



SHORT NOTE

Karyotypic Data of Five Ant Taxa from the Brazilian Atlantic Rainforest

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Abstract

The Brazilian Atlantic rainforest is an endangered biome and a biodiversity hotspot. Ant cytogenetic studies from this biome showed remarkable chromosomal diversity among species, and provided useful insights on phylogeny, chromosomal evolution, and taxonomy. In this study, we karyotyped five ant taxa from the Atlantic rainforest. The observed chromosome numbers were *Pheidole germani* 2n=22, *Pheidole* sp. *flavens* group 2n=20, *Brachymyrmex admotus* 2n=18, *Camponotus atriceps* 2n=40, and *Odontomachus haematodus* 2n=44. The data obtained for *Pheidole* spp. represent the first chromosomal records for the genus in Brazil and provide information on the chromosomal evolution of *Pheidole*. Karyotypic information from *B. admotus* brings the genus back to the cytogenetic scenario after decades of neglect. Similar karyotype was observed among the *C. atriceps* from different populations already studied, corroborating its status as a good species. Chromosomal variations were observed between populations of *O. haematodus*, which need to be investigated in further researches. This study increased the cytogenetic knowledge of ants from the Brazilian Atlantic rainforest.

The Brazilian Atlantic rainforest is a biodiversity hotspot with a notable number of endemic species; however, anthropogenic actions have led to the substantial reduction and fragmentation of most of this biome, and also the loss of this biodiversity (Morellato & Haddad, 2000; Paviolo et al., 2016). Many unknown species were, or may be extinct even before their description (Morellato & Haddad, 2000). In this scenario, cytogenetics is a key tool in discovering biodiversity of different organisms (Di-Nizo et al., 2017; Cioffi et al., 2018) and generates useful information that can be used in conservation strategies for endangered species (Mariano et al., 2008; Potter & Deakin, 2018). Considering ant communities, even with area regeneration strategies, only pristine native areas can maintain a substantial proportion of biodiversity (Silva et al., 2007).

Ants show a significant karyotype diversity and the variation in the chromosome number observed for the

Neotropical region is 2n=4 in *Strumigenys louisianae* Roger, 1863 to 2n=120 in *Dinoponera lucida* Emery, 1901 (reviewed by Mariano et al., 2019). Both species are from the Brazilian Atlantic rainforest, with *D. lucida* endemic and endangered of this biome. The minimum interaction theory (MIT), the most widely used in ants, proposes that centric fissions are the main rearrangements that explain this significant chromosomal variation among species (Imai et al., 1994). However, inversions, translocations, and Robertsonian fusions have also been important in the origin of numerical and structural chromosomal polymorphisms and chromosome evolution in ant species (reviewed by Lorite & Palomeque, 2010; Mariano et al., 2019; Teixeira et al., 2020; Micolino et al., 2020).

We described the karyotypes of five ant taxa, from four genus and three subfamilies, which live in the Brazilian Atlantic rainforest, suggesting insights on chromosomal evolution for some of these species. Additionally, a comparative



approach with other available populations was performed for *Camponotus atriceps* (Smith, 1858) and *Odontomachus haematodus* (Linnaeus, 1758).

Colonies were collected from the southeastern Brazilian Atlantic Forest in the cities of Viçosa, in the Horto Botânico of Universidade Federal de Viçosa, and in a rural area at Ubá (Table 1, Fig 1) (ICMBio/SISBIO accession number 32459). Adult vouchers were identified by Dr. Jacques Delabie and deposited in the ant collection at the Laboratório de Mirmecologia do Centro de Pesquisas do Cacau (CPDC/Brazil),

in Bahia, Brazil, under records #5815 and #5817. Mitotic chromosomes were obtained from the cerebral ganglia of larvae after meconium elimination, using hypotonic colchicine solution (0,005%) and fixatives according to Imai et al. (1988) and submitted to 4% Giemsa staining. Chromosomes were measured, arranged in order of decreasing size and classified as m=metacentric ($r=1-1.7$), sm=submetacentric ($r=1.7-3$), st=subtelocentric ($r=3-7$) and a=acrocentric ($r>7$), according to Levan et al. (1964). Chromosomes were organized using Adobe Photoshop® CS6 and measured using Image Pro Plus®.

