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CHROMOSOMAL VARIATION AND CYTOGENETICS OF *PLEBEIA LUCII* AND *P. PHRYNOSTOMA* (HYMENOPTERA: APIDAE)

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Abstract

Plebeia (Hymenoptera: Apidae) is a poorly defined genus and its classification and systematics are controversial. Tools such as cytogenetics may contribute to clarify the relationships among the species. The aim of this study was to characterize the karyotypes of the species *Plebeia lucii* Moure, 2004 and *Plebeia phrynostoma* Moure, 2004. For this purpose conventional staining, C-banding and fluorochrome techniques were performed. The same chromosome number (2n = 34) was observed for both species. The karyotypic formula of *P. lucii* was 2K = 22 A^M + 12A. A heteromorphic pair was observed with euchromatic and heterochromatic regions of different sizes on the 2 homologs. The presence of a secondary constriction was observed in this same pair. In *P. phrynostoma* the karyotypic formula was 2K = 18A^M + 10A + 6M and did not show polymorphisms or secondary constrictions. The DAPI fluorochrome marked portions of the heterochromatic arm and the regions close to the centromere in some chromosomes of both species. CMA_3 marked the heteromorphic pair in *P. lucii* and some points in other chromosomes, while it stained 2 pairs of chromosomes in *P. phrynostoma*. Despite the similarity in chromosome number, these species show variation both in morphology and in composition of chromatin which may reflect a phylogenetic position in different clades.

Key Words: Meliponini, karyotype, C-banding, fluorochrome, chromosomal polymorphism

Resumo

O gênero *Plebeia* não é um grupo taxonômico bem definido e apresenta problemas quanto a sua classificação e sistemática. As ferramentas citogenéticas podem auxiliar no esclarecimento das relações entre as espécies deste gênero. O principal objetivo deste estudo foi caracterizar o cariótipo das espécies de *Plebeia lucii* Moure, 2004 e *Plebeia phrynostoma* Moure, 2004. Para isso foram usadas técnicas de coloração convencional, bandeamento C e fluorocromos. O mesmo número cromossômico (2n = 34) foi observado em ambas espécies. A fórmula cariotípica de *Plebeia lucii* foi 2K = 22A^M + 12A. Foi observado um par heteromórfico com as regiões eucromáticas e heterocromáticas localizadas em diferentes porções dos dois homólogos. A presença de uma constrição secundária também foi observada neste mesmo par. Em *Plebeia phrynostoma* a fórmula cariotípica foi 2K = 18A^M + 10A + 6M e não foi observado nem polimorfismos nem contrições secundárias. O fluorocromo DAPI marcou regiões dos braços heterocromáticos e regiões próximas ao centrômero em alguns cromossomos de ambas as espécies. O CMA_3 marcou o par heteromórfico de *P. lucii* e alguns pontos em outros cromossomos, enquanto marcou dois pares de cromossomos em *P. phrynostoma.* Apesar da similaridade no número cromossômico, estas espécies mostraram variação tanto na morfologia quanto na composição da cromatina o que pode ser reflexo da posição filogenética delas em diferentes clados.

Palavras Chave: Meliponini, cariótipo, Banda C, fluorocromo, polimorfismo cromossômico

Bees are insects of the Hymenoptera order and exhibit a great diversity in size, color, behavior, sociability, etc (Silveira et al. 2002) amongst which we emphasize the Meliponini. The individuals this tribe are known as "indigenous stingless bees" because of their atrophied stinger (Kerr et al. 2001). They are found in the Neotropical region and also in Malaysia, India, Indonesia, Africa and Australia.

Plebeia ,one of the 33 genera of the Meliponini tribe, has 38 described species according to Moure

(2007). These bees occupy regions of tropical and subtropical South and Central America. They are popularly known as "mirim" bees because of their reduced size, and generally they are not aggressive and are easy to handle. In Brazil 18 *Plebeia* species are known to occur, but probably there are several others not yet described. Camargo & Pedro (1992) suggested that the genus, *Plebeia*, is not well defined and that the relationships among the taxa remain uncertain. Furthermore, controversies about classification and systematics are often related.

Rasmussen & Cameron (2010), in trying to clarify the phylogenetic relationship within the tribe Meliponini, analyzed—among other Meliponini—8 species of *Plebeia* by Bayesian inference using sequences of 5 nuclear genes. From these results Rasmussen & Cameron (2010) proposed the existence of 2 clades within the genus. By comparing the types of nests of some of the species analyzed in this study, we observed that in one clade the species present the nest shaped as bunch, while the species in the other clade present their nest shaped as a honeycomb. However, information on the nest structure for all species is not available, and some of them are mentioned only as *Plebeia* sp., which may reflect problems with their classification, which other authors already mentioned.

