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Cytogenetic survey of eight ant species from the Amazon rainforest

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Abstract. The scarce information regarding ant diversity in the state of Amapá and lack of cytogenetic data of species from the Amazon region can hide ant biodiversity information that may be detectable with affordable cytogenetic techniques. In this study, we describe the karyotypes of eight ant taxa collected from Amazonian localities in French Guiana and Brazil. Chromosome numbers ranged from $2n = 18$ to $2n = 68$. The following chromosome numbers were observed for each species: *Azteca* sp. group *chartifex* $2n = 28$; *Dolichoderus bidens* (Linnaeus, 1758) $2n = 18$; *Gnamptogenys tortuolosa* (Smith, 1858) $2n = 44$; *Camponotus renggeri* Emery, 1894 $n = 20$; *Pseudomyrmex unicolor* (Smith, 1855) $2n = 68$ and $n = 34$; *Apterostigma* sp. *pilosum* complex $2n = 46$; *Odontomachus bauri* Emery, 1892 $2n = 44$, and *Wasmannia auropunctata* (Roger, 1863) $2n = 32$. The karyotypes of *P. unicolor*, *G. tortuolosa*, and *O. bauri* are reported here for the first time. Our data enabled comparisons between chromosomal data of some species from Amazon and Atlantic rainforests. We also highlight the methods used for the ant chromosome classification.

Keywords: karyotype, chromosome evolution, biodiversity, Formicidae, Neotropics, taxonomy.

INTRODUCTION

The classical cytogenetic approach utilizes a single dye, orcein or Giemsa (Liehr 2017), without previous trypsin-treatment, for the study of chromosomes and has also been denoted as beta karyology by White (reviewed by Petitpierre 2009). The low cost of classical cytogenetics allows more extensive sampling and plays a vital role in the discovery and understanding of diver-

sity in different organisms (Zacharopoulou *et al.* 2017; Di-Nizo *et al.* 2017; Cioffi *et al.* 2018).

In hymenopteran cytogenetics, chromosomes can be obtained from live larvae using a stereomicroscope and chemicals, even from distant localities such as those in Amazonia. The technique provided by Imai *et al.* (1988) enables the use of artisanal procedures with rustic material such as empty pill packs to keep the ganglia in hypotonic solution and syringes for their dissociation on the slides. Important taxonomic insights may be achieved from karyotype information and, according to Schubert (2011), efforts must be made to avoid losing such data. The resolution of sampling issues is particularly important in population-level approaches for understanding taxonomic problems (Petitpierre 2011; Cioffi *et al.* 2018; Chèvre *et al.* 2018). To date, classical cytogenetic studies are routinely performed for many organisms (Petitpierre 2009; Liehr 2017), thus supporting the accuracy and validity of their results.

Karyotype configuration can be useful for species delimitation, as karyotypes with structural and/or numerical differences may not pair properly during meiosis (King 1993). This kind of chromosomal variation can affect fertility in heterozygotes and, in extreme cases, lead to sterility caused by gamete aneuploidy. Remarkable examples of chromosome number distinctness in closely related species or within the same species have been reported. For instance, in the Cervidae species *Muntiacus muntjak* (Zimmermann, 1780), females possess $2n = 6$ chromosomes and males possess $2n = 7$, and *Muntiacus reevesi* (Ogilby, 1839) has a distinct chromosomal organization of $2n = 46$ (Wurster and Benirschke 1970). Recent examples of intraspecific chromosomal variations in ants have been observed from different populations within the species. For instance, different cytotypes have been found in *Holcoponera striatula* (Mayr, 1884) (as *Gnamptogenys striatula*) ($2n = 32, 34$), *Holcoponera moelleri* Forel, 1912 (as *Gnamptogenys moelleri*) ($2n = 34, 44$) (Teixeira *et al.* 2020), and *Mycetophylax morschi* (Emery, 1888) ($2n = 26, 28, 30$) (Micolino *et al.* 2019).

