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Cytogenetic Studies on Workers of the Neotropical Ant *Wasmannia auropunctata* (Roger 1863) (Hymenoptera: Formicidae: Myrmicinae)

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Abstract. *Wasmannia auropunctata* is known as one of the worst invasive ants in the World. A cytogenetic study was conducted on two native populations from southeastern Bahia, Brazil. The analysis of the chromosomes observed in mitotic metaphases was made by a combination of methods: Giemsa conventional staining, chromomycin A3 (CMA3) and 4-6-diamidino-2-phenylindole (DAPI) fluorochrome staining, and acridine orange banding. The workers have all the karyotype 2n=32, with ten pairs of metacentric and six pairs of acrocentric chromosomes. One chromosome arm of the pair ten was positive for CMA3 and acridine orange, suggesting the occurrence of a nucleolar organizing region. This region is an interesting marker because is very conservative and seems to constitute an interesting specific taxonomic character. The pericentromeric region of many chromosomes was stained with DAPI, evidencing the occurrence of AT bases rich heterochromatin.

Résumé. Études Cytogénétiques sur les Ouvrières de la Fourmi Néotropicale *Wasmannia auropunctata* (Roger 1863) (Hymenoptera: Formicidae : Myrmicinae). *Wasmannia auropunctata* est connue comme étant l'une des fourmis les plus invasives du monde. Une étude cytogénétique a été réalisée dans deux populations natives du sud-est de Bahia, Brésil. L'analyse des chromosomes en métaphases mitotiques a été effectuée selon diverses méthodes: coloration conventionnelle de Giemsa, fluorochromes chromomycine A3 (CMA3) et 4-6-diamidino-2-phenylindole (DAPI), et mise en evidence de bandes par l'acridine orange. Les ouvrières ont toutes le caryotype 2n=32, avec dix paires de chromosomes métacentriques et six acrocentriques. Un bras chromosomique de la dixième paire s'est montré positif pour le CMA3 et l'acridine orange, suggérant l'existence d'une région organisatrice du nucléole. Cette région est un marqueur spécifique de choix car elle est très conservatrice et constitue ainsi un intéressant caractère taxonomique. La région péricentromérique de beaucoup de chromosomes a été marquée par le DAPI, mettant en évidence l'existence d'une hétérochromatine riche en bases AT.

Keywords: Karyotype, ant, DAPI and CMA bands, acridine orange, NORs.

Cytogenetic studies with ants started in the 1960s and led to the karyotype description of about 750 morpho-species (Lorite & Palomeque 2010), which rendered extended discussions on karyotype phylogeny and development in Formicidae (Imai *et al*. 1988; Lorite & Palomeque 2010). However, according to Delabie & Mariano (2005), the amount of information about the chromosome structure in the Formicidae remains small when compared to the number of species described (up to 12,000). The studies about ants from Neotropics are still incipient (see, for example, Mariano *et al*. 2001, 2011) and there is no cytogenetic study in the tribe Blepharidattini Wheeler & Wheeler 1991 (*sensu* Bolton 2003), which includes both the genera *Blepharidatta* Wheeler 1915 and *Wasmannia* Forel 1893. The Attini Forel 1892 are considered as being the Blepharidattini sister-group (Schultz & Meier 1995; Diniz *et al*. 1998) and cytogenetic investigations in this tribe concern around two dozen species in different genera (Barros *et al.* 2010; Lorite & Palomeque 2010).

Fluorochrome staining of metaphasic chromosomes is used to obtain information about chromatin constitution (Guerra & Souza 2002). Chromosome positives for CMA3 (chromomycin A3) and DAPI (4',6-diamidino-2-phenylindole) were evidenced in several Hymenoptera, such as bees of the genera *Melipona* Illiger 1806, *Partamona* Schwarz 1939 and *Trigona* Jurine 1807 (Rocha *et al*. 2002; Costa *et al*. 2004; Brito *et al*. 2005) and in the ants *Tapinoma*

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erraticum (Latreille 1798) [=*T. nigerrima* (Nylander 1856)] (Lorite *et al*. 1996, 1997) and few species of the genera *Anochetus* Mayr 1861 and *Odontomachus* Latreille 1804 (Santos *et al.* 2010). Acridine orange is also used to provide the standard of chromosomal banding (Verma *et al*. 1977), and there is at least one record of its use in ants with *Dinoponera lucida* Emery, 1901 (Barros *et al.* 2009).

