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First Record of a B Chromosome in *Polybia fastidiosuscula* Saussure (Vespidae) and Investigation of Chromatin Composition Through Microsatellite Mapping

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Abstract
The characterization of karyotypes is an important aspect in understanding the structure and evolution of genomes. *Polybia* is a genus of social wasps of the family Vespidae. This genus has 58 species, but for only 8 of these chromosome number and morphology have been reported in the literature. The aim of this study was to describe and characterize the *Polybia fastidiosuscula* Saussure karyotype, presenting the first case of a B chromosome in Vespidae. In addition, we investigated the chromatin composition of this species through C-banding, base-specific fluorochrome staining, and physical mapping of 7 microsatellites and 18S rDNA. Four colonies of *P. fastidiosuscula* from Minas Gerais and Paraná states, Brazil, were analyzed. The chromosome number identified was 2n = 34, and 2 colonies presented a B chromosome. We characterized the chromatin composition of this species, analyzing the existence of different microsatellite-rich heterochromatic regions which are also enriched with AT or GC base pairs. We suggest an intraspecific origin of the B chromosome based on the homology of the heterochromatic composition with A chromosomes and also verify that the TTAGG and TCAGG sequences are not telomeric, but only microsatellites that occur in the centromeres of most chromosomes, as well as GAG and CGG.

Introduction

Polybia belongs to the tribe Epiponini of the family Vespidae [Richards, 1978] and is characterized as a swarm-founding wasp genus [Carpenter and Marques, 2001]. *Polybia* comprises 58 species distributed from the southern United States to Northern Argentina [Richards, 1978]. The broad geographical distribution permits the comparison of populations from different regions, which sets *Polybia* as an interesting model group for cytogenetic studies.

Cytogenetic techniques are useful to describe, classify, and characterize chromosome sets. Furthermore, they permit investigating the distribution and composition of chromatin along the chromosomes [Menezes et al., 2014; Bai et al., 2018]. Fluorescence in situ hybridization (FISH)

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is a valuable technique that physically maps specific chromosomal sequences [Larracuente and Ferree, 2015], such as ribosomal DNA (rDNA) or other regions of repetitive DNA.

A eukaryote genome generally has numerous regions of repetitive DNA [Britten and Kohne, 1968] which are classified into dispersed repetitive DNA (transposons and retrotransposons) and tandem repetitive DNA (satellite DNAs, microsatellites, minisatellites, and multigene families such as rDNA) [reviewed in López-Flores and Garrido-Ramos, 2012]. Repetitive DNAs, such as microsatellites and ribosomal genes, are used as molecular markers and allow for investigating the composition and organization of chromatin, as well as chromosomal evolution in various organisms, including insects [Butcher et al., 2000; Brito et al., 2005; Lopes et al., 2014; Menezes et al., 2014; Cunha et al., 2018; Piccoli et al., 2018; Santos et al., 2018; Travenzoli et al., 2019].

In *Polybia,* only 8 out of 58 species have been investigated cytogenetically so far, and most studies just describe the number and morphology of the chromosome set: *P. jurinei* (n = 5), *P. rejecta* (n = 15), *P.* sp. 1 (n = 16), *P.* sp. 2 (n = 17), *P. occidentalis* (n = 17), *P. paulista* (n = 17), *P. scutellaris* (n = 17), and *P. sericea* (n = 27) [Pompolo and Takahashi, 1987, 1990; Menezes et al., 2014]. These studies show a large variation in chromosome number, which indicates a high rate of genomic reorganization during karyotype evolution in this genus.