Table 1. Ant species from the Brazilian Atlantic rainforest karyotyped in this study. Collection site, sample size (number of colonies/individuals), diploid chromosome number, and karyotypic formula.

Ant species	Locality	Coordinates	Col./Ind.	2n	Karyotypic formula
Subfamily Myrmicinae					
<i>Pheidole germaini</i> Emery, 1896*	Viçosa, Minas Gerais, Brazil	-20.757041, -42.873516	1/8	22	18m+2sm+2st
<i>Pheidole</i> sp. (<i>flavens</i> group)	Viçosa, Minas Gerais, Brazil	-20.757041, -42.873516	1/4	20	18m+2sm
Subfamily Formicinae					
<i>Brachymyrmex admotus</i> Mayr, 1887*	Viçosa, Minas Gerais, Brazil	-20.757041, -42.873516	1/6	18	16m+2sm
<i>Camponotus atriceps</i> (Smith, 1858)	Viçosa, Minas Gerais, Brazil	-20.757041, -42.873516	1/5	40	4sm+34st+2a
Subfamily Ponerinae					
<i>Odontomachus haematodus</i> (Linnaeus, 1758)	Ubá, Minas Gerais, Brazil	-21.128880, -42.937646	1/5	44	8sm+18st+18a

* first cytogenetic report

Pheidole germaini Emery, 1896 (*tristis* group) showed $2n=22$; $2K=18m+2sm+2st$ (Fig 2A). This is the first record of occurrence of *P. germaini* for the state of Minas Gerais since published records are restricted to the states of Mato Grosso and Mato Grosso do Sul (Guénard et al., 2017). *Pheidole* is a monophyletic hyperdiverse genus with over 1,100 species described on all continents (except Antarctica) and possibly originated in New World (Moreau, 2008; Bolton, 2020). Karyotype data are available for 75 taxa and most of them showed $2n=20$ with representatives in the Old and New World (reviewed by Lorite & Palomeque, 2010). *Pheidole spininodis* Mayr, 1887 and *Pheidole subarmata* Mayr, 1884 (as *P. cornutula*), also included in the *tristis* group, presented $2n=20$ metacentric chromosomes. The former has two larger metacentric pairs compared to the other chromosomes and the latter has three pairs (Goñi et al., 1983). *Pheidole germaini* showed only a single larger metacentric pair, in addition to one submetacentric and another subtelocentric pair, both of medium size. Considering the karyotype configuration observed in *P. germaini*, it is more likely that chromosomal fission involving the large metacentric pair resulted in two acrocentric pairs, according to MIT, increasing the chromosomal number from $2n=20$ to 22. Subsequent differential heterochromatin growth in the short arms of the acrocentric chromosomes seems to have originated a subtelocentric and a submetacentric pair observed in *P. germaini*.

Pheidole sp. (*flavens* group) revealed $2n=20$; $2K=18m+2sm$ (Fig 2B). The same karyotype was observed in *Pheidole dentigula* Smith, 1927 from Florida, USA, also included in the *flavens* group (Crozier, 1970). Further studies in Brazil will investigate karyotypic variations in this group and the understanding of chromosome evolution.

Brachymyrmex admotus Mayr, 1887 showed $2n=18$; $2K=16m+2sm$ (Fig 2C). *Brachymyrmex* is a monophyletic genus with 40 described species and taxonomically neglected due to small body size (3 mm), soft mesosoma, and superficially similar external morphology among species (Ortiz-Sepulveda et al., 2019). To date, only a single unidentified taxon of this genus from São Paulo, Brazil, was karyotyped (Crozier, 1970), showing this genus was also overlooked cytogenetically. The karyotype of *B. admotus* is similar to *Brachymyrmex* sp., despite that the latter species showed only metacentric chromosomes based in visual comparison (Crozier, 1970). In this study, mensuration of chromosome arms was performed making chromosomal classification more accurate. Considering the difficulties in species identification in *Brachymyrmex* due to their complex morphology and their extremely small size, molecular cytogenetics can bring important insights in taxonomy as observed in other ant species (Aguir et al., 2017; Micolino et al., 2019).

