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# Hsc-FA and NOR bandings on chromosomes of the giant ant *Dinoponera lucida* Emery, 1901 (Hymenoptera: Formicidae)

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**Abstract.** The distribution of the threatened ant *Dinoponera lucida* is limited to the south of the State of Bahia, farthest east of Minas Gerais and north of Espírito Santo, Brazil. Recent cytogenetic studies carried out in 15 sites distributed along the range of the ant indicated high chromosome number variation in the populations of Bahia, 2n=106 to 120 chromosomes, while the populations of Espírito Santo presented a constant number, with 2n=118 chromosomes. This study aimed at describing the banding pattern of *D. lucida* chromosomes, in some populations of Espírito Santo and Bahia, applying the Hsc-FA technique (secondary constriction heterochromatin-associated bands by fluorescence using acridine) and identifying the nucleolar organizing region (active NORs) impregnated with $AgNO_3$ which is especially important for the cytogenetic characterization of a species. The utilization of these two techniques showed positive interstitial markings on the region of the long arm of the pair $AM^t$, similar to those obtained with the $CMA_3$ fluorochrome and with the FISH technique complementing the cytogenetic data of this species.

**Key words:** cytogenetics, simple NOR, fluorochrome, ant, Formicidae.

## Introduction

Cytogenetics is an important tool especially in evolutionary and taxonomic studies (Macgregor, 1993), as chromosomal alterations may influence the adaptation and speciation of organisms (Hoffmann, Rieseberg, 2008). Over 500 species of ants (Hymenoptera: Formicidae) have already been cytogenetically studied according to Mariano (2004), and diploid chromosome number varies from 2n=2 to 120 chromosomes in the Myrmeciinae *Myrmecia croslandi* Taylor, 1991 (Crosland, Crozier, 1986) and the Ponerinae *Dinoponera lucida* Emery, 1901 (Mariano et al., 2008), respectively.

The chromosome banding techniques are important to locate specific regions of chromosomes. This would be impossible only with conventional staining with Giemsa, which allows, for example, the comparison at population and species level, besides helping homologues chromosome pairing. Fluorochromes

are substances which fluoresce when stimulated by proper wave lengths and are divided into two categories according to their affinity with DNA, that is, they can be AT or GC specific. These substances are important in order to get to know the nature of chromatin (Verma, Babu, 1995; Sumner, 2003). Among the cytogenetic tools, the acridine orange (AO) fluorochrome is non specific; it intercalates in the DNA and can bind to the nucleic acid in two ways: by intercalating between the base pairs, which results in the emission of a green-yellowish fluorescence, or by binding to phosphate groups and emitting a red-orange fluorescence (Sumner, 1990). Verma, Babu (1995) call the use of this fluorochrome, RFA, since it produces a R band using Acridine Orange fluorochrome in human chromosomes. Almeida, Carvalho (2004) changed the name of the technique to Hsc-FA (secondary constriction heterochromatin-associated bands by fluorescence using acridine) when working with corn and green pepper chromosomes, as they obtained a different pattern from that observed in human chromosomes. This technique allowed the identification, at cytological level, of heterochromatin segments associated with active and inactive sites of rDNA. This fluorochrome was used in chromosomes of the ant *Wasmannia auropunctata* (Roger, 1863) and marking was detected in the same chromosomes marked with $CMA_3$ fluorochrome (Souza, 2007).

NORs are DNA regions responsible for rRNA transcription and they determine the formation of the nucleolus during the interphase. A characteristic of the rDNA is that this region is frequently rich in GC base pairs (Miller, 1981). The technique used for the detection of active NORs is the silver nitrate impregnation in metaphase and in interphase nucleus (Howell, Black, 1980). This technique was applied to chromosomes of certain ants, such as *M. croslandi* (Imai et al., 1992) and *Tapinoma nigerrimum* (Nylander, 1856) (Lorite et al., 1997).

The giant ant *D. lucida* is included in the Brazilian official list of threatened species of the fauna especially due to the fragmentation, loss of habitat and peculiarities of the biology of the species (Ministério do Meio Ambiente, 2003; Campiolo, Delabie, 2008). This ant is only found in the southern part of Bahia, in the farthest east of Minas Gerais and in the north of Espírito Santo (Campiolo, Delabie, 2008). Recent studies on this species carried out in 15 sites distributed in the range of the ant in the states of Espírito Santo (ES) and Bahia (BA) showed the occurrence of a large variation in the chromosome numbers: in Bahia they vary between 2n = 106 to 2n = 120 chromosomes, whereas in the populations of Espírito Santo this number is constant with 2n=118 chromosomes. The *D. lucida* chromosomes are all very small with the exception of a chromosome pair classified as $AM^t$, which presents heterochromatin on the short arm and on the extremity of the long arm (Mariano et al., 2004; Mariano et al., 2008).

