Chromosome Evolution in the Genus *Partamona* (Apidae: Meliponini), with Comments on B Chromosome Origin

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Keywords

Banding patterns · Cytogenetic markers · Molecular phylogeny · Stingless bee · Supernumerary chromosomes

Abstract

The genus *Partamona* includes 33 species of stingless bees, of which 11 were studied cytogenetically. The main goal of this study was to propose a hypothesis about chromosomal evolution in *Partamona* by combining molecular and cytogenetic data. Cytogenetic analyses were performed on 3 *Partamona* species. In addition, the molecular phylogeny included mitochondrial sequences of 11 species. Although the diploid number was constant within the genus, $2n = 34$, B chromosomes were reported in 7 species. Cytogenetic data showed karyotypic variations related to chromosome morphology and the amount and distribution of heterochromatin and repetitive DNA. The molecular phylogenetic reconstruction corroborated the monophyly of the genus and separated the 2 clades (A and B). This separation was also observed in the cytogenetic data, in which species within each clade shared most of the cytogenetic characteristics. Furthermore, our data suggested that the B chromosome in the genus *Partamona* likely originated from a common an-

cestor of the species that have it in clade B and, through interspecific hybridization, it appeared only in *Partamona rustica* from clade A. Based on the above, *Partamona* is an interesting genus for further investigations using molecular mapping of B chromosomes as well as for broadening phylogenetic data.

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Introduction

Partamona Schwarz, 1939 is a stingless bee genus that is found from southern Mexico to southern Brazil, in both forest and urban environments [Pedro and Camargo, 2003]. These bees show great uniformity in their morphological features; therefore, the species have been identified by a combination of characteristics, such as the shape of teeth in the mandible, integument coloring, and the color of hair and wings [Pedro and Camargo, 2003]. The attributes of the nest entrance and nesting substrate have also been used to identify species in this genus [Camargo and Pedro, 2003].

The homogeneity reported for *Partamona* species is not restricted to morphological and behavioral traits, and

the genus has frequently been described as a group of bees with a stable karyotype. The same diploid number ($2n = 34$) was found in all studied *Partamona* species, *P. ailyae*, *P. chapadicola*, *P. cupira*, *P. helleri*, *P. mulata*, *P. nhambi-quara*, *P. peckolti*, *P. rustica*, *P. seridoensis*, *P. sooretamae*, and *P. vicina* [Brito et al., 1997, 2003, 2005; Brito-Ribon et al., 1999; Marthe et al., 2010; Tosta et al., 2014; Gonçalves et al., 2020; Lopes et al., 2020]. Numerical changes in the karyotype have been reported regarding the presence of supernumerary chromosomes in *P. cupira*, *P. helleri*, and *P. rustica*, based on conventional cytogenetic analyses [Brito et al., 1997; Marthe et al., 2010; Martins et al., 2013, 2014; Tosta et al., 2014] as well as in *P. gregaria*, *P. criptica*, *P. seridoensis*, and *P. chapadicola*, using a molecular RAPD-SCAR marker [Correia et al., 2014; Tosta et al., 2014; Machado et al., 2016].

Despite the karyotype constancy, differences in the heterochromatin distribution, DAPI/CMA₃, and 18S rDNA patterns were observed among *Partamona* species [Brito et al., 2003, 2005; Marthe et al., 2010; Martins et al., 2014; Gonçalves et al., 2020; Lopes et al., 2020]. Differences in cytogenetic banding patterns have also been detected in other insect groups with the same diploid number [Gokhman and Kuznetsova, 2006], as well as in amphibians [Kasahara et al., 2003], fish [Vicari et al., 2008], plants [Hasterok et al., 2006], and mammals [Labaroni et al., 2014], contributing to taxonomic and systematic classification.

Chromosome analysis data, combined with DNA sequence information, have resulted in the establishment of patterns in chromosome evolution based on phylogenetic hypotheses within different taxonomic groups, such as ants [Cardoso et al., 2014, 2018; Micolino et al., 2019] and wasps [Paladino et al., 2015; Fusu, 2017; Menezes et al., 2017]. The available molecular phylogenetic inference regarding the genus *Partamona* is not well resolved and includes only a few species [Rasmussen and Cameron, 2010], most of which do not have available cytogenetic data. Therefore, there is a gap in knowledge regarding the phylogenetic evolutionary scenario in this genus, covering representatives of species with and without B chromosomes.

