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# Cytogenetic characterization of *Austroplebeia australis*: evolutionary hints from a stingless bee outside the Neotropical region

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**Abstract** – Cytogenetic data on stingless bees outside the Neotropical region are still scarce, with chromosome numbers known for only a handful of species, without any further cytogenetic investigations. Our goal was to broaden the cytogenetic characterization of *Austroplebeia australis* from Australia to contribute to the cytogenetic knowledge of the Meliponini tribe. The following techniques were performed: Giemsa-staining, C-banding, DAPI/CMA<sub>3</sub>, and fluorescence in situ hybridization (FISH) with 18S rDNA, GA<sub>(15)</sub>, GAG<sub>(10)</sub>, CAA<sub>(10)</sub>, CGG<sub>(10)</sub>, and TTAGG<sub>(6)</sub> probes. *Austroplebeia australis* has  $2n = 36$  chromosomes and high heterochromatin content. DAPI<sup>+</sup> coincided with euchromatic regions, whereas CMA<sub>3</sub><sup>+</sup> marked the heterochromatin. 18S rDNA clusters were identified in four chromosome pairs, and microsatellites were mainly present in the euchromatin. It has been hypothesized that  $2n = 36$  is the ancestral karyotype of the Meliponini tribe, and *A. australis* retains this plesiomorphic number. A large amount of heterochromatin is a common pattern observed in other stingless bees although it evolved independently in each genus. Interestingly, CMA<sub>3</sub><sup>+</sup> heterochromatin trait is shared with solitary bee species of the Euglossini and Emphorini tribes, whereas the Neotropical Meliponini species have heterochromatin DAPI<sup>+</sup>. The predominance of microsatellite sequences in euchromatin is a trend observed in Neotropical Meliponini, whereas TTAGG represents the telomeric sequence of bees and ants. This is the first study to investigate the cytogenetic patterns of a stingless bee outside the Neotropical region using different banding techniques. This body of work broadens Meliponini cytogenetic data and contributes to the identification of general evolutionary trends in this tribe.

**Base-specific fluorochromes / Euchromatin / Karyotype evolution / Fluorescence in situ hybridization / Heterochromatin / Microsatellite probe**

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## 1. INTRODUCTION

The Meliponini tribe, commonly known as stingless bees, contains more than 500 described species with great morphological and behavioral

diversity (Camargo and Pedro 2013; Michener 2007). Distributed over the tropical and subtropical regions, it represents a monophyletic group in which the species of the Neotropical lineage form a sister clade with the species of the Afrotropical and Indo–Malay/Australasia lineages (Rasmussen and Cameron 2010). Interestingly, the Indo–Malay/Australasia clade is polyphyletic because of two genera—*Austroplebeia* (Australia) and *Lisotrigona* (Asia)—that group together inside the Afrotropical clade (Rasmussen and Cameron 2010).

The species diversity of the Indo–Malay and Australasia regions reaches almost 100 species divided into 15 genera, of which only two are found in Australia: *Tetragonula* and *Austroplebeia* (Dollin and Dollin 1997; Michener 2007; Rasmussen 2008). The *Austroplebeia* genus is endemic to Australia and New Guinea and is represented by five species: *Austroplebeia assingtoni* (Cockerell, 1905), *Austroplebeia australis* (Friese, 1898), *Austroplebeia cincta* (Mocsary, 1898), *Austroplebeia cassiae* (Cockerell, 1910), and *Austroplebeia magna* (Michener, 2007) (Dollin et al. 2015; Halcroft et al. 2016).

Majority of the cytogenetic data on stingless bees is available for the Neotropical region, in which the diploid number varies from  $2n=16$  to  $2n=34$ . The scarce data on the other Meliponini branches indicates a variation of  $2n=28–36$  in the Afrotropical region and  $2n=36–40$  in the Indo–Malay/Australasia region (reviewed in Cunha et al. 2021a). The latter records are restricted to description of the diploid number of *Tetragonula minangkabau* ( $2n=40$ ) (Hoshiba and Imai 1993) and *A. australis* ( $2n=36$ ) (Pereira et al. 2020).

Therefore, the goal of the present study was to broaden the cytogenetic characterization of *Austroplebeia australis* using classical and molecular techniques in order to contribute to the cytogenetic knowledge of the Meliponini tribe. The use of new cytogenetic tools has enabled more detailed and accurate descriptions of Meliponini karyotypes and has contributed to the understanding of the underlying mechanisms that lead to chromosomal diversification in this group of bees (Andrade-Souza et al. 2018; Piccoli et al. 2018; Travanzoli et al. 2019a; Cunha et al. 2020; Pereira et al. 2020, 2021; Novaes et al. 2021a, b).