Karyological information is currently available for approximately 800 species of ants distributed across the world (reviewed by Lorite and Palomeque 2010; Cardoso *et al.* 2018; Mariano *et al.* 2019). Neotropical ant species have been targeted for cytogenetic studies since the first surveys conducted by Crozier (1970) in South America, including Brazil, and by Góñi *et al.* (1983) in Uruguay. Pioneering studies in ant cytogenetics in Brazil were performed by Fadini and Pompolo (1996) and Mariano *et al.* (2000) and, since then, there has been a steady increase in the number of cytogenetic researches in ants using different approaches. Thus far, more than

180 ant taxa have been cytogenetically studied in the Neotropics, most of them from the Atlantic rainforest in Brazil (reviewed by Mariano *et al.* 2019). In the Amazonian region, karyological information is limited to that obtained from species restricted to French Guiana and Brazil (reviewed by Aguiar *et al.* 2020).

In this study, we describe the karyotypes of eight ant species from the Amazon rainforest using a comparative approach with available population data, as our contribution toward understanding the evolutionary pattern of ant diversity in the Neotropics.

MATERIALS AND METHODS

Ant colonies were collected by active search in French Guiana at Kourou and Sinnamary, and in Brazil at Oiapoque, state of Amapá and Açaílândia, state of Maranhão (Table 1). Adult voucher specimens were deposited into the ant collection at the Laboratório de Mirmecologia do Centro de Pesquisas do Cacau (CPDC/Brazil) in Bahia, Brazil, under records #5802, #5803, and #5816.

Mitotic chromosomes were obtained from the cerebral ganglia of the larvae after meconium elimination, as described by Imai *et al.* (1988). The chromosome number and morphology of metaphases were analyzed using conventional 4% Giemsa staining. Chromosomes were arranged in order of decreasing size and based on the ratio of the chromosome arm lengths ($r = \text{long arm}/\text{short arm}$), i.e., on the centromeric position, according to the classification proposed by Levan *et al.* (1964). The chromosomes were measured and classified as $m = \text{metacentric}$ ($r = 1-1.7$), $sm = \text{submetacentric}$ ($r = 1.7-3$), $st = \text{subtelocentric}$ ($r = 3-7$), and $a = \text{acrocentric}$ ($r > 7$). Chromosomes were organized using Corel Photopaint X3 and measured using Image Pro Plus.

Reflexions on the nomenclature used to classify ant chromosomes

Imai (1991) proposed a detailed chromosomal nomenclature based on heterochromatin location; however, a classification based on this type of chromatin is impractical because large (detectable) heterochromatic blocks are not present in many ant groups. Additionally, the use of chromosome measurements diminishes subjectivity and enables karyotype comparisons between populations or species.

Analysis of the karyotypes of *Acromyrmex* spp. (reviewed by Barros *et al.* 2021) using the nomenclature of Levan *et al.* (1964) allowed for the detection of dissimilarities in the karyotypic formula caused by the

Table 1. Ant species collected from the Amazon rainforest and analyzed using classical cytogenetics. Collection sites, sample sizes (numbers of colonies/individuals), diploid (2n) and haploid (n) chromosome numbers, and karyotype formula.

Ant species	Locality	Col./Ind.	2n (n)	Karyotype formula 2n / (n)
Subfamily Dolichoderinae				
<i>Azteca</i> sp. group <i>chartifex</i>	La Montagne des Singes, Kourou, FG	1/8	28	10m + 4sm + 6st + 8a
<i>Dolichoderus bidens</i> (Linnaeus, 1758)	Chácara du Rona, Oiapoque-AP, BR	2/11	18	14m + 4sm
Subfamily Ectatomminae				
<i>Gnamptogenys tortuolosa</i> (Smith, 1858) *	Sinnamary, FG	1/4	44	12m + 17sm + 15st
Subfamily Formicinae				
<i>Camponotus rengersi</i> Emery, 1894	Campus Agronomique, Kourou, FG	1/4	(20)	(2sm + 17st + 1a)
Subfamily Myrmicinae				
<i>Apterostigma</i> sp. <i>pilosum</i> complex	La Montagne des Singes, Kourou, FG	2/6	46	6m + 18sm + 16st + 6a
<i>Wasmannia auropunctata</i> (Roger, 1863)	Chácara du Rona, Oiapoque-AP, BR	1/5	32	16m + 10sm + 6st
Subfamily Ponerinae				
<i>Odontomachus bauri</i> Emery, 1892 *	Açailândia-MA, BR	1/7	44	6sm + 24st + 14a
Subfamily Pseudomyrmecinae				
<i>Pseudomyrmex unicolor</i> (Smith, 1855) *	Campus Agronomique, Kourou, FG	2/5	68 (34)	56m + 12sm (56m+12sm) (28m + 6sm)

