Zoologischer Anzeiger 293 (2021) 273–281

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

Research paper

Cytogenetic studies on the social parasite *Acromyrmex ameliae* (Formicidae: Myrmicinae: Attini) and its hosts reveal chromosome fusion in *Acromyrmex*

Luísa Antônia Campos Barros [a, *], Hilton Jeferson Alves Cardoso de Aguiar [a], Gisele Amaro Teixeira [b], Danival José de Souza [c], Jacques Hubert Charles Delabie [d, e], Cléa dos Santos Ferreira Mariano [d, f]

[a] Universidade Federal do Amapá, Campus Binacional - Oiapoque, BR 156, n°3051 Bairro Universidade, 68980-000, Oiapoque, Amapá, Brazil
[b] Programa de Pós-graduação em Biologia Celular e Estrutural, Universidade Federal de Viçosa, Viçosa, MG, 36570-000, Brazil
[c] Universidade Federal do Tocantins, Campus Gurupi, 77410-530, Gurupi, TO, Brazil
[d] Laboratório de Mirmecologia, CEPEC/CEPLAC, Itabuna, BA, CP 7, 45600-000, Brazil
[e] Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, Ilhéus, BA, 45650-000, Brazil
[f] Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, BA, 45650-000, Brazil

ARTICLE INFO

Article history:
Received 29 June 2020
Received in revised form
22 June 2021
Accepted 23 June 2021
Available online 26 June 2021

Corresponding editor: Sven Bradler

Keywords:
Chromosome evolution
Comparative cytogenetics
Biodiversity
Leaf-cutting ants
Social parasitism
Robertsonian translocation

ABSTRACT

The ant *Acromyrmex ameliae* is a social parasite of two leaf-cutting ant subspecies: *Acromyrmex subterraneus subterraneus* and *A. subterraneus brunneus*. Cytogenetic data are available for 14 species of *Acromyrmex* and all of them possess 2n = 38 chromosomes. In this study, chromosome number, heterochromatin detection, and detection of AT and GC-rich blocks of colonies of *A. ameliae* and its hosts were carried out. Additionally, the detection of nucleolus organizer regions and 18S rDNA clusters in chromosomes of the parasite and physical mapping of telomeres were undertaken. The same chromosome number and morphology were detected for the hosts 2n = 38 (10m + 14sm + 12st + 2a), while the females and males of the social parasite *A. ameliae* presented 2n = 36 (10m + 16sm + 8st + 2a) and n = 18 (5m + 8sm + 4st + 1a). In both *A. ameliae* and its hosts, the terminal region on the short arm of the largest subtelocentric pair is heterochromatic GC-rich, and this region corresponded to the 18S rDNA clusters in the parasite. The short arms of several chromosomes were heterochromatin-rich. The telomeric probe hybridized telomeres on all chromosomes of the parasite and was not detected in intrachromosomal regions. Through a comparative cytogenetic analysis, we hypothesize that the karyotype of *A. ameliae* (2n = 36) originated from a chromosomal rearrangement that reduced the number of chromosomes from 38 to 36; as available data on the genus *Acromyrmex* show that all other species possess 38 chromosomes, representing 45% of the 33 valid species in this genus. The mechanism of the chromosome rearrangement is discussed. Thus, the chromosome number observed in *A. ameliae* is a derivation from the genus. Our data show variation in the chromosomal number in *Acromyrmex* and suggest that analyses of the karyotypes of parasite species can yield novel insights with regards to the evolution of this genus.

1. Introduction

Social parasitism is defined as the coexistence of two species of social insects in the same nest, in which one species develops at the expense of the other (Wilson, 1971; Buschinger, 1986) and is considered one of the most intriguing phenomena in ants and a few social wasps. This type of relationship is estimated to have evolved independently at least 30 times in ants (Hölldobler & Wilson, 1990), and to date, more than 300 parasitic species have been described (Rabeling et al., 2019). There is no other animal group where this phenomenon occurs so frequently (Tinaut & Ruano, 1999; Stuart, 2002; Smith et al., 2007).

* Corresponding author.
E-mail address: luisa@unifap.br (L.A.C. Barros).

https://doi.org/10.1016/j.jcz.2021.06.012
0044-5231/

In ants, social parasitism has been reported in six subfamilies: Dolichoderinae, Formicinae, Myrmeciinae, Pseudomyrmecinae, Myrmicinae (reviewed by Tinaut & Ruano, 1999) and Ectatomminae (Hora et al., 2005; Feitosa et al., 2008), among which, inquilinism is considered the most frequent type (Buschinger, 2009). In inquilinism *sensu* Wilson (1971), parasitic species spend their whole life cycle in the host's nest and frequently do not produce a worker caste, but instead invest resources and effort in producing only gynes and males. Therefore, they depend entirely on the host species and accordingly ensure that the queen of the host colony remains alive (Buschinger, 1986).

