

## MORPHOLOGICAL CHANGES IN *T. cruzi* ISOLATES FROM *Triatoma vitticeps* AFTER TREATMENT WITH BENZNIDAZOLE.

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*Trypanosoma cruzi*, the etiologic agent of Chagas disease, presents considerable heterogeneity between isolated populations within the wild and domestic cycles. Molecular, biologic and biochemical studies were performed to evaluate the parasite diversity on samples isolated from specimens of *Triatoma vitticeps* in two sites in the municipality of Santa Maria Madalena and one site in Conceição de Macabu in the state of Rio de Janeiro. In this study the effect of benznidazole against *T. cruzi* silvatic isolates was performed showing a time- and a dose-dependent action and the effects observed by scanning and transmission electron microscopy.

Epimastigotes of *T. cruzi* (2x10<sup>6</sup> parasites / mL of SMM10 and SMM88 samples) were incubated or not (control) for 24 hours at 28 ° C with a dose of benznidazole corresponding to values of IC<sub>50</sub> previously determined for each isolate of *T. cruzi* diluted in LIT medium. After this procedure the samples were fixed in 2.5% glutaraldehyde with 4% paraformaldehyde in 0.1 M cacodylate buffer and pós-fixed with a 1% osmium tetroxide solution in the same buffer. After that the samples were processed according to routine for scanning and transmission electron microscopy.

By scanning electron microscopy we observe in SMM 10 and SMM 88 samples the deformations in cell body, presenting the parasites the most rounded shape and retraction in the body surface. The flagellum were very elongated and often times with rigid appearance (Figures 1-2). In untreated material, there is a typical morphology, including the presence of rosettes (Figure 3).

The ultrastructural analysis of epimastigotes by transmission electron microscopy revealed that treatment with benznidazole caused mitochondrial swelling in many cells with loss of matrix and cristas while others were observed the disruption of mitochondrial morphology (Figure 4). The cytoplasm shows intense vacuolization, presence of myelin figures and structures as autofagossoma-like (Figures 4, 5 and 6). In the most of the cells was not observed any change in subpericulares microtubules layer however some cells showing a complete cytoplasmic disorganization. Furthermore, it was observed changes in nuclear morphology with abnormal chromatin condensation and nuclear envelope alterations (Figure 7). The control group had morphology characteristic of the reference strains known.

Our results corroborate other descriptions in the literature and contribute towards the knowledge and records of the profile of some additional wild isolates of *T. cruzi* in regions not yet affected by the disease.

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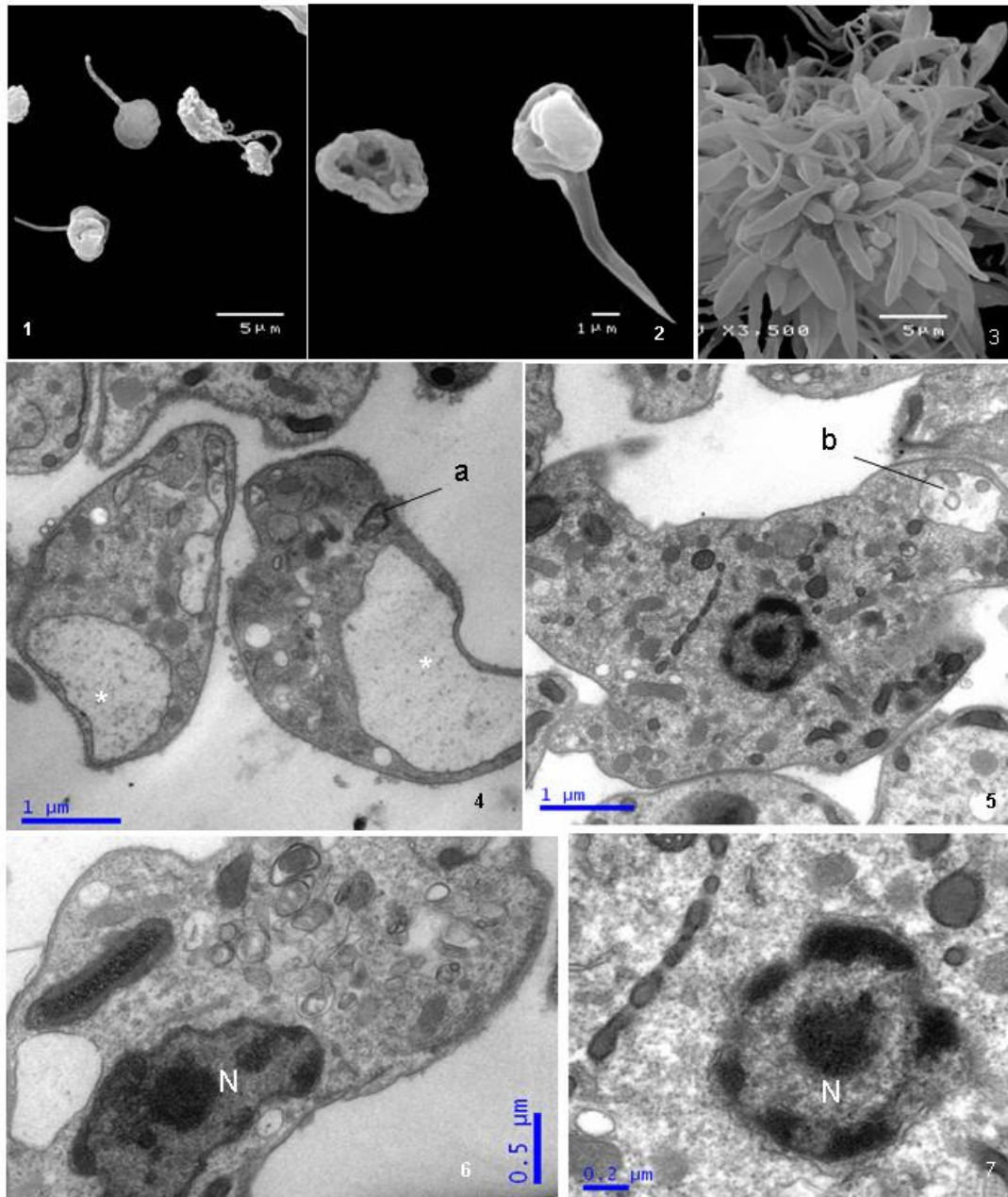


Fig. 1 - 2. Ultrastructural aspects of epimastigotes of *Trypanosoma cruzi* isolated from *Triatoma vitticeps* after in vitro incubation of benznidazole (IC50) by scanning electron microscopy.

Figure 3. Rosettes of *Trypanosoma cruzi* isolated from *Triatoma vitticeps* of control group untreated with benznidazole visualized by scanning electron microscopy.

Figures 4 - 7. Morphological changes observed with transmission electron microscopy after treatment with benznidazole (IC50) showing mitochondrial swelling (\*), presence of myelin figures (a), structures as autofagossoma-like(b), cytoplasmic disorganization and abnormal chromatin condensation