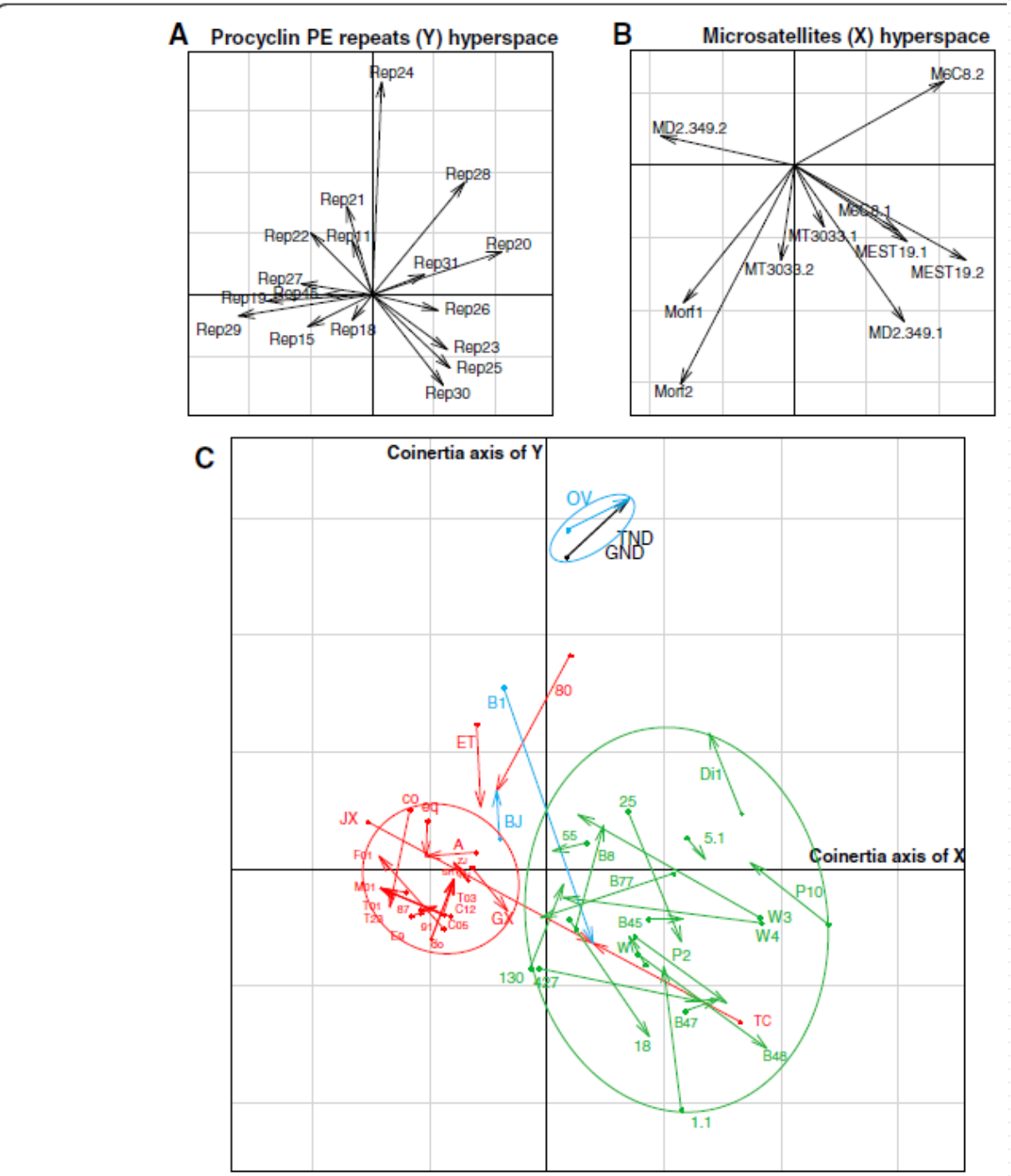
RAPD's

Fig. 4 Microsatellites and four maxicircle genes were used to characterize nine *Trypanosoma* spp. Venezuelan isolates. Seven isolates were shown to be closely related to *T. evansi* reference strains, while two were classified as *T. equiperdum*

Table 1 Summary of the Venezuelan *T. evansi* and *T. equiperdum* strains characterized in this study