## 2. MATERIAL AND METHODS

Larvae of two colonies (total of 68 individuals, 36 from one colony and 32 from the second) of *A. australis* were sampled at the University of Western Sydney, Australia. Adult specimens of queens, males, and workers were deposited in the scientific collection of the Entomology Museum at Universidade Federal de Viçosa, Minas Gerais, Brazil. The cerebral ganglia of the larvae at the end of the last instar stage were used to obtain mitotic chromosomes (Imai et al. 1988). The C-banding technique was used to determine heterochromatic regions (Sumner 1972). Sequential staining with fluorochromes DAPI/CMA<sub>3</sub> (4'-6-diamidino-2-phenylindole/chromomycin A<sub>3</sub>) was performed following Schweizer (1980). Images were obtained using an Olympus BX60 epifluorescence microscope with the Q-Color3 Olympus® image software.

The 18S ribosomal DNA and five microsatellites were used as probes in the fluorescence in situ hybridization (FISH) (Pinkel et al. 1986). The 18S rDNA probe was amplified using primers 18SF1 (5'-GTCATATGTTGTCTCAAA GA-3') and 18SR1.1 (3'-TCTAATTTTTTC AAAGTAAACGC-5'). This probe was labeled with digoxigenin-11-dUTP (Roche Applied Science) and the signal was detected with anti-digoxigenin-rhodamine (Roche Applied Science). Repetitive DNA probes GA<sub>(15)</sub>, GAG<sub>(10)</sub>, CAA<sub>(10)</sub>, CGG<sub>(10)</sub>, and TTAGG<sub>(6)</sub> were marked directly with Cy3 during synthesis (Sigma). The metaphases were photographed using an Olympus BX53 microscope with an Olympus DP73F camera using the CellSens Imaging software. An average of 10 metaphases was analyzed to determine the cytogenetic patterns.

## 3. RESULTS

*Austroplebeia australis* has  $2n=36$  (Fig. 1a) and a large amount of heterochromatin in its karyotype (Fig. 1b). The centromeres are not well defined; therefore, it was not possible to determine the karyotypic formula. Fourteen chromosome pairs are composed of a major

part of heterochromatin and a small portion of euchromatin, whereas four pairs are completely euchromatic (Fig. 1b). The first pair has a size polymorphism between the homologous chromosomes (Fig. 1) that was observed in individuals of both colonies, 20 individuals from one colony and 14 from the second. Fluorochrome staining indicated the richness of AT bases in the euchromatic regions (DAPI<sup>+</sup>) (Fig. 1c) and CG bases in the heterochromatin (CMA<sub>3</sub><sup>+</sup>) (Fig. 1d–e).

FISH analysis using the 18S rDNA probe revealed four pairs of chromosomes bearers of ribosomal clusters in the terminal region (Fig. 2a). Microsatellite GA<sub>(15)</sub>, GAG<sub>(10)</sub>, CAA<sub>(10)</sub>, and CGG<sub>(10)</sub> probes were identified in the euchromatic region of the chromosomes (Fig. 2b–e). The microsatellite TTAGG<sub>(6)</sub> probe corresponds to the telomeric sequence of *A. australis* (Fig. 2f).