The goal of this study was to propose a phylogenetic hypothesis for chromosome evolution in the genus *Partamona*. For this, we surveyed several aspects of chromosome features in 13 *Partamona* species, 3 analyzed in the present paper, including chromosome number and morphology, presence/absence of B chromosomes, differential banding patterns (C- and DAPI/CMA₃ bandings), and mapping of 18S rDNA clusters. The molecular

phylogeny included 11 *Partamona* species, which allowed us to make new comments about the origin and evolution of the B chromosome in this genus.

Materials and Methods

Biological Material

Larvae of 3 *Partamona* species were collected to carry out the cytogenetic analyses: *P. gregaria* from Novo Progresso, Pará, *Partamona auripennis* from Redenção, Pará, and *P. rustica* from Lontra, Minas Gerais. Adult workers of 11 *Partamona* species were collected for molecular analyses (online suppl. Table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000520552). Adult individuals of *Paratrigona* sp. 1, *Geotrigona* sp. 1, *Geotrigona* sp. 2, *Trigona* sp. 1, *Trigona* sp. 2, and *Melipona quadrifasciata* were included as outgroups. Voucher specimens were deposited at the Laboratory of Molecular Biology of Insects of the Universidade Federal de Viçosa, Minas Gerais, Brazil.

Cytogenetic Analyses

Chromosomes were obtained from the brain ganglion of the larvae in the last instar stage [Imai et al., 1988]. After Giemsa staining, the chromosome number of each individual was determined, and chromosome morphology was defined according to the chromosome arm ratios proposed by Levan et al. [1964]. C-banding was performed according to Sumner [1972]. The metaphases were photographed under an Olympus BX-60 microscope coupled with a Q-Color3 camera.

Fluorescence in situ hybridization (FISH) was performed according to Pinkel et al. [1986]. The 18S rDNA probe was obtained by PCR amplification using 18S F (5'-GTCATATGCTTGTCTCAAAGA-3') and 18S R (3'-TCTAATTTTTCAAAGTAAACGC-5') primers, labeled with digoxigenin-11-dUTP (Roche Applied Science), and the signal was detected with anti-digoxigenin-rhodamine (Roche Applied Science). Staining with chromomycin A₃/4',6-diamidino-2-phenylindole (CMA₃/DAPI) fluorochromes was conducted following the methods described by Schweizer [1980]. The results of the fluorescence techniques were photographed with an Olympus BX-53 epifluorescence photomicroscope coupled to an Olympus XM10 camera and assembled using the Adobe Photoshop® 21.1.1 image-editing program. The idiograms of the karyotypes were drawn using Easy Idio software [Diniz and Melo, 2006].

Molecular Analyses

Total DNA was extracted according to the method described by Waldschmidt et al. [1997]. Partial sequences of the cytochrome oxidase subunit 1 (COI) and cytochrome B (CytB) genes were amplified using the COIdegF (TAAACTTCWGGATGWCCAAAAAATCA) and COIdegR (TATCWACHAATCATAAAAAATMTTGG) primers and CytbF (TCGTTTAATTCAAYATAAATGGAGC) and CytbR (ATTACWCCTCCTAATTTATTWGGGAAT) primers, respectively [Batalha-Filho et al., 2010]. Sequences were deposited in GenBank with accession numbers OK636162–OK636200.

Consensus sequences from the forward and reverse strands of each sample were assembled using the CodonCode Aligner package v.5 (Copyright, 2002–2015 CodonCode Corporation). The sequences of each gene fragment were separately aligned in the pro-

Fig. 1. Giemsa-stained karyotypes of the *Partamona* species. **a** *P. gregaria*. **b** *P. auripennis*. **c** *P. rustica*. Chromosome arm ratios are shown in parentheses. Scale bar, 10 μ m.

gram Mega v6.06 [Tamura et al., 2013] using the MUSCLE method, and the data were concatenated into a single matrix using the SequenceMatrix program [Vaidya et al., 2011].