Strain (host)	Microsatellite Genotyping and Coinertia analysis	Maxicircle gene
T. evansi		
TeAp-Cedral05 (capybara)	Closely related to *T. evansi* references strains from South America (Brazil and Colombia) and Asia (China).	n.a.
TeAp-Cedral12 (capybara)		n.a.
TeAp-ElFrío01 (capybara)		n.a.
TeAp-Mantecal01 (horse)		n.a.
TeGu-Terecay03 (donkey)		n.a.
TeGu-Terecay01 (donkey)		n.a.
TeGu-Terecay323 (donkey)		n.a.
T. equiperdum		
TeAp-N/D1 (horse)	Closely related to the *T. equiperdum* STIB841/ OVI strain	Positive for *cytb, cox1, a6 and nd8*
TeGu-N/D1 (horse)	Closely related to the *T. equiperdum* STIB841/OVI strain.	Positive for *cytb, cox1, a6 and nd8*

na: not amplified by PCR. The nomenclature, natural hosts and molecular characteristics of the nine Venezuelan strains included in this study are presented. The geographical origin of the strains has been previously reported [52]



Coinertia Analysis combining microsatellites and Procyclin repeats

T. brucei brucei

T. evansi strains

T. equiperdum: Ovi –Te Ap-N/D1 and Te Gu-N/D1

Fig. 1 Coinertia analysis by the Hill-Smith method combining microsatellites and Procyclin PE repeats. **a** and **b** scatterplots represent the coefficients of the combinations of the variables for each data matrix to define the coinertia axes. Separate analyses find axes maximizing inertia in each hyperspace. These axes are projected in the scatterplot (**c**) on which the *Trypanosoma* spp. isolates and reference strains are also projected. The beginning of the arrows is the position of the isolate described by the microsatellite data matrix and the end of the arrow is the position of the isolate described by the procyclin PE repeats. Arrows of the same species were grouped in ellipses of 95 % of variance observed, identifying three groups: *T. evansi* (red), *T. brucei brucei* (green) and *T. equiperdum* (blue). *T. evansi* and *T. equiperdum* isolates that fell outside the major groups were not used to calculate the confidence ellipses. The analysis explained 53.68 % in the microsatellites hyperspace and 22.16 % in the Procyclin PE repeats hyperspace of the observed inertia with a Rv Escoufier similarity coefficient of 0.424415. **C05**: TeAp-Cedral05; **C12**:TeAp-Cedral12; **T03**: TeGu-Terecay03; **F01**: TeAp-ElFrio01; **M01**: TeAp-Mantecal01; **T23**: TeGu-Terecay323; **T01**: TeGu-Terecay01; **TND**: TeAp-N/D1; **GND**: TeGu-N/D1; **E9**: E9/CO; **87**: 2187; **91**: 2191; **A**: A; **do**: dog; **eq**: equi; **co**: coati; **SH**: SH; **ZJ**: ZJ; **NJ**: NJ; **GX**: GX; **JX**: JX; **TC**: TC; **ET**: ET; **80**: KETRI 2480; **OV**: STIB841/OVI; **B1**: BoTat-1.1; **BJ**: BJ; **5.1**: AnTat-5/1; **55**: LM 55; **18**: LM 118; **84**: LM 184; **25**: LM 225; **P10**: KP10; **130**: PTAG 130 (IPR-01130); **P2**: KP2; **Di1**: DiTat-1; **B8**: B8/18; **W3**: SW3/87; **W4**: SW4/87; **W**: SW 161/87; **B45**: STIB 345; **B77**: STIB-777.AE; **1.1**: AnTat-1/1; **427**: EATRO-427; **B47**: STIB247.LFB; **B48**: STIB348