#### 4. DISCUSSION

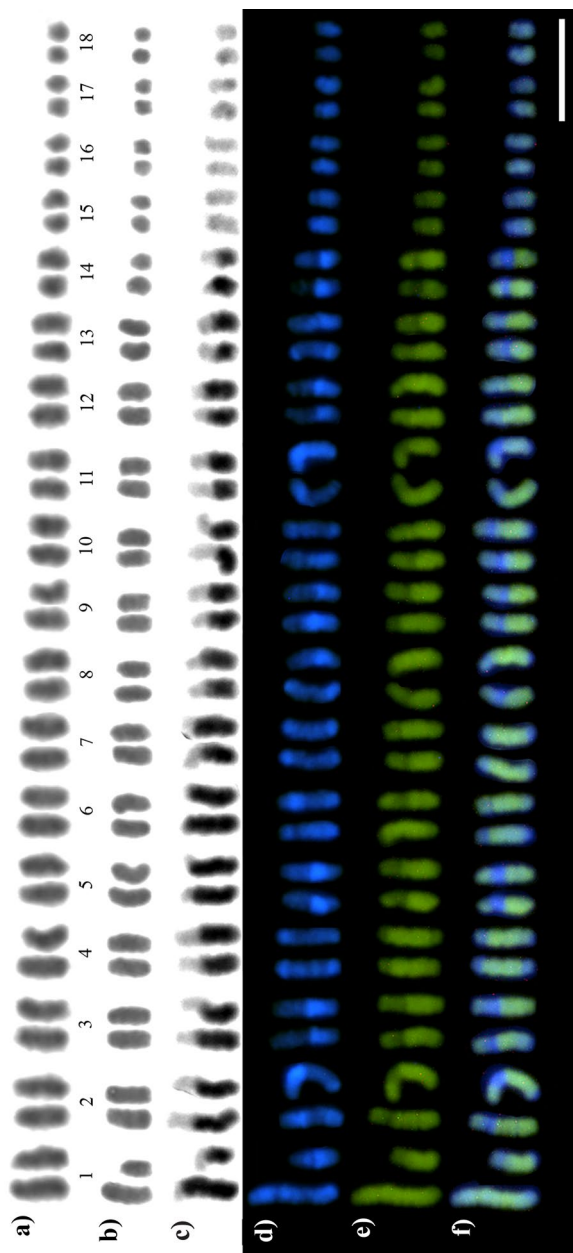
To date, majority of the cytogenetic studies on stingless bees have focused on the Neotropical region (Cunha et al. 2021a). Our work on the cytogenetic banding patterns of an Australian species showed not only some shared chromosomal features but also a few differences with species of the New World.

The  $2n=36$  chromosome number found in *A. australis* has been hypothesized to be the ancestral chromosome number of the Meliponini tribe (Travanzoli et al. 2019b). The genus *Austroplebeia* shares some morphological traits with the African genus *Plebeina* (Michener 2007), which may reflect its close phylogenetic relationship with the Afrotropical clade, emerging as the result of a dispersal event from the Afrotropical region to the Indo–Malay and Australasia regions (Rasmussen and Cameron 2010). We also observed a resemblance of the diploid number with some Afrotropical genera, such as *Plebeina* ( $2n=36$ ), *Cleptotrigona* ( $2n=36$ ), and *Meliponula* ( $2n=34–36$ ) (Kerr and Araújo 1957; Kerr 1972). On the other hand, the chromosome number decreased in other African

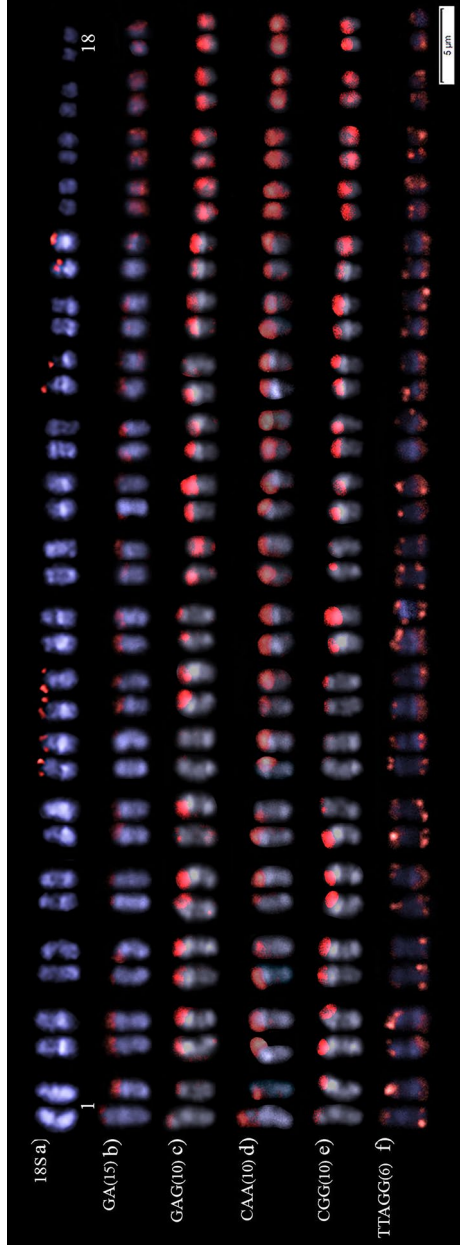
genera, *Dactylurina* ( $2n=34$ ) and *Hypotrigona* ( $2n=28–30$ ) (Kerr 1972), as well as in the Neotropical Meliponini clade ( $2n=16–34$ ) (reviewed in Cunha et al. 2021a), whereas the number of chromosomes increased in the Australasia genus *Tetragonula* ( $2n=40$ ) (Hoshiba and Imai 1993), which requires the cytogenetic characterization of other Indo–Malay/Australasia bees to determine the apomorphic condition of the fission events that occurred in this genus.

