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Research Article

Karyotypic description of four species of *Trigona* (Jurine, 1807) (Hymenoptera, Apidae, Meliponini) from the State of Mato Grosso, Brazil

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Abstract

The genus *Trigona* contains at least 31 species, but there have been few cytogenetic studies of this group. In this work, four species of *Trigona* (*T. branneri*, *T. chanchamayoensis*, *T. hyalinata,* and *T. recursa*) from the municipality of Cuiabá, in the State of Mato Grosso, Brazil, were studied. In all of the species, the females had 2n = 34 chromosomes and the males had n = 17. The C-banding patterns showed that the karyotypes of these species consisted mainly of acrocentric and pseudoacrocentric chromosomes. These cytogenetic findings should useful in future phylogenetic studies of this group.

Key words: cytogenetics, karyotype, Meliponini, stingless bees, *Trigona*.

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Introduction

The Brazilian state of Mato Grosso has three distinct biomes ("Cerrado", Amazon rain forest and Pantanal wetlands) and an important faunal and floral diversity. However, there is very little information on the apifauna of Mato Grosso and still no list of the bee species that occur in this state.

The tribe Meliponini (stingless bees) is highly diversified in the Neotropics, with 52 genera and more that 300 species found in the Americas (Velthuis, 1997). These bees are of considerable ecological and economic importance because they are responsible for the pollination of 40-90% of plant species, depending on the ecosystem (Kerr *et al.,* 1996).

Bees of the genus *Trigona*, popularly known as *xupé*, *mombuca* and *sanharão*, show a variety of defense behaviors and a range of nesting habits, with nests being built on branches of plants or on walls, anthills or underground. This group of bees is widespread in the Neotropics (from Mexico to Argentina), in Asia (from India and Sri-Lanka to Taiwan), in the Pacific region (Carolina and Solomon islands, southern Indonesia and New Guinea), and in some regions of Australia (Michener, 2000). Some species of *Trigona* are important in the pollination of certain palm species of the Brazilian Cerrado (Oliveira, 1998) and may have an important role in nutrient dispersal since they are frequently seen collecting animal feces.

To date, only five species of *Trigona* have been studied cytogenetically and include *T. fuscipennis, T. recursa* (Tarelho, 1973), *T. barrocoloralensis* and *T. minangkabau* (Hoshiba and Imai, 1993) and *T. spinipes* (Brito and Pompolo, 1997). These investigations have been limited to a description of chromosome numbers based on acetic-orcein staining, which does not allow efficient visualization of the chromosomal morphology. The aim of this study was to cytogenetically characterize four species of *Trigona* in order to obtain data that could be useful for future phylogenetic studies of this group.

Material and Methods

The four species of *Trigona* studied (*T. branneri, T. chanchamayoensis, T. hyalinata* and *T. recursa*) were collected from nests in and around Cuiabá, capital of the State of Mato Grosso, Brazil. Adult specimens were identified to the species level by Dr. Jesus Santiago Moure, Dr. Gabriel Rodrigues de Melo and Maria Christina de Almeida, MSc (all in the Department of the Universidade Federal do Paraná, Curitiba, PR, Brazil).

The cytogenetic mounts were prepared from the cerebral ganglia of post-defecating larvae as described by Imai *et al*. (1988). Constitutive heterochromatin was detected as

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described by Sumner (1972), with the modifications of Pompolo and Takahashi (1990), and by staining with distamycin-chromomycin A_3 (DA/CMA_3) using an adaptation of the method of Schmid (1980). On average, 20 metaphases were analyzed per slide in a total of 40 slides per species. The best metaphases were photographed in a LEICA DMLS photomicroscope using IMAGELINK film and printed on Kodak F3 Kodabrome paper.

After pairing, the chromosomes were classified as acrocentric (A), totally euchromatic acrocentric (A^e), pseudoacrocentric (A^M) or totally heterochromatic metacentric ($\overline{M}^h$), based on the location of the constitutive heterochromatin and on the nomenclature proposed by Imai (1999).

Results and Discussion

The chromosomal number for the females of the four species was identical (2n = 34), with n = 17 for the males, as also observed for *T. fuscipennis, T. recursa* (Tarelho, 1973) and *T. spinipes* (Brito and Pompolo, 1997) (Figures 1 and 2). The karyotypic formulas based on the C-banding patterns (Imai, 1991) are shown in Table 1. These findings differed from those obtained for *T. spinipes* in which most of the chromosomes are metacentric ($\overline{M}^c$), but agreed with the main types of chromosomes obtained for Meliponini species (Hoshiba and Imai, 1993; Menezes, 1997; Moreira, 1997; Caixeiro, 1999).