Bayesian inference was performed online using the CIPRES Science Gateway server [Miller et al., 2010] with the program MrBayes v.3.2.1 [Ronquist et al., 2012]. Two independent runs were used, each with 4 Monte Carlo Markov Chain (MCMC) replications. For this, the mixed model was implemented [Ronquist and Huelsenbeck, 2003] with an invariant ratio and Gamma correction for the 2 gene fragments. For the tree generated from the concatenated data, 12,000,000 generations of the MCMC were used with trees sampled every 1,000 generations. The convergence of the Markov chains was verified using the program Tracer v.1.5 [Rambaut and Drummond, 2009]. Twenty-five percent of the initial MCMC generations were excluded, and the remainder was used to calculate a consensus tree. The frequency of each branch in the majority consensus represented the value of the PP (posterior probability) of the Bayesian formula. The tree resulting from the analysis was edited in FigTree v. 1.3.1 [Rambaut, 2009].

Results

Karyotype Analyses

The diploid number determined for the 3 analyzed species was $2n = 34$, without the presence of B chromosomes. Male individuals ($n = 17$) were found in *P. gregaria*. The karyotype formulae were $2k = 28m + 6sm$ in *P. gregaria*, $2k = 20m + 12sm + 2st$ in *P. auripennis*, and $2k = 28m + 6sm$ in *P. rustica* (Fig. 1). In *P. gregaria*, the heterochromatin was restricted to the centromeric and pericentromeric regions (Fig. 2a), whereas it was ob-

served along the short arm of all chromosomes of *P. auripennis* (Fig. 2b). In *P. rustica*, heterochromatin was distributed in the centromeric and pericentromeric regions, as well as along one chromosome arm, with less intense staining (Fig. 2c).

18S rDNA clusters were observed in 6 chromosomes of *P. gregaria* (Fig. 2d), 2 chromosomes of *P. auripennis* (Fig. 2e), and 4 chromosomes of *P. rustica* (Fig. 2f). In addition, CMA₃⁺ markings were identified in 6 chromosomes of *P. gregaria*, 2 chromosomes of *P. auripennis*, and 4 chromosomes of *P. rustica*, whereas DAPI⁺ regions overlapped with the heterochromatin regions (online suppl. Fig. S1).

An idiogram of the species' karyotypes was drawn to facilitate visualization of cytogenetic patterns (Fig. 3a). Although *P. auripennis* was not sampled for molecular analysis, its cytogenetic patterns are characteristic of clade A.

Molecular Analyses

The mean partial sequence sizes of the *COI* and *CytB* genes were 593 and 598 bp, respectively. Phylogenetic reconstruction, based on the *COI* and *CytB* sequences, confirmed the monophyly of the genus *Partamona* (online suppl. Fig. S2) and showed the division into 2 clades (A and B). Clade A was formed by the species *P. ailyae*, *P. rustica*, *P. mulata*, *P. testacea*, and *P. vicina*, and clade B included the species *P. chapadicola*, *P. seridoensis*, *P. cupira*, *P. helleri*, *P. gregaria*, and *P. nhambiquara* (Fig. 3b).

Fig. 2. *Partamona* metaphases subjected to C-banding and FISH using 18S rDNA. **a, d** *P. gregaria*. **b, e** *P. auripennis*. **c, f** *P. rustica*. The arrows indicate hybridization signals. Scale bars, 10 μ m (**a–c**) and 5 μ m (**d–f**).

Discussion

Cytogenetic information available for *Partamona* currently comprises data of 13 karyotyped species out of the 33 species described in this genus. The diploid number of *P. gregaria*, *P. auripennis*, and *P. rustica* ($2n = 34$) was similar to the other species in this genus (Table 1), as well as in the closely related Meliponini [reviewed in Cunha et al., 2021a], confirming the chromosome number conservatism in this group of bees. On the other hand, variations in chromosome morphology among species are related to cytogenetic rearrangements that do not alter the diploid number.

The minimum interaction theory has been proposed to explain karyotype evolution in the Hymenoptera order [Imai et al., 1988, 1994; Hoshiba and Imai, 1993]. According to this theory, chromosomes pass through fission events that increase the number of smaller chromosomes, with a concomitant increase in heterochromatin, which would lead to a decrease in deleterious interactions during the cell cycle. Considering that *Partamona* species and closely related Meliponini genera share the same diploid number ($2n = 34$), fission events and, consequently, increases in the number of chromosomes would not be the main model of karyotype evolution as predicted by

the minimum interaction theory. Recently, alternative hypotheses have proposed a handful of rearrangements to explain karyotype evolution in the Meliponini tribe [Tavares et al., 2017; Travenzoli et al., 2019; Cunha et al., 2021b].