The karyotypic formula of *A. australis* could not be determined due to the uncertainty of the centromere position. While the karyotypic structure of the Afrotropical species is not known (reviewed in Cunha et al. 2021a), the Australasia *Tetragonula* karyotype is composed of metacentric and acrocentric chromosomes (Hoshiba and Imai 1993). Therefore, more data will be needed to infer the ancestral chromosome structure of the Meliponini tribe. On the other hand, in the Neotropical clade, hindered centromeres have also been observed in the genus *Melipona*, which can be cytogenetically subdivided into two groups: “low heterochromatin content” and “high heterochromatin content” (Rocha and Pompolo 1998; Rocha et al. 2002). In the *Melipona* “high heterochromatin” group, the heterochromatin growth is believed to have hindered the centromeres of all chromosomes (Cunha et al. 2020; Pereira et al. 2021). The same explanation cannot be applied to *A. australis* because its euchromatic chromosomes also do not have visible centromeres (Fig. 1). Immunolabeling of centromere proteins would help to elucidate this puzzle in both genera.

Although *Austroplebeia* and *Melipona* share the presence of high heterochromatin content in the karyotype, the sequences that compose each heterochromatin are exclusive and are thought to have evolved independently in each genus (Pereira et al. 2020; Cunha et al. 2021b). The presence of completely euchromatic chromosomes is also observed in other stingless bees, such as *Scaptotrigona barrocoloradensis* (Hoshiba and Imai 1993), *Trigona* spp. (Costa et al. 2004), and *Schwarziana quadripunctata* (Cunha et al. 2021b), and could be associated with rearrangements, such as chromosome



**Figure 1** Mitotic metaphasic chromosomes of *Austroplebeia australis*: Giemsa-staining without the polymorphic trait **a)**, Giemsa-staining showing the polymorphic first pair **b)**, C-banding **c)**, DAPI **d)**, CMA<sub>3</sub> **e)**, and merged DAPI/CMA<sub>3</sub> **f)**. Scale bar = 10 μm.



**Figure 2** Fluorescence in situ hybridization patterns of *Austroplebeia australis*: 18S rDNA **a)**, GA<sub>(15)</sub> **b)**, GAG<sub>(10)</sub> **c)**, CAA<sub>(10)</sub> **d)**, CGG<sub>(10)</sub> **e)**, and TTAGG<sub>(6)</sub> **f)**. Chromosomes were counterstained with DAPI (blue), and hybridized probes are shown in red. Scale bar = 5 μm.



translocations, that do not alter chromosome number; hence, the most commonly observed pattern in Meliponini is the presence of one heterochromatic and one euchromatic chromosome arm (Costa et al. 2004; Godoy et al. 2013; Miranda et al. 2013; Cunha et al. 2021b; Elizeu et al. 2021). The presence of a polymorphic pair is another trait shared with some *Melipona* species (Andrade-Souza et al. 2018; Piccoli et al. 2018; Travanzoli et al. 2019a), but also with *S. quadripunctata* (Cunha et al. 2021b), and could have been generated through heterochromatin amplification due to unequal crossing over.

The fluorochrome CMA<sub>3</sub> usually corresponds to the nucleolus organizing region in Neotropical stingless bees (Duarte et al. 2009; Godoy et al. 2013; Martins et al. 2014; Silva et al. 2018), differing only in the genus *Melipona*, where the “high heterochromatin content” species have all the euchromatin CMA<sub>3</sub><sup>+</sup> (Lopes et al. 2008, 2011; Cunha et al. 2018). Contrary to *Melipona*, *A. australis* has heterochromatin CMA<sub>3</sub><sup>+</sup> (Fig. 1), the same pattern that is observed in the solitary species *Euglossa carolina* (Euglossini) (Fernandes et al. 2013) and *Melitoma segmentaria* (Emphorini) (Cristiano et al. 2014). Interestingly, this trait was thought to differentiate these solitary species from social stingless bees (Meliponini) (Cristiano et al. 2014).