Phylogenetic Analysis based on Maxicircle genes

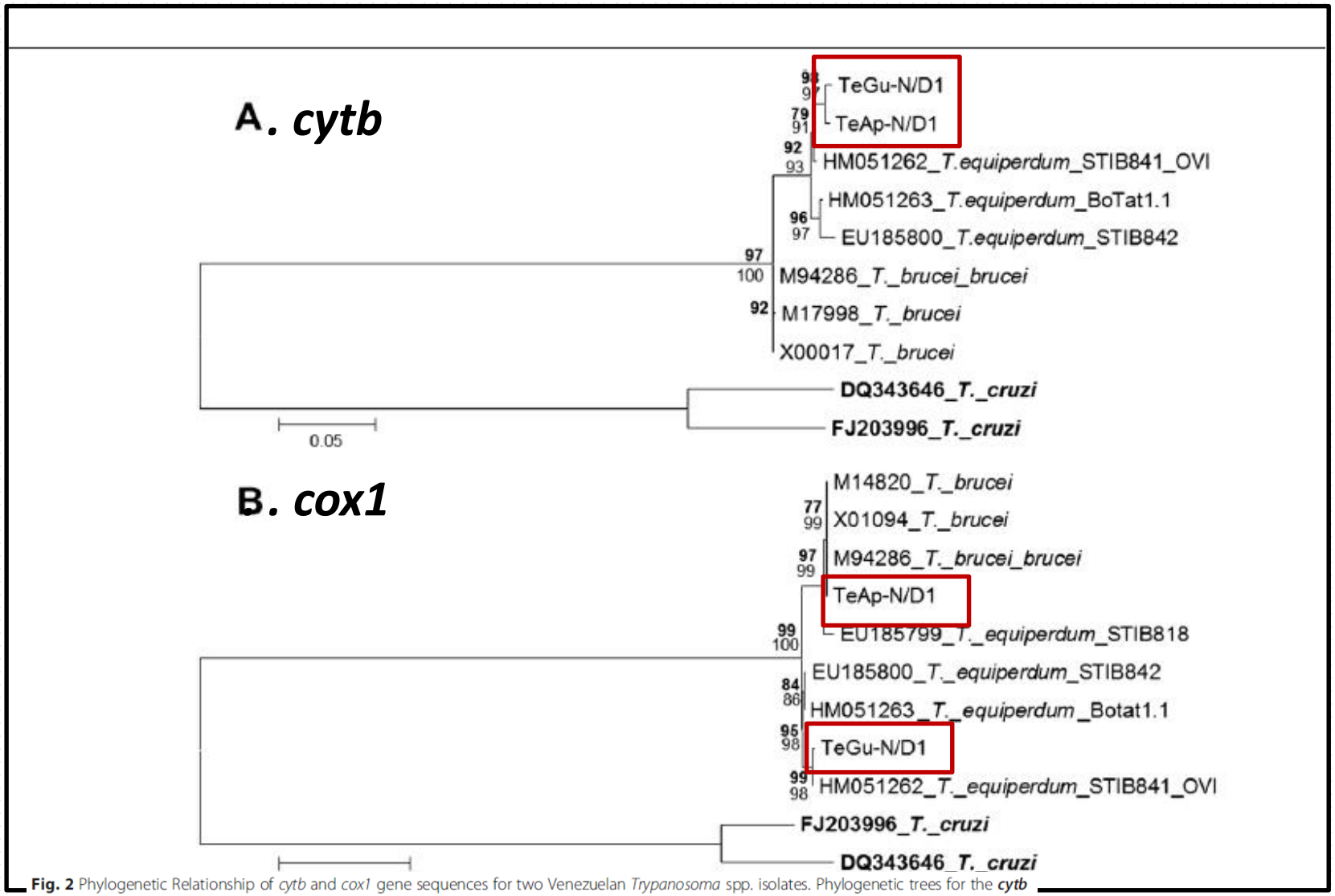


Fig. 2 Phylogenetic Relationship of *cytb* and *cox1* gene sequences for two Venezuelan *Trypanosoma* spp. isolates. Phylogenetic trees for the ***cytb*** (**a**) and ***cox1*** (**b**) gene sequences were inferred by Maximum Parsimony (**MP**, bold numbers) and Maximum Likelihood (ML) methods in MEGA 5.1 ®, 500 replicates (bootstrap > 75 % are shown). *T. cruzi* was used as outgroup. The tree topology shown corresponds to the ML method derived from previous evolutionary sequence alignment. *cytb* = Hasegawa-Kishino-Yano (HKY) + Gamma distribution. ***cox1*** = HKY + Invariant sites

