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# Analysis of metabolic activity in cystic cells of *Triatoma rubrofasciata* (Hemiptera: Triatominae) and its capacity to occupy different environments

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Spermatogenesis is composed of three distinct phases: spermatocytogenesis, which is the proliferation phase; meiosis, which is the division phase; and spermiogenesis, which is the differentiation phase (Johnson *et al.* 1997).

In insects the spermatogenesis is cystic (Dumser 1980). In cystic spermatogenesis, the meiotic divisions are synchronous within a given cyst (Smith 1916). This phenomenon has been confirmed in the subfamily Triatominae (Silistino-Souza *et al.* 2011; Alevi *et al.* 2015).

Cystic spermatogenesis germ cells are contained within a cyst and form a simple layer of cells of mesodermal origin (Dumser 1980). These cells that involve the spermatogonial cysts are termed cystic cells, which undergo polyploidisation events and can display elevated metabolic rate (Cruz-Landim 2001; Schmidt & Dorn 2004).

The size and number of nucleoli and pre-nucleolar bodies depend on the functional characteristics of the cells and may reflect metabolic and functional differences (Tavares & Azeredo-Oliveira 1997). Therefore, the metabolic rate of cystic cells can be evaluated by characterising the nucleolar pattern.

The nucleolar pattern of 16 species of triatomines was analysed (Tavares & Azeredo-Oliveira 1997; Severi-Aguiar & Azeredo-Oliveira 2005; Costa *et al.* 2008; Mendonça *et al.* 2010; Bardella *et al.* 2012; Alevi *et al.* 2013; Pereira *et al.* 2015) and most species had only a corpuscle impregnated by silver ions, demonstrating low metabolic activity. However, evaluating the metabolic rate of new species can assist in the understanding of the functional dynamics of cystic cells.

*Triatoma rubrofasciata* (De Geer) (Hemiptera: Triatominae) is considered of global epidemiological importance, since it presents pantropical distri-

bution (has been captured in approximately 45 countries (Galvão *et al.* 2003), highlighting Angola, Congo (Katanga), Guinea (Conakry), Saudi Arabia, Sierra Leone, South Africa, Tanzania (Dujardin *et al.* 2015) that are on the African continent and was found infected with *Trypanosoma cruzi* protozoan (Sherlock & Serafim 1974; Brazil & Silva 1983), the etiological agent of Chagas disease.

Thus, knowing more about the biology of this vector species is of utmost importance to assist in understanding the ability of *T. rubrofasciata* being able to occupy so many countries with different environmental conditions. Therefore, we describe the nucleolar pattern of the cystic cells of *T. rubrofasciata*, with emphasis on the analysis of cellular metabolic activity.

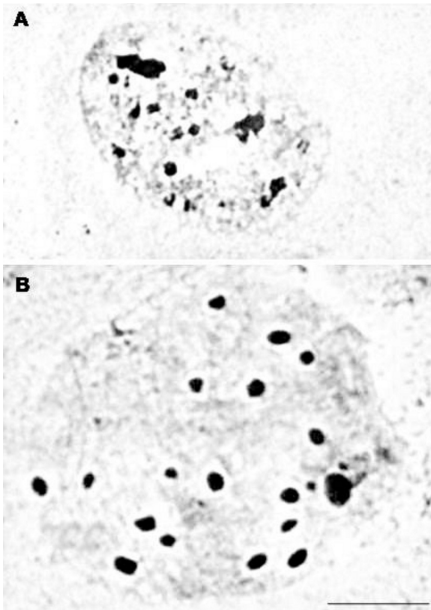
Four adult males coming from the National and International Reference Laboratory in Taxonomy of Triatominae, FIOCRUZ, Rio de Janeiro, Brazil, were analysed cytogenetically. Seminiferous tubules were first shredded and squashed on a slide, which was then placed in liquid nitrogen. The preparation was stained by impregnation with silver ions (Howell & Black 1980).

Based on the analysis of slides it was observed that cystic cells were polyploid and showed many nucleolar marks (Fig. 1A, B), which suggests that the cells of *T. rubrofasciata* have a high metabolic activity.

We reviewed the literature and grouped all the species that also had cystic cells analysed by means of silver ions (Table 1). From 16 species that have been analysed, only three have similar results to *T. rubrofasciata*.

