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Research Article

Cytogenetic data of *Partamona peckolti* (Hymenoptera, Apidae, Meliponini) by C banding and fluorochrome staining with DA/CMA$_3$ and DA/DAPI

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Abstract

The stingless bees of the *Partamona* genus have been studied taxonomically, ecologically and behaviourally, but cytogenetic studies are still rare. The objective of this study was to obtain cytogenetic data to contribute to *Partamona peckolti* species characterization. Heterochromatin was localized in all chromosome pericentromeric regions but some blocks could be visualized on some large chromosomes arms. A large heterozygous DA-CMA$_3$-positive band was observed on one large chromosome arm, but was completely absent when C banding was applied before fluorochrome staining, with only one small positive band being visualized. Sequential DA-CMA$_3$-NOR staining of interphase nuclei provided coincident positive responses. This suggests that DA-CMA$_3$-positive bands of *P. peckolti* correspond to nucleolar organizer regions, as previously confirmed for another *Partamona* species by FISH.

Key words: Meliponini, *Partamona*, karyotype, C-banding, fluorochromes.

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Introduction

The first karyotype evolution hypothesis elaborated for Meliponini bees postulated that the different chromosome numbers observed today are the product of polyploidy events from a basic number, n = 8 of *Melipona* species (Kerr *et al*., 1952). Several Meliponini genus have been studied in detail at the cytogenetic level aiming at the comprehension of the karyotype evolution of stingless bees such as: *Leurotrigona* (Pompolo and Campos, 1995); *Frieseomelitta* (Moreira, 1997), *Plebeia* (Caixeiro and Pompolo, 1998) and *Melipona* (Rocha and Pompolo, 1998; Rocha *et al*., 2002). These modern cytogenetic data have shown interespecific differences that cannot sustain the Polyploidy Hypothesis mentioned. Alternatively another karyotype evolution theory, the Minimum Interaction Theory (Imai *et al.* 1986, 1988, 1994), postulated for Australian ants, has also been accepted as a possible model for the karyotype evolution of Meliponini bees (Pompolo, 1992). This theory states that primitive karyotypes had small numbers of large chromosomes and, as time went by, theses chromosomes got smaller and increased in number by centric fission that would prevent deleterious interactions within the interphase nucleus, such as translocations.

Detailed study of each Meliponini genus at the cytogenetic level will provide the necessary data to verify if this Theory could also be applied to the stingless bees or whether it would be necessary to postulate another hypothesis to understand the karyotype evolution of this important group which is responsible for the pollination of 40 to 90% of wild plant species, depending on the ecosystem (Kerr, *et al*. 1996).

The objective of the present scientific note was to contribute to the knowledge of the *Partamona* genus at the cytogenetic level. According to Pedro (1998), 32 species of *Partamona* have been described so far, but few have been characterized cytogenetically yet: *P. personi* (Tarelho, 1973); *P. mulata*, *P. vicina*, *P. ayilae*, *Partamona* sp.n. (Brito-Ribon, *et al*., 1999); and *Partamona* sp. aff. *nigrior* (Brito, 1998), all presenting 2n = 34 e n = 17 chromosomes. There is also *Partamona helleri* (Costa *et al*., 1992, Brito *et al*. 1997, Tosta *et al*., 1998) which presents 2n = 34 chromosomes and a B chromosome system varying from 0 to 4.

In this short communication, we describe the karyotype of *Partamona peckolti,* a species that occurs in the tropical rainforests of central America, the Caribbean

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and Pacific coasts, Colombia and Equador (Camargo, 1980), presenting the chromosome number, C banding pattern and fluorochrome AT and GC specific staining.