Abbreviations: * first cytogenetic report; BR = Brazil, FG = French Guiana; Brazilian states: AP = Amapá, MA = Maranhão.

variations in short arm size due to differential heterochromatin growth. Among the *Atta* spp., differences were not detected even with chromosome classification using chromosomal measurements (Barros *et al.* 2014), but variations could be identified by karyomorphometric comparison with the leaf-cutting ant *Amoimyrmex striatus* (Roger, 1863) (Cristiano *et al.* 2013). *Amoimyrmex striatus*, in addition to two other species, currently belongs to the new genus *Amoimyrmex* (Cristiano *et al.* 2020).

The nomenclature of Levan *et al.* (1964) is typically used for chromosomal classification of different organisms such as plants (Winterfeld *et al.* 2018; Sadeghian *et al.* 2019), spiders (Araújo *et al.* 2020), beetles (Şendoğan and Alpagut-Keskin 2016), bees (Lopes *et al.* 2021), wasps (Tavares and Teixeira 2021), velvet worms (reviewed by Duarte *et al.* 2020), and fishes (Brandão *et al.* 2018). Recent ant cytogenetic studies have focused on measurements of chromosomes (Barros *et al.* 2010, 2014, 2016; Cristiano *et al.* 2013, 2017, Santos *et al.* 2016, Micolino *et al.* 2019, 2020; Teixeira *et al.* 2020). We suggest the use of the standardized chromosomal nomenclature employing measurements described by Levan *et al.* (1964) in Formicidae as well as in Hymenoptera, thereby allowing for comparisons between the species and populations. We also suggest the use of less condensed chromosomes and care with centromeric location (primary constriction) to diminish subjectivity in chromosome measurements. This chromosome classification based on measurements

will also facilitate access to data on ant cytogenetics by researchers working on other organisms and could likely contribute to a better understanding of ant chromosomal diversity and evolution.

RESULTS AND DISCUSSION

We analyzed the chromosomes of eight ant species, eight genera, and six subfamilies. Our analysis presents the first karyological records for *Pseudomyrmex unicolor* (Smith, 1855), *Gnamptogenys tortuolosa* (Smith, 1858), and *Odontomachus bauri* Emery, 1892. Three species have already been described for the Atlantic rainforest, and showed karyotypic similarities. Unique karyotypes were detected in two different species complexes, suggesting genera revision.

Subfamily Dolichoderinae

Azteca sp. group *chartifex* presented 2n = 28, 10m + 4sm + 6st + 8a (Figure 1A). Previously, karyological data for only five taxa from the genus *Azteca* were available; four of these taxa were characterized as 2n = 28 and one, *Azteca alfari* Emery, 1893, as 2n = 26 (reviewed by Mariano *et al.* 2019). The karyotype of *Azteca chartifex* Emery, 1896 from French Guiana is 2n = 28, 10M + 18A (Mariano *et al.* 2019). If we group the chromosomes of *Azteca*

