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Composition of constitutive heterochromatin of *Pseudonannolene strinatii* Mauriès, 1974 (Diplopoda, Spirostreptida) analyzed by AT/CG specific fluorochromes

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ABSTRACT. Triple staining with fluorochromes (DA/DAPI/CMA) and C-banding were used to characterize the composition of *Pseudonannolene strinatii* heterochromatin. C-banding showed C+ bands of different labeling intensity on chromosomes 1 and 2 in some cells. Fluorochrome staining revealed DAPI+ regions corresponding to the C-banding pattern, indicating that the heterochromatin of this species is abundant in AT-rich sequences.

Key words: Diplopoda, Cytogenetics, Heterochromatin, Fluorochromes

INTRODUCTION

The cytogenetics of diplopods has seen a tremendous development over the last few years, with approximately 80 species having been analyzed cytogenetically in this group. The application of differential cytogenetic techniques to the characterization of chromosomes of diplopods is still rare, mainly due to technical difficulties in obtaining mitotic chromosomes (Fontanetti et al., 2002).

Diplopods are conservative in terms of general karyotypic evolution, and knowledge is lacking about which types of chromosome polymorphisms exist in myriapods, as well as about the groups of rearrangements that occurred during the evolution of their karyotypes (White, 1979).

The genus *Pseudonannolene* is found in many Brazilian caves, constituting troglophilic populations across the country (Trajano et al., 2000). The species *Pseudonannolene strinatii* Mauriès, 1974 is widely distributed in caves of the Speleologic Province of the Ribeira Valley, which encompasses the south of the State of São Paulo and the north of the State of Paraná, and is one of the seven species of the genera that have been studied cytogenetically.

The present study contributes to the chromosomal characterization of this species by describing the composition and some peculiarities of constitutive heterochromatin determined by C-banding and simultaneous staining with fluorochromes specific for AT- and CG-rich regions.

MATERIAL AND METHODS

Specimens of *P. strinatii* Mauriès, 1974 were collected from different caves in the municipality of Iporanga, south of São Paulo, by K.A. Campos and colleagues during different seasons.

For chromosome preparation, the individuals were fasted for one week and then injected with 0.08% colchicine. After a period of approximately 16 h (overnight), the specimens were then dissected and the middle intestine was removed, hypotonized in tap water for 10 min, and fixed in Carnoy I fixative. The slides were prepared using the cell suspension method which consists of centrifugation after previous dissociation in 45% acetic acid, followed by two changes of the fixative and staining with 3% Giemsa.

C-banding was performed according to Sumner (1972) and fluorochrome staining of AT- and CG-rich regions was performed according to Schweizer (1980), with small modifications.

RESULTS

Pseudonannolene strinatii has a chromosome number of 2n = 16 and an XY/XX type sex determination mechanism (Campos and Fontanetti, 2004) (Figure 1A). C-banding demonstrated a large amount of heterochromatin in the chromosomes of this species, corresponding to about 65% of the diploid genome, with each pair showing specific labeling (Figure 1B). A more detailed analysis of the C-banding pattern of *P. strinatii* chromosomes revealed intensely stained C+ blocks on chromosomes 1 and 2 in some cells (arrows in Figure 1C), intercalated with other less intensely stained blocks.