Material and Methods

The biological material was collected from a nest associated with a Bromeliacea plant, in San Francisco de Las Pampas village (00°25'182" S and 79°00'183" W), Cotopaxi province, Ecuador. Slides with metaphases were prepared from 30 post-defecant larvae cerebral ganglion according to the technique described by Imai *et al.* (1988). Chromosome banding techniques were applied as C banding according to Sumner (1972) and fluorochrome sequential staining DA - DAPI - Chromomycin A_3 (Schweizer, 1980). Sequential treatment, C banding followed by DA - DAPI - CMA_3 staining was done using the techniques in the same way as done separately. Another sequential staining with DA - CMA_3 followed by AgNOR treatment was also applied using the Schweizer (1980) protocol and silver impregnation as described by Howell and Black (1980). The metaphases were photographed in photomicroscope Olympus BX60, with FUJI HR II ISO 40 and KODAK GOLD ULTRA ISO 100 films. The karyotypes were mounted in increasing order of the chromosome length.

Results and Discussion

Partamona peckolti presented 2n = 34 chromosomes as observed in other species of the genus (Tarelho, 1973; Brito 1998; Brito-Ribon *et al.*, 1999) and conventional staining revealed a secondary constriction in the first pair of the karyotype not observed in any other *Partamona* species studied cytogenetically (Brito 1998, Brito-Ribon *et al.*, 1999) (Figure 1A).

The C-banding pattern showed heterochromatin in the pericentromeric region of all chromosomes, as already observed in *P. helleri* (Brito, 1998) and *Partamona* sp.n. (Brito-Ribon *et al.*, 1999) as well as a large hetercromatic block on the first karyotype pair, similarly to *P. aylae*, *P. mulata* and *P. vicina* (Brito-Ribon *et al.*, 1999) (Table 1 and Figure 1B).

Sequential staining with DA-DAPI-CMA_3 revealed a wide positive band in one large chromosome with both fluorochromes (Figure 2A and B, arrows). A similar wide CMA_3-positive band has already been observed in other *Partamona* species (Table 1). However, this large band disappeared when C banding was applied before sequential staining with DA-DAPI-CMA_3 (Figure 3A, B and C). Another small DA/CMA_3^+ band seen with or without the C banding treatment which presented the shape and richness in GC base pairs led us to believe that we were evidencing a nucleolar organizer region (NOR) (Figure 3 arrowhead). This correlation between CMA_3^+ bands and NORs is quite common among animals and in insects has been observed in: the grasshoppers *Eyprepocnemis plorans* and *Locusta migratoria* (Camacho, *et al*., 1991); the wasp *Trypoxylon albitarse* (Araújo *et al*., 2000; 2002); the lady beetle *Olla v-nigrum* (Maffei *et al*., 2001a); and in other Meliponini bees such as *Melipona* (Rocha and Pompolo, 1998; Rocha *et al*., 2002); *Plebeia* (Caixeiro, 1999) and *Friesella*