In general, morphological characters are used for species classification, so the use of other tools, such as cytogenetics, may be useful not only to expand information about the karyotype, but also to contribute to the understanding of the relationships within the genus and to assist in the identification of the species.

In the genus *Plebeia*, 7 of 38 described species were analyzed cytogenetically (Caixeiro 1999; Rocha et al. 2003). The first cytogenetic studies of the genus *Plebeia* focused on the determination of chromosome numbers of individual species. The following findings were reported: in *Plebeia droryana*, n = 9 (Kerr 1952), n = 18 (Kerr 1972; Tarelho 1973) and 2n = 34 (Hoshiba & Imai 1993); in *Plebeia emerina*, n = 18 and in *Plebeia remota* n = 18 (Kerr 1972).

Further analysis performed by Caixeiro (1996; 1999) revealed that the chromosome number in *Plebeia* is n = 17 and 2n = 34 for the studied species, which differed from the values found in earlier works by Kerr. Despite the similarity in the number of chromosomes, Caixeiro (1996, 1999) showed differences in morphology and heterochromatin content among the studied species. Thus, with the increase of the number of cytogenetically analyzed species, a better understanding of the characteristics of the genus can be obtained to help in understanding the processes that led to changes in karyotype of the group and the relationships among species. Therefore, in this work we used methods of conventional staining, C banding and fluorochromes to describe the karyotypes of *P. lucii* and *P. phrynostoma,* and to verify the number, morphology and heterochromatic content of the chromosomes. Thus, through comparisons of karyotypes between the various species, we searched for a better understanding of the evolution of the karyotypes within the genus, *Plebeia*.

Material and Methods

Biological Material

Larvae from 3 colonies (identified as PL08, PL36 and 795) of *P. lucii* from Viçosa, Minas Gerais and from 2 colonies of *P. phrynostoma*, originating from the state of Espirito Santo, were used to obtain the metaphasic chromosomes. All colonies were maintained in the Central Apiary of the Federal University of Viçosa.

The study was conducted at the Laboratory of Insect Cytogenetics, of the Federal University of Viçosa. The mitotic metaphasic chromosomes were obtained according to the methodology of Imai et al. (1988) from cerebral ganglia of larvae at the stage of defecation.

Cytogenetic Techniques

For conventional staining the slides were covered with a 4% solution of Giemsa and Sörensen buffer (0.06 M, pH 6.8) for 30 min. They were then washed in water and allowed to dry at room temperature.

The methodology used for the C-banding was based on that of Sumner (1972) and sequential staining with the fluorochromes CMA_3/Distamycin/DAPI was performed according to Schweizer (1980). The slides were examined by an Olympus BX60 light microscope, and images were captured and subsequently analyzed. Classification of chromosomes was performed according to criteria proposed by Imai (1991), which take into consideration the location of heterochromatin blocks visualized by the C-banding technique. On average 10 metaphases were analyzed per slide.

The Programs Corel Photo-Paint® and CorelDraw® (version 12, Corel Corporation, 2003) and Image Pro Plus™ (version 4.5, Media Cybernetics 2001) were used for measurement and analysis of images. The colored version of Figs. 3 and 4 can be found in supplementary material for this article in Florida Entomologist 96(4) (December 2013) online at http://purl.fcla.edu/fcla/entomologist/browse

Results and Discussion

The species *P. lucii* (Fig. 1) and *P. phrynostoma* (Fig. 2) showed chromosome numbers of 2n = 34 for females. Regarding the chromosome number observed for *Plebeia*, some differences are reported in literature. Kerr (1952, 1972) and Tarelho (1973) found different values for *Plebeia droryana*. This species was later analyzed and the