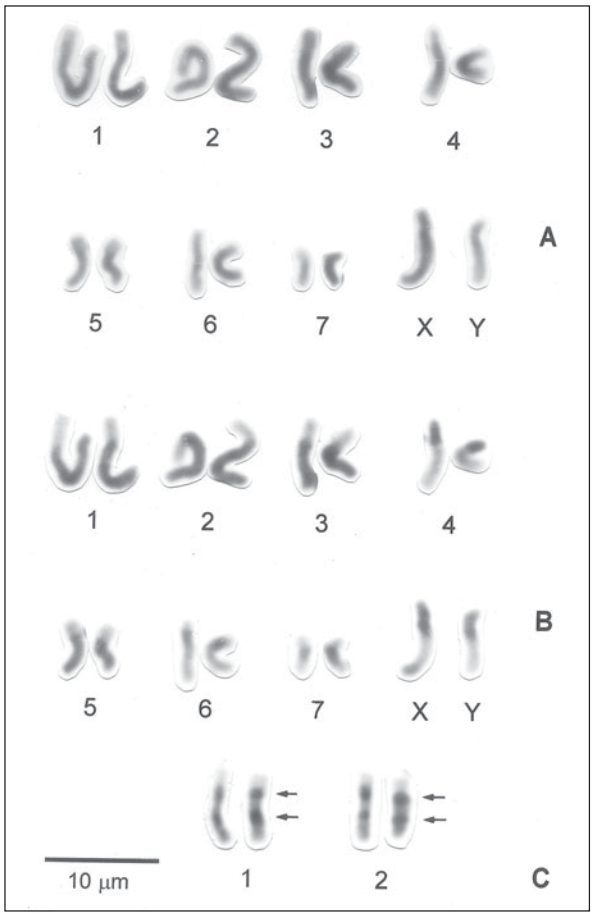

Figure 1. Karyotype of *Pseudonannolene strinatii*. **A.** Conventional staining with Giemsa; **B.** C-banding technique and **C.** C-banding technique of chromosome pairs 1 and 2 in some cells (arrows indicate intensely stained C+ blocks intercalated with other less intensely stained blocks).

DAPI staining corresponded to the C-banding pattern (Figure 2A), and also confirmed the existence of chromosomes containing blocks of different labeling intensity in some cells (Figure 3A). No positive labeling was observed with CMA (Figures 2B and 3B).

DISCUSSION

Large amounts of constitutive heterochromatin in the chromosomes of diplopods have been described for two European species analyzed by C-banding, with the heterochromatin accounting for about 60% of the total genome (Vitturi et al., 1997). Similarly, large amounts of constitutive heterochromatin comprising about 65% of the genome have also been reported for two *Pseudonannolene* species (Campos and Fontanetti, 2004; Souza et al., in press).

In *P. strinatii*, the heterochromatic regions of chromosomes 1 and 2 were found to be intercalated with dark and grayish C+ blocks in some cells. Data in the literature indicate the existence of grayish C+ segments in the chromosomes of grasshoppers (King and John, 1980)

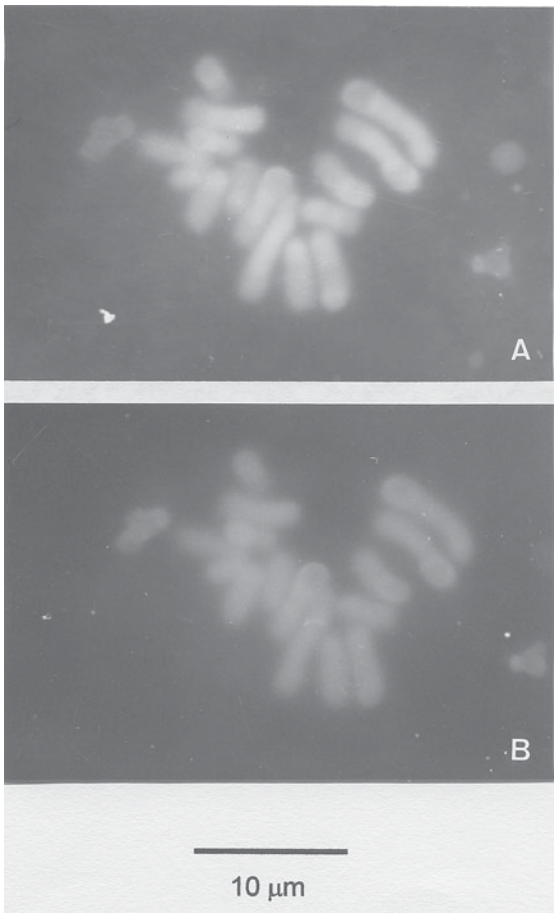

Figure 2. Fluorochrome staining CMA/DA/DAPI of *Pseudonannolene strinatii*. **A.** DAPI staining and **B.** CMA staining.

and in the telomeric regions of the chromosomes of amphibians of the family Hylidae (King, 1980; King et al., 1990). In this respect, the authors discuss the existence of intrachromosomal differences in the heterochromatin of the species, indicating particular sites characterized by highly specific DNA profiles and thus differing from all other C+ bands of the complement (John et al., 1986; King, 1991).