To date, only the Neotropical genera *Friesella* (Elizeu et al. 2021), *Melipona* (Andrade-Souza et al. 2018; Cunha et al. 2018; Silva et al. 2018; Pereira et al. 2021), *Partamona* (Martins et al. 2014; Gonçalves et al. 2021; Lopes et al. 2020; Novaes et al. 2021a, b), and *Trigona* (Barboza and Costa 2021; Tavares et al. 2021) have been studied using rDNA FISH probes. Collectively, these results are still incipient in determining the general evolutionary trends of ribosomal genes in the Meliponini tribe.

The predominance of microsatellite sequences in euchromatin is a trend observed in the Neotropical Meliponini species (Piccoli et al. 2018; Travanzoli et al. 2019a; Lopes et al. 2020; Barboza and Costa 2021; Elizeu et al. 2021; Novaes et al. 2021b), indicating that the heterochromatic portions may be composed of

other repetitive classes, such as satellite DNAs and transposable elements (Piccoli et al. 2018; Cunha et al. 2020; Pereira et al. 2020, 2021). The TTAGG<sub>(6)</sub> sequence is the telomeric motif found in both bees and ants (Sahara et al. 1999; Travanzoli et al. 2019a; Micolino et al. 2020; Barros et al. 2021; Novaes et al. 2021b).

This is the first study to investigate the cytogenetic patterns of a stingless bee outside the Neotropical region using different banding techniques, in addition to determining the chromosome number; such data for African lineages are still lacking. This body of work broadens Meliponini cytogenetic data and with a cumulative dataset will contribute to the identification of general evolutionary trends in this tribe.

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## AUTHOR CONTRIBUTION

Study conception and design: NMT, LVT, RMB, BO, and DML. Data collection and analyzes: NMT, MSC, and RMB. Supervision: LAOC, and DML. Literature review and the first draft of the manuscript: NMT and MSC. All authors commented on previous versions and approved the final manuscript.

## DATA AVAILABILITY

Not applicable.