VIRULENCE STUDIES OF *T. evansi* and *T. equiperdum* strains

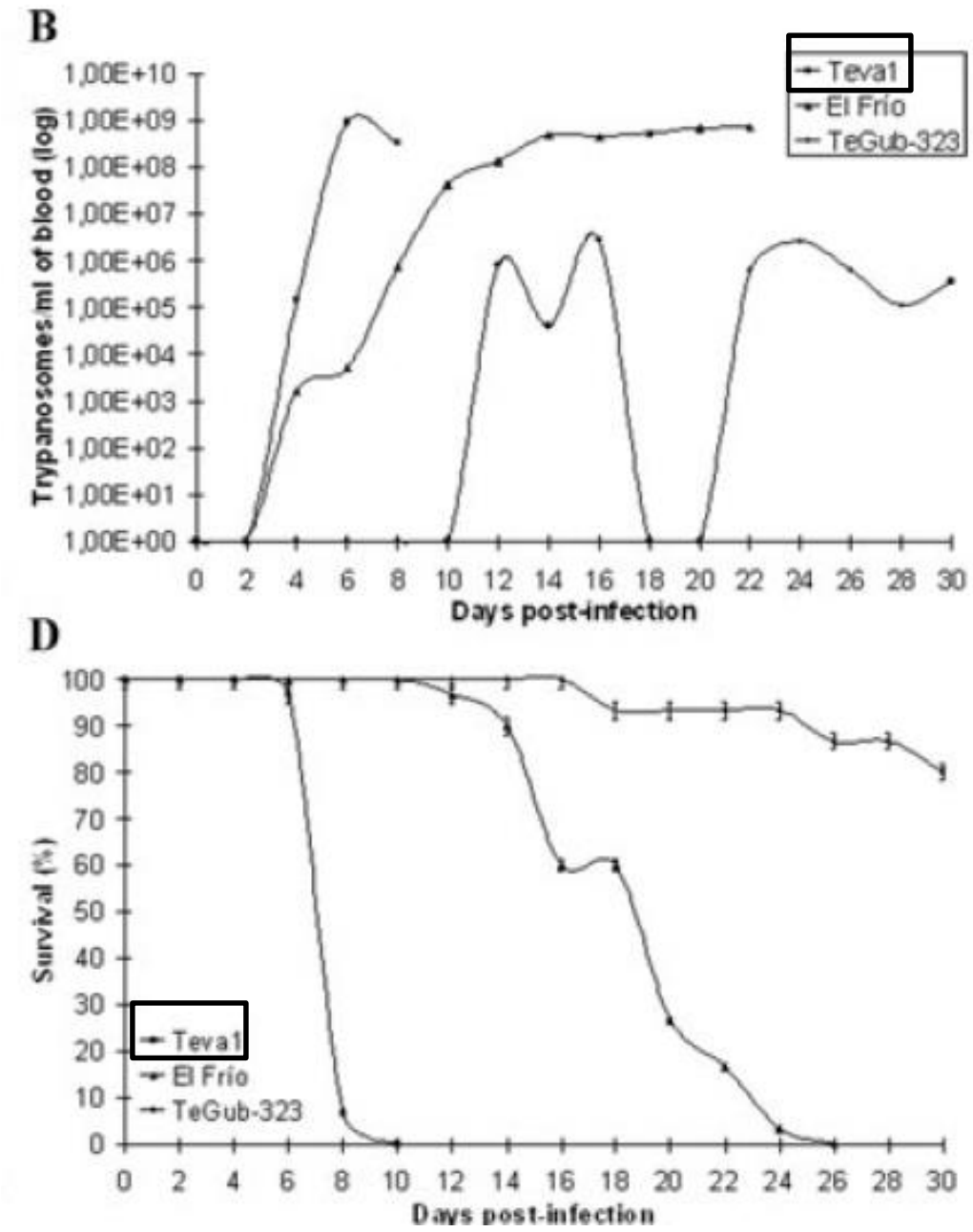
Secretome of Animal Trypanosomes

From a Standard Method toward New Diagnostic and Therapeutic Targets

Philippe Holzmuller,[a] Pascal Grébaut,[a] Jean-Benoît Peltier,[b] Jean-Paul Brizard,[c] Trina Perrone,[d] Marisa Gonzatti,[e] Zakaria Bengaly,[f] Michel Rossignol,[b] Pedro María Aso,[e] Philippe Vincendeau,[g] Gérard Cuny,[a] Alain Boulangé[a] and Roger Frutos[a]

Animal Biodiversity and Emerging Diseases: Ann. N.Y. Acad. Sci. 1149: 337–342 (2008).