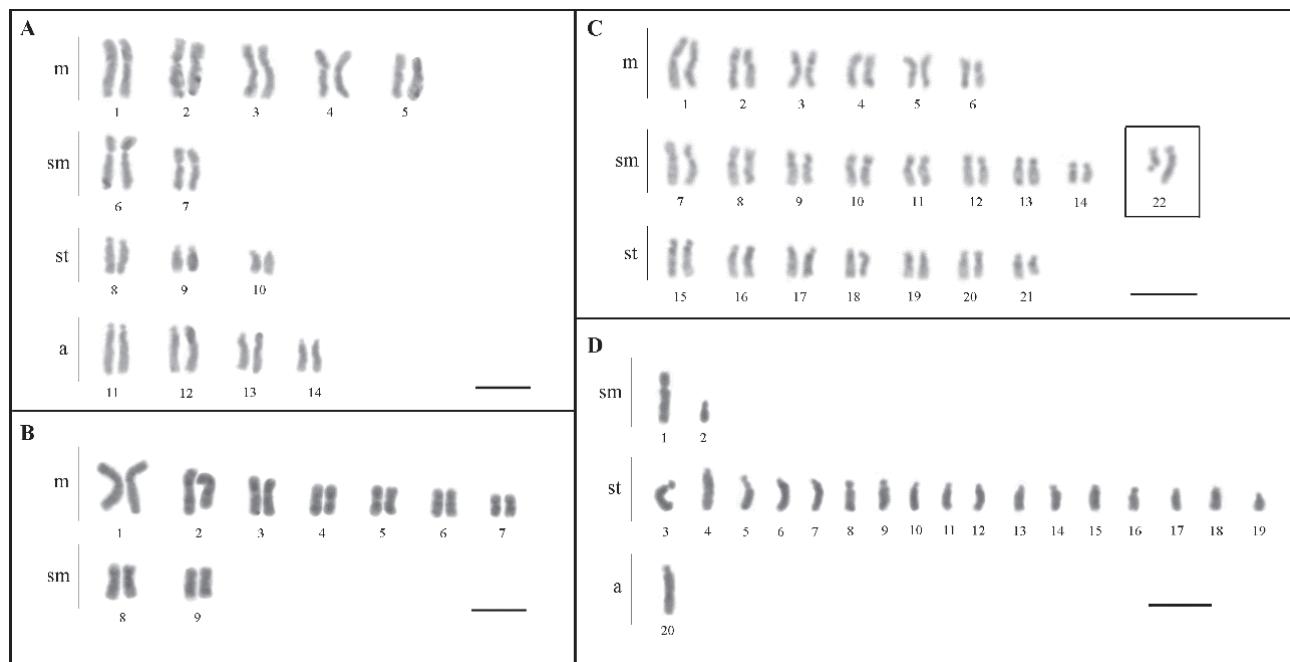


Figure 1. Karyotypes of ant species from subfamilies: Dolichoderinae - (A) *Azteca* sp. group *chartifex* ($2n = 28$), (B) *Dolichoderus bidens* ($2n = 18$); Ectatomminae - (C) *Gnamptogenys tortuolosa* ($2n = 44$); and Formicinae - (D) *Camponotus renggeri* ($n = 20$). Box in (C) show size heteromorphism of the long arm of pair 22 in *G. tortuolosa*, with one homologous submetacentric and the other subtelocentric chromosome. Scale bars = 5 μm .

sp. group *chartifex* from the present study into two categories, partially in accordance with Imai *et al.* (1988), as with *Az. chartifex*, the karyotypic formula is 14M + 14A. This seems to indicate differences in chromosome morphology between the two taxa, which corroborates the morphological data. Data from molecular cytogenetic studies may contribute to corroborate these two taxa.

Colonies of *Dolichoderus bidens* (Linnaeus, 1758) were found in carton nests built on the abaxial surface of leaves of the family Musaceae. The behavior of the workers was particularly aggressive. There are several records of *D. bidens* in French Guiana (Franco *et al.* 2019) and a single record in the neighboring Brazilian state of Amapá, in Serra do Navio, the center of the state (Kempf 1959). To date, there has been no report of *D. bidens* inhabiting areas between these regions, which are approximately 400 km apart.

Dolichoderus bidens showed a karyotype of $2n = 18$, 14m + 4sm (Figure 1B) in our study. Heterochromatic blocks around the centromeric/pericentromeric area of the chromosomes were identified (Figure 2A). Until now, the karyotype of *D. bidens* was only available for specimens collected in the Atlantic rainforest of Ilhéus, Bahia (Santos *et al.* 2016). Our results for the specimens collected from the Amazon rainforest showed similarities between these two rainforest populations, with subtle

variations due to measurement divergences. In contrast, in a recent study, *Dolichoderus imitator* Emery, 1894 showed remarkable karyotypic differences between the population from the Amazon rainforest ($2n = 46$) and that from the Atlantic rainforest ($2n = 38$) (Santos *et al.* 2016; Aguiar *et al.* 2020).