The nucleolus is the most prominent structure in a cell nucleus. It is the site of ribosomal RNA (rRNA) transcription, pre-rRNA processing and ribosome subunit assembly (Olson *et al.* 2002). The analysis of the nucleolar pattern of cystic cells from

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**Fig. 1. A, B,** Cystic cells of *Triatoma rubrofasciata*. Note the presence of many nucleolar blocks. Scale bar: 10  $\mu$ m.

*T. lenti* and *T. melanocephala* allowed Alevi *et al.* (2013) to report that the cells of *T. lenti* have a high metabolic activity, since they present many nucleolar markings. In the same perspective, in this study we observed that cystic cells of *T. rubrofasciata*

also underwent polyploidisation events and have many nucleolar markings, resulting in high metabolic activity.

The event of polyploidisation present in all cystic cells has been described for cells of other tissues of the triatomines, such as salivary glands (Anhê & Azeredo-Oliveira 2008; Anhê *et al.* 2014) and Malpighian tubules (Alvarenga *et al.* 2011, 2012). This event is critical to the nutritional role that these cells exert on the cells in meiotic division (Schmidt & Dorn 2004), because it allows certain regions of the genome to be endoreplicated.

We suggest that polyploidy in conjunction with high metabolic activity of *T. rubrofasciata* are important factors for the occupation of 45 countries by the species (Galvão *et al.* 2003) and assisted in the occupation of home environments. This same characteristic is observed for *T. infestans* (Bardella *et al.* 2012), the species of triatomine vectors of most importance in the Americas.

*Triatoma rubrofasciata* and *T. infestans*, which live in human households (Silveira & Rezende 1994) and have many nucleolar marks and wide geographical distribution (Galvão *et al.* 2003), whereas the endemic species such as *T. vandae* (Galvão *et al.* 2003) show only a nucleolar marking. This confirms that the high metabolic activity of *T. rubrofasciata*, a vector species of great importance, is an extremely important phenomenon for

**Table 1.** Cytogenetic characteristics of cystic cells of triatomines.

Species	Nucleolar markings in cystic cell	References
<i>Triatoma brasiliensis</i>	One corpuscle	Tavares & Azeredo-Oliveira (1997)
<i>Triatoma delpontei</i>	One corpuscle	Tavares & Azeredo-Oliveira (1997)
<i>Triatoma infestans</i>	Many corpuscles	Bardella <i>et al.</i> (2012)
<i>Triatoma infestans melanosoma</i>	Many corpuscles	Bardella <i>et al.</i> (2012)
<i>Triatoma klugi</i>	One corpuscle	Costa <i>et al.</i> (2008)
<i>Triatoma lecticularia</i>	One corpuscle	Tavares & Azeredo-Oliveira (1997)
<i>Triatoma lenti</i>	Many corpuscles	Alevi <i>et al.</i> (2013)
<i>Triatoma maculata</i>	One corpuscle	Mendonça <i>et al.</i> (2010)
<i>Triatoma matogrossensis</i>	One corpuscle	Bardella <i>et al.</i> (2012)
<i>Triatoma melanocephala</i>	One corpuscle	Alevi <i>et al.</i> (2013)
<i>Triatoma platensis</i>	Many corpuscles	Severi-Aguiar & Azeredo-Oliveira (2005)
<i>Triatoma protracta</i>	One corpuscle	Severi-Aguiar & Azeredo-Oliveira (2005)
<i>Triatoma pseudomaculata</i>	One corpuscle	Mendonça <i>et al.</i> (2010)
<i>Triatoma rubrovaria</i>	One corpuscle	Tavares & Azeredo-Oliveira (1997)
<i>Triatoma rubrofasciata</i>	Many corpuscles	This paper
<i>Triatoma sordida</i>	Two corpuscles	Tavares & Azeredo-Oliveira (1997)
<i>Triatoma tibiamaculata</i>	One corpuscle	Severi-Aguiar & Azeredo-Oliveira (2005)
<i>Triatoma vandae</i>	One corpuscle	Pereira <i>et al.</i> (2015)
<i>Triatoma williami</i>	One corpuscle	Pereira <i>et al.</i> (2015)

the occupation of different environments, because it supports all the transcriptional activity necessary for reproduction of species in new environments.

Thus we suggest that cystic cells of *T. rubrofasciata* have high metabolic activity and relate this phenomenon to the vectorial capacity of these insects to inhabit different countries.

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