In addition to the A chromosome complement, some species have extra chromosomes, known as B chromosomes [Camacho et al., 2000]. B chromosomes have been reported in several species of Hymenoptera [Imai, 1974; Imai et al., 1977; Palomeque et al., 1990; Werren, 1991; Costa et al., 1992; Araújo et al., 2000; Stouthamer et al., 2001; Lopes et al., 2008; Barth et al., 2011; Gokhman et al., 2014b], but until now, they have not been observed in the family Vespidae. Since they are not part of the A chromosome complement, these chromosomes are cited as dispensable, because they generally neither possess major effect genes nor recombine with the normal chromosome complement [Beukeboom, 1994; Jones, 1995]. They follow a non-mendelian inheritance model and are present in approximately 15% of eukaryotes [Camacho et al., 2000]. Most B chromosomes are heterochromatic and composed of repetitive DNA sequences, which vary in both repeat type and copy number [Camacho et al., 2000].

Due to the scarcity of data for the family Vespidae and the importance of cytogenetics in different areas, this study characterizes the *Polybia fastidiosuscula* karyotype, reporting the occurrence of a B chromosome. In addition, we investigated the chromatin composition of this species using C-banding, staining with base-specific fluorochromes, and physical mapping of microsatellites and 18S rDNA, providing information that may contribute to the understanding of karyotype evolution in this genus.

Table 1. Colonies of *Polybia fastidiosuscula*, collection sites, geographical coordinates, and diploid number

Colony	Location	Geographic coordinates	Diploid number
1	Viçosa, MG	20°45′31.3″S 42°52′05.5″W	2n = 34
2	Palotina, PR	24°17′03.7″S 53°50′27.3″W	2n = 34+1B
3	Viçosa, MG	20°48′07.3″S 42°51′30.5″W	2n = 34
4	Viçosa, MG	20°45′31.3″S 42°52′05.5″W	2n = 34+1B

Materials and Methods

Four colonies of *P. fastidiosuscula* were collected from Minas Gerais (MG) and Paraná (PR) states, Brazil (Table 1). Adult individuals from each colony were identified by Dr. Orlando Tobias Silveira, from the Emilio Goeldi Museum, Belém, Pará. The vouchers were deposited in the scientific collection of the Apiário Central da Universidade Federal de Viçosa, MG. In order to obtain mitotic metaphase chromosomes, we used prepupal brain ganglia according to Imai et al. [1988]. The brain ganglia underwent treatment with 1% sodium citrate containing 0.005% colchicine for 90 min. Conventional staining was performed with Giemsa (5% in Sörensen buffer). For visualization of the content and distribution of heterochromatin, the C-banding method proposed by Sumner [1972] was used, with modifications (0.2 N HCl for 6 min, 5% barium hydroxide for 8 min, 2× SSC at 60°C for 2 min).

In order to investigate the chromatin composition, sequential staining with DAPI and CMA_3 fluorochromes was performed following the protocol of Schweizer [1980] and fluorescence in situ hybridization (FISH) according to Pinkel et al. [1986]. For FISH, microsatellite probes $(TAT)_{10}$, $(GA)_{15}$, $(GAG)_{10}$, $(CGG)_{10}$, $(CAA)_{10}$, $(TTAGG)_6$, and $(TCAGG)_6$ were used (labeled with Cy3 at the 5′ end during synthesis; Sigma, St. Louis, MO, USA) and an 18S rDNA probe obtained by PCR amplification using 18S F (5′-GTCATATGCTTGTCTCAAAGA-3′) and R (3′-TCTA-ATTTTTTCACAAGAAACGC-5′) rDNA primers [Pereira, 2006]. This probe was labeled by the indirect method using digoxigenin-11-dUTP (Roche, Mannheim, Germany) during amplification. Probe detection was performed with anti-digoxigenin-rhodamine (Roche Applied Science), and the slides were mounted in Fluoroshield with DAPI (Sigma, F6057).