Homologies were observed among the euchromatic acrocentric (A^e) chromosomal pairs of *T. chanchamayoensis*, *T. hyalinata* and *T. recursa*, as already reported for *T. spinipes* (Brito and Pompolo, 1997). Homologies were also noted for the totally heterochromatic metacentric pair ($\overline{M}^h$) of *Trigona branneri*, *T. chanchamayoensis* and *T. recursa* (Figures 1 and 2).

Table 1 - Chromosomal types in four species of *Trigona* based on the nomenclature proposed by Imai (1991).

Species	Chromosomal type			
	A	A^M	$\overline{M}^h$	Ae
T. branneri	7	8	1	-
T. chanchamayoensis	9	6	1	1
T. hyalinata	2	14	-	1
T. recursa	6	9	1	1

A: acrocentric, A^e: totally euchromatic acrocentric, AM: pseudoacrocentric, $\overline{M}^h$: totally heterochromatic metacentric.

Staining with DA/CMA_3 revealed the presence of positive spots on four non-homologous chromosomes of *T. branneri* (Figure 3A,B) that resembled those already observed in *T. spinipes* (Brito and Pompolo, 1997). In the other chromosomes of *T. branneri*, there was a homogenous pattern of staining with DA/CMA_3 that coincided with the positive C-bands (Figures 1A and 3A,B). In *T. chanchamayoensis*, a marked reaction was seen with DA/CMA_3 (Figure 4A), while in *T. recursa*, the staining with CMA_3 revealed no GC-rich regions in the chromosomes (Figure 4B). The positive reactions seen were probably associated with nucleolus organizing regions (NORs), as observed in

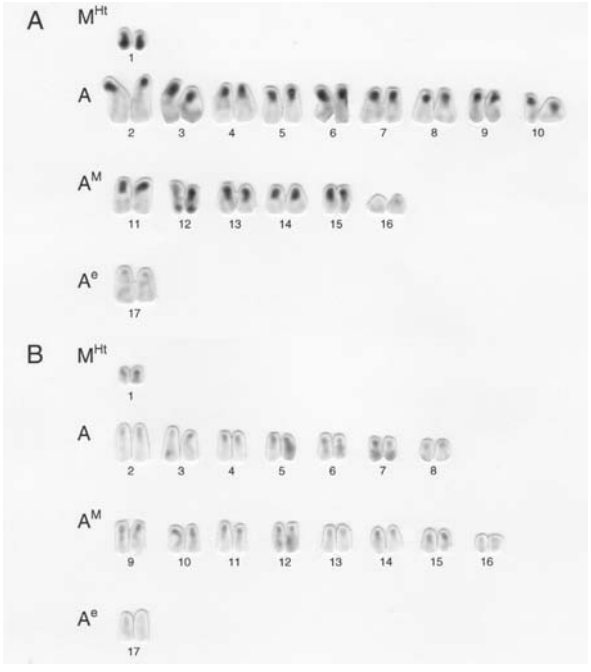

Figure 1 - Karyotypes assembled from metaphases of female *Trigona chanchamayoensis* (A) and *Trigona branneri* (B) following C-banding. The nomenclature is based on that proposed by Imai (1991).

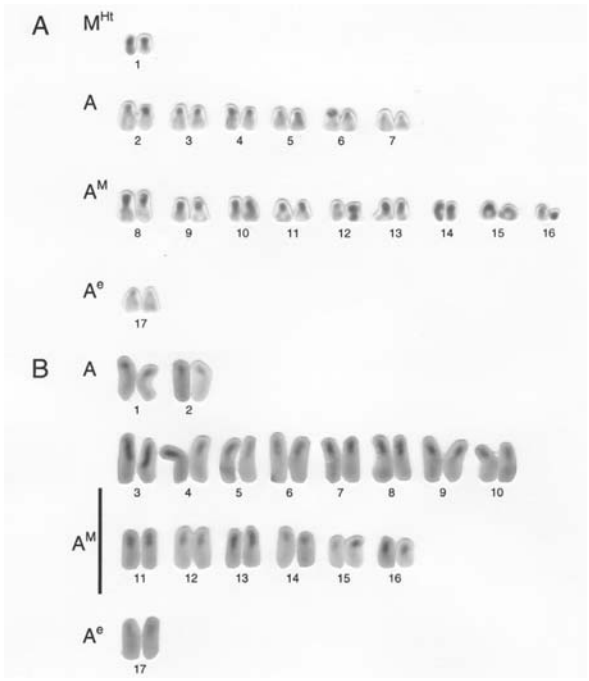

Figure 2 - Karyotypes assembled from metaphases of female *Trigona recursa* (A) and *Trigona hyalinata* (B) following C-banding.