Comparison of *T. evansi* and *T. equiperdum* Infectivity and Virulence in rats.

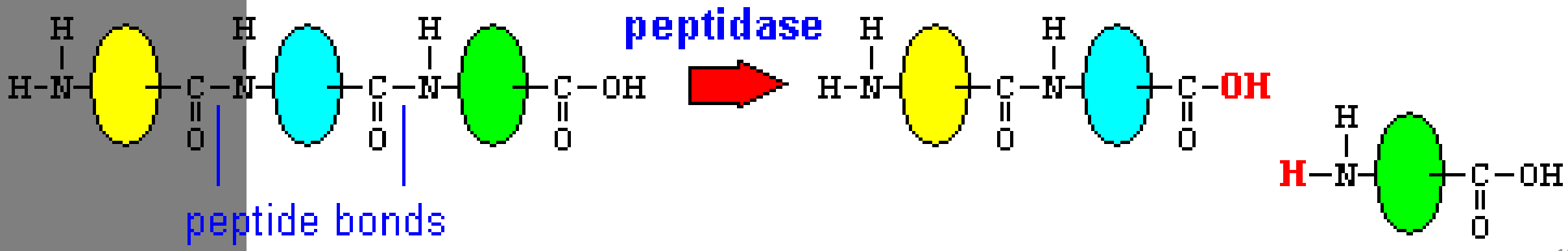


Teva1= TeAp-N/D1

Cysteine Peptidases

- The most common class in protozoa.
- Role in host-parasite “cross-talk”

- Tissue invasion.
- Nutrient degradation
- Virulence factors
- Role in pathogenesis.