The "little fire ant" *Wasmannia auropunctata* (Roger 1863) is currently widely distributed all over the tropics (Wetterer & Porter 2003). It is an ant of considerable economical and environmental importance (Errard *et al.* 2005; Foucaud *et al.* 2010) actually considered among the World's worst invasive species (Lowe *et al.* 2000). This ant has called recently the attention of scientific community due to its very original reproductive mechanism that is a combination of three different genetic systems: haplodiploidy, thelytoky, and male clonality (Fournier *et al.* 2005; Foucaud *et al*. 2007). This Myrmicinae ant deserves thus much

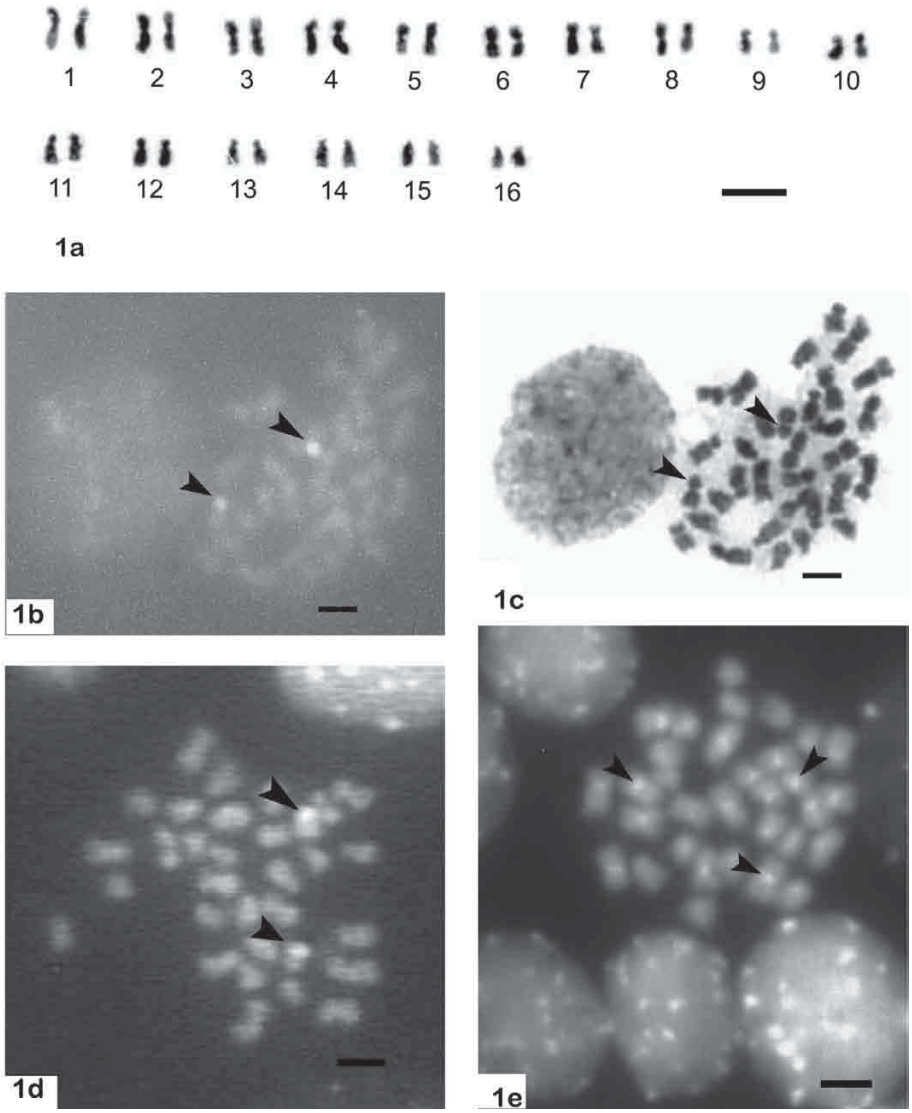

Figure **1**
Cytogenetic studies in workers (2n) *Wasmannia auropunctata.* (**a**) Karyotype, 2n=32. Chromosome metacentric pairs: 1 to 10 (M); acrocentric pairs: 11 to 16 (A). Bar = 5 µm. **b-e**: Brain cell metaphases: (**b**) CMA_3 positive reaction in an arm of the sister chromatid of the pair 10 (arrowheads), Bar = 2.2 µm. (**c**) Same metaphase that (**b**) stained with Giemsa showing the chromosome pair 10 (arrowheads), Bar = 2.2 µm. (**d**) Acridine orange positive reaction in an arm of the sister chromatid in the pair 10 (arrowheads), Bar = 3 µm. (**e**) Pericentromeric regions of the chromosomes evidenced by DAPI (arrowheads), Bar = 3 µm.

more studies in a range of perspectives, cytogenetics being only one of them. The aim of this study was to characterize the *W. auropunctata* worker karyotype focusing number, morphology and heterochromatic chromosomal markings.