Subfamily Ectatomminae

Gnamptogenys tortuolosa, which is included in the Neotropical *sulcata* group, presented $2n = 44$, 12m + 17sm + 15st (Figure 1C). As observed previously by Imai (1991), using standard Giemsa staining, all chromosomes showed heterochromatic blocks restricted to the pericentromeric region and the short arms of subtelocentric pairs (Figure 2B). Cytogenetic data for 14 taxa of *Gnamptogenys* are available, including representatives of the *mordax*, *striatula*, and *rastrata* Neotropical groups (reviewed by Teixeira *et al.* 2020). This is the first chromosomal record for the *sulcata* group. The high chromosome number ($2n > 12$, according to Imai *et al.* 1994) and the high number of subtelocentric pairs with heterochromatin in the short arms suggest that centric fission rearrangements could have played an important role during the evolution of *G. tortuolosa*, as other spe-

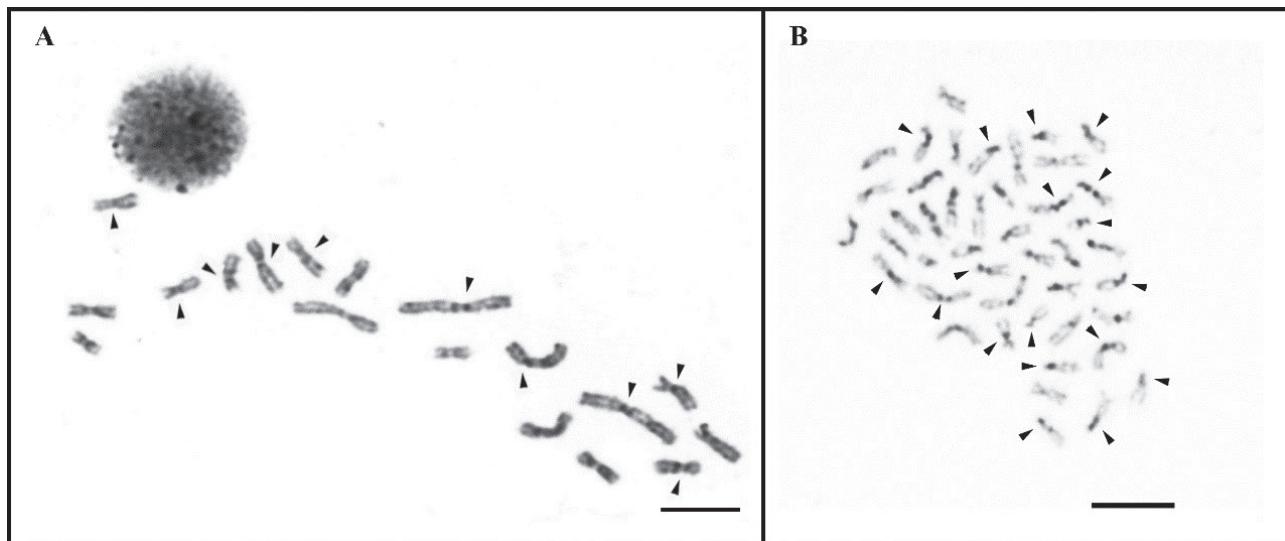


Figure 2. Metaphases showing heterochromatic blocks (arrowheads) via 4% Giemsa staining in (A) *Dolichoderus bidens* ($2n = 18$) on centromeric and pericentromeric regions and (B) *Gnamptogenys tortuolosa* ($2n = 44$) on pericentromeric regions of all chromosomes and short arms of subtelocentric pairs. Scale bar = 5 μ m.

cies of *Gnamptogenys* do not typically have a large number of chromosomes (Teixeira *et al.* 2020). Mariano *et al.* (2015) have proposed that centric fissions are important in the evolution of this genus and, although there are scarce cytogenetic data concerning the *sulcata* group, these fissions appear to play an important role in this group.

Heteromorphism involving the long arm of chromosome pair 22 was observed in *G. tortuolosa*, which resulted in differences in the morphology of homologous chromosomes, with one chromosome being submetacentric and the other subtelocentric (Figure 1C, box). The two chromosome variants are different in size and, therefore, processes that duplicate or delete chromatin could have been involved in the origin of this heteromorphism.

Subfamily Formicinae

The nest of *Camponotus renggeri* Emery, 1894 collected during the present study was found on fallen rotten wood. In Oiapoque, north of the state of Amapá, Brazil, we also observed underground nests, as previously reported by Ronque *et al.* (2016). It is important to note that it is rarer to find *C. renggeri* nests in rainforest areas than in savannah regions, including the Amazonian savannahs (Aguiar, Barros personal observation).