We analyzed 20 individuals per colony (1 slide per individual) and 10 metaphases per slide using the microscope Olympus BX-60 coupled with a QColor3 Olympus® imaging system for Giemsa and C-band photos, and the Olympus BX53 epifluorescence microscope with the Olympus DP73F camera using Cell Sens Imaging software for DAPI/CMA_3 and FISH. Chromosomes were mea-

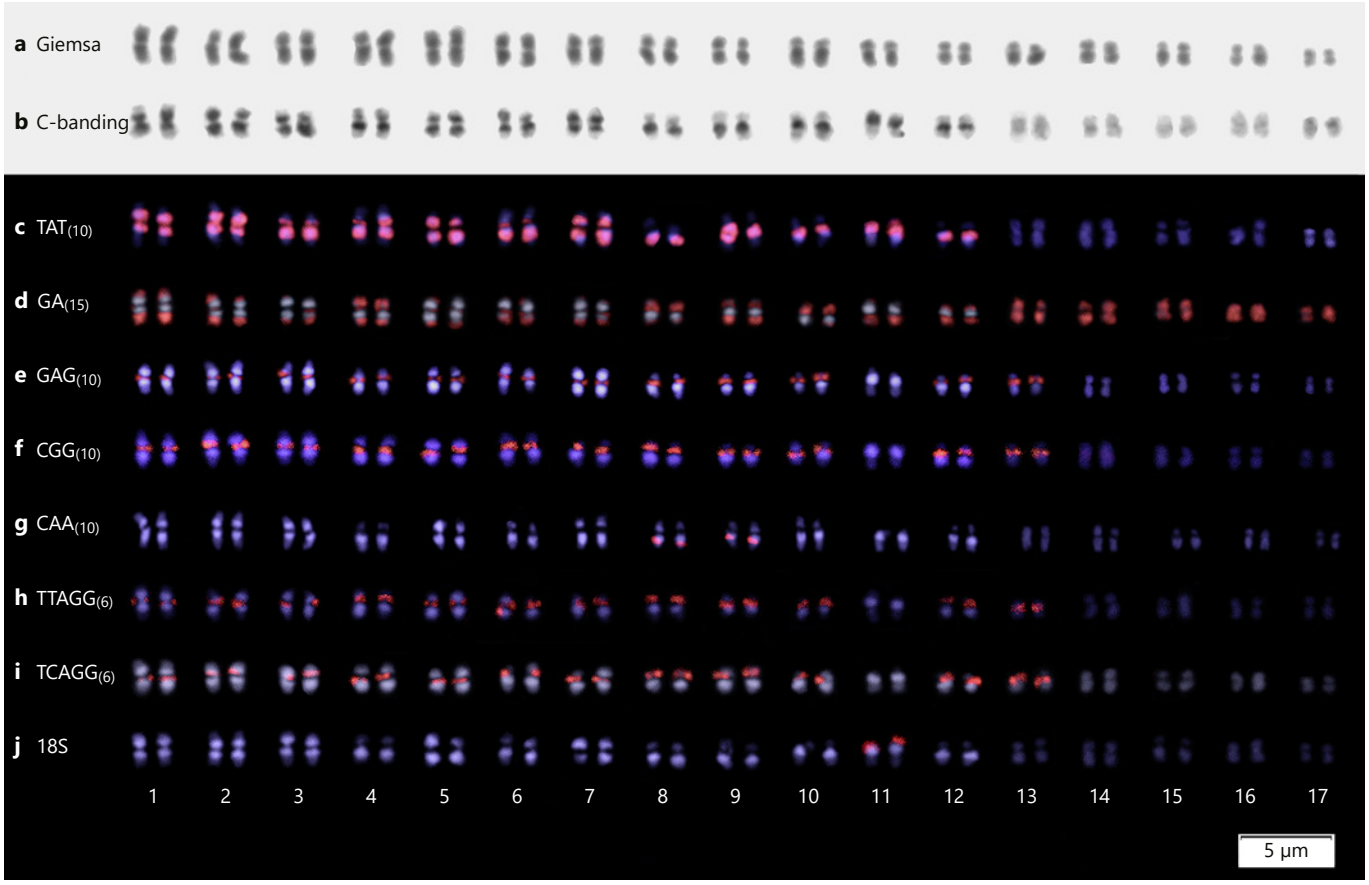

Fig. 1. Female karyotype of *Polybia fastidiosuscula*. **a** Giemsa staining. **b** C-banding. **c–i** FISH with microsatellites $(TAT)_{10}$ (**c**), $(GA)_{15}$ (**d**), $(GAG)_{10}$ (**e**), $(CGG)_{10}$ (**f**), $(CAA)_{10}$ (**g**), $(TTAGG)_6$ (**h**), and $(TCAGG)_6$ (**i**). **j** FISH with 18S rDNA. Chromosomes are stained with DAPI (blue), and hybridized probe signals are in red.