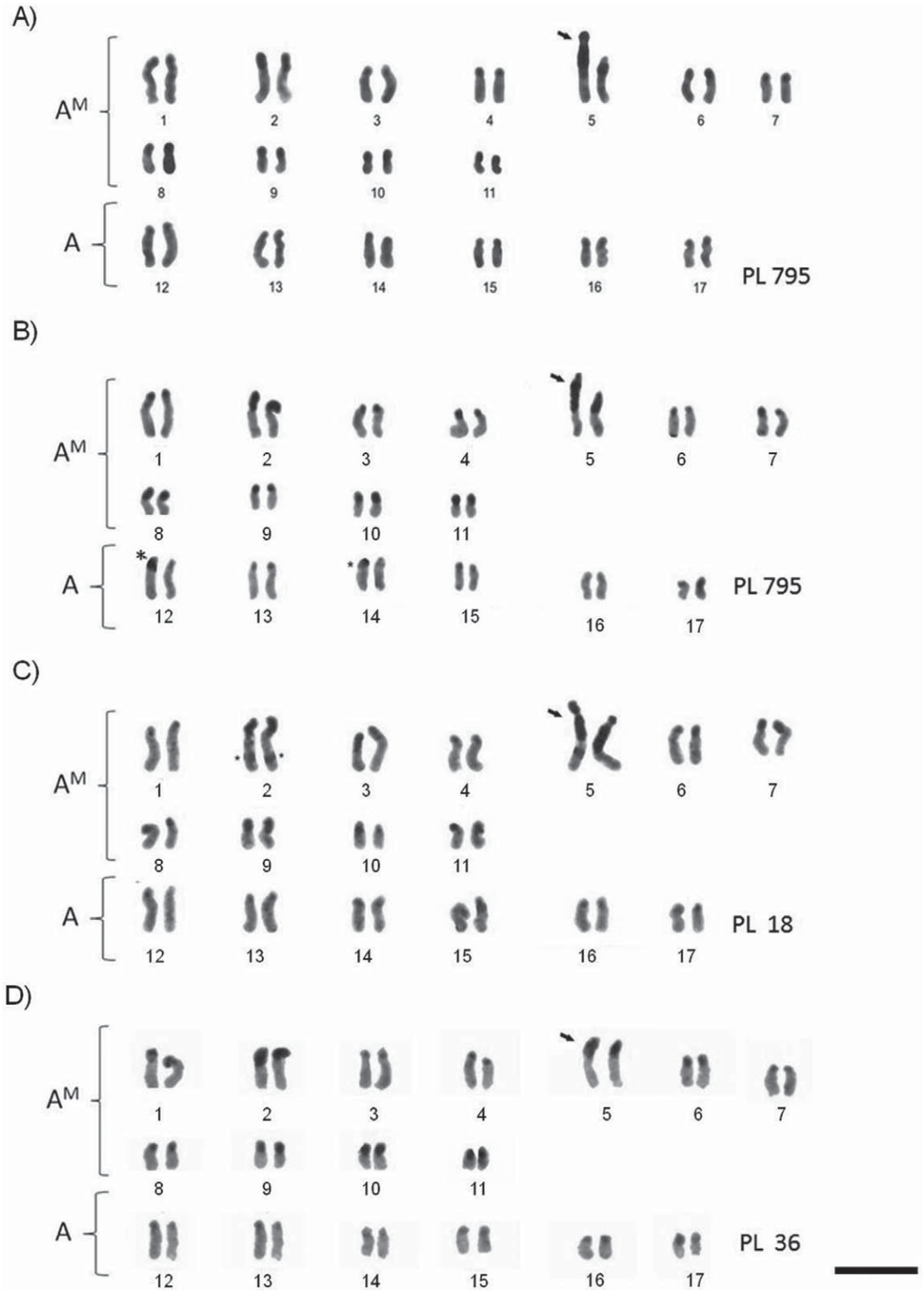

Fig. 1. Karyotype of *Plebeia lucii.* Conventional staining (A) and C-banding (B, C and D). The arrow indicate the polymorphic pair. Scale bar = 10 µm.