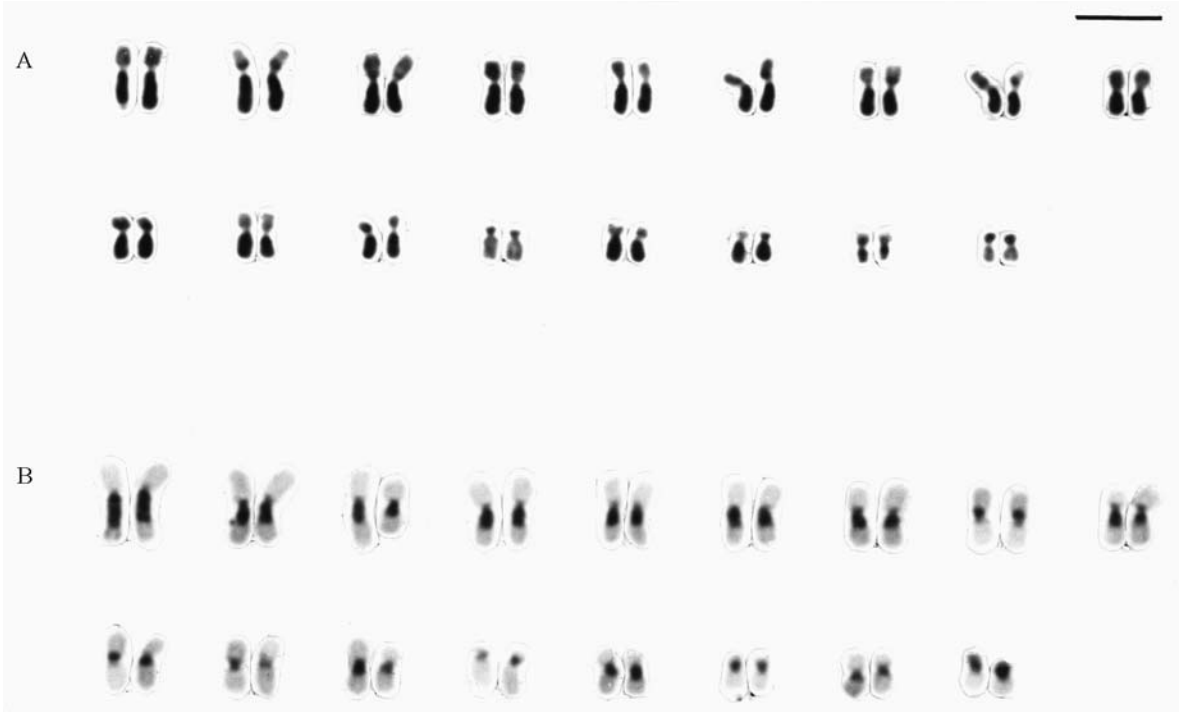

Figure 1 - *Partamona peckolti* karyotypes mounted from metaphases submitted to: A) Conventional staining, and B) C banding. Bar = 5 µm.

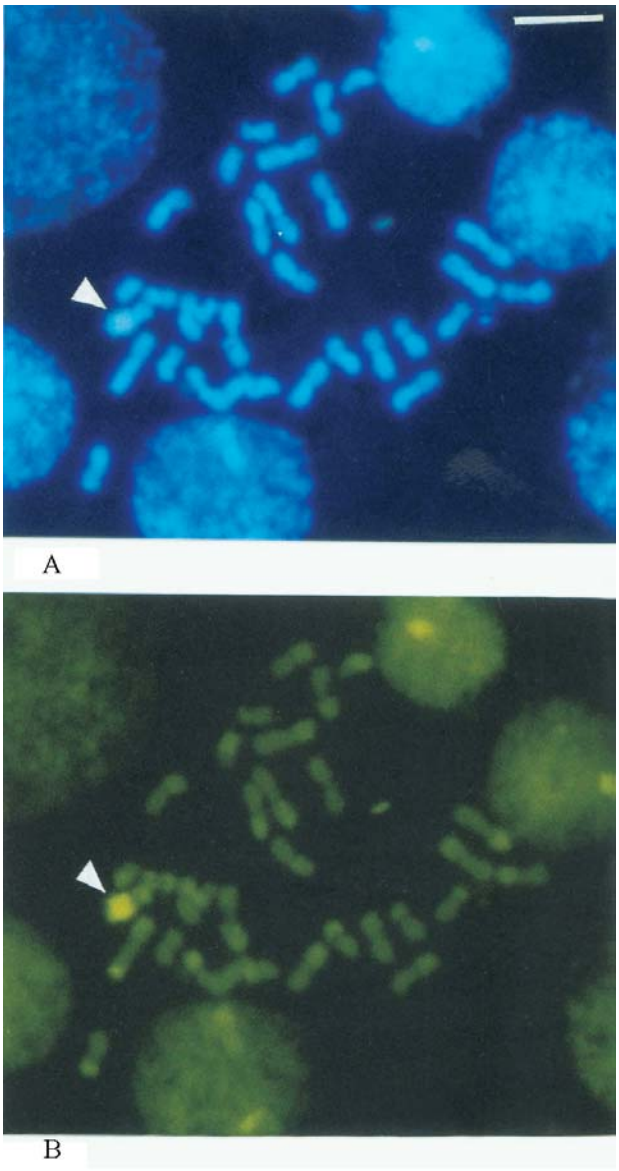

Figure 2 - *Partamona peckolti* metaphase submitted to sequential staining with: a) DA-DAPI, and B) CMA_3. The arrow points at a large DA-DAPI-CMA_3-positive band. Bar = 5 µm.