sured using the Image-Pro Plus 4.5 software, arranged according to size, and classified based on the long (q) and short (p) arm ratio index as metacentric (M), submetacentric (SM), and subtelocentric (ST) [Levan et al., 1964].

Results

All analyzed colonies of *P. fastidiosuscula* presented 2n = 34 chromosomes and a karyotype formula 2K = 30M + 2SM + 2ST . In 2 colonies, the presence of 1 B chromosome (2n = 34 + 1B) was observed (Fig. 1, 2). The B chromosome was not morphologically classified due to the absence of a visible centromere. In colony 2 (Palotina, PR), the B chromosome was present in 31% of the analyzed individuals, and in colony 4 (Viçosa, MG), it was present in 68% of the analyzed individuals, with no variation within each individual.

Twelve chromosome pairs had heterochromatin in the centromeric and/or pericentromeric regions, 5 chromosome pairs were euchromatic (Fig. 1b, 2b), and the B chromosome was completely heterochromatic (Fig. 2b).

The distribution of microsatellites varied across chromosomes. The TAT microsatellite showed positive signals in the pericentromeric regions of 12 chromosome pairs coinciding with heterochromatic regions (Fig. 1c, 2c). The B chromosome was also positively labeled by this probe, but was not marked by any other probe utilized (Fig. 2). The CAA microsatellite marked the heterochromatic regions of the major arms of only 2 chromosome pairs (pairs 8 and 9) (Fig. 1g, 2g).

The microsatellite GA was found in the euchromatic regions of all chromosomes, constituting the terminal regions of 12 pairs and the entire length of 5 pairs of chromosomes (Fig. 1d, 2d).

