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Cytogenetics of four species of Spirostreptidae (Diplopoda, Spirostreptida)

Juliana Aparecida Preto de Godoy, Pedro Henrique Braga Pierozzi, Carmem Silvia Fontanetti *

Department of Biology, São Paulo State University (UNESP), Bioscience Institute (IB), Av. 24A, No. 1515, Bela Vista, 13506-900 Rio Claro, São Paulo, Brazil

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Abstract

Considering an estimated number of millipedes of approximately 80,000, cytogenetic studies on these animals are rare, as only a total of 70 species have their karyotypes described. The present study reports on the chromosomal number of four Brazilian diplopods of the family Spirostreptidae: *Urostreptus atrobrunneus* with $2n = 24$, XY; *Gymnostreptus olivaceus* $2n = 12$, XY and *Alloporus araraquarensis* and *A. principes*, $2n = 18$, XY. The C-banding pattern and NOR staining of *U. atrobrunneus*, *G. olivaceus* and *A. araraquarensis* are described.

Keywords: Millipede; NOR; C-banding; Chromosomes; Heterochromatin

1. Introduction

Diplopods form a complex taxonomic group with a great diversity of species, constituting one of the largest classes in terms of numbers of myriapods, distributed in all zoogeographic regions. These animals have been poorly studied in many aspects, especially systematically.

Cytogenetic techniques have helped elucidate taxonomic issues in many groups, including diplopods (Fontanetti, 1996c; Campos and Fontanetti, 2001). However, in comparison with other taxa, only few studies report the cytogenetics of diplopods. Most of the information about the chromosomes of diplopods is based on conventional staining techniques especially in works conducted between the 60s and 80s by Asian researchers (Achar, 1983a,b, 1984a,b, 1985, 1986, 1987; Achar and Chowdaiah, 1979, 1980; Chowdaiah, 1966a,b,c, 1967, 1969; Chowdaiah and Kanaka, 1969, 1974, 1979).

Regarding Brazilian species, Fontanetti and colleagues have catalogued the chromosomal number and the sex determination system in millipedes, currently totalling 17 species. This is a very low number considering the estimated 2000–3000 species found in Brazil (Fontanetti, 1990, 1991, 1996a,b,c, 1998, 2000; Fontanetti et al., 2002, 2003; Campos and Fontanetti, 2004, 2005; Souza et al., 2005; Pierozzi and Fontanetti, 2006).

This group is of special interest due to the large quantity of constitutive heterochromatin present in some species, representing more than 50% of the genome (Vitturi et al., 1997; Campos and Fontanetti, 2004; Souza et al., 2005; Pierozzi and Fontanetti, 2006). The heterochromatin is one of the most studied portions of the genomic material from a cytogenetic and molecular point of view. Many authors have proposed several functions of heterochromatin such as help chromosome pairing and segregation, facilitate chromosome rearrangement, reduce recombination rate (Brutlag, 1980; Grewal and Elgin, 2002), protect euchromatic regions (Hsu, 1975) and during speciation (Yunis and Yasmineh, 1971; Hatch et al., 1976; Bush et al., 1977; Pathak and Wurster-Hill, 1977). But, according to Sumner (2003) the evidences for some of these hipothesis is weak. However, the main functions credited to the constitutive heterochromatin are the determination of the architecture of the interphase nucleus and its possible effect, direct or indirect, on gene expression (Hilliker and Appels, 1980; Grewal and Elgin, 2002).

The diploid chromosomal number in diplopods ranges from $2n = 8$ (although this number is questionable according to White, 1979) to $2n = 30$. The most commonly found sex determination mechanism is XY in males and XX in females (White, 1979; Fontanetti et al., 2002).

* Corresponding author.
E-mail address: fontanet@rc.unesp.br (C.S. Fontanetti).

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Most of the knowledge on the chromosomes of diplopods is based on conventional staining methods. The use of other differential staining techniques in diplopods is rare, especially due to technical difficulties in obtaining mitotic chromosomes.

The purpose of this work was to characterize the chromosomes of four species of Spirostreptidae using conventional and differential staining techniques, to provide a framework for future studies on the dynamics of the karyotypic evolution in this group.

2. Materials and methods

A total of 6 specimens of *Urostreptus atrobrunneus* Pierozzi and Fontanetti, 2006 was collected by A. Mesa and C.B. Portugal in February 2002, in the Fausto Santomauro highway, km 3 North/SP (22°42′30″S, 47°41′18″W). Seven specimens of *Gymnostreptus olivaceus* Schubart, 1944 were collected in December 2003, by A. R. Miyoshi, in Rio Claro/SP, Unesp Bela Vista campus (22°23′42.83″S, 47°32′43″W) and in October 2004, by J.A.P. Godoy, in Monte Alegre/SP (22°40′56.83″S, 46°40′53.21″W). *Alloporus principes* Brolemann, 1902 was collected in November 1985, by A. Mesa and F.A. Mello, in a Brazilian savannah (cerrado) in the city of Corumbataí, Rio Claro/SP (22°14′29″S, 47°41′18″W), totalling 5 specimens. Four specimens of *Alloporus araraquarensis* Schubart, 1950 were collected in March 2005, by R.A. Prado, in Descalvado/SP (21°54′14.6″S, 47°37′11.53″W).