Additionally, the C-banding, CMA₃⁺, and 18S rDNA markings revealed other differences in *Partamona* karyotypes, showing that they are not homogeneous in their chromatin composition (Table 1). These differences allowed the separation of the 2 clades, which species shared most of the cytogenetic characteristics, suggesting that they may have followed different evolutionary pathways. This division corroborated the molecular phylogeny (Fig. 3).

Phylogenetic reconstruction based on *COI* and *CytB* sequences confirmed the monophyly of the genus *Partamona* [Camargo and Pedro, 2003; Rasmussen and Cameron, 2010]. However, the phylogenetic relationships inferred by molecular data differed from those based on morphological data and did not corroborate the previous morphological groups [Camargo and Pedro, 2003; Pedro and Camargo, 2003]. We associated the 2 clades observed in the molecular phylogeny with the available cytogenetic literature (Table 2) to comment on the origin and evolution of B chromosomes in this genus.

Fig. 3. a Idiograms of the heterochromatin and CMA₃⁺/18S rDNA distribution patterns in *Partamona*. **b** Concatenated Bayesian phylogeny of the *COI* and *CytB* genes showing the division of *Partamona* species into clades A and B.

The supernumerary chromosomes are regarded as parasitic elements that accumulate in the host genome, regardless of possible negative impacts on fitness [reviewed in Camacho, 2005]. They do not follow mendelian

segregation, and there are 2 possible scenarios for their origin: (1) intraspecific origin from the A chromosome complement of the host species, or (2) interspecific origin as a result of hybridization of different species [Camacho

Table 1. Cytogenetic data on diploid number, heterochromatin patterns, and number of chromosomes marked with CMA₃ and 18S rDNA probe available for the genus *Partamona*

Species	2n	Heterochromatin distribution	CMA ₃ ⁺ chromosomes, <i>n</i>	18S carrying chromosomes, <i>n</i>	References
Cupira group					
<i>P. ailyae</i>	34	Along one chromosomal arm	8	2	Brito-Ribon et al., 1999; Gonçalves et al., 2020
<i>P. cupira</i>	34	–	7	–	Marthe et al., 2010
<i>P. helleri</i>	34	Centromeric and pericentromeric regions, and a few terminal blocks	5 to 12	6 to 8	Brito et al., 1997, 2005; Martins et al., 2009, 2013; Gonçalves et al., 2020
<i>P. mulata</i>	34	Along one chromosomal arm	2	–	Brito-Ribon et al., 1999
<i>P. rustica</i>	34	Along one chromosomal arm, with chromosomes showing 3 color patterns	4	4	Present study
<i>P. sooretamae</i>	34	Along one chromosomal arm, with some completely euchromatic chromosomes	16	2	Gonçalves et al., 2020
Nigrior group					
<i>P. auripennis</i>	34	Along one chromosomal arm	2	2	Present study
<i>P. chapadicola</i>	34	Along one chromosomal arm, with chromosomes showing 3 color patterns	4	6	Lopes et al., 2020
<i>P. seridoensis</i>	34	Blocks mainly in the pericentromeric regions, and a few terminal blocks	8	7	Brito et al., 2005
Musarum group					
<i>P. gregaria</i>	34	Centromeric and pericentromeric regions	6	6	Present study
<i>P. vicina</i>	34	Along one chromosomal arm	2	–	Brito-Ribon et al., 1999
Bilineata/epiphytophila group					
<i>P. nhambiquara</i>	34	Along one chromosomal arm, with chromosomes showing 3 color patterns	4	4	Brito-Ribon et al., 1999; Lopes et al., 2020
<i>P. peckolti</i>	34	Pericentromeric regions of all chromosomes	1	–	Brito et al., 2003
The species were separated into groups based on the morphological proposal of Pedro and Camargo [2003].					

et al., 2000]. Due to a lack of homology with the A complement in *P. helleri* [Martins et al., 2013] and the presence of the same genetic marker (RAPD-SCAR) in other *Partamona* species [Correia et al., 2014; Machado et al., 2016], the B chromosome in this genus is believed to have been recently transferred through several interspecific hybridizations [Tosta et al., 2014] or, alternatively, originated in the common ancestor of the species that share the homology of the B RAPD-SCAR sequence [Machado et al., 2016].