Camponotus (Myrmotherix) atriceps, exhibited $2n=40$; $2K=4sm+34st+2a$ (Fig 2D). Similar karyotypes were observed for individuals collected in the Atlantic rainforest at Ilhéus,

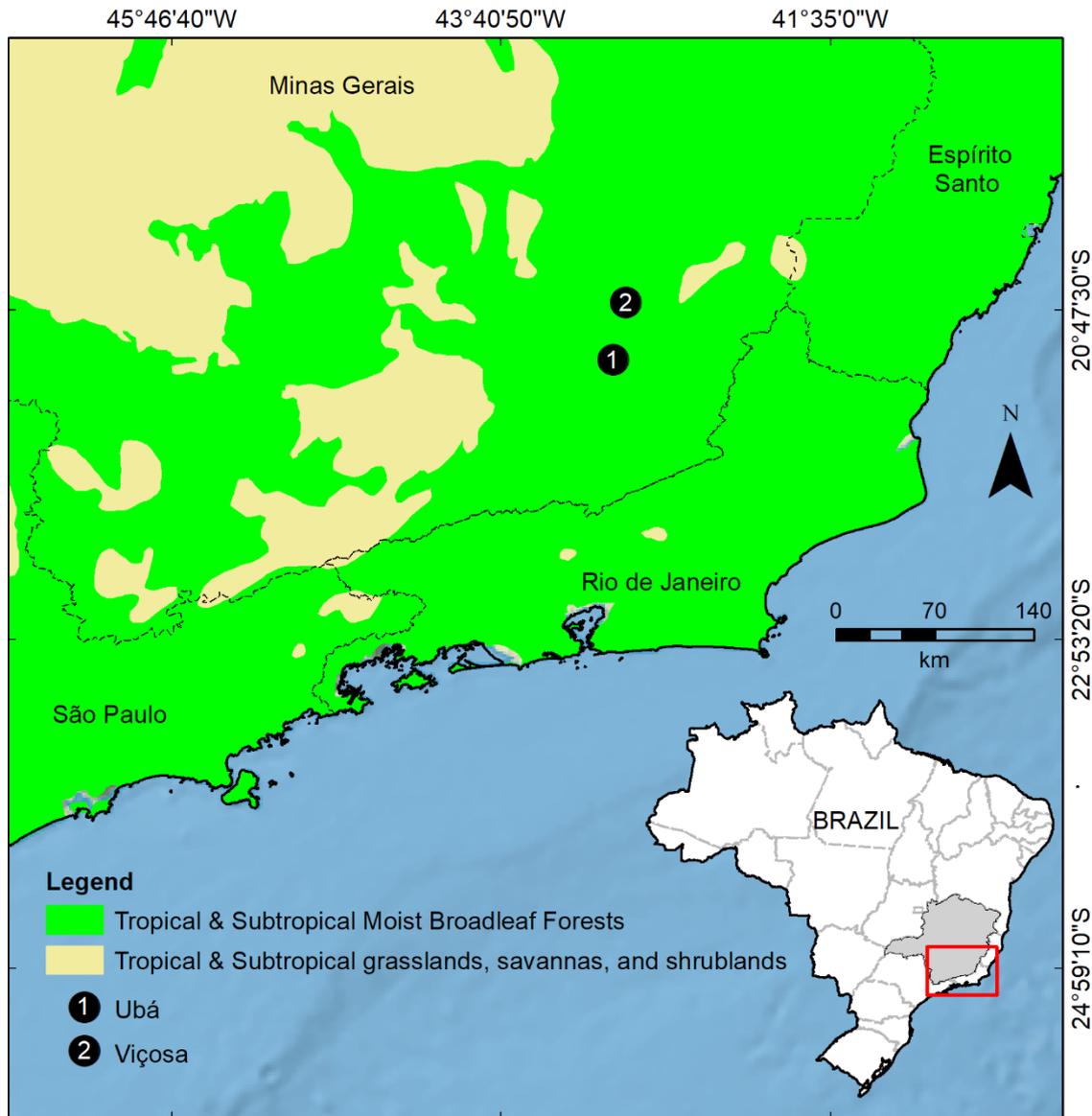


Fig 1. Map of the sampling localities of the ant taxa cytogenetically studied in this report from the Atlantic rainforest. Modified from Dinerstein et al. (2017).

Bahia (Mariano et al., 2001) and in the Brazilian Cerrado (Aguiar et al., 2017). Although Mariano et al. (2001) used a chromosomal classification different to the present study, it is possible to recognize the same chromosomal pairs in the two localities from the Atlantic rainforest. It suggested karyotype stability of *C. atriceps* since several chromosomal polymorphisms were observed for different populations of *Camponotus (Myrmothrix)* spp. (Aguiar et al., 2017). A secondary constriction was observed in the short arm of the second submetacentric pair of *C. atriceps* (Fig 2D, arrow), which possibly corresponds to the nucleolus organizing region. This region coincides with the location of GC-rich bands observed for the same species from Cerrado, and with ribosomal genes mapped for other *Camponotus* spp. (Aguiar et al., 2017).

Odontomachus haematodus presented $2n=44$; $2K=8sm+18st+18t$ (Fig 2E). The monophyletic genus *Odontomachus*

includes 74 species described (Larabee et al., 2016; Bolton, 2020), with 13 taxa studied cytogenetically (reviewed by Santos et al., 2010; Mariano et al., 2019). The karyotype formula varies among species, specifically, the number of acrocentric chromosomes, due to differential growth of heterochromatin chromosomal evolution of the genus (Aguiar et al., 2020). Although the karyotype formula is the same, variations were observed between the karyotype of *O. haematodus* from the Atlantic rainforest (present study) and the Amazon rainforest (Aguiar et al., 2020). The former has the first submetacentric pair with almost twice the size of the other submetacentric chromosomes, while the latter karyotype has all submetacentric pairs with a similar size. Duplications/deletions of chromosomal segments and other complex rearrangements may be involved in the origin of this difference.

