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Analysis of spermiogenesis like a tool in the study of the triatomines of the Brasiliensis subcomplex

Analyse de la spermiogenèse comme un outil dans l'étude des triatomes du sous-complexe Brasiliensis

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ABSTRACT

The specific identification and systematic of triatomines have been based fundamentally on morphological observations. These organisms are classified into complexes and specific subcomplexes, principally for morphological parameters and geographical disposition. The use of cytogenetic analyzes has been represented as a tool in systematic and taxonomy of triatomines. Thus, the present work, through the analysis of spermiogenesis, aims to characterize this stage of spermatogenesis in triatomines little studied, and especially to compare it among the species *Triatoma lenti* and *T. sherlocki*, to assist in the diagnosis of differentiation of these insects. The presence of the heteropyknotic corpuscle is shown as a diagnostic tool to differentiate *T. sherlocki* and *T. lenti*, since it is absent in *T. lenti*. The analysis of the spermiogenesis in *T. sherlocki* also allowed us to address morphological differences between elongating cells, which were relatively smaller and more filamentous when compared to *T lenti*. Furthermore, the flagellum was observed in all stages of cell differentiation and elongation. This structure, which helps in the locomotion of the sperm, is hardly observed in cytogenetic analysis, especially throughout spermiogenesis. Thus, although other comparative approaches should be taken, this paper allowed emphasizing the analysis of spermiogenesis as an important cytotaxonomic tool that assists in the differentiation of morphologically related species, such as *T. lenti* and *T. sherlocki*.

RÉSUMÉ

L'identification spécifique et systématique des triatomes a été fondamentalement basée sur des observations morphologiques. Ces organismes sont classés en sous-complexes et complexes spécifiques, principalement en ce qui concerne les paramètres morphologiques et la disposition géographique. L'utilisation de l'analyse cytogénétique a été présentée comme un outil pour la systématique et la taxonomie des triatomes. Ainsi, le présent

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travail, à travers l'analyse de la spermiogenèse, vise à caractériser cette étape de la spermatogenèse chez des triatomes peu étudiés, et surtout de la comparer entre les espèces *Triatoma lenti* et *T. sherlocki*, pour aider au diagnostic de différenciation de ces insectes. La présence du corpuscule heteropyknotic est présenté comme un outil de diagnostic pour différencier *T. lenti* de *T. sherlocki* lent, car il est absent chez *T. lenti*. L'analyse de la spermiogenèse chez *T. sherlocki* a également permis d'aborder les différences morphologiques entre les cellules d'allongement, qui étaient relativement petites et plus filamenteuses par rapport à *T lenti*. En outre, le flagelle a été observé dans toutes les étapes de la différenciation cellulaire et l'élongation. Cette structure, qui aide à la locomotion du sperme, est à peine observée dans l'analyse cytogénétique, en particulier tout au long de la spermiogenèse. Ainsi, bien que d'autres approches comparatives doivent être prises en considération, la présente étude a permis de mettre l'accent sur l'analyse de la spermiogenèse comme un outil important de cytotaxonomie, qui contribue à la différenciation d'espèces morphologiquement liées, telles que *T. lenti* et *T. sherlocki*.

1. Introduction

The specific identification and systematics of triatomines have been based fundamentally on morphological observations, mainly by means of optical [1] and electron scanning microscopy [2,3]. These organisms are classified into complexes and specific subcomplexes [4].

Triatoma lenti and *T. sherlocki* are hemipterans that belong to the Brasiliensis subcomplex [4]. This subcomplex is present in South America and consists of nine species, i.e., *T. brasiliensis*, *T. juazeirensis*, *T. melanica*, *T. melanocephala*, *T. petrochiae*, *T. lenti*, *T. sherlocki*, *T. tibiamaculata* and *T. vitticeps* [4]. However, the parameters used to group the subcomplex in triatomines were principally morphological and geographical disposition.

Costa et al. proposed the *T. brasiliensis* complex using egg morphology [3], morphological [3], biological [5], isoenzymes [6], and ecological [5] data. This complex comprises the subspecies *T. b. brasiliensis* and *T. b. macromelanosoma*, and species *T. juazeirensis* and *T. melanica*. Mendonça et al. (2009), by means of a phylogenetic reconstruction, proposed the recent inclusion of the *T. sherlocki* to this complex [7]. Thus, the complex *T. brasiliensis* shows up as a monophyletic group.