Stingless bees of the genus *Melipona* have C+ bands of different labeling intensity that can be detected by C-banding, with the phenomenon being attributed to variations in the amount of heterochromatin among different chromosome regions (Rocha et al., 2002).

Fluorochrome staining of *P. strinatii* chromosomes suggests the presence of a large number of AT-rich sequences in constitutive heterochromatin, since DAPI labeling showed a pattern similar to the C-banding pattern. The chromosomes of bees of the genus *Melipona* belonging to the chromosome II group are characterized by a large amount of heterochromatin, and the application of AT-specific fluorochromes (quinacrine mustard and DAPI) has revealed that the heterochromatic regions of these species are uniformly stained by these fluorochromes (Rocha et al., 2002). In the two European diplopod species mentioned earlier, DAPI staining

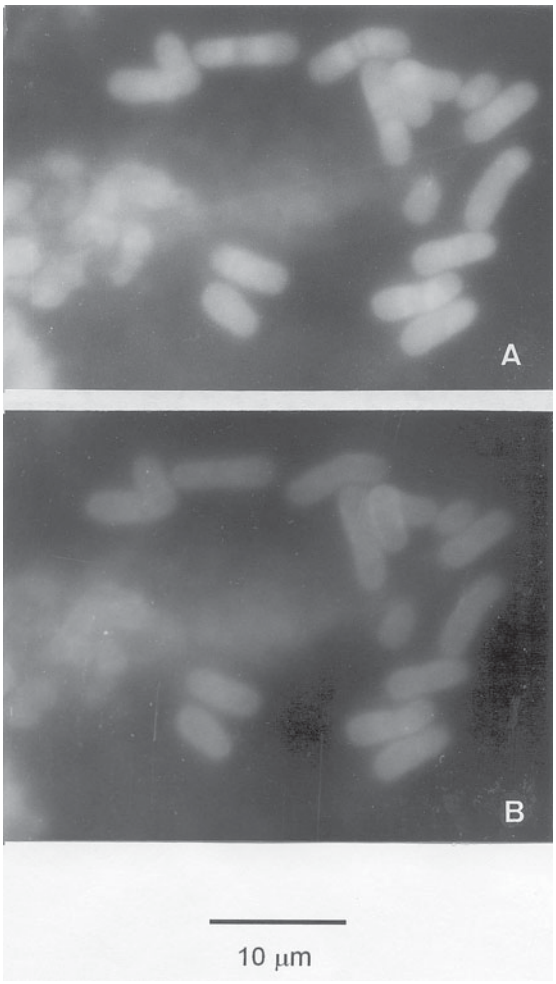

Figure 3. Fluorochrome staining CMA/DA/DAPI of some cells of *Pseudonannolene strinatii*. **A.** DAPI staining (note the presence of chromosomes containing blocks of different labeling intensity, as seen with C-banding technique) and **B.** CMA staining.

also corresponded to the C-banding pattern, suggesting that the repetitive DNA of these two species has the same base composition (Vitturi et al., 1997).

The results demonstrate a similarity in chromosome composition between *P. strinatii* and the other two diplopod species not only in terms of the large amount of constitutive heterochromatin in their chromosomes as mentioned above, but also in terms of the DAPI+ staining corresponding to heterochromatic regions.

These data confirm the assertion that diplopods are conservative in terms of general karyotype evolution (White, 1979), considering that these species belong to three different orders with various geographic distributions. However, studies involving a larger number of species using the same cytogenetic techniques as employed here, as well as more refined methods, are necessary to better understand chromosome characteristics and the direction of karyotype evolution in this group.

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