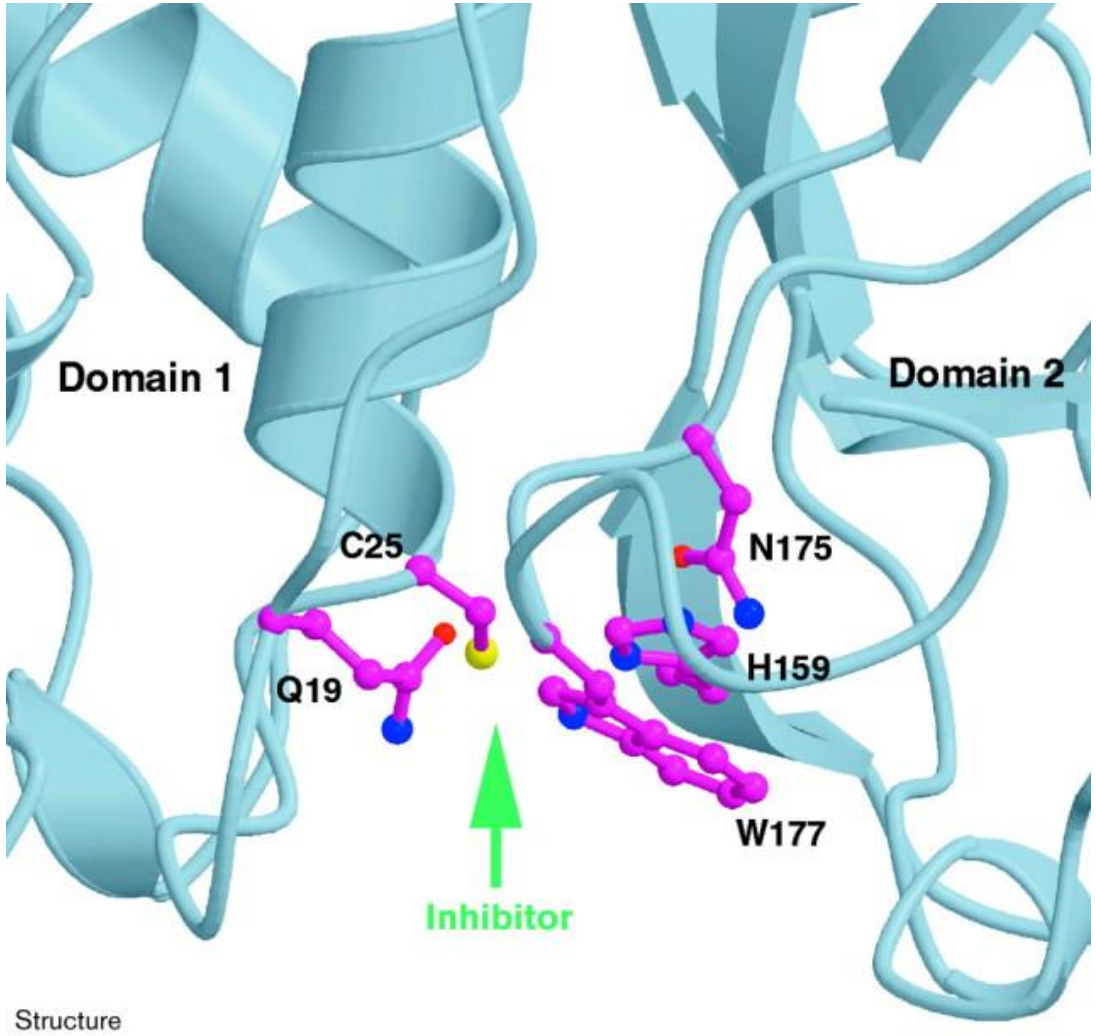


Cruzain Active site

A target within the target: probing cruzain's P1′ site to define structural determinants for the Chagas' disease protease

Linda S Brinen[1]*, Elizabeth Hansell[2], Jianming Cheng[3], William R Roush[3], James H McKerrow[3] and Robert J Fletterick[4]