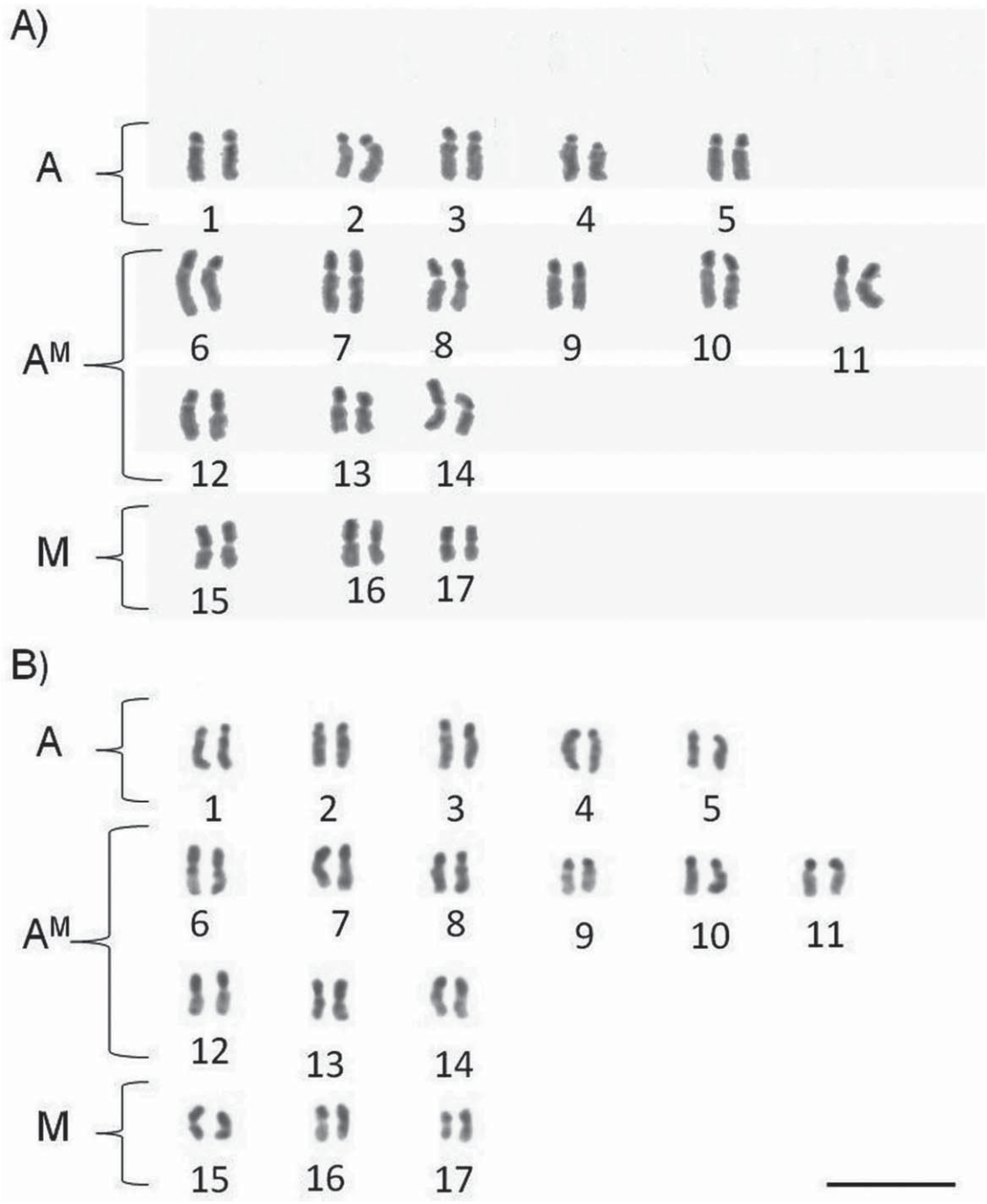

Fig. 2. Karyotype of *Plebeia phrynostoma.* Conventional staining (A) and C-banding (B). Scale bar = 10 µm.

chromosome number was determined to be 2n = 34 (Caixeiro 1996, 1999). The same number was confirmed for other species of *Plebeia* and analysis showed the number of 2n = 34 to be constant for all *Plebeia* species studied (Caixeiro 1999). This reported differences may been caused by the crushing technique used by Kerr and Tarelho to obtain metaphase chromosomes, which is less secure than the methodology proposed by Imai and used by Caixeiro (1999) and also in the pres-

ent work. Thus, among the *Plebeia* species already studied the same number was observed (Caixeiro 1996; 1999), which indicates constancy within the genus, as also occurs in stingless bees in general (Rocha et al. 2003).

The C-banding technique revealed that most of the chromosomes in the 2 species had one heterochromatic arm. In *P. lucii* 22 pseudo-acrocentric (A^M) and 12 acrocentric (A) chromosomes were observed, generating the karyotypic formula $2K = 22A^M + 12A$ (Fig. 1B, 1C and 1D). Pair 5 presented a long arm with a pronounced heterochromatin accumulation that can be visualized in interphase nuclei as 2 very evident marks by C-banding. In the colonies analyzed, this pair showed a sizable polymorphism of the heterochromatic arm (Figs. 1 and 3). In one of the colonies the polymorphic pair presented heterochromatic arms of different sizes (Fig. 1B), while in others only chromosomes with the largest heterochromatic arm (Fig. 1C) or only with the smaller arm were found (Fig. 1D).

Polymorphism in the size of the heterochromatic arm was also observed in 4 species of *Plebeia* studied by Caixeiro (1999). In general only one pair showed polymorphism, except in the species *P. droryana* in which 2 pairs presented differences. This suggests that this type of polymorphism is common within the genus or it may represent an ancient characteristic of the karyotype since it is shared by several species.