This study aimed at describing the banding pattern of *D. lucida* chromosomes by applying the Hsc-FA technique and also identifying the nucleolar organizing region (active NORs) impregnated with $AgNO_3$, which is especially important for the cytogenetic characterization of any organism.

## Material and Methods

The metaphases were obtained from the brain ganglia of pharate larvae of last instar (Imai et al., 1988) and submitted to the Hsc-FA (Verma, Babu, 1995) and NOR banding techniques (Howell, Black, 1980). In the Hsc-FA technique, the colonies used were from the localities of Linhares, Viana, Cariacica and Domingos Martins (State of Espírito Santo)

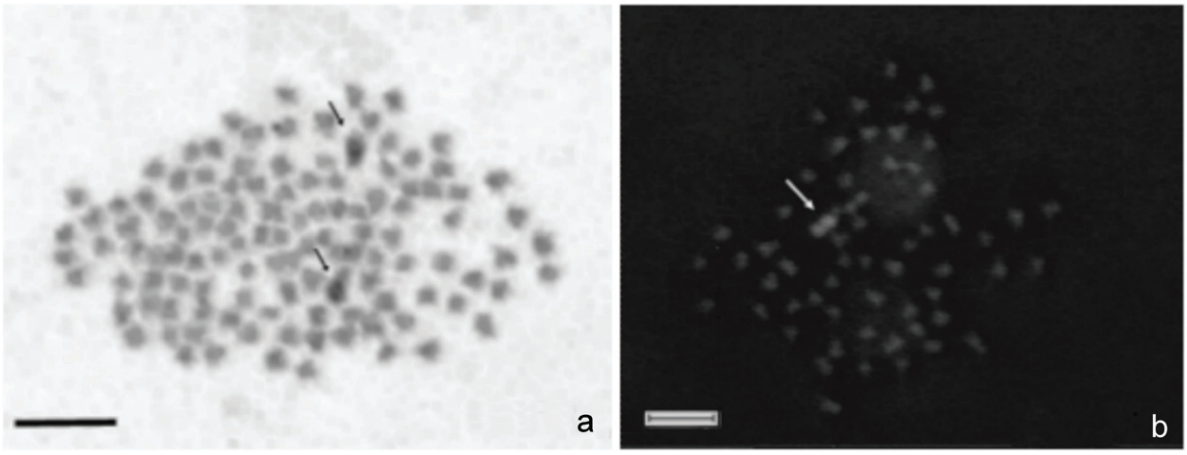


**Fig. 1. a** - Metaphase of female submitted to the $AgNO_3$ impregnation technique (Marechal Floriano, State of Espírito Santo). **b** - metaphase of male submitted to acridine orange fluorochrome (Linhares, State of Espírito Santo). The arrows show positive markings in the $AM^t$. Bar = 5µm.

and Barrolândia (State of Bahia). For the NOR banding technique, the analyzed populations came from the localities of Barrolândia, Ibirapuã and Mucuri (State of Bahia), Linhares, Marechal Floriano and Santo Antônio do Canaã (State of Espírito Santo).

The metaphases were examined with a BX 60 microscope connected to the image capture program, Image Pro Plus®. In the Hsc-FA technique the slides were analyzed by epifluorescence microscopy using a WB filter.

## Results and Discussion

The NOR banding technique and the Hsc-FA technique indicated positive marking in the interstitial region of the long arm of chromosomes $AM^t$ (Fig. 1). This marking pattern is similar to the one obtained with $CMA_3$ fluorochrome and with the FISH technique (Mariano et al., 2008).

The NOR banding technique enabled the identification of the nucleolar organizing region. For the interphase nuclei two markings were observed for females and one for males (Fig. 2). The difficulty in obtaining metaphases with a better quality of the NOR banding technique occurs, probably, due to the reduced size of the chromosomes which become upset after being submitted to the technique. Studies on the Australian ant *M. croslandi* (Imai et al., 1992) raise the possibility that, somehow, the ribosomal gene chromatin was not easily accessible, making it difficult to the argentophilic proteins to associate with chromatin and later that the silver binds itself with these proteins.

