Tissues, Pathology, and Diagnostic Microscopy

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Spatial distribution of heterochromatin bodies in vectors of Chagas' disease as studied by confocal microscopy

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Most interphase cells of the insects *Triatoma infestans* and *Panstrongylus megistus*, vectors of the Chagas' disease, are characterized by high levels of polyploidy and the presence of constitutive heterochromatin bodies assembled in chromocenters, which are maintained during all the insect life [1, 2]. In *T. infestans* the chromocenters are contributed by three pairs of autosomes and the sex chromosomes whereas in *P. megistus* the chromocenter is contributed by the Y chromosome [2, 3]. These chromocenters contain AT-rich/GC-poor DNA and H3K9 trimethylation; in addition, they display histone hypoacetylation and non-methylated DNA [4, 5] and reveal transcription inertness [1]. In higher vertebrates it is generally accepted that most heterochromatin concentrates close to the nuclear periphery, although gene transcription may also occur at this region [6-8]. However, it was suspected that in the case of *T. infestans* and *P. megistus* the localization of the chromocenters could not always be restricted to the nuclear periphery. In the present work confocal microscopy was found to be necessary for studies concerned with the spatial distribution of these heterochromatic bodies.

Whole-mounted Malpighian tubules of *T. infestans* and *P. megistus* 5th instar male nymphs were subjected to fluorescent Feulgen reaction and examined under a Carl Zeiss LSM510 META confocal scanning laser microscope using λ = 543 nm (81%) and C-Apochromatic 63x water immersion objective and pixel depth of 8 bits. The images were captured and processed using the software Carl Zeiss LSM Image Examiner – Advanced Imaging Microscopy release 4.0.

Conspicuous chromocenters were highlighted in the fluorescent images of the Feulgen-stained nuclei of *T. infestans* and *P. megistus* (Figures 1 and 2). The analysis of the orthogonal optical sections of the nuclei revealed that in 91% of the cases analyzed in *T. infestans* the chromocenters appeared positioned near one side of the nuclear periphery. In 85.7% of the cell nuclei of *P. megistus,* the chromocenter appeared at the inner part of the nuclei.

It is concluded that the spatial distribution of the chromocenter bodies in the cell nuclei of *T. infestans* and *P. megistus* can differ when comparing the two species from each other and vary inside the nuclei of the same species. This situation was probably related to cell physiological events other than those related to cell cycle, or the topology was maintained from previous cell cycles [9], as Malpighian tubule cells in late 5th instar nymphs no longer undergo DNA replication [1, 2].

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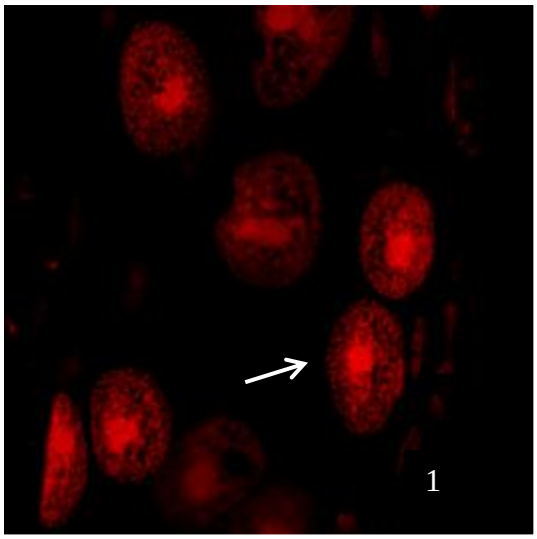

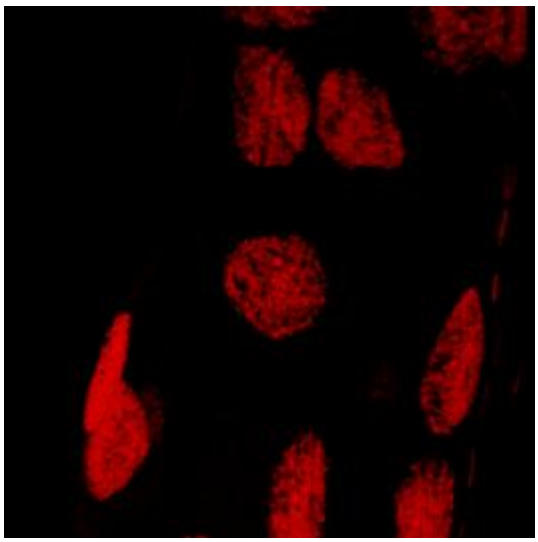

Figures 1 and **2**.Feulgen-stained fluorescent images of *T. infestans* (1) and *P. megistus* nuclei (2). Arrows indicate chromocenters. Bars, 10 µm