In pair 5, along with the polymorphism a lighter region was observed, that may represent a secondary constriction at the end of the chromosome (Figs. 1 and 3); and this phenomenon was

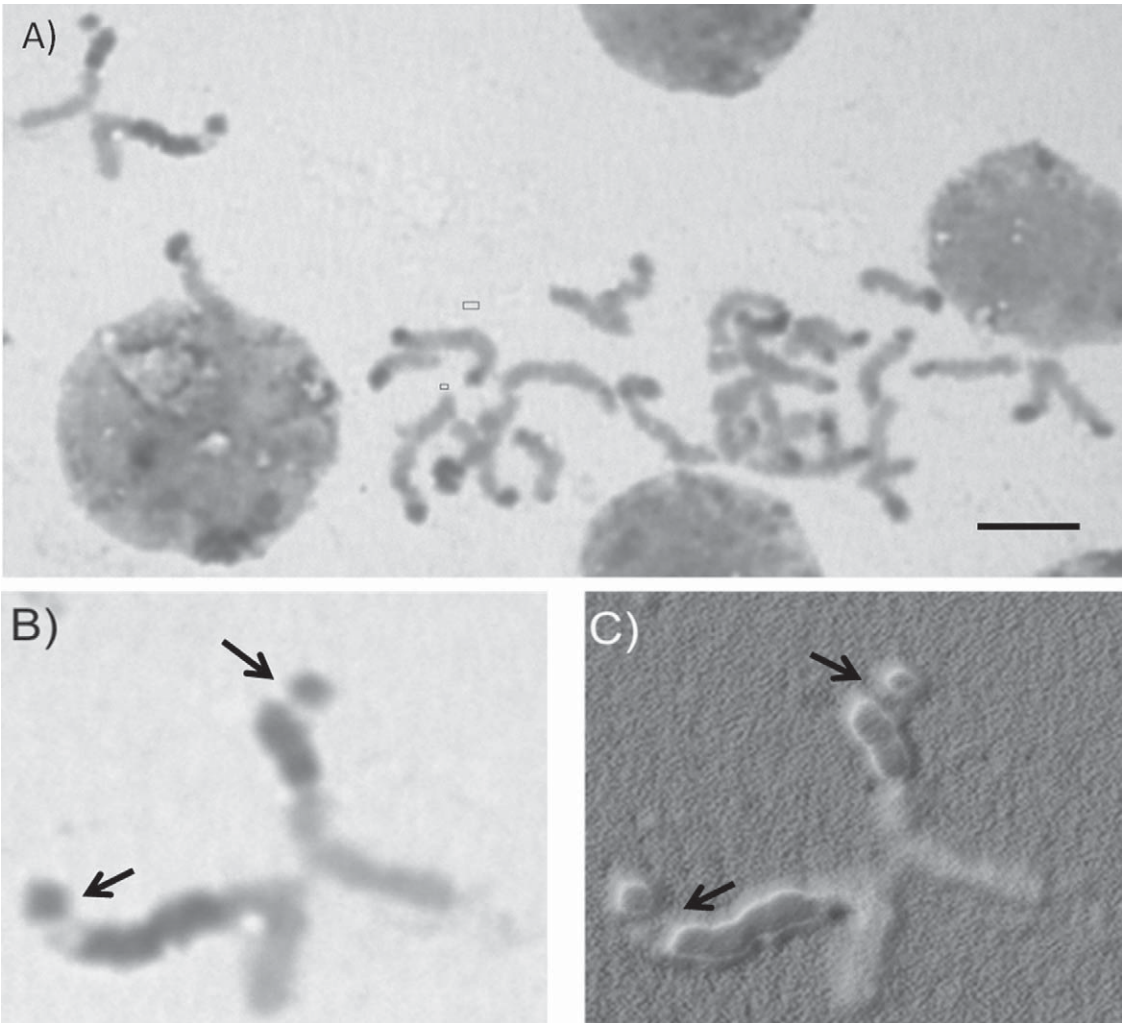

Fig. 3. Metaphase of *Plebeia lucii* by C-banding (A). Heteromorphic pair in detail, specifically the secondary constriction and accumulation of heterochromatin differential (B and C). Scale bar = 5 µm. . The colored version of this figure can be found in supplementary material for this article in Florida Entomologist 96(4) (December 2013) online at http://purl.fcla.edu/fcla/entomologist/browse .

observed also by Caixeiro (1999) in *P. droryana* and *Plebeia* sp.