schrottkyi (Mampumbu, 2002). The location of these regions by the classical technique of Howell and Black (1980) is difficult on Meliponinae chromosomes that was effectively achieved only in *Tetragonisca angustula* (Menezes, 1997), *Melipona* (Maffei *et al*., 2001b; Rocha *et al*., 2002); *Plebeia* (Maffei *et al*., 2001b) and *Friesella schrottkyi* (Mampumbu, 2002). Conversely, correspondence of DA-CMA_3 and AgNOR staining could be observed at interphase nuclei (Figure 4A and B). As the correlation among positive staining with DA-CMA_3; AgNOR and fluorescent in situ hybridization with 18S rDNA probe has been observed for two another *Partamona* species, *P. helleri* and *P.* aff. *nigrior* (Brito, 1988), we indeed accepted that structure (Figure 3C) as a NOR.

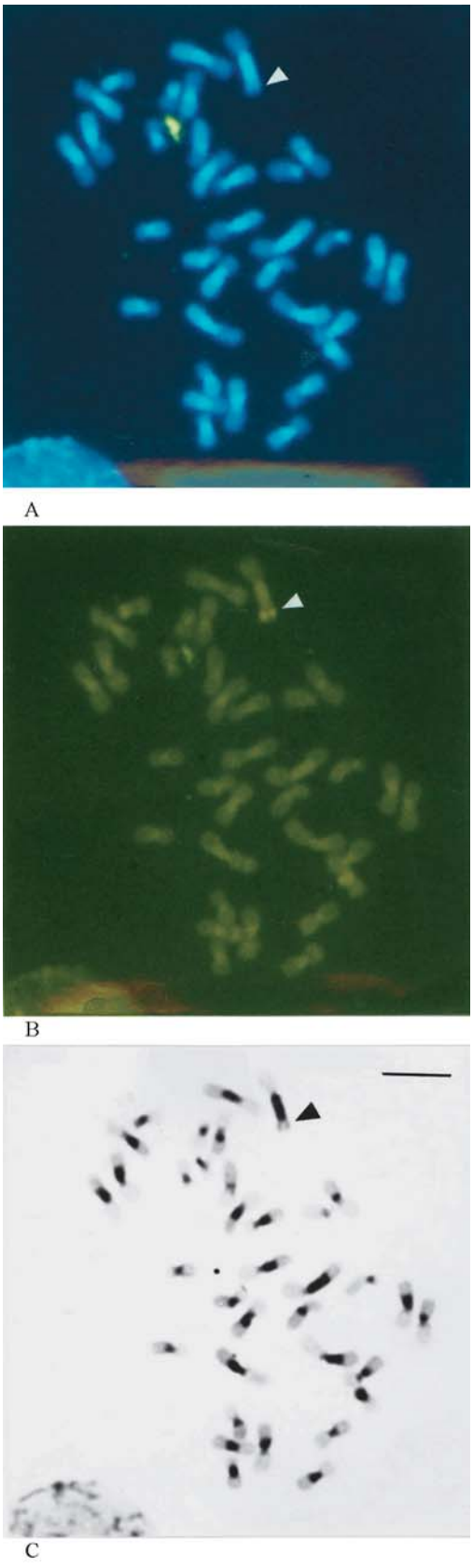

Figure 3 - *Partamona peckolti* metaphase submitted to C banding followed by sequential staining. A) DA-DAPI; B) CMA_3; C) GIEMSA. The arrow points at a NOR-like CMA_3-positive band. Bar = 5 µm.

Table 1 - Comparative data obtained for the *Partamona* genus with C-banding; DA/CMA_3^+ staining and $AgNO_3$ impregnation. *: Not assayed.