Structure 2000, **8**:831–840

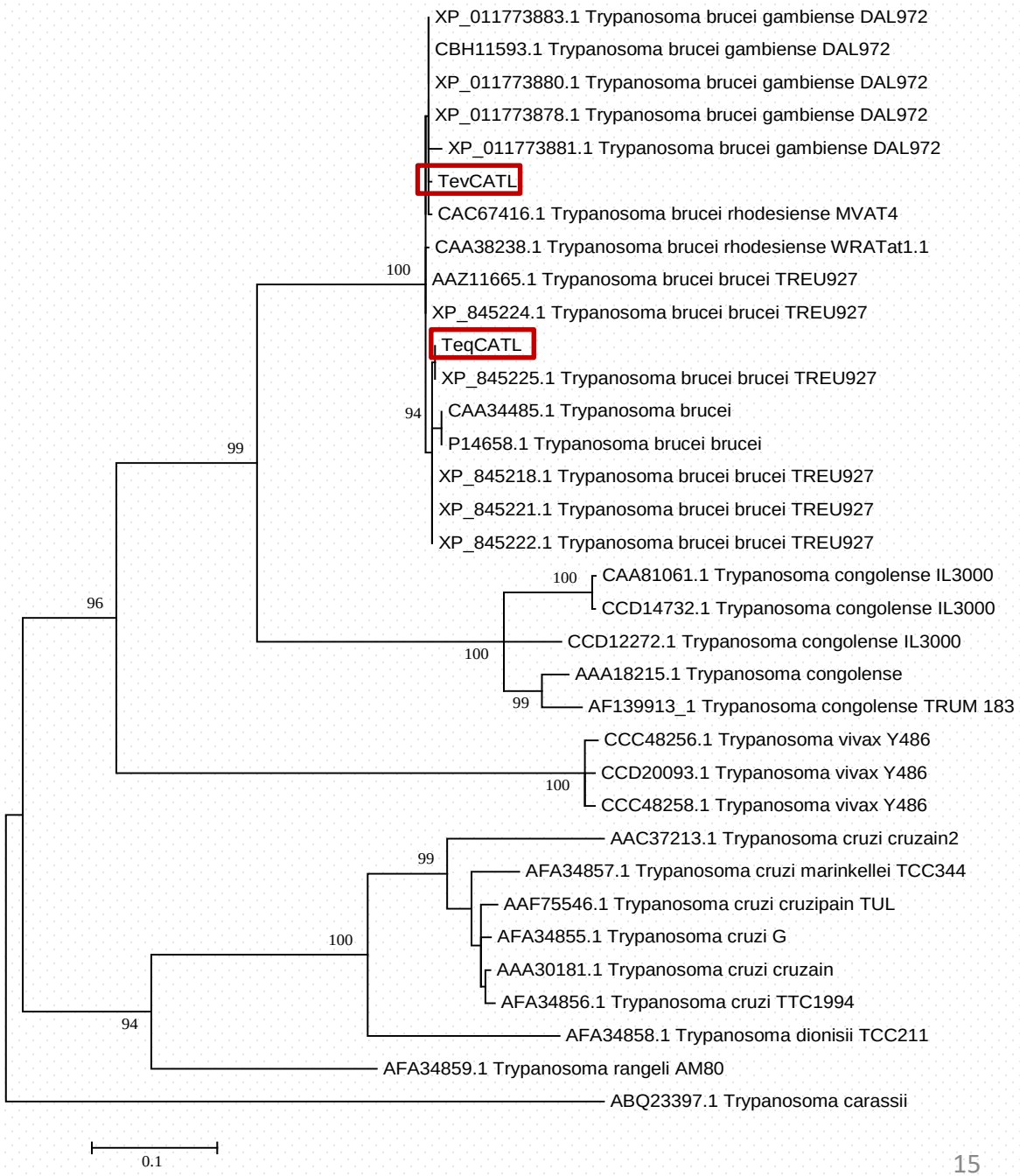


Structure

Phylogenetic Relationship based on CATL protein sequences of Venezuelan *Trypanosoma* spp. isolates.

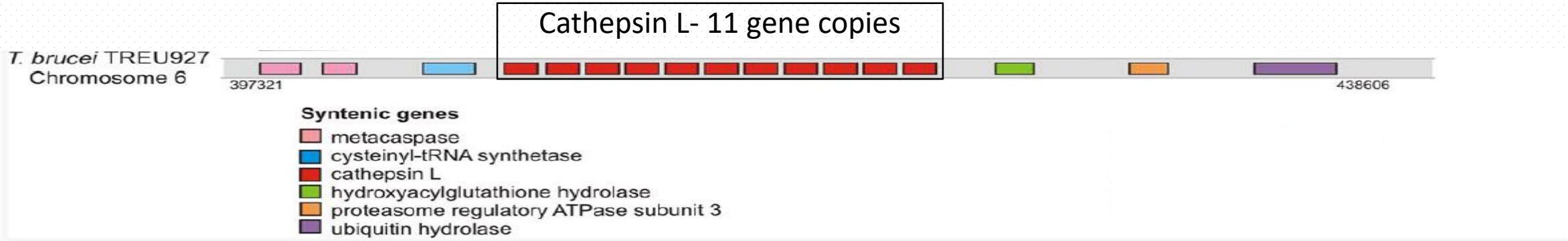
Inferred by Maximum Parsimony and Maximum Likelihood using MEGA 5.2 ®.

T. carassii was used as outgroup



The TevCATL translated aminoacid sequence showed polymorphism when compared to the *catL* genes in the *T. evansi* STIB805 akinetoplastic reference strain (Carnes et al. , 2015).

The TeqCATL translated aminoacid sequence is almost identical to one of the *T. brucei* (TREU927) *catL* genes.



Repertoire, Genealogy and Genomic Organization of Cruzipain and Homologous Genes in *Trypanosoma cruzi*, *T. cruzi*-Like and Other Trypanosome Species

Luciana Lima[1], Paola A. Ortiz[1], Flávia Maia da Silva[1], João Marcelo P. Alves[1,2], Myrna G. Serrano[2], Alane P. Cortez[1,3], Silvia C. Alfieri[1], Gregory A. Buck[2], Marta M. G. Teixeira[1]*

Conclusions and Perspectives:

- Based on coinertia analysis and maxicircle gene sequence phylogeny, the TeAp-N/D1 and TeGu-N/D1 strains constitute the first confirmed *T. equiperdum* strains isolated in Latin America.
- Virulence and infectivity studies carried out in rats and mice showed that these *T. equiperdum* strains are highly virulent and caused 100% mortality within 10 days. The *T. evansi* strains showed low to moderate virulence and produced either chronic or acute infections.
- By both zymography and the CatL fluorometric assay, peptidase activity was five-six fold lower in the *T. equiperdum* crude extracts, as compared to *T. evansi*, while comparable levels of CatB were observed for the two species.
- BLASTP analysis of the translated sequences of the *T. evansi* and *T. equiperdum* CATL sequences showed 99% identity to the *T.b. gambiense* (DAL972) and *T.b.brucei* (TREU927) peptidases.
- These studies support the view that regulation of the *T. evansi* and *T. equiperdum* CatL activity may be an important and determining factor in the host-parasite cross-talk that determines infectivity and virulence.

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Thank you!!