Generally, secondary constrictions are related to nucleolus organizing regions (NORs) (Wagner et al. 1993). One way to confirm this would be to use more specific techniques for visualization of NORs, such as Ag-NOR banding, the use of rDNA probes or the fluorochrome CMA_3. Caixeiro (1999) performed the Ag-NOR and fluorochrome techniques and compared the data obtained by both techniques in bees. For the 4 *Plebeia* species the heterochromatic arm of the heteromorphic pair was marked by CMA_3, reinforcing the idea that this region contains the nucleolus organizer. Furthermore, positive correlation was observed between markers Ag-NOR and CMA_3 in interphase nuclei, which showed the prevalence of G and C bases in the region of the nucleolus. In 2 of the species (*P. remota* and *P.* sp.) correlations were observed only in some markings, which can be explained by the fact that the Ag-NOR shows only active NORs while CMA_3 can stain both active and inactive NORs (Caixeiro 1999).

In *P. phrynostoma* the karyotype presented 18 pseudo-acrocentric chromosomes (A^M), 10 acrocentric chromosomes (A) and 6 metacentric chromosomes (M), with heterochromatin located mainly in the centromeric and terminal regions. The karyotypic formula for this species was determined to be $2K = 18A^M + 10A + 6M$ (Fig. 2).

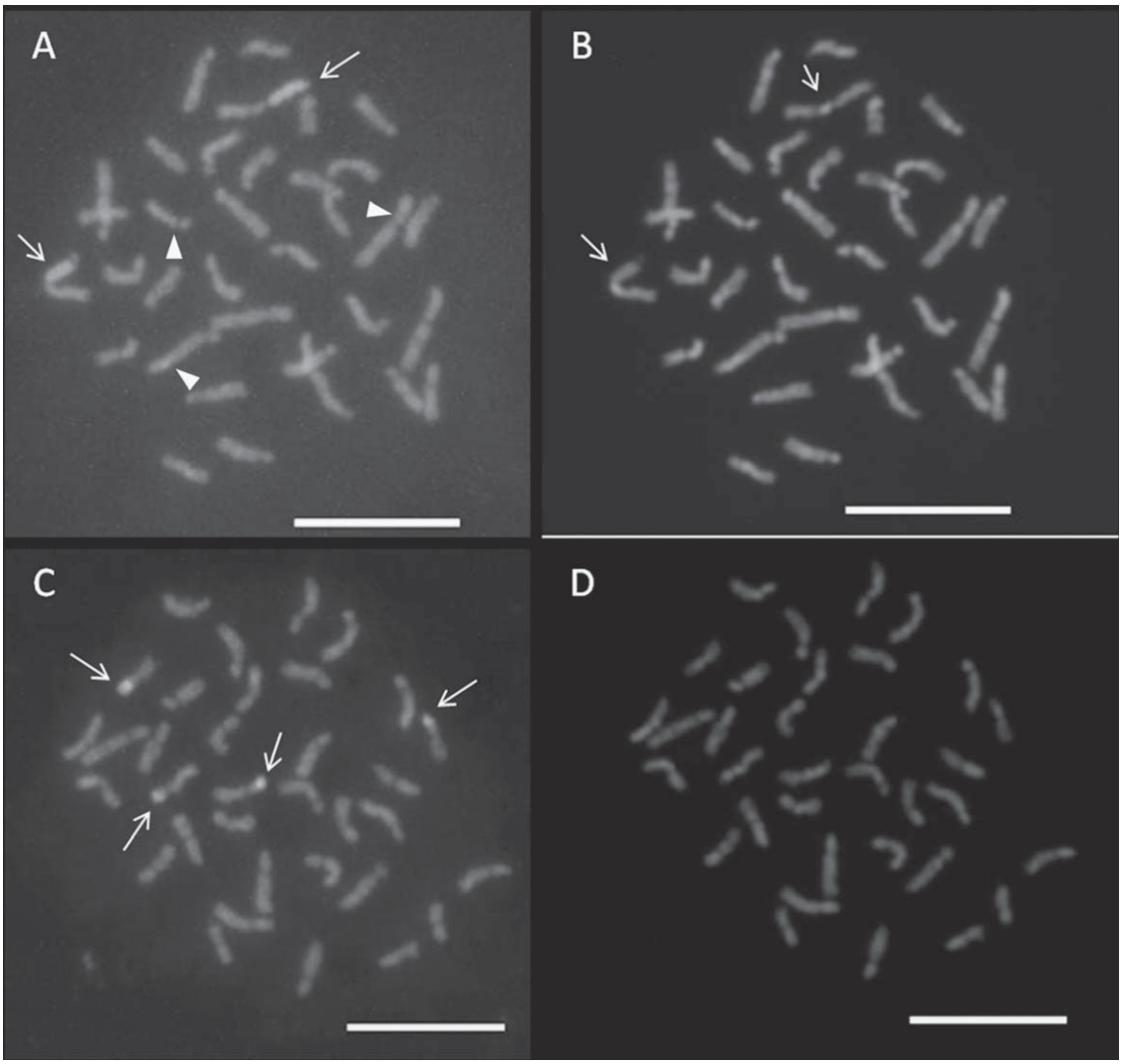

Fig. 4. Sequential staining with CMA_3 in metaphases of *Plebeia lucii* (A – CMA_3, B – DAPI) and *Plebeia phrynostoma* (C - CMA_3, D – DAPI). The arrows indicate positive regions. The asterisk indicates weak markings. Scale bar = 20 µm. The colored version of this figure can be found in supplementary material for this article in Florida Entomologist 96(4) (December 2013) online at http://purl.fcla.edu/fcla/entomologist/browse .

No polymorphisms or chromosomes with large heterochromatic regions were observed in *P. phrynostoma* as encountered in *P. lucii*. Secondary constrictions were also not seen in *P. phrynostoma.*

For the 2 species, a predominance of pseudo-acrocentric chromosomes was observed. The large number of pseudo-acrocentric chromosomes has been considered to be evidence of chromosomal fissions with subsequent accumulations of heterochromatin, events explained by the Minimum Interaction Theory proposed by Imai (1986, 1988, 1994). Imai suggested that there is a tendency for chromosomal fission to occur during evolution to reduce the size of chromosomes and thus prevent the occurrence of exchanges and inversions between nonhomologous chromosomes. Thus, more derived karyotypes should present smaller and more numerous chromosomes. After the fissions involved in this process, an increase of heterochromatin in each region of breakage would occur (Imai et al. 1988). In the Meliponini tribe Rocha et al. (2003) verified that species with lower chromosome numbers have metacentric chromosomes, while species with higher chromosome numbers have predominantly acrocentric and pseudo-acrocentric chromosomes. Intermediate karyotypes had chromosomes of the 3 types with average proportions.