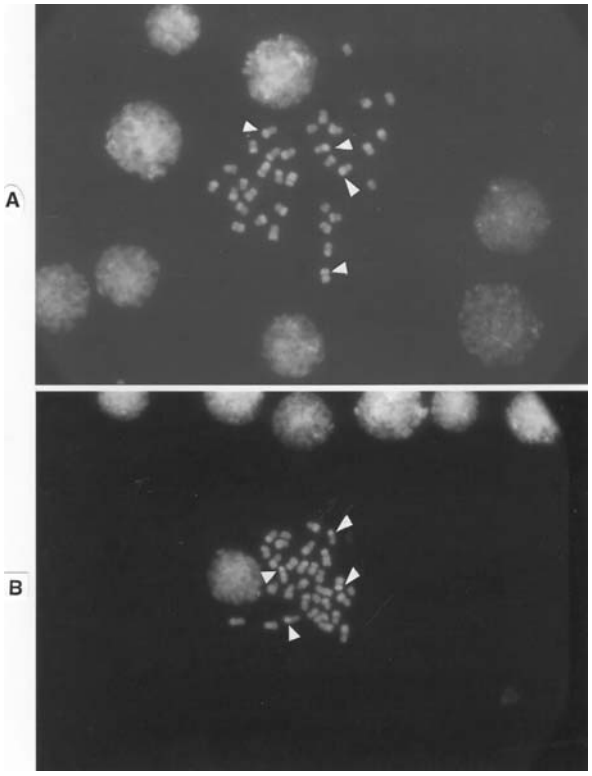

Figure 3 - Metaphases of female *Trigona branneri* after staining with DA/CMA_3.

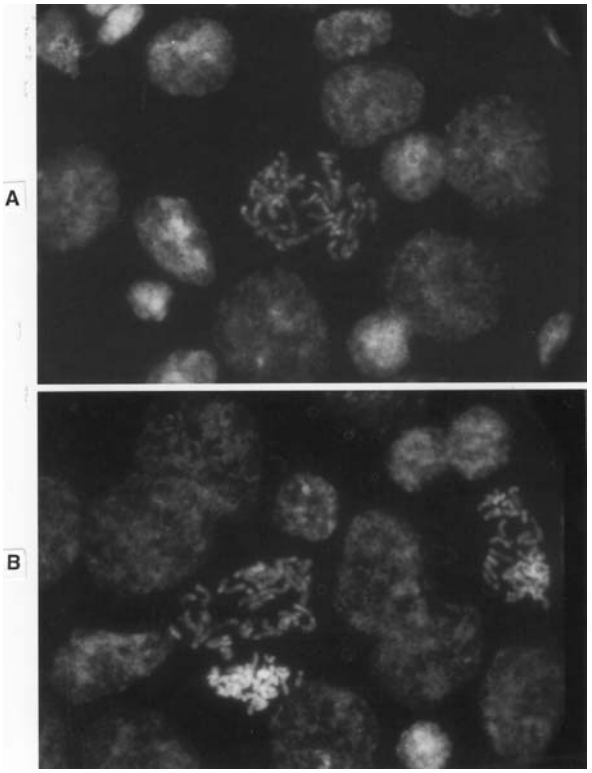

Figure 4 - Metaphases of female *Trigona chanchamayoensis* (A) and *Trigona recursa* (B) after staining with DA/CMA_3.

other insects such as Orthoptera (Camacho *et al*., 1991), Coleoptera (Maffei *et al*., 2001), and in other Hymenoptera, including Formicidae (Palomeque *et al*., 1988; Imai *et al.*, 1992), Sphecidae (Araújo *et al*., 2000) and Meliponini (Brito *et al.,* 1999, 2003; Caixeiro, 1999; Rocha, 2000). Some authors have suggested that the number of NORs revealed by impregnation with silver nitrate or by CMA_3 staining is an important taxonomic character. Other methods that detect NORs, such as fluorescent *in situ* hybridization (FISH) with rDNA probes, need to be applied to *Trigona* species, especially *T. recursa*.

Since there are more than 300 species in the Meliponini, with only about 25% of them having been studied cytogenetically, more genetic data are necessary for this tribe. Studies of the genus *Trigona* in other biomes should contribute to our knowledge of the karyotypic diversity among these bees.

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