The marking pattern observed with the Hsc-FA technique was the same observed with the $CMA_3$ fluorochrome, which marks regions rich in GC base pairs, a pattern also observed by Souza (2007) in the Neotropical ant *W. auropunctata*. Several organisms including order Hymenoptera (Lorite et al., 1997, Mampumbu, 2002; Brito et al., 2005) have their nucleolar organizing regions rich in GC base pairs. Generally, the $CMA_3$ fluorochrome markings correspond to silver nitrate impregnation markings for most vertebrates (Schmid, Guttenbach, 1988).

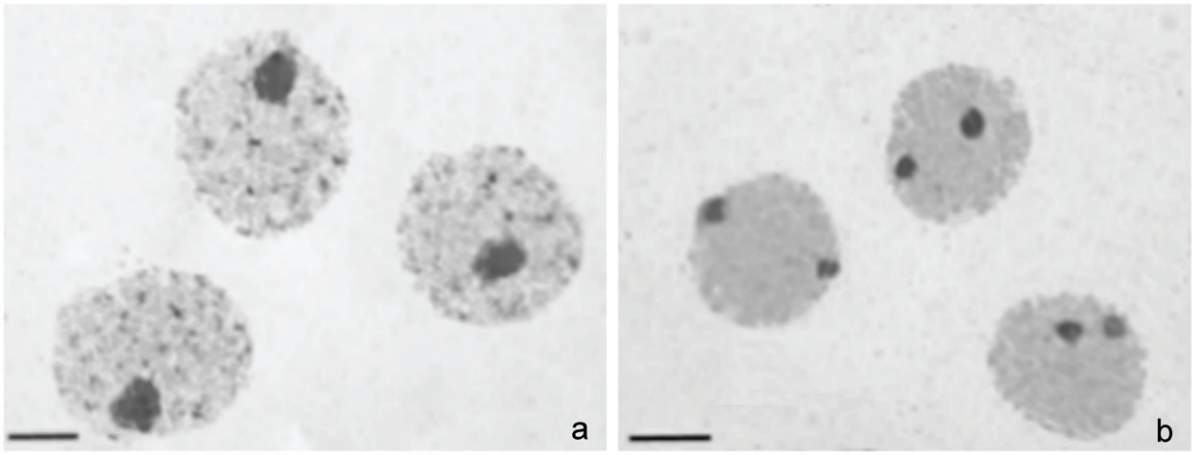


**Fig. 2.** *Dinoponera lucida*, population of Viana, State of Espírito Santo. **a** - interphase nuclei of male. **b** - female submitted to AgNO3 impregnation technique. Bar = 5µm.

However, it is important to highlight that not all regions rich in GC base pairs correspond to NOR. Fontana (1994) studied four species of sturgeons and observed that there was no correspondence between the NORs identified by silver nitrate impregnation and the $CMA_3$ fluorochrome. This also occurs with mammals such as marsupials, rats, mice, gorillas and pigs (Schmid, Guttenbach, 1988). In some species of bees of the genera *Melipona*, which possess high heterochromatin content, the eurochromatin is limited to the extremities of chromosomes and is rich in GC base pairs as it presents positive marking when treated with $CMA_3$. However, *Melipona compressipes* (Fabricius, 1804), when submitted to the FISH technique with the rDNA probe, is marked on only one of the extremities of a chromosome pair (Rocha et al., 2002).

The advantage that AO has in relation to $CMA_3$ regards the time in which data can be obtained, since it is not necessary to wait 15 days for the analyses of the slides. After the slides are treated, they can be observed through fluorescence microscopy and image capture systems. Since this fluorochrome is unspecific, it stains regions rich in GC base pairs in different ways according to their non denaturation. This way, the region rich in GC base pairs will be intercalated between the base pairs by the AO, whereas the fluorochrome will bind to the phosphate groups in the other regions of the chromosomes, thus providing a difference in the color of the emitted fluorescence.

*Dinoponera lucida* possesses ribosomal genes on a pair of chromosomes in females and one chromosome in males, which was confirmed in direct (FISH) and indirect (NOR banding and fluorochromes) methods. Considering that the ant hypothetical ancestral would have a reduced number of chromosomes (Imai et al., 2001), simple NOR is probably a plesiomorphy and can be considered as the ancestor of multiple NORs. The presence of a single NOR associated with the high number of chromosomes supports morphologic and behavioral data establishing that this species possesses a set of plesiomorphic characteristics. *Dinoponera gigantea* (Perty, 1833) presents multiple NORs detected by the FISH technique (Aguiar, Pompolo, personal communication), which indicates the occurrence of chromo-

somal rearrangements involving these genes. The results obtained in this study complement the *D. lucida* cytogenetic data (Mariano et al., 2008) and also promote the use of the Hsc-FA technique, which appears useful to complement cytogenetic studies in a range of organisms.

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