## DECLARATIONS

**Ethical Approval** Not applicable

**Consent to Participate** Not applicable.

**Consent for Publication** Not applicable.

**Conflict of Interest** The authors declare no competing interests

## REFERENCES

- Andrade-Souza V, Duarte OMP, Martins CCC, Santos IS, Costa MGC, Costa MA (2018) Comparative molecular cytogenetics in *Melipona* Illiger species (Hymenoptera, Apidae). *Sociobiology* 65(4):696–705
- Barboza VP, Costa MA (2021) Cytogenetic analysis in *Trigona spinipes* Fabricius (Hymenoptera, Meliponina) reveals intraspecific variation. *Neotrop Entomol*. <https://doi.org/10.1007/s13744-021-00853-7>
- Barros LAC, Aguiar HJAC, Teixeira GA, Souza DJ, Delabie JHC, Mariano CDSF (2021) Cytogenetic studies on the social parasite *Acromyrmex ameliae* (Formicidae: Myrmicinae: Attini) and its hosts reveal chromosome fusion in *Acromyrmex*. *Zool Anz* 293:273–281
- Camargo JMF, Pedro SRM (2013) Meliponini Lepeletier, 1836, in Moure, J. S., Urban, D. and Melo, G. A. R. (Eds), Catalogue of bees (Hymenoptera, Apoidea) in the Neotropical region. Online version available at <http://www.moure.cria.org.br/catalogue>. Accessed 20 Jan 2021
- Costa KF, Brito RM, Miyazawa CS (2004) Karyotypic description of four species of *Trigona* (Jurine, 1807) (Hymenoptera, Apidae, Meliponini) from the State of Mato Grosso. *Brazil Genet Mol Biol* 27:187–190
- Cristiano MP, Simões TG, Lopes DM, Pompolo SG (2014) Cytogenetics of *Melitoma segmentaria* (Fabricius, 1804) (Hymenoptera, Apidae) reveals differences in the characteristics of heterochromatin in bees. *Comp Cytogenet* 8(3):223–231
- Cunha MS, Travenzoli NM, Ferreira RP, Cassinella EK, Silva HB, Salomão TMF, Lopes DM (2018) Comparative cytogenetics in three *Melipona* species (Hymenoptera: Apidae) with two divergent heterochromatic patterns. *Genet Mol Biol* 41:806–813
- Cunha MS, Campos LAO, Lopes DM (2020) Insights into the heterochromatin evolution in the genus *Melipona* (Apidae: Meliponini). *Insectes Soc* 67(3):391–398
- Cunha MS, Cardoso DC, Cristiano MP, Campos LAO, Lopes DM (2021a) The Bee Chromosome database (Hymenoptera: Apidae). *Apidologie* 52(2):493–502
- Cunha MS, Soares FAF, Clarindo WR, Campos LAO, Lopes DM (2021b) Robertsonian rearrangements in Neotropical Meliponini karyotype evolution (Hymenoptera: Apidae: Meliponini). *Insect Mol Biol*. <https://doi.org/10.1111/imb.12702>
- Dollin AE, Dollin LJ (1997) Australian stingless bees of the genus *Trigona* (Hymenoptera: Apidae). *Invertebr Taxon* 6:861–896
- Dollin AE, Dollin LJ, Rasmussen C (2015) Australian and New Guinean stingless bees of the genus *Austroplebeia* Moure (Hymenoptera: Apidae) a revision. *Zootaxa* 4047(1):01–73
- Duarte OMP, Martins CCC, Waldschmidt AM, Costa MA (2009) Occurrence of multiple NORs and intraspecific karyotype variation in *Scaptotrigona xanthotricha* Moure (Hymenoptera, Meliponini). *Genet Mol Res* 8:831–839
- Elizeu AM, Travenzoli NM, Ferreira RP, Lopes DM, Tavares MG (2021) Comparative study on the physical mapping of ribosomal genes and repetitive sequences in *Friesella schrottkyi* (Friese 1900) (Hymenoptera: Apidae, Meliponini). *Zool Anz* 292:225–230
- Fernandes A, Werneck HA, Pompolo SG, Lopes DM (2013) Evidence of separate karyotype evolutionary pathway in *Euglossa* orchid bees by cytogenetic analyses. *An Acad Bras Ciênc* 85:937–944
- Godoy DC, Ferreira RP, Lopes DM (2013) Chromosomal variation and cytogenetics of *Plebeia lucii* and *P. phrynostoma* (Hymenoptera: Apidae) *Fla Entomol* 96(4):1559–1566
- Gonçalves GC, Dalbosco AM, Barth A, Miranda EA, Costa MA (2021) Comparative cytogenetic analysis of three species of the genus *Partamona* (Apidae, Meliponini). *Apidologie* 52(1):80–88
- Halcroft MT, Dollin A, Francoy TM, King JE, Riegler M, Haigh AM, Spooner-Hart RN (2016) Delimiting the species within the genus *Austroplebeia*, an Australian stingless bee, using multiple methodologies. *Apidologie* 47(1):76–89
- Hoshiba H, Imai HT (1993) Chromosome evolution of bees and wasps (Hymenoptera, Apocrita) on the basis of C-banding pattern analyses. *Jpn J Entomol* 61(3):465–492
- Imai HT, Taylor RW, Crosland MW, Crozier RH (1988) Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis. *Jpn J Genet* 63(2):159–185
- Kerr WE (1972) Numbers of chromosomes in some species of bees. *J Kans Entomol Soc* 45(1):111–122
- Kerr WE, Araújo VP (1957) Contribuição ao estudo citológico dos Apoidea. I. Espermatogênese em três espécies africanas. *Garcia de Orta* 5(3):431–433
- Lopes DM, Pompolo SG, Campos LAO, Tavares MG (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(1):49–52
- Lopes DM, Fernandes A, Praça-Fontes MM, Werneck HA, Resende HC, Campos LAO (2011) Cytogenetics of three *Melipona* species (Hymenoptera, Apidae, Meliponini). *Sociobiology* 58(1):185–194
- Lopes DM, Travenzoli NM, Fernandes A, Campos LA (2020) Different levels of chromatin condensation in *Partamona chapadicola* and *Partamona nhambiquara* (Hymenoptera, Apidae). *Cytogenet Genome Res* 160(4):206–213
- Martins CCC, Waldschmidt AM, Costa MA (2014) Unprecedented record of ten novel B chromosomes