The colony of *C. renggeri* from the Amazon rainforest showed $n = 20$, $2sm + 17st + 1a$ (Figure 1D). Colonies from other localities, such as the Amazonian savannah

located at Macapá and the savannahs of Cerrado in the states of Mato Grosso (Aguiar *et al.* 2017) and Goiás (Vieira and Santana 2020), also showed $n = 20$ chromosomes. The presence of a secondary constriction on the short arm of a subtelocentric chromosome of medium size (pair 5) suggests the presence of rDNA clusters. Two chromosome-rDNA bearer pairs, a submetacentric pair and a subtelocentric pair of medium size, have previously been reported for this species (Aguiar *et al.* 2017). This is in contrast to that observed in the sister species *Camponotus rufipes* (Fabricius, 1775) and *Camponotus (Myrmotherix)* spp., which show a single submetacentric rDNA-bearer pair (Aguiar *et al.* 2017). Several chromosomal polymorphisms are associated with *Camponotus (Myrmotherix)* spp., but no variation was observed among the males analyzed in this study.

Subfamily Myrmicinae

Wasmannia auropunctata Roger (1863) presented $2n = 32$, $16m + 10sm + 6st$ (Figure 3A). Its karyotype showed the same chromosome number and similar morphology to that of the Atlantic rainforest population (Souza *et al.* 2011). Although Souza *et al.* (2011) used a different chromosome classification method (Imai 1991), without the use of chromosome measurements, the karyotype is similar to that obtained in this study, being possible to recognize all chromosome pairs. A chromosomal polymorphism was detected in ants from French Guiana (Aguiar *et al.* 2020, see Figure 5b, since the kar-