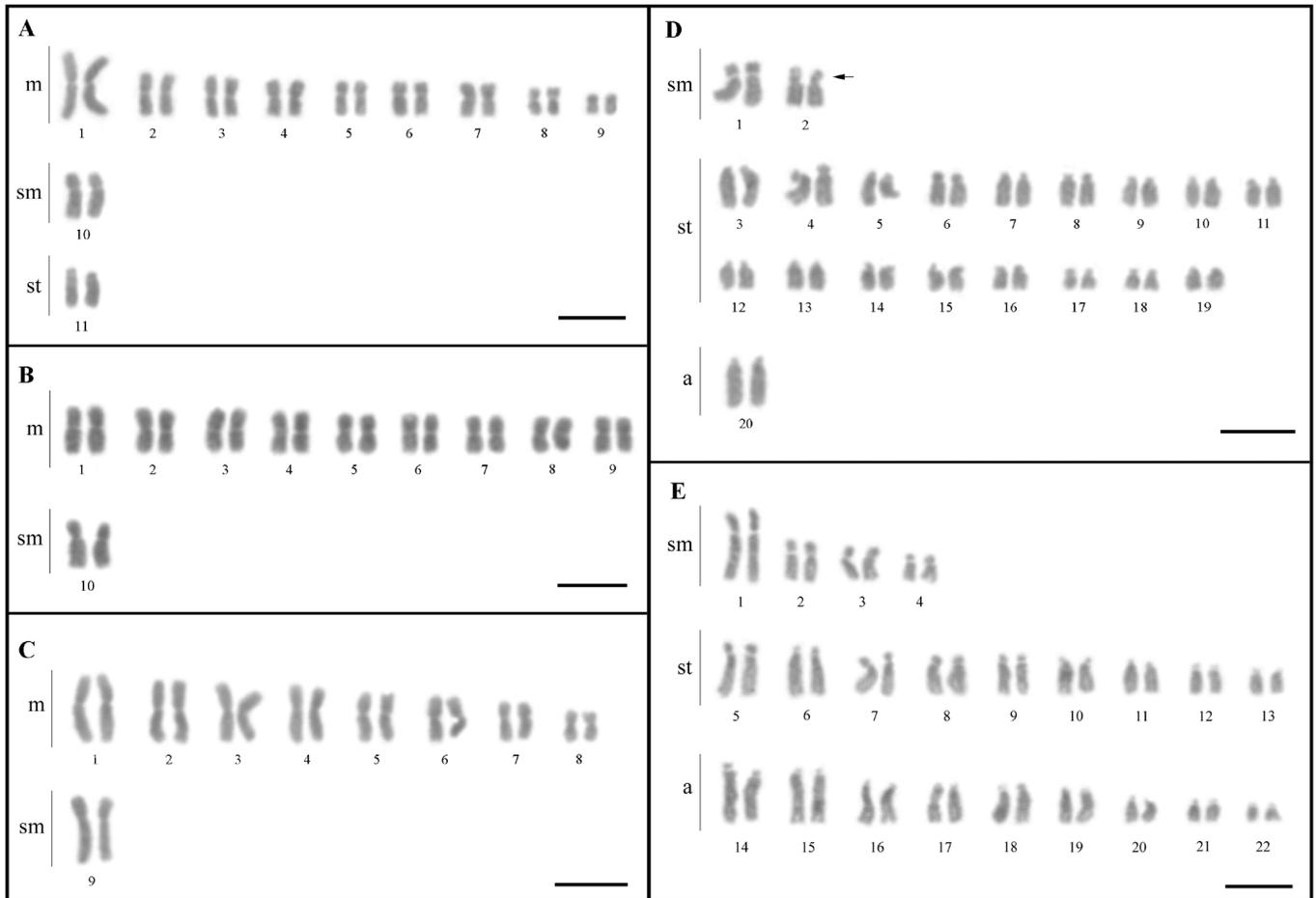


Fig 2. Female karyotypes of ant species from the Atlantic rainforest: (A) *Pheidole germaini* ($2n=22$), (B) *Pheidole* sp. (*flavens* group) ($2n=20$), (C) *Brachymyrmex admotus* ($2n=18$), (D) *Camponotus atriceps* ($2n=40$), and (E) *Odontomachus haematodus* ($2n=44$). The arrow in (D) indicates the secondary constriction in pair 2. Scale bars = 5 μ m.

In short, our data for *P. germaini* and *Pheidole* sp. *flavens* group represent the first chromosomal record for the genus in Brazil. Karyotypic information obtained for *B. admotus* enhances the chromosomal data for this genus. Concerning *C. atriceps*, our study shows a similar karyotype to that already published of another population of this ant, corroborating its status as a good species. The increase in the number of populations studied as well as the use of molecular cytogenetics will provide new insights into the origin of the chromosomal variation observed in *O. haematodus*. This study increased the cytogenetic knowledge of ants from the Atlantic rainforest. Cytogenetic studies concerning neglected ant genera or rare species, especially small ones, can bring novelties on ant biodiversity.

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Authors' Contribution

GA Teixeira - Conceptualization, methodology, resources, investigation, data curation, visualization and writing
 LAC Barros - Conceptualization, methodology, resources, investigation, data curation, visualization, writing, supervision, project administration and funding acquisition
 DM Lopes - Methodology, writing, visualization and funding acquisition
 HJAC Aguiar - Conceptualization, methodology, resources, investigation, visualization, writing, supervision, project administration and funding acquisition

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