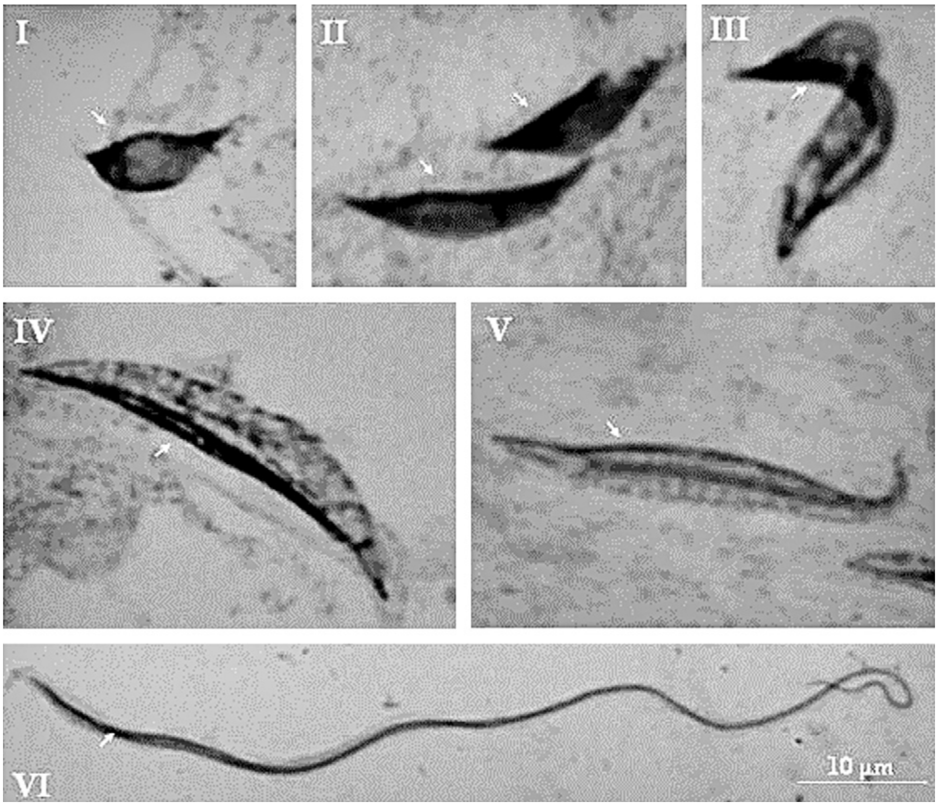

Fig. 1. Spermiogenesis of *Triatoma lenti* (I–VI). Note a peripheral heteropcnotic filament in all cells (arrows).

It is possible that *T. brasiliensis* species complex may encompass other species such *T. lenti*. However, new approaches, such as molecular analysis, should be performed such as in *T. sherlocki*, to propose the grouping of this species in the complex [7,8].

T. lenti is an endemic triatomine of the States of Bahia, Goias, and Roraima [9]. The species is considered a potential vector for Chagas disease of secondary importance and it was found infected with the etiologic agent, the protozoan *Trypanosoma cruzi* [10]. *T. sherlocki* is an endemic species of Bahia [9]. It presents unique morphological characteristics that resemble those of *T. lenti*, as reduced hemelytra, reddish orange colored rings on the femur, and spots in the connective [11]. It was also reported that these organisms live in sympatry in some regions [12]. *T. sherlocki* has been found in human habitations, infected with *T. cruzi*, indicating a process of domiciliation of this vector [13]. Although both species were found infected with *T. cruzi*, only *T. sherlocki* presents epidemiological importance, because *T. lenti* is an exclusively sylvatic species [14].

The use of cytogenetic analyzes has been represented as a tool in systematic and taxonomy of cryptic species of triatomine [15,16]. In addition, it has been shown recently that cytogenetic is very important in the study of subcomplexes, since karyotype analysis was used to propose the exclusion of *T. melanocephala*, *T. vitticeps*, and *T. tibiamaculata* from the Brasiliensis subcomplex for approaching the triatomines in South America [17].

Thus, the present work, through the analysis of spermiogenesis, aims to characterize this stage of spermatogenesis in triatomines little studied, and especially to compare it among the species *T. lenti* and *T. sherlocki*, to assist in the diagnosis of differentiation of these insects.

2. Material and methods

In this study, five males of each species was used, assigned by the 'Triatominae Insectarium' installed at the Department of Biological Sciences, Faculty of Pharmaceutical Sciences, Araraquara campus. The seminiferous tubules of adult males, after being torn and fixated on cover slip, underwent the cytogenetic technique of lacto-acetic orcein [18]. The biological material was analyzed by a Jenaval light microscope (Zeiss) coupled with a digital camera and an image analyzer Axio Vision LE 4.8 (Copyright© 2006–2009 Carl Zeiss Imaging Solutions Gmb H). The images were magnified by a factor of 1000.