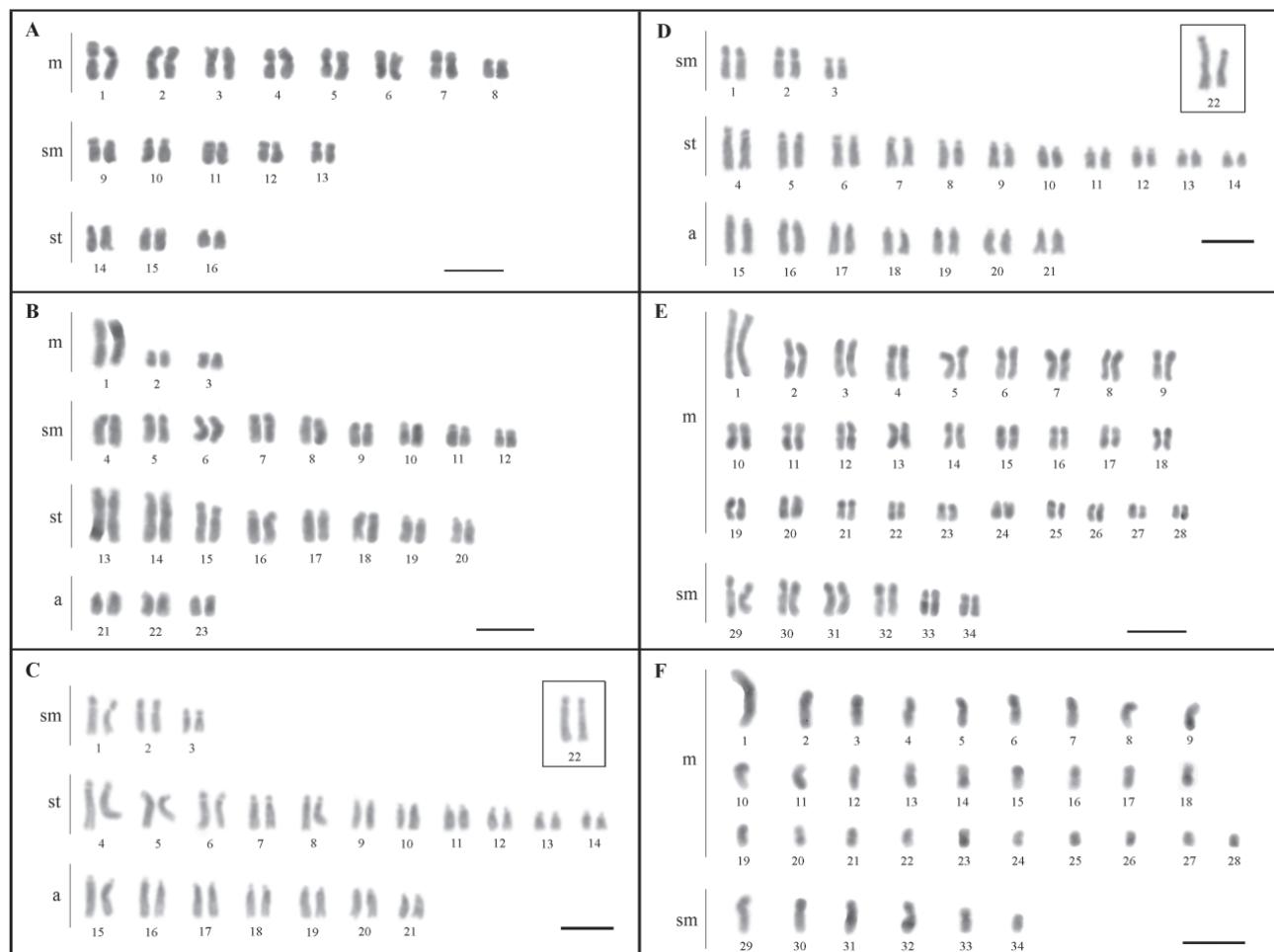


Figure 3. Karyotypes of ant species from subfamilies: Myrmicinae - (A) *Wasmannia auropunctata* ($2n = 32$), (B) *Apterostigma* sp. *pilosum* complex ($2n = 46$); Ponerinae - (C), (D) *Odontomachus bauri* ($2n = 44$); and Pseudomyrmecinae - (F), (G) *Pseudomyrmex unicolor* ($2n = 68$, $n = 34$). The boxes show polymorphism for subtelocentric chromosome pair 22 in *O. bauri*: (C) homozygous individual with small arms and (D) heterozygous individual with a distinctive large subtelocentric chromosome. Scale bars = 5 μ m

yotype is incorrectly written in Table 1); however, ants collected at Oiapoque, Brazil (about 200 km away) did not show karyotype variations. The comparison between the karyotypes of specimens from these two localities provided insights into the polymorphism observed in French Guiana. A submetacentric chromosome, which corresponds to the largest chromosome of the karyotype in ants from French Guiana, is absent in specimens from Oiapoque, so we can infer that this particular chromosome originated from a chromosomal rearrangement that need to be further investigated.

The *Apterostigma* sp. *pilosum* complex was characterized as $2n = 46$, $6m + 18sm + 16st + 6a$ (Figure 3B). The chromosome number among *Apterostigma* ranges from $2n = 20$ to $2n = 46$ (Mariano *et al.* 2019). The genus *Apterostigma* contains six taxa that have been cytoge-

netically analyzed, but only half of the species have been taxonomically described. The *Apterostigma pilosum* complex is composed of nine similar species and is considered to be taxonomically difficult to resolve (Lattke 1997). Some species were placed in synonymy of *Apterostigma mayri* Forel, 1893 by Weber (1958). *Apterostigma mayri* and *Apterostigma* sp. *pilosum* complex showed distinct chromosome numbers of $2n = 24$ and $2n = 46$, respectively, although both are included within the *pilosum* complex (Lattke 1997). The karyotypes with a lower chromosome number show more meta/submetacentric chromosomes when compared to species with higher chromosome numbers, including members of the *Apterostigma* sp. *pilosum* complex. This suggests that centric fission rearrangements seem to be a part of the chromosomal evolution of the genus *Apterostigma*. A taxon

from French Guiana showed a distinct and intermediate number of chromosomes ($2n = 32$) (Mariano *et al.* 2011) compared to that in the *Apterostigma* sp. described here. Cytogenetic data highlight the need for revision of the *pilosum* complex and the genus *Apterostigma*.