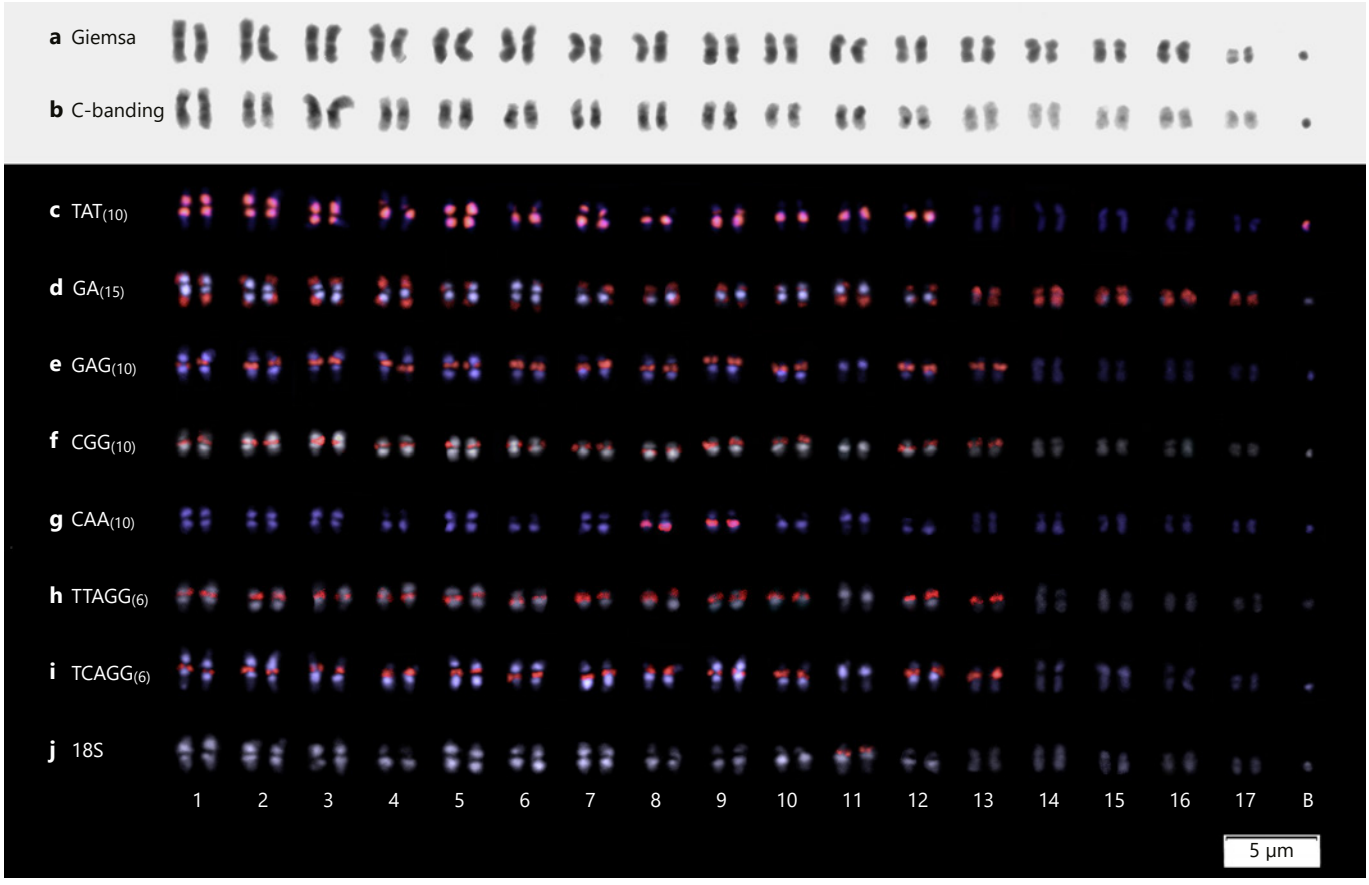

Fig. 2. Female karyotype of *Polybia fastidiosuscula* with the presence of a B chromosome. **a** Giemsa staining. **b** C-banding. **c–i** FISH with microsatellites $(TAT)_{10}$ (**c**), $(GA)_{15}$ (**d**), $(GAG)_{10}$ (**e**), $(CGG)_{10}$ (**f**), $(CAA)_{10}$ (**g**), $(TTAGG)_6$ (**h**), and $(TCAGG)_6$ (**i**). **j** FISH with 18S rDNA. Chromosomes are stained with DAPI (blue), and hybridized probe signals are in red.

Microsatellites GAG, CGG, TTAGG, and TCAGG marked the centromeric regions of 12 chromosome pairs, in which 11 pairs were heterochromatic and one euchromatic (Fig. 1e, f, h, i; 2e, f, h, i). The 18S rDNA was located near the centromeric region of chromosome pair 11 (Fig. 1j, 2j).

Both DAPI and CMA_3 fluorochromes marked heterochromatic regions, however, at different locations (Fig. 3). Both DAPI and the TAT microsatellite marked the pericentromeric regions of 12 chromosomal pairs and the B chromosome (Fig. 3). Positive CMA_3 markings were observed in the centromeric regions of 12 pairs of chromosomes, as well as the GAG, CGG, TTAGG, and TCAGG microsatellites (Fig. 3). We did not observe variations in the results obtained for the 4 colonies analyzed with any of the techniques, except for the presence of the B chromosome in 2 of the colonies.

Discussion

In this study, we describe the *P. fastidiosuscula* karyotype and the presence of a B chromosome (2n = 34 + 1B), increasing the number of species of *Polybia* with karyotypic description from 8 to 9. The species karyotypes in this genus show a wide variation regarding the chromosome number (n = 5 to n = 27) [Pompolo and Takahashi, 1987, 1990; Menezes et al., 2014]. This variation also occurs in the tribe Epiponini and suggests a high rate of genomic reorganization during karyotype evolution [Menezes et al., 2014]. Despite numerical variation, chromosome morphology does not show large variations in Epiponini, with most pairs classified as metacentric [Pompolo and Takahashi, 1987, 1990; Menezes et al., 2014], as also observed in *P. fastidiosuscula*.

In 2 of the colonies analyzed, we observed the presence of a B chromosome. This chromosome was identified by

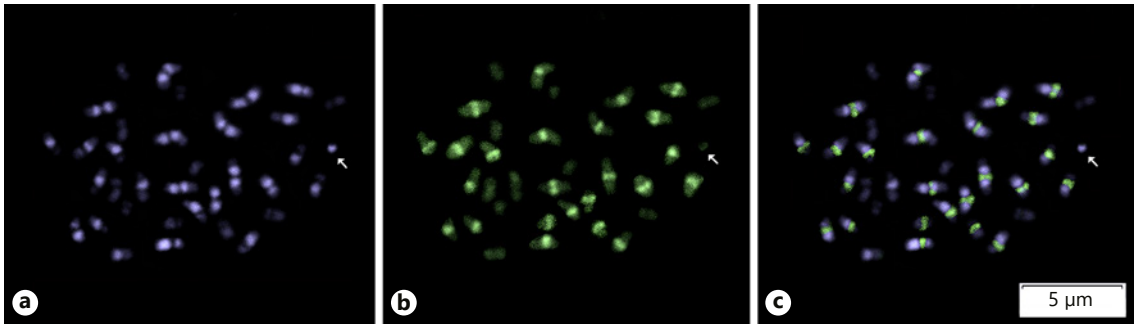

Fig. 3. Mitotic metaphase chromosomes of a female *Polybia fastidiosuscula* stained with DAPI (**a**), CMA_3 (**b**), and DAPI/CMA_3 (**c**). DAPI- and CMA_3-positive regions show bright fluorescence. Arrows indicate the B chromosome.

its peculiar characteristics such as small size in relation to the A chromosome complement, variation in frequency (not present in every individual of each population), absence of homologues, and completely heterochromatic composition. Such characteristics are common to B chromosomes [see Beukeboom, 1994; Camacho et al., 2000; Houben et al., 2014].

B chromosomes are reported in thousands of animal species, plants, and fungi [reviewed in Houben et al., 2014]. In Hymenoptera, B chromosomes have already been recorded in ants [Imai, 1974; Imai et al., 1977; Palomeque et al., 1990], bees [Costa et al., 1992; Lopes et al., 2008; Barth et al., 2011], and parasitoids of the families Pteromalidae, Sphecidae, Trichogrammatidae, and in certain Eulophidae [Werren, 1991; Araújo et al., 2000; Stouthamer et al., 2001; Gebiola et al., 2012; Gokhman et al., 2014b]. However, this is the first record for Vespidae.

The presence of the B chromosome varied between *P. fastidiosuscula* colonies (from 0 to 1) and within the colonies itself (31% of individuals in colony 2 and 68% of individuals in colony 4), but was uniformly observed within individuals. Variations in the frequency and quantity of B chromosomes have already been reported in other Hymenoptera species, such as *Partamona helleri* (0–4 B chromosomes), *Partamona cupira* (0–1 B chromosome), *Trypoxylon albitarse* (0–2 B chromosomes), *Podomyrma adelaidae* (0–7 B chromosomes), and *Pnigalio gyamiensis* (0–6 B chromosomes) [Imai et al., 1977; Costa et al., 1992; Araújo et al., 2000; Marthe et al., 2010; Gokhman et al., 2014b]. Such variations arise because their transmission follows a non-mendelian inheritance, adopting their own evolutionary and species-specific pathways [Camacho et al., 2000]. Additionally, this B chromosome was present in *P. fastidiosuscula* colonies collected in different regions in Brazil (MG and PR states), showing that its presence is not restricted to a geographical location and, despite the distance (1,300 km), gene flow must be occurring at the same time throughout its distribution.

In general, B chromosomes are composed of noncoding DNA and are not essential for the normal development of species [reviewed in Houben et al., 2014]. However, in the parasitic wasps *Nasonia vitripennis* [Werren, 1991] and *Trichogramma kaykai* [Stouthamer et al., 2001], the presence of the B chromosome, called PSR (paternal sex ratio), causes a change in the population's sex ratio [Werren, 1991]. In *T. albitarse* it is known that the B chromosome is undergoing a process of integration into the A genome [Araújo et al., 2001]. The effects of the B chromosome on *P. fastidiosuscula* are still unknown and to address this problem, detailed cytogenetic, fitness, and physiological studies and behavioral analysis of this species are necessary.

Regarding the chromatin composition, only 1 microsatellite was found in euchromatic regions. Generally, microsatellites have been found in euchromatic regions of insect chromosomes, for example, in locusts [Ruiz-Ruano et al., 2015], bees [Piccoli et al., 2018; Santos et al., 2018; Travenzoli et al., 2019], and ants [Barros et al., 2018]. However, our results show that most of the microsatellites analyzed are located in heterochromatic regions in *P. fastidiosuscula*, suggesting that they can be found in the entire chromatin depending on the species [Milani and Cabral-de-Mello, 2014].

The heterochromatin in *Polybia* presented different compositions of microsatellites, one rich in repetitive DNA sequences TAT and CAA observed in the pericentromeric region, and the other rich in CGG, GAG, TTAGG, and TCAGG, identified in the centromeric region of most chromosomal pairs. The distribution of these microsatellites in the centromeric region suggests that such sequences may play an important role in the organizational and

functional structure of the centromere and, consequently, in the genome of this species [Raskina et al., 2008]. The centromeric regions of chromosomes are formed by a highly heterogeneous composition of repetitive DNA, usually poorly conserved among species [Tyler-Smith and Floridia, 2000], and even within species. In sugarcane, the repetitive DNA sequences from the SCEN family (consisting of 140-bp repetitive units) are found at the centromere of certain chromosomes [Nagaki et al., 1998]. This may explain why these microsatellite sequences were not found in all *P. fastidiosuscula* centromeres.

The TTAGG sequence is part of the telomere of many insects [Vítková et al., 2005], but exceptions have been observed [Mravinac et al., 2011]. According to Frydrychová et al. [2004], TTAGG is an ancestral sequence found in telomeres of insects, but it has been lost over evolutionary time. The TTAGG telomeric repeat has been found in the lower Hymenoptera [Gokhman and Kuznetsova, 2018], suggesting its ancestral nature for the order. This motif is generally lost in Apocrita (= higher Hymenoptera) [Gokhman et al., 2014a; Menezes et al., 2017], but independently reappeared in the superfamilies Formicoidea and Apoidea (ants and bees) [Sahara et al., 1999; Lorite et al., 2002; Korandová et al., 2014; Pereira et al., 2018; Travenzoli et al., 2019].

An alternative telomeric sequence found in some insects, for example, is the TCAGG repeat in some beetles of the families Tenebrionidae, Mycetophagidae, and Meloidae [Mravinac et al., 2011]. Distribution of the TTAGG and TCAGG telomeric repeats among insects is discussed in detail in a recent review [Kuznetsova et al., 2020]. Our data show that these 2 sequences, TTAGG and TCAGG, are present in *P. fastidiosuscula* chromosomes, but not in the telomeres. These sequences were found in the centromeric regions of most chromosomes, so we believe they are not telomeric sequences in this species, but only repetitive ones that occur at the centromeres of some chromosomes. One possibility is that they may have been ancestral telomeric sequences that were lost during evolution through rearrangements, such as fusions and inversions, and are now restricted to the centromeric region. During the rearrangements, the telomeric sequence may have been replaced by another unknown sequence.