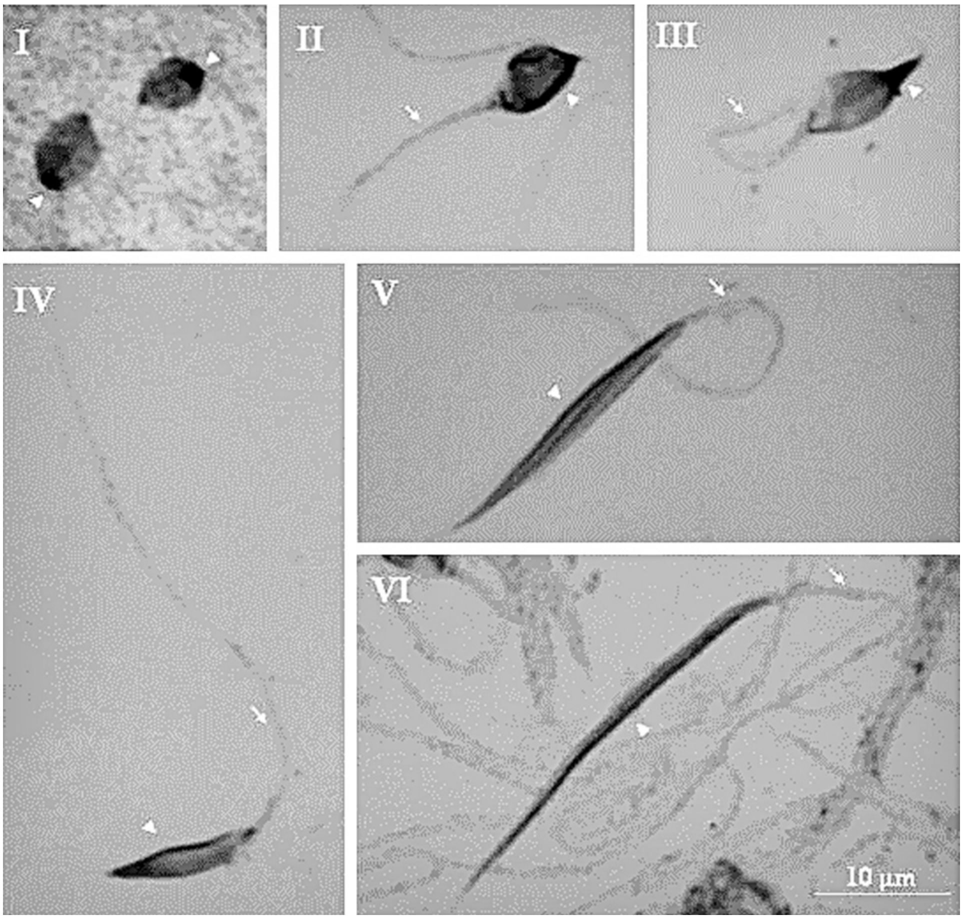

Fig. 2. Spermiogenesis of *Triatoma sherlocki* (I–VI). I: note the heteropyknotic corpuscle (arrowheads); II–VI: note a peripheral heteropcnotic filament in all cells (arrowheads) and the flagellum (arrows).

3. Results

By using the cytogenetic technique of lacto-acetic orcein, it was observed the elongation of the spermatid until differentiation into spermatozoa (Fig. 1(I–VI) and Fig. 2(I–VI)). During spermiogenesis of *T. lenti*, it was possible to detect a peripheral heteropycnotic filament in all cells (arrows). Already in *T. sherlocki*, the early spermatids showed a heteropyknotic corpuscle (Fig. 2I, arrowhead) that took the cell periphery during cell elongation (Fig. 2II–VI, arrowheads). During cell elongation of *T. sherlocki*, it was possible to observe the flagellum (Fig. 2II–VI, arrows). Note that the cells of this species are smaller and more tapered.

4. Discussion

Spermatogenesis occurs in the seminiferous tubules and consists, respectively, of three major divisions, out more, spermatocitogenesis, meiosis and spermiogenesis, which results in the production of sperm [19]. Data on spermatogenesis in triatomines is commonly discussed in the literature, but focus mainly on meiosis, more specifically on the behavior of chromosomes.

By analyzing the spermiogenesis on *T. lenti* and *T. sherlocki*, we observed that both have a heteropyknotic filament on the periphery of the cells. This characteristic was also observed in *T. pseudomaculata* [20]. These organisms also share characteristics of egg morphology [21] and karyotypic, since all have 22 chromosomes [20,22,23].

The analysis of the initial spermatid of *T. sherlocki* allowed the visualization of a heteropyknotic corpuscle. This characteristic was also observed in *T. klugi* [24], *T. maculata* [20] and *T. melanosoma* [25]. *T. maculata*, *T. sherlocki*, and *T. melanosoma* also share characteristics of egg morphology [21]. Furthermore, all species have the diploid chromosome set $2n = 22A$ $(20A + XY)$ [20,22,25].

The presence of the corpuscle is shown as a diagnostic tool to differentiate *T. sherlocki* and *T. lenti*, since it is absent in *T. lenti*. The analysis of the spermiogenesis in *T. sherlocki* also allowed us to address morphological differences between elongating cells, which were relatively smaller and more filamentous when compared to *T lenti*. Furthermore, the flagellum was observed in all stages of cell differentiation and elongation. This structure, which helps in the locomotion of the sperm, is hardly observed in cytogenetic analysis, especially throughout spermiogenesis.

Thus, although other comparative approaches should be taken, this paper allowed emphasize the analysis of spermiogenesis as an important cytotaxonomic tool that assists in the differentiation of morphologically related species, such as *T. lenti* and *T. sherlocki*.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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