Subfamily Ponerinae

Odontomachus bauri showed $2n = 44$, $6sm + 24st + 14a$ (Figure 3C-D). This species is included in the *haematodus* group, and all the studied species have the same chromosome number, $2n = 44$ (reviewed in Santos *et al.* 2010). However, variations in chromosomal morphology exist among species and provide insights into the mode of karyotypic evolution in this group (Aguiar *et al.* 2020). Differential heterochromatin growth after centric fission events may have played a role in the chromosomal evolution of the *haematodus* group according to Imai *et al.* (1994). The *O. bauri* karyotype, according to the morphological variations due to heterochromatin growth on short arms, is derived within the *haematodus* group (see Aguiar *et al.* 2020) and corroborates the molecular phylogenetic position (Larabee *et al.* 2016).

The long arm of the second subtelocentric pair of *O. bauri* collected from the Amazon rainforest showed a size polymorphism that was observed in individuals of the same colony. Homozygous individuals harbored two smaller subtelocentric chromosomes (Figure 3C, box). Only heterozygous individuals showed a distinctive large subtelocentric chromosome (Figure 3D, box). No individuals with two large subtelocentric chromosomes were observed. This type of chromosome size polymorphism has been observed in several ant species (e.g., Barros *et al.* 2013; Teixeira *et al.* 2020) and can originate from unequal crossing-over or translocations that cause visible chromosomal deletions or duplications (Schubert and Lysak 2011; Barros *et al.* 2013).

Subfamily Pseudomyrmecinae

Pseudomyrmex unicolor has been reported from Serra do Navio in the state of Amapá (Kempf 1959); however, it was also reported by different researchers in French Guiana (Franco *et al.* 2019) highlighting the scarcity of myrmecological studies in the state of Amapá. *Pseudomyrmex unicolor* was characterized as having $2n = 68$, $56m + 12sm$ and $n = 34$, $28m + 6sm$ (Figure 3E, F); a similarly high chromosome number is present in *Pseudomyrmex gracilis* (Fabricius, 1804) ($2n = 70$) obtained from the Atlantic rainforest. Cytogenetic information is available for seven *Pseudomyrmex* spp. ranging from $2n$

= 24 to $2n = 70$ (Sposito *et al.* 2006). Despite having high chromosome numbers, only metacentric and submetacentric chromosomes were detected in *P. unicolor*. Polyploidy does not appear to be an important factor in the chromosomal evolution of ants (Lorite and Palomeque 2010) and, thus far, there is no evidence indicating polyploidization among *Pseudomyrmex* spp. (Tsutsui *et al.* 2008; Ardila-Garcia *et al.* 2010).

The presence of heterochromatin blocks on the short arms of chromosomes of *P. unicolor* suggests that the “heterochromatic growth” after centric fissions (Imai *et al.* 1994) occurred during the chromosomal evolution of this species. Although this process is not well understood (Hirai *et al.* 1994), it may involve distinct mechanisms that enlarge the size of heterochromatic blocks on the chromosomes, such as slippage saltatory amplification, which contributes to an increase in the amount of DNA; unequal crossing-over, which extends the heterochromatin among homologous regions; and also distribution by ectopic recombination among non-homologous chromosomes (Hirai 2020). The dispersion of rDNA on terminal regions indicates the involvement of different mechanisms (Hirai 2020). The increase in heterochromatin after chromosome fission has been previously suggested as a mechanism of chromosomal evolution in leaf-cutting ants of the genus *Acromyrmex* (Barros *et al.* 2016).

FINAL REMARKS

As there are few ant cytogenetic studies at the population level, we conducted the karyotypic analysis of some ant species from the Amazon rainforest and carried out a comparative analysis with the populations of the Atlantic rainforest to detect karyotypic similarities and dissimilarities between them. Despite its simplicity, classical cytogenetics can reveal chromosomal variations that may affect the ability of a species to generate fertile progeny. This study highlights the need for a taxonomic revision of the *Apterostigma pilosum* complex and the *Azteca chartifex* group. Structural variations provide insights into the chromosomal evolution responsible for the polymorphisms detected in this study in *W. auropunctata* and *O. bauri*, as well as the heteromorphism in *G. tortuolosa*.

GEOLOCATION INFORMATION

Ant colonies were collected from the following locations in French Guiana: *La Montagne des Sing-*

es, Kourou (5.07225, -52.69407), Campus Agronomique, Kourou (5.17312, -52.65480), and Sinnamary (5.28482, -52.91403). Colonies were collected in Brazil at Oiapoque, Amapá (3.84151, -51.84112), and Açaílândia, Maranhão (-4.84200, -47.29667) (Table 1).

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