Our phylogenetic analyses allowed us to shed some light on this issue. Within clade A, only *P. rustica* had B chromosomes, whereas most species of clade B had these extra elements, except *P. nhambiquara* (Table 2). Therefore, not all species with B chromosomes group together in the same clade, which does not corroborate the common ancestor hypothesis [Machado et al., 2016]. Instead of several hybridizations [Tosta et al., 2014], one interspecific hybridization could explain its presence only in *P. rustica* from clade A, which shares the same RAPD-SCAR

Table 2. B chromosome information for the *Partamona* species analyzed in the molecular phylogeny

Clade	Species	B chromosomes ^a	References
A	<i>P. ailyae</i>	B ⁻ cytogenetic B ⁻ SCAR sequence	Brito-Ribon et al., 1999 Correia et al., 2014; Machado et al., 2016
	<i>P. rustica</i>	B ⁺ cytogenetic B ⁺ SCAR sequence	Correia et al., 2014; Tosta et al., 2014; Machado et al., 2016
	<i>P. mulata</i>	B ⁻ cytogenetic B ⁻ SCAR sequence	Brito-Ribon et al., 1999 Correia et al., 2014; Machado et al., 2016
	<i>P. vicina</i>	B ⁻ cytogenetic B ⁻ SCAR sequence	Brito-Ribon et al., 1999 Correia et al., 2014; Machado et al., 2016
B	<i>P. nhambiquara</i>	B ⁻ cytogenetic B ⁻ SCAR sequence	Lopes et al., 2020 Correia et al., 2014
	<i>P. gregaria</i>	B ⁻ cytogenetic B ⁺ SCAR sequence	Present study Correia et al., 2014; Machado et al., 2016
	<i>P. chapadicola</i>	B ⁻ cytogenetic B ⁺ SCAR sequence	Lopes et al., 2020 Correia et al., 2014; Machado et al., 2016
	<i>P. seridoensis</i>	B ⁻ cytogenetic B ⁺ SCAR sequence	Brito et al., 2005 Correia et al., 2014; Machado et al., 2016
	<i>P. cupira</i>	B ⁺ cytogenetic B ⁺ SCAR sequence	Marthe et al., 2010 Correia et al., 2014; Tosta et al., 2014; Machado et al., 2016
	<i>P. helleri</i>	B ⁺ cytogenetic B ⁺ SCAR sequence	Correia et al., 2014; Tosta et al., 2014; Machado et al., 2016

^a Presence (B⁺) or absence (B⁻) of B chromosomes based on cytogenetic or RAPD-SCAR sequence data.

sequence with clade B species. The overall cytogenetic data of this species also share some resemblance with clade B (Fig. 3a). On the other hand, the origin of the supernumerary chromosomes in clade B can be traced to the common ancestor of the species, except for *P. nhambiquara* which do not have B chromosomes. Further investigations are needed to clarify the polytomy between *P. gregaria* and *P. nhambiquara*, and to confirm the pleiomorphic condition of the absence of B chromosomes in this clade.

We observed a discrepancy regarding the presence of B chromosomes in some *Partamona* species between the cytogenetic and RAPD-SCAR data (Table 2). The non-mendelian segregation of the B chromosomes and their presence in low numbers in most of the species' populations could explain this discrepancy of B⁻ with conventional cytogenetics and B⁺ when we expand the sampling with the RAPD-SCAR sequence. Since this molecular marker was originally designed for *P. helleri*, further cytogenetic analyses should investigate its cytological presence in other *Partamona* species to rule out the hypoth-

esis that it is present in the A chromosomes of some B-lacking species.

Bee chromosome evolution has traditionally been considered stable because of the apparent high karyotype conservatism of several taxa. However, the presence of variation, particularly structural alterations in repetitive DNA distribution patterns, has challenged this idea. Unlike the morphological and behavioral characteristics that are not very informative in systematic studies in the genus *Partamona*, the cytogenetic data are congruent with the molecular information, thus providing potential sources of phylogenetic information for this group of bees.

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Statement of Ethics

Ethical approval is not required for this type of research.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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Author Contributions

Study conception and design: C.M.N., A.F., and D.M.L. Data collection and analyses: C.M.N., M.S.C., A.F., and H.A.W. Supervision: L.A.O.C. and D.M.L. Literature review and first draft of the manuscript: C.M.N. and M.S.C. All authors commented on previous versions and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article or its supplementary material files. Further inquiries can be directed to the corresponding authors.

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