Partamona species	Pericentromeric C^+ bands	1st pair with broadly heterocromatic long arm	Majority of pairs with heterocromatic short arms	Large heteromorphic DA/CMA_3^+ band	Positive band correlation C^+-DA/CMA_3^+	Positive nucleolus staining correlation DA/CMA_3^+/$AgNO_3$	References
P. aff. nigrior	yes	no	no	no	no	yes	Brito (1998)
P. ayilae	no	yes	yes	no	*	*	Brito-Ribon (1999)
P. helleri	yes	no	no	yes	yes	yes	Brito (1998)
P. mulata	no	yes	yes	yes	*	*	Brito-Ribon (1999)
P. peckolti	yes	yes	no	yes	no	yes	**Present work**
P. sp.n.	yes	no	no	no	*	*	Brito-Ribon (1999)
P. vicina	no	yes	yes	yes	*	*	Brito-Ribon (1999)

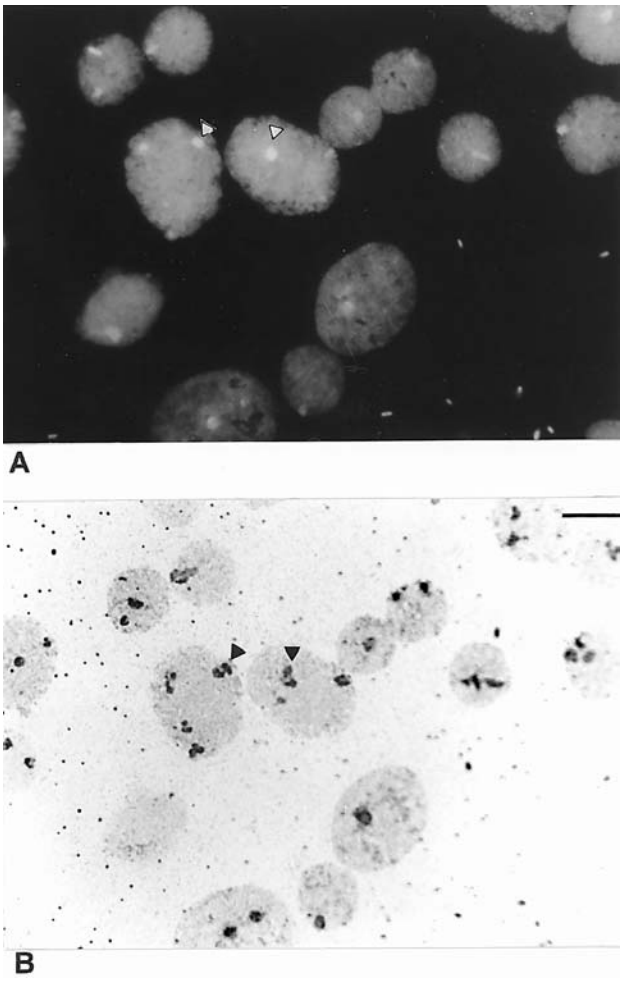

Figure 4 - Interphase nucleus submitted to: A) DA-CMA_3 staining, and B) AgNOR staining. The arrows point at coincident positive staining points. Bar = 5 μm.

Many studies have been carried out for a better understanding of the evolution of the *Partamona* genus: taxonomy revision (Pedro, 1998), provisioning and oviposition process behavior of nine species (Azevedo, 2001); molecular study of B chromosomes of *P. helleri* (Tosta, 2001); mitochondrial genome characterization of *P. mulata* and *P. helleri* (Brito and Arias, 2001). In this context, Cytogenetics can still contribute greatly to the knowledge of the relations among *Partamona* species to further cytotaxonomy treatment of the data. Nevertheless, to achieve this purpose, more karyotype characterizations are needed as many *Partamona* species persist even without their chromosome number known. Furthermore, the better comprehension of the relations among *Partamona* species, especially at the cytogenetic level, will contribute to the comprehension of the karyotype evolution of the Meliponinae bees as a whole.

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