Five reports describing obligate inquiline social parasitism have been published among leaf-cutting ants (reviewed by Rabeling et al., 2019). According to Emery's Rule (Sumner et al., 2004), social parasites and their hosts must share a common ancestor and would be phylogenetically closely related, even being sister groups. The situation is not that simple: Emery's rule appears to fit in the case of *Acromyrmex insinuator* Schultz et al., 1998 (parasite) and *Acromyrmex echinatior* (Forel, 1899) (host), but not initially for the parasite *Acromyrmex fowleri* Rabeling et al., 2019 (presented as *Pseudoatta* sp. in Sumner et al., 2004) and its host *Acromyrmex rugosus* (Smith, 1858). In the latter case (*A. fowleri/A. rugosus*), preliminary molecular evidence (partial sequences of the *COI* and *COII* genes) may have led to the erroneous conclusion that social parasitism in the genus *Acromyrmex* is polyphyletic and that the parasite *A. fowleri* and its host *A. rugosus* do not form sister groups (Sumner et al., 2004). However, recent studies based on multiple nuclear and genomic markers have, nevertheless, indicated that *A. fowleri* is, in fact, a close relative of its host *A. rugosus* (Rabeling et al., 2019) and that Emery's rule holds true for the species.

Acromyrmex ameliae De Souza et al., 2007 is a social parasite hosted by *Acromyrmex subterraneus subterraneus* (Forel, 1893) and *A. subterraneus brunneus* (Forel, 1912), which shares several morphological similarities (De Souza et al., 2007). The gynes and males of *A. ameliae* are invariably smaller than those of its hosts, and *A. ameliae* (De Souza et al., 2007) and *A. insinuator* (Bekkevold and Boomsma, 2000) are among the few inquilines known to have a worker caste. Only the workers of the minor caste of *A. ameliae* are produced; these have discrete but apparent differences from the hosts' workers (De Souza et al., 2007).

Cytogenetic studies indicate that the social parasites *Leptothorax kutteri* Buschinger, 1966 (n = 23 or 25), *Leptothorax goesswaldi* Kutter, 1967 (n = 28), and *Leptothorax pacis* (Kutter, 1945) (n = 26) are phylogenetically closer when compared with their common host *Leptothorax acervorum* (Fabricius, 1793) (n = 13), and they are likely to be closely related (reviewed by Buschinger, 1990).

Cytogenetic data are currently available for 14 species of *Acromyrmex* from Brazil, Uruguay, Panama, and French Guiana (Table 1). All the species have a chromosome number of 2n = 38. However, in the congener "*Acromyrmex striatus*" the chromosome number is 2n = 22 (Cristiano et al., 2013; Teixeira et al., 2017; Pereira et al., 2018), which is the same as that in all species of *Atta* studied to date and was considered as the sister group of the remaining leaf-cutting ants (Cristiano et al., 2013). Recent studies using molecular phylogeny proposed a new genus of leaf-cutting ants, *Amoimyrmex* Cristiano, Cardoso & Sandoval, which includes *Amoimyrmex striatus* (Roger, 1863) and other two species (Cristiano et al. 2020). A comparison of the karyotypic formulae of *Am. striatus* and *Atta* spp. indicated morphological differences with respect to two chromosome pairs, as well in the banding pattern and the nucleolus organizing regions (NOR) bearing chromosome pair (Cristiano et al., 2013; Barros et al., 2014; Teixeira et al., 2017).

For obtaining new insights into the chromosome evolution in the genus *Acromyrmex*, in this study, we describe the karyotypes of the social parasite *A. ameliae* and its hosts *A. subterraneus subterraneus* and *A. subterraneus brunneus* and compare these with the karyotypes of other species of *Acromyrmex*.

2. Materials and methods

2.1. Colonies collection and rearing conditions

All colonies were collected in eucalypt plantations at Itapoã Farm (V. & M. Florestal Ltda) (19°17′S 44°29′W), Paraopeba, state of Minas Gerais, Brazil, in August 2007.

The early separation of the host and social parasite queens was necessary since the distinction between the species at larval stages is as yet not possible. A total of eight small colonies ("subcolonies") previously parasitized were artificially formed. Subcolonies contained given volume of the fungus garden, workers and a queen of either the host or the parasite. Four small colonies had several *A. ameliae* queens (parasite colonies, all polygynous): one had a single *A. subterraneus brunneus* queen (host colony, monogynous), and three had a single or several *A. subterraneus subterraneus* queens (host colonies; monogynous or polygynous). This procedure warranted that newly produced brood came from a unique type of queen (parasite or host). *A. ameliae* queens were taken from four parasitized colonies: three of them from *A. subterraneus subterraneus* and one from *A. subterraneus brunneus.* Besides, five colonies of *A. ameliae* