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Karyotype diversity of stingless bees of the genus *Frieseomelitta* (Hymenoptera, Apidae, Meliponini)

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Abstract. *Frieseomelitta* (Ihering, 1912) is a genus of stingless bees, distributed in the Nearctic and Neotropical regions. Specimens can be found in forests, cerrado, caatinga and mountainous regions. This genus has 16 species, of which 13 are recorded in Brazil. Cytogenetics has contributed to evolutionary studies of some Hymenoptera groups and although many *Frieseomelitta* species have been described, few species have been studied cytogenetically. The present study aims to contribute to the knowledge of the karyotype diversity of this genus, seeking to understand the possible evolutionary mechanisms that occurred in the diversification of the karyotype of this genus. *Frieseomelitta portoi* and *Frieseomelitta trichocerata* and *Frieseomelitta doederleini* showed diploid karyotypes with 2n = 30 chromosomes, similarly to all the species previously analyzed in the genus. Unprecedentedly, *Frieseomelitta longipes* showed 2n = 34. These results confirm that the frequent diploid number of 30 chromosomes is typical of this genus. The finding of 2n = 34 chromosomes in *F. longipes* comprises the first record of a diploid chromosome number different from 2n=30 in this group, which suggests that it can be the result of a recent chromosome change event. An interspecific comparative analysis was developed involving present and previous studies, as well as a discussion on the mechanisms involved in the karyotypic evolution in the genus.

Keywords: heterochromatin, karyotype evolution, centric fission, chromosome, variation.

INTRODUCTION

Frieseomelitta (Ihering, 1912) is a Neartic and Neotropical genus of stingless bees, and occurs from Mexico to southeast Brazil (Camargo and Pedro, 2013). These bees present small to medium size, with body measuring about 10 mm (Silveira *et al.*, 2002). According to Camargo and Pedro (2013), 16 species are known to be valid in the genus *Frieseomelit-*

ta, of which 13 are found in Brazilian territory: *Frieseomelitta dispar* (Moure, 1950), *Frieseomelitta doederleini* (Friese, 1900), *Frieseomelitta flavicornis* (Fabricius, 1798), *Frieseomelitta francoi* (Moure, 1946), *Frieseomelitta freiremaiai* (Moure, 1963), *Frieseomelitta languida* (Moure, 1989), *Frieseomelitta longipes* (Smith, 1854), *Frieseomelitta meadewaldoi* (Cockerell, 1915), *Frieseomelitta paranigra* (Schwarz, 1940), *Frieseomelitta portoi* (Friese, 1900), *Frieseomelitta silvestrii* (Friese, 1902), *Frieseomelitta trichocerata* (Moure, 1988) and *Frieseomelitta varia* (Lepeletier, 1836). Cytogenetic analysis has been a widely used tool in evolutionary and taxonomic studies in some groups of Hymenoptera, however, for the genus *Frieseomelitta*, the chromosomal analysis are hitherto restricted the species *F. doederleini* (Kerr and Silveira, 1972; Tarelho, 1973; Rocha *et al*., 2003; Santos *et al*., 2018), *F. languida* (Rocha *et al*., 2003), *F. varia* (Kerr, 1969; Kerr and Silveira, 1972; Tarelho, 1973; Rocha *et al*., 2003; Santos *et al*., 2018), *F. dispar* (Carvalho and Costa, 2011; Santos *et al*., 2018), *F. francoi* (Carvalho and Costa, 2011; Santos *et al*., 2018), *Frieseomelitta meadewaldoi* and *Frieseomelitta* sp. n. (Santos *et al*., 2018).

During the karyotype analyzes of several species of bees, Kerr (1972a, b) recorded the haploid chromosome number n = 15 chromosomes in the species *F. varia*, *F. doederleini* and *F. ghilianii*. Rocha *et al.* (2003) reported the same chromosome number and described the karyotype of the species *F. doederleini*, *F. languida* and *F. varia* as part of an analysis of different genera of stingless bees. Carvalho & Costa (2011) also described the karyotypes of *F. dispar* and *F. francoi*, and Santos *et al.* (2018) developed a comparative analysis of the hybridization patterns of microsatellite DNA probes in karyotypes of five species, *F. dispar, F. doederleini e F. francoi, F. meadewaldoi*, *Frieseomelitta* sp. n. and *F. varia*. In addition, other karyotypic features were reported. In all these cases the chromosome number was consistently n = 15 or 2n = 30. These differ from the chromosome numbers determined for other less closely related genera, such as n = 9 for *Melipona*, and n = 17, common to several of the other genera of stingless bees. However, groups closely related to *Frieseomelitta* such as genus *Duckeola* also have shown n = 15 chromosomes (Kerr 1972a, b).

The taxonomy and phylogeny of *Friesomelitta* is still not well resolved, and there are some species with broad geographic distribution. In this context, the present study aimed to contribute to the knowledge of the karyotype diversity of this genus, including characterizing samples of new points in the distribution of species and new species. Furthermost, we search for data that may aid in the taxonomic resolution of the group and in the understanding of the possible evolutionary mechanisms that occurred in the diversification of the karyotype of the group.

MATERIAL AND METHODS

We analyzed samples of four species from different localities of Brazil, *Frieseomelitta doederleini*, from the municipality of Canavieiras, state of Bahia, (15º 61 'S, 39º42' W), *Frieseomelitta longipes*, from the municipality of Belém, state of Pará, 1:30 'S, 48 ° 73' W); *Frieseomelitta portoi*, from the municipality of Rio Branco, state of Acre (9º98 'S, 67º90' W); and *Frieseomelitta* aff. *trichocerata*, from the municipality of Juína, state of Mato Grosso (11º52 'S, 60º50' W). Taxonomist of bees identified the collected specimens and adult specimens of each of the species were mounted on entomological pins and deposited in the entomological collection of the Universidade Estadual de Santa Cruz, Ilheus, BA.

The slide preparations were made from cells of the cerebral ganglia of specimens in the prepupa stage, according to the protocol described by Imai *et al.* (1988). The prepared slides were stained conventionally with 3% Giemsa/Sorensens's Buffer and the selected metaphases were photographed on an Olympus CX-41 microscope with attached Olympus C7070 digital camera.

Staining with the base-specific fluorochromes 4,6-diamidino-2-phenylindole (DAPI) and chromomycin A3 (CMA_3) to evidence the chromosomal regions rich in AT (DAPI) and CG (CMA_3), respectively, were performed according to Schweizer (1980), with modifications proposed by Guerra and Souza (2002). Coverslips were mounted on slides with antifading Vectashield (Vector Laboratories, Burlingame, USA). The images were captured on a Leica DMRA2 epifluorescence microscope using the Leica IM50 software (Leica Microsystems Imaging Solutions Ltda, Cambridge, UK).

To allow comparison with previous studies, we followed chromosomal nomenclature proposed by Imai (1991). (M) Metacentric chromosome: the arms of approximately equal sizes and euchromatic, the heterochromatin restricted to the centromeric region; (A) Acrocentric chromosome: centromeric region and short heterochromatic arms; (A^M) Pseudoacrocentric chromosome: centromeric region, middle or long heterochromatic arms and short eucrotic arm.

The Karyograms were organized with the use of Adobe Photoshop® CS6 13.0x 64 software. From the karyotypes, the chromosome pairs, diploid (2n) and haploid (n) numbers and karyotype formulas (2k) were defined.

RESULTS AND DISCUSSION

The chromosome number found for the species *F. doederleini*, *F. portoi* and *F.* aff. *trichocerata* was 2n = 30 for females. In *F. longipes*, females showed 2n = 34 chromosomes (Table 1, Fig. 1). Kerr and Silveira, 1972 registered 2n = 30 chromosomes for *F. doederleini* and *F. varia* and Rocha *et al.* (2003) found 2n = 30 chromosomes for *F. doederleini*, *F. languida*, *F. varia*. The same number was found by Carvalho and Costa (2011) for *F. dispar* and *F. portoi*. Together these results indicate that the frequent diploid number of 30 chromosomes is characteristic of the genus. The finding of 2n = 34 chromosomes in *F. longipes* comprises the first record of a diploid chromosome number different from 30 in this genus. In the species analyzed here, the following karyotypic formulas were found: *F. doederleini*, 2K = 4M + 4A + 22A^{M}, *F. portoi*, 2K = 4M + 26A, *F.* aff. *Trichocerata*, 2K = 6M + 20A + 4A^{M}, and *F. longipes*, 2K = 8M + 12A + 14A^{M} (Tab. 1).

The karyotypic formula observed in *F. doederleini* is similar to that cited by Rocha *et al.*, (2003) for another population of the same species, evidencing intraspecific karyotypic stability.

The predominance of acrocentric and pseudoacrocentric (A^{M} - which contains a long heterochromatic arm) chromosomes in karyotypes was consistent with that observed in previous studies for other species of the genus, such as *F. dispar. F. francoi*, *F. languida*, and *F. varia* (Rocha *et al.*, 2003; Carvalho and Costa, 2011). However, in *F. portoi* and *F.* aff. *trichocerata* was observed a reduced number of A^{M} chromosomes.

The classical cytogenetics using conventional Giemsa staining and C-banding allowed observing heterochromatin in studies of several meliponine genera such as *Friesella* (Mampumbu, 2002), *Leurotrigona* (Pompolo and Campos, 1995), *Melipona* (Hoshiba, 1988; Rocha & Pompolo, 1998; Rocha *et al.*, 2002); *Nannotrigona* (Hoshiba & Imai, 1993), *Partamona* (Costa *et al.*, 1992; Martins *et al.*, 2012), *Plebeia* (Caixeiro & Pompolo, 1999), *Tetragonisca* (Barth *et al.*,), *Trigona* (Hoshiba and Imai, 1993; Costa, 2004; Domingues *et al.*, 2005), among others. The distribution of heterochromatin has still been the focus of comparative studies in stingless bees (e.g. Travenzoli *et al.*, 2019), and for being variable

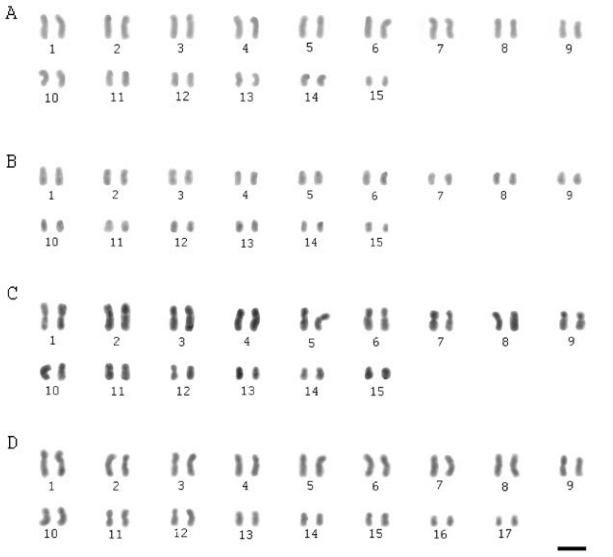

Figure 1. Karyotypes of workers of *Frieseomelitta* species stained with Giemsa: (A) *F. doederleini* (B) *F. portoi*; (C) *F.* aff. *trichocerata*; (D) *F. longipes*. Bar = 10um.

Table 1. Available karyotype data of Frieseomelitta species. 2n = diploid number, 2k = diploid karyotype formula, M = metacentric, A = acrocentric, AM = pseudoacrocênctrico chromosomes.

Species	Sampling location	2n	2k	References
F. dispar	Ilhéus/BA	30	4M + 2M + 4A + 20A^{M}	Carvalho & Costa (2011)
F. doederleini	-	30	-	Kerr & Silveira, (1972)
	Santana do Seridó/RN	30	4M + 4A + 22A^{M}	Rocha *et al.*, (2003)
	Canavieiras/BA	30	4M + 4A + 22A^{M}	Present study
F. francoi	Cairú/BA	30	4M + 2M + 4A + 20A^{M}	Carvalho & Costa (2011)
F. languida	Divinópolis/MG	30	4M + 4A + 22A^{M}	Rocha *et al.*, (2003)
F. longipes	Belém/PA	34	8M + 12A + 14A^{M}	Present study
F. portoi	Rio Branco/AC	30	4M + 26A	Present study
F. aff. *trichocerata*	Juína/MT	30	6M + 20A + 4A^{M}	Present study
F. varia	-	30	-	Kerr & Silveira, (1972)
	Divinópolis/MG	30	4M + 4A + 22A^{M}	ROCHA *et al.*, (2003)

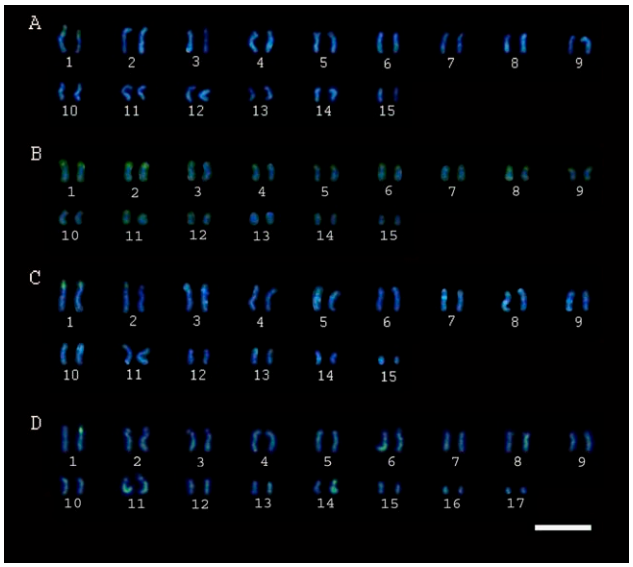

Figure 2. Karyotypes of workers of *Frieseomelitta* species, stained with CMA_3/DAPI fluorochromes: (A) *F. doederleini*; (B) *F. portoi*; (C) *F. trichocerata*; (D) *F. longipes*. Bar = 10um.

among genera and species is a useful cytological information for the characterization of species or populations of these bees.

Imai *et al.* (1988) have suggested that the tandem addition of terminal heterochromatin in chromosomes is a relatively rapid way of restoring telomeric stability after centric fission events, leading to the formation of A^M chromosomes. Elimination of any excess heterochromatin addition may follow this addition by deletion mechanisms, as a tendency to reduce non-specific heterochromatin interactions of the long pseudoacrocentric chromosomes. The high content of heterochromatin found, however, contrasts with the numerical or even structural stability observed in *Frieseomelitta*, especially considering species such as *F. doederlaini*, analyzed in different studies and localities of its geographic distribution.

The CMA_3/DAPI fluorochrome staining in *F. doederleini*, showed that the centromeric region of the metacentric, acrocentric and the heterochromatic arm of the pseudoacrocentric chromosomes are rich in AT base pairs ($DAPI^+$). This same region was also weakly stained with CMA_3 (Fig. 2A). These results were similar to the findings of Rocha *et al.* (2003) for *F. varia* and this marking pattern may be related to the presence of a nucleolus organizing region. These regions are often labeled by CMA_3 in karyotypes of bees. NOR banding or in situ hybridization of ribosomal probes are likely to confirm this location.

In the *F. portoi*, CMA_3^+/$DAPI^-$ bands were identified in short heterochromatic arms in chromosomal pairs 1, 2, 3, 4, 5, 6, 8, 9, 11, 12 and 14. Pairs 7, 10 and 13 were totally CMA_3^-/$DAPI^+$ in this species (Fig. 2B). *Frieseomelitta* aff. *trichocerata* showed CMA_3^+/$DAPI^-$ bands in chromosomal pairs 1, 2, 3, 5, 7, 8, 10 and 11, and pairs 4, 6, 9, 12, 13, 14 and 15 were homogeneously CMA_3^-/$DAPI^+$ (Fig. 2C).

We observed in the four species analyzed here that the first pair showed the CMA_3^+/$DAPI^-$ label on the short arm. Although in *F. longipes* only one of the homologues was labeled by CMA_3, suggesting the presence of a heteromorphism, this labeling, which may be associated with the presence of NOR, seems to be common in the genus. This can be confirmed in further analyzes that include new species. Diverging from the other species, *F. longipes* had the long arms of pairs 6 and 11 and pair 14 strongly labeled CMA_3^+/$DAPI^-$ (Fig. 2D).

The results obtained in the present analysis together with the previous karyotypic descriptions show that the karyotype differentiation in *Frieseomelitta* mostly involved minor structural alterations such as heterochromatin gain and loss. However, numerical change occurred in the differentiation of *F. longipes*, possibly due to centric fission. Since this is the only record of a chromosome number other than 2n = 30 in this genus, this is possibly a derived characteristic in this group. The lack of a better taxonomic definition and a more resolved and complete phylogeny for *Frieseomelitta* leaves this question open. If a derived position *F. longipes* is found, the recent origin of this numerical difference will be confirmed.

From the observation of the high frequency of the chromosome number 2n = 30 (n = 15) in the *Frieseomelitta*, it is possible to suggest that this chromosome number is basal for this genus. The n = 15 chromosomes was also found in the species *Duckeola ghiliani* Kerr, 1972a, b; Kerr and Silveira, 1972). According to the Meliponini phylogeny proposed by Rasmussen and Cameron (2010) *Duckeola* is closely related to the genus *Frieseomelitta*. However, without a more detailed phylogenetic assessment, the hypothesis that the 2n = 34 found in *F. longipes*, alternatively, represents the basal condition for this group can not be discarded, since this chromosome number is found in most genera of neotropical Meliponini (Rocha *et al.*, 2003). More complete phylogenetic analyzes, including a representative sampling of *Friesemelitta* diversity, however, would be necessary to better clarify these questions.

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REFERENCES

Barth A, Fernandes A, Pompolo SG, Costa MA (2011) Occurrence of B chromosomes in *Tetragonisca* Latreille, 1811 (Hymenoptera, Apidae, Meliponini): a new contribution to the cytotaxonomy of the genus. Genetics and Molecular Biology 34(1): 77–79

Caixeiro APA, Pompolo SG. 1999. Caracterização citogenética de heterocromatina constitutiva e sua implicação na evolução do cariótipo de espécies do gênero *Plebeia* (Hymenoptera, Apidae, Meliponini). Genetics and Molecular Biology 22 (Supplement 3): 31-32.

Carvalho AF, Costa MA. 2011. Cytogenetic characterization of two species of *Frieseomelitta* Ihering, 1912 (Hymenoptera, Apidae, Meliponini). Genetics and Molecular Biology (Impresso), v. 34, p. 237-239.

Camargo JMF, Pedro SRM. 2013. Meliponini Lepeletier, 1836. In Moure JS, Urban D and Melo GAR (Orgs). In Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region - online version. Available at http://www.moure.cria.org.br/catalogue. Accessed Agosto/14/2016.

Cockerell TDA. 1915. The Real *Trigona dorsalis* Smith rediscovered (Hym.) Entomological News, 26: 30-32.

Costa MA, Pompolo SG, Campos LAO. 1992. Supernumerary chromosomes in *Partamona cupira* (Hymenoptera, Apidae, Meliponinae). Revista Brasileira de Genetica 15, 801–806.

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. Genetics and Molecular Biology. Vol. 27 nº 2 São Paulo.

Crozier RH. 1975. Hymenoptera. In: B. John (ed), Animal Cytogenetics 3. Insecta 7. Berlin, Grebuder, Borntraeger. 95p.

Domingues AMT, Waldschmidt AM, Andrade SE, Souza VA, Alves RMO, Junior JCS, Costa MA. 2005. Karyotype characterization of *Trigona fulviventris* Guérin, 1835 (Hymenoptera, Meliponini) by C banding and fluorochrome staining: Genetics and Molecular Biology, 28, 3, 390-393.

Fabricius JC. 1798. Suplementum Entomologiae Systematicae, Vol. 5 – Hafniae, Apud Proftet Stortch, 572pp.

Friese HFAKL. 1900. Neue Arten der Bienengattungen *Melipona* III., und *Trigona* Jur. Természetrajzi Fuzetek, 23(11): 381-394.

Friese HFAKL. 1902. Neue Meliponiden (Hym). Zeitschrift fur Systematische Hymenopterologie und Dipterologie, 2: 382-383.

Guerra MS, Souza MJ. 2002. Como Observar Cromossomos: Um Guia de Técnicas em Citogenética Vegetal, Animal e Humana. Ribeirão Preto, São Paulo, Brazil.

Hoshiba H. 1988. Karyological analysis of a stingless bee *Melipona favosa* (Apidae, Hymenoptera). Cytologia 53:153-156.

Hoshiba H, Imai HT. 1993. Chromosome evolution of bees and wasps (Hymenoptera, Apocrita) on the basis of C banding pattern analyses. Japanese Journal of Entomology 61:465-492.

Imai HT. 1988. Modes of spontaneous chromosomal mutation and karyotypes evolution in ants with reference to he minimum intraction hypothesis. Japanese Journal of Genetics 63: 159-185.

Imai HT. 1991. Mutability of constitutive heterochromatin (c-bands) during eukaryotic chromosomal evolution and their cytological meaning. Japanese Journal of Genetics 66: 653-661.

Imai HT, Taylor RW, Crosland MWJ, Crozier RH. 1988. Modes of spontaneous evolution in ants with reference to the minimium interaction hypothesis. Japanese Journal of Genetics 66: 159-185.

Imai HT, Maruyama T, Gojobori T, Inoue Y., Crozier RH. 1986. Theoretical bases for karyotype evolution. The minimum-interaction hypothesis. American Naturalist 128: 900-920.

Imai HT, Taylor RW, Crozier RH. 1994. Experimental bases for the minimium interaction theory. Chromosome evolution in ants of the *Myrmecia pilosula* species complex (Hymenoptera: Formicidae: Myrmecinae). Japanese Journal of Genetics 69: 137-182.

Imai HT, Brown WL, Kubota JRM, Young HS, Tho YP. 1984. Chromosome observations of tropical ants in western Malasya. Annual Report National Institute of Genetics 63: 66-69.

Imai HT, Taylor RW. 1989. Chromosomal polymorphism involving telomere fusion, centromeric inactivation and centromere shipt in the ant *Myrmecia* (*pilosula*) n=1. Chromosoma 98: 456-460.

Imai HT, Crozier RH, Taylor RW. 1977. Karyotype evolution in Australlian ants. Chromosoma 53: 341-393.

Imai HT, Kubota M. 1975. Chromosome polymorphism in the ant, *Pheidole nodus*. Chromosoma 51: 391-399.

Imai HT. 1991. Mutability of constitutive heterochromatin (c-bands) during eukaryotic chromosomal evolution and their cytological meaning. Japanese Journal of Genetics 66: 653-661.

Kerr WE. 1972a. Numbers of Chromosomes in Some Species of Bees. Journal of the Kansas Entomological Society, 45 (1): 111-122.

Kerr WE. 1972b. Karyotypic Evolution of Bees and Corresponding Taxonomic Implications. Evolution, 45 (1): 197-202.

Kerr WE, Silveira ZV. 1972. Karyotypc evolution of bees and corresponding taxonomic implications. Evolution 26: 197-202.

Lepeletier de Saint-Fargeau A. 1836. Histoire Naturalle des Insects Hyménopteres. nº 10, Bis. Librairie Encyclopédique de Roret, Paris. 547pp.

Mampumbu AR. 2002. Análise citogenética da heterocromatina e da NOR em Populações de abelhas sem ferrão *Friesella schrottkyi* (FRIESE, 1900) (Hymenoptera: Apidae: Meliponini). Dissertação de Mestrado, Unicamp, Campinas.

Martins CCC, Diniz D, Sobrinho-Scudeler PE, Foresti F, Campos LAO, Costa MA. 2012. Investigation of *Partamona helleri* (Apidae, Meliponini) B chromosome origin. An approach by microdissection and whole chromosome painting. Apidologie 44, 75–81

Moure JS. 1946. Contribuição para o Conhecimento dos Meliponinae (Hym. Apoidea). Revista de Entomologia, 17(3): 437-443.

Moure JS. 1950. Notas Sobre Alguns Meliponinae Bolivianos (Hymenoptera, Apoidea). Dusenia, 2(1): 70-80.

Moure JS. 1963. Uma Nova Espécie de "*Trigona* (*Frieseomelitta*)" do Norte do Distrito Tupi (Hymenoptera, Apoidea). Revista Brasileira de Biologia, 23(1): 39-43.

Moure JS. 1989. Espécies Novas de Abelhas da Região Central do Estado de Minas Gerais, Brasil (Hymenoptera, Apoidea). Acta Biológica Paranaense 18(1, 2, 3, 4): 115-127.

Moure JS. 1988. Uma Nova Espécie de *Frieseomelitta* do Oeste da Amazônia (Hymenoptera, Apoidea). Acta Biológica Paranaense 17(1, 2, 3, 4): 141-145.

Oliveira FF. 2003. Revisão do gênero *Frieseomelitta* von Ihering, 1912 (Hymenoptera, Apidae, Meliponinae), com notas bionômicas de algumas Espécies. Tese de Doutorado, Universidade Federal do Paraná, Curitiba, 327p.

Pompolo SG, Campos LAO. 1995. Karyotypes of two species of stingless bee, *Leurotrigona muelleri* and Leurotrigona pusilla (Hymenoptera, Meliponinae). Revista Brasileira de Genetica 18:181-184.

Rasmussen C, Cameron SA. 2010. Global stingless bee phylogeny supports ancient divergence, vicariance, and long-distance dispersal. Biological Journal of the Linnean Society, 99: 206–232.

Rocha MP, Pompolo SG. 1998. Karyotypes and heterochromatin variation (C-bands) in *Melipona* species (Hymenoptera, Apidae, Meliponinae). Genetics and Molecular Biology V. 21, p. 41-45.

Rocha MP, Pompolo SG, Dergam JÁ, Fernandes A, Campos LAO. 2002. DNA characterization and karyotypic evolution in the bee genus *Melipona* (Hymenoptera, Meliponini). Hereditas 136:19-37.

Rocha MP, Pompolo SG, Campos LAO. 2003. Citogenética da tribo Meliponini (Hymenoptera, Apidae). In: Melo, G. A. R. and Santos I. A. (eds) Apoidea Neotropica: Homenagem aos 90 anos de Jesus Santiago Moure. UNESC, Criciúma, pp 311-320.

Santos JM, Diniz, D, Rodrigues TAS, Cioffi, MB, Waldschmidt, AM. 2018. Heterochromatin distribution and chromosomal mapping of microsatellite repeats in the genome of *Frieseomelitta* stingless bees (Hymenoptera: Apidae: Meliponini). Florida Entomologist. 101(1):33-39.

Schweizer D. 1980. Simultaneous fluorescent staining of R bands and specific heterochromatic regions (DA-DAPI bands) in human chromosomes. Cytogenetics and Cell Genetics 27:190-193.

Silveira FA, Melo GAR, Almeida EAB. 2002. Abelhas Brasileiras – Sistemática e Identificação. Editora Composição e Arte, Minas Gerais, Brasil. 1ª edição. Pg 86.

Smith F. 1854. Catalogue of Hymenopterous Insects in the Collection of the British Museum: Part II, Apidae. Printed by Order of the Trustees, London. pp. 199-465.

Schwarz HF. 1940. Additional Species and Records of Stingless Bees (Meliponidae) from British Guiana. American Museum Novitates, 1078: 1-12p.

Travenzoli, N. M., Barbosa, I. C. de O., Carvalho-Zilse, G. A., Salomão, T. M. F., & Lopes, D. M. (2019) Karyotypic description and repetitive DNA chromosome mapping of *Melipona interrupta* Latreille, 1811 (Hymenoptera: Meliponini). Caryologia 72(2), 91-95.