Thus, according to the pattern and types of chromosomes found, the Minimum Interaction Theory appears to be the mechanism that best explains the evolution of the karyotype in *P. lucii* and *P. phrynostoma*.

Regarding to the base composition of AT and GC observed by the fluorochromes, DAPI and CMA_3, respectively, we observed that for the 2 species the heterochromatic portions were generally DAPI-positive (Figs. 4B and 4D); CMA_3 revealed in *P. lucii* 2 markings (Fig. 4A) and other weaker markings in others chromosomes, while *in P. phrynostoma* CMA3 revealed 4 markings (Fig. 4C). The 2 markings observed in *P. lucii* coincide with the heteromorphic pair in the region of a secondary constriction, confirming that this is the nucleolus organizer region.

The 2 studied species presented the same chromosome number, but there are some differences in their karyotype. These differences are mainly due to a polymorphism in the heterochromatic arm, the presence of a secondary constriction on pair 5 and the number of CMA_3 markings. *Plebeia lucii* and *P. phrynostoma* differ in nest construction, and some authors suggested that they would be in different clades. Despite the observed differences, it is necessary to study a larger number of *Plebeia* species with respect to both genetics and ecology in order to make conclusions about the processes that led to the divergence of the 2 clades.

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REFERENCES CITED

BARTH, A., FERNANDES, A., POMPOLO, S. G., AND COSTA, M. A. 2011. Occurrence of B chromosomes in *Tetragonisca* Latreille, 1811 (Hymenoptera, Apidae, Meliponini): a new contribution to the cytotaxonomy of the genus. Genet. Mol. Biol. 34(1): 77-79.

CAIXEIRO, A. P. A. 1996. Contribuição ao estudo citogenético do gênero *Plebeia* (Hymenoptera, Meliponinae). Monograph. Departamento de Biologia Geral. Universidade Federal de Viçosa, Viçosa, Brazil. 33 pp.

CAIXEIRO, A. P. A., AND POMPOLO, S. G. 1999. Caracterização citogenética da heterocromatina constitutiva e sua implicação na evolução do cariótipo do gênero *Plebeia* (Hymenoptera: Apidae, Meliponinae). Genet. Mol. Biol. 22 (Supplement 3): 31-32.

CAMARGO, J. M. F., AND PEDRO, S. R. M. 1992. Systematics, phylogeny and biogeography of the Meliponinae (Hymenoptera, Apidae): A minireview. Apidologie 23: 509-522.