The number and location of 18S rDNA sites in *P. fastidiosuscula* coincides with ribosomal gene markings in 3 species of the same tribe, *Metapolybia decorata*, *M. docilis*, and *M. cingulata* [Menezes et al., 2013, 2014], indicating a conserved chromosomal localization in the tribe. The only heterochromatic pair not labeled by the CGG, GAG, TTAGG, and TCAGG microsatellites in the centromeric region was the pair labeled by the 18S rDNA probe. However, that region may have been lost, because it is often formed by a highly heterogeneous composition of repetitive DNAs, even between chromosomes of a particular species [Tyler-Smith and Floridia, 2000].

DAPI and CMA_3 fluorochromes show regions rich in AT and GC base pairs, respectively, and, in *P. fastidiosuscula*, both were observed in the heterochromatin. The sites rich in AT base pairs coincided with heterochromatic regions, including the B chromosome, indicating that the composition of the B chromosome is similar to the A-chromosome heterochromatin, suggesting its possible origin. The CMA_3^+ bands were observed in the centromeric heterochromatic regions, as well as the GAG, CGG, TTAGG, and TCAGG microsatellites. These CMA_3^+ markers differed from other *Polybia* species analyzed and were similar to *Metapolybia* species [Menezes et al., 2013, 2014], highlighting a common occurrence of this trait between genera. Moreover, CMA_3^+ regions in Epiponini are involved in different types of chromosomal rearrangements, indicating intense evolutionary dynamics of these regions [Menezes et al., 2014].

Our data indicate homology between the heterochromatic composition of the B chromosome and the A-chromosome complement. Based on positive DAPI and TAT markings, we show that the B chromosome and A-chromosome complement of *P. fastidiosuscula* have the same heterochromatic composition. There are 2 major hypotheses of origin of B chromosomes: intraspecific (originated as by-products of rearrangements occurring between A chromosomes) and interspecific (originated from A chromosomes of related species through hybridization) [Camacho et al., 1997, 2000; Araújo et al., 2000]. The combination of these 2 hypotheses to explain the emergence of B chromosomes has also been proposed [Li et al., 2017]. Therefore, we suggest that the B chromosome of *P. fastidiosuscula* originated through fragmentation of a heterochromatic portion (pericentromeric region) of an A chromosome, following the hypothesis of intraspecific origin. Corroborating results were found in fish (*Steindachnerina insculpita*) [Sampaio et al., 2015], bees (*Melipona rufiventris*) [Lopes et al., 2008], and wasps (*T. albitarse*) [Araújo et al., 2000]. Considering that genetic flow occurs between populations of *P. fastidiosuscula*, the origin of the B chromosome is possibly the same for the 2 colonies studied.

Following the hypothesis of intraspecific origin, it is likely that the TAT microsatellite accumulated before the appearance of the B chromosome. During an unstable cell division, the fragmentation of a heterochromatic pericentromeric region of an A chromosome may have generated

a small B chromosome. Mitotic and meiotic analyses in fish species showed that the small size of B chromosomes can reflect instability during cell divisions [Sampaio et al., 2015]. This chromosomal fragmentation may have been a recent event since the B chromosome still has the same heterochromatic characteristics (presence of the TAT microsatellite) as the A chromosome that produced it.

In this study, we describe the *P. fastidiosuscula* karyotype with the presence of a B chromosome, and we suggest its intraspecific origin based on its heterochromatic composition. In addition, our study provides data on the organization of microsatellite sequences in the *P. fastidiosuscula* genome, highlighting the existence of different heterochromatic compositions. We also verified that the TTAGG and TCAGG microsatellites are not telomeric sequences in *P. fastidiosuscula*. The next step is to use a larger number of species to understand the distribution patterns of these microsatellites in *Polybia* and thus to track the karyotype evolution in the genus.

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Statement of Ethics

Ethical approval is not required for this type of research.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

P.M. and D.M.L. designed the study, collected the samples and performed experiments and analyses; L.A.O.C. contributed reagents/analytic tools. All authors wrote, read, and approved the final manuscript.

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