Materials and methods

Eight colonies of *W. auropunctata* from southeast Bahia, Brazil, were used for the cytogenetic studies: six colonies come from experimental fields of CEPLAC (three from the Zoo-Botanical Reserve and three from "G" area) at Ilhéus (14°45'S 39°13'W) and two from native forest remnants at Una (15°18'S 39°07'W). The distance between the two localities is near 80 Km.

The metaphases were obtained from brain cells of 160 worker larvae of last instars, at the beginning of metamorphose (Imai *et al*., 1988). Some were stained with Giemsa (Imai *et al*., 1988); others with fluorochromes chromomycin A (CMA3) and DAPI (Schweizer, 1980), and a last series with acridine orange (Verma *et al*., 1977). Metaphase pictures were captured with a video-camera Q Color 3 connected to a microscope Olympus BX 60 with an immersion lens (100 x). The epifluorescence system (filters WB and WU) was used to observe metaphases under fluorescence. Some pictures were taken with AGFA films (conventional staining) and TMAX (fluorochromes). The morphological classification of the chromosomes followed Imai's (1991) terminology based on the chromosome heterochromatine localization.

Results

The number of chromosomes was 2n = 32 in all the individuals and in the two localities, with ten pairs metacentric and six pairs acrocentric (Fig. 1a). One of the arms of the sister chromatids of the tenth pair was positive for CMA3 (Fig. 1b) and acridine orange (Fig. 1c, d). The pericentromeric region of the majority of the chromosomes was evidenced with DAPI (Fig. 1e).

Discussion

This study is the first cytogenetic description of a species of the Blepharidattini tribe. The chromosome number found in *W. auropunctata* is 2n = 32, with the karyotype formula (M: metacentric, A: acrocentric) 2K = 20M + 12A. It is in the range of the chromosomal numbers recorded for Attini species, the sister group of Blepharidattini, with the extremes observed in the genera *Mycocepurus* Forel 1893 (2n = 8) and *Mycetarotes* Emery 1913 (2n = 54) (Barros *et al.* 2010).

Hirai *et al*. (1994, 1996) described nucleolar organizing regions (NORs) interspecific numeric variation in ants of the genus *Myrmecia* Fabricius, 1804, while CMA3 staining, which shows G-C base pairs, was also recorded in studies with hymenopterans. The correlation between CMA3 positive bandings and NORs is common among insects (Brito *et al*. 2003). Lorite *et al*. (1997) detected CMA3 staining showing NOR in the proximal portion of the short arms of chromosome 6 of the ant *T. nigerrimum*. Brito *et al*. (2003, 2005) evidenced DA/CMA3 positive reaction interpreted as NORs in *Partamona* bees. The acridine orange fluorochrome stained the same regions that the CMA3 in the *W. auropunctata* chromosomes and it is the first time that this fluorochrome is used in cytogenetic studies on ants. In agreements to these results, it is assumed that the chromosomal regions stained with CMA3 and acridine orange in *W. auropunctata* correspond to NORs. The detection of NORs in *W. auropunctata* chromosomes, evidenced with fluorochrome CMA3, is especially interesting because these regions are species-specific markers, since they are considered as well preserved regions (Alberts *et al*. 2004), making them useful for taxonomic and phylogenetic purposes.

DAPI staining allows the detection of chromosomal regions rich in AT base-pairs. Cytogenetic studies involving some hymenopterans show that DAPI stains mainly chromosomal parts different from the centromeric region. Brito *et al*. (2003) and Rocha *et al*. (2002, 2003) found DAPI-positive reactions in different parts of the chromosomes of bees of the genera *Frieseomelitta* Ihering 1912, *Melipona* or *Partamona.* However, in *W. auropunctata*, the fluorescence obtained with DAPI only marked the pericentromeric regions of great part of the chromosomes. This result was rather related with those of Rocha *et al*. (2003) for *Frieseomelitta varia* (Lepeletier 1836).

Due to the fact that the three kinds of individuals living in *W. auropunctata* nests (males, reproductive females, workers) are depending of a different genetic mechanism for their perpetuation, cytogenetic studies with chromosomal markers on other *W. auropunctata* colonies and *Wasmannia* species from other habitats and localities need to be carried out in order to provide additional information about evolutionary mechanisms in these ants.

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