The collected animals were starved for 1 week and then injected with 0.08% colchicine. After approximately 16 h (overnight), the specimens were anesthetized and dissected in physiological solution. The gonads and the midgut were removed, brought to a hypotonic state by incubation in tap water for 5 and 10 min, respectively, and then fixed in Carnoy I (3:1 ethylic alcohol and glacial acetic acid). The slides were prepared by cellular suspension, by centrifuging, or by smashing. The material was stained with 3% Giemsa. In some cells, sequencial staining are used: conventional staining with Giemsa, C-banding and silver nitrate staining.

C-banding was prepared according to Sumner (1972) and stained with silver nitrate according to Howell and Black (1980).

The centromeric index and arm ration of chromosome pairs were obtained according to Guerra (1986).

3. Results

Urostreptus atrobrunneus presents $2n = 24$ and the sex determination system is XY in males. The diploid number includes three pairs of acrocentrics (pairs 4, 6 and 11), four pairs of metacentrics (pairs 3, 5, 8 and 10) and four pairs of

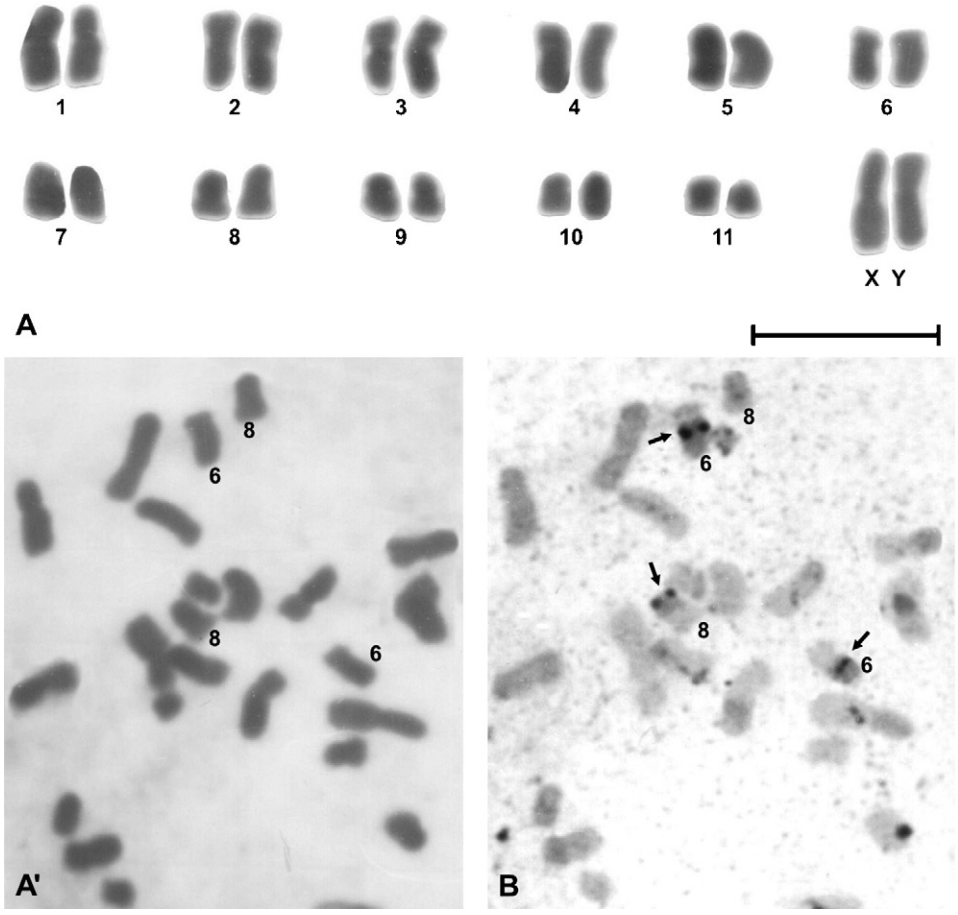

Fig. 1. Sequencial staining in mitotic nucleus of *Urostreptus atrobrunneus*. (A, A′) Conventional staining with Giemsa. (B) Silver nitrate staining. Arrows = NORs. Bar = 10 μm.