- in the stingless bee *Partamona helleri* (Apidae, Meliponini). *Apidologie* 45(4):431–439
- Michener CD (2007) *The Bees of the World*, 2nd edn. The Johns Hopkins University Press, Baltimore, pp 66–75
- Micolino R, Cristiano MP, Cardoso DC (2020) Karyotype and putative chromosomal inversion suggested by integration of cytogenetic and molecular data of the fungus-farming ant *Mycetomoellerius iheringi* Emery, 1888. *Comparat Cytogenet* 14(2):197–210
- Miranda RV, Fernandes A, Lopes DM (2013) Karyotype description of *Cephalotrigona femorata* Smith (Hymenoptera: Apidae) and the C-banding pattern as a specific marker for *Cephalotrigona*. *Sociobiology* 60(1):131–134
- Novaes CM, Cunha MS, Werneck HA, Fernandes A, Campos LA, Lopes DM (2021a) Chromosome evolution in the genus *Partamona* (Apidae: Meliponini), with comments on B chromosome origin. *Cytogenet Genome Res* 161(10–11):520–528
- Novaes CM, Cunha MS, Clarindo WR, Tosta VC, Salomão-Fernandes TM, Lopes DM (2021b) Inter- and intra-population B chromosome variability in *Partamona helleri* (Apidae: Meliponini). *Apidologie* 52:1334–1345
- Pereira JA, Salomão TMF, Lopes DM (2020) Different repetitive DNA sequences make up heterochromatin in Meliponini. *Apidologie* 51(5):855–860
- Pereira JA, Travanzoli NM, Oliveira MP, Werneck HA, Salomão TMF, Lopes DM (2021) Molecular cytogenetics in the study of repetitive sequences helping to understand the evolution of heterochromatin in *Melipona* (Hymenoptera, Meliponini). *Genetica* 149(1):55–62
- Pinkel D, Straume T, Gray JW (1986) Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. *Proc Natl Acad Sci USA* 83(9):2934–2938
- Piccoli MCA, Bardella VB, Cabral-de-Mello DC (2018) Repetitive DNAs in *Melipona scutellaris* (Hymenoptera: Apidae: Meliponidae): chromosomal distribution and test of multiple heterochromatin amplification in the genus. *Apidologie* 49(4):497–504
- Rasmussen C (2008) Catalog of the Indo-Malayan/Australasian stingless bees (Hymenoptera: Apidae: Meliponini). *Zootaxa* 1935(1):1–80
- Rasmussen C, Cameron SA (2010) Global stingless bee phylogeny supports ancient divergence, vicariance, and long distance dispersal. *Biol J Linn Soc* 99(1):206–232
- Rocha MP, Pompolo SG (1998) Karyotypes and heterochromatin variation (C-bands) in *Melipona* species (Hymenoptera, Apidae, Meliponinae). *Genet Mol Biol* 21:41–45
- Rocha MP, Pompolo SG, Dergam JA, Fernandes A, Campos LAO (2002) DNA characterization and karyotypic evolution in the bee genus *Melipona* (Hymenoptera, Meliponini). *Hereditas* 136(1):19–27
- Sahara K, Marec F, Traut W (1999) TTAGG telomeric repeats in chromosomes of some insects and other arthropods. *Chromosome Res* 7(6):449–460
- Schweizer D (1980) Simultaneous fluorescent staining of R bands and specific heterochromatic regions (DA-DAPI bands) in human chromosomes. *Cytogenet Genome Res* 27(2–3):190–193
- Silva AA, Rocha MP, Pompolo SG, Campos LAO, Tavares MG (2018) Karyotypic description of the stingless bee *Melipona quinquefasciata* Lepelletier, 1836 (Hymenoptera, Meliponini) with emphasis on the presence of B chromosomes. *Comparat Cytogenet* 12(4):471–482
- Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res* 75:304–306
- Tavares MG, Ferreira RP, Travanzoli NM, Lopes DM (2021) Karyotypic variation in the stingless bee *Trigona spinipes* (Hymenoptera: Apidae: Meliponini) from different geographical regions of Brazil. *Apidologie* 52:1358–1367
- Travanzoli NM, Lima BA, Cardoso DC, Dergam JA, Fernandes-Salomão TM, Lopes DM (2019a) Cytogenetic analysis and chromosomal mapping of repetitive DNA in *Melipona* species (Hymenoptera, Meliponini). *Cytogenet Genome Res* 158(4):213–224
- Travanzoli NM, Cardoso DC, Werneck HA, Fernandes-Salomão TM, Tavares MG, Lopes DM (2019) The evolution of haploid chromosome numbers in Meliponini. *PLoS One* 14(10)

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