COSTA, M. A., POMPOLO, S. G., AND CAMPOS, L. A. O. 1992. Supernumerary chromosomes in *Partamona helleri* (Hymenoptera, Apidae, Meliponinae). Rev. Brasileira Genet. 15: 801-806.

GUERRA, M. S. 1988. Introdução à Citogenética Geral. Guanabara Koogan, Pernambuco. 139 pp.

HOSHIBA, H., AND IMAI, H. T. 1993. Chromosome evolution of bees and wasps (Hymenoptera, Apocrita) on the basis of C-banding pattern analyses. Japanese J. Entomol. 61: 465-492.

IMAI, H. T. 1991. Mutability of constitutive heterochromatin (C-bands) during eukaryotic chromosomal evolution and their cytological meaning. Japanese J. Genet. 66: 635-661.

IMAI, H. T., MARUYAMA, T., GOJOBORI, T., INOUE, Y., AND CROZIER R. H. 1986. Theoretical bases for karyotype evolution. The minimum-interaction hypothesis. American Nat. 128: 900-920.

IMAI, H. T., TAYLOR, R. W., CROSLAND, M. W. J., AND CROZIER, R. H. 1988. Modes of spontaneous evolution in ants with reference to the minimum interaction hypothesis. Japanese J. Genet. 63: 159-185.

IMAI, H. T., TAYLOR, R. W., AND CROZIER, R. H. 1994. Experimental bases for the minimum interaction theory. Chromosome evolution in ants of the *Myrmecia pilosula* species complex (Hymenoptera: Formicidae: Myrmecinae). Japanese J. Genet. 69: 137-182.

KERR, W. E. 1952. A variação do número de cromossomas na evolução dos Hymenoptera. Sci. Genet. 4: 182-190.

KERR W. E. 1972. Numbers of chromosomes in some species of bees. J. Kansas Entomol. Soc. 45: 11-122.

KERR W. E., CARVALHO, G. A., AND NASCIMENTO, V. M. 1996: Abelha Uruçu. Biologia, Manejo e Conservação. Fundação Acangaú, Belo Horizonte. 146 pp.

KERR, W. E., CARVALHO, G. A., SILVA, A. C., AND ASSIS, M. G. P. 2001. Aspectos pouco mencionados da biodiversidade amazônica. Parcerias Estratégicas, Brasília, Brazil. pp. 20-41.

LOPES, D. M., POMPOLO, S. G., CAMPOS, L. A. O., AND TAVARES, M. G. 2008. Cytogenetic characterization

of *Melipona rufiventris* Lepeletier 1836 and *Melipona mondury*Smith 1863 (Hymenoptera, Apidae) by C banding and fluorochromes staining. Genet. Mol. Biol. 31: 49-52.

MOURE, J. S., URBAN, D., AND MELO, G. A. R. 2007. Catálogo de abelhas (Hymenoptera, Apoidea) da Região Neotropical. Soc. Brasileira Entomol. Curitiba.

RASMUSSEN, C., AND CAMERON, S. A. 2010. Global stingless bee phylogeny supports ancient divergence, vicariance, and long distance dispersal. Biol. J. Linnean Soc. 99: 206-232.

ROCHA, M. P., POMPOLO, S. G., DERGAM, J. A., FERNANDES, A., AND CAMPOS, L. A. O. 2002. DNA characterization and karyotypic evolution in the bee genus *Melipona* (Hymenoptera, Meliponini). Hereditas 136: 19-27.

ROCHA, M. P., POMPOLO, S. G., AND CAMPOS, L. A. O. 2003. Citogenética da tribo Meliponini (Hymenoptera, Apidae), pp. 311-320 *In* G. A. R. Melo and I. Alves-Dos-Santos [eds.], Apoidea Neotropica: Homenagem aos 90 anos de Jesus Santiago Moure. UNESC, Criciúma, Brazil.

SILVEIRA, F. A., MELO, G. A. R., AND ALMEIDA, E. A. B. 2002: Abelhas Brasileiras. Sistemática e Identificação. Fundação Araucária, Belo Horizonte. 253 pp.

SUMNER, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. 75: 304-306.

SUMNER, A. T. 2003. Chromosomes: organization and function. London, Blackwell Publishing. 287 pp.

TARELHO, Z. V. S. 1973: Contribuição ao estudo citogenético dos Apoidea. Tesis M.Sc. Universidade de São Paulo, Ribeirão Preto, Brasil.

WAGNER, R. P., MAGUIRE, M. P., AND STALLINGS, R. L. 1993. Chromosomes-A Synthesis. New York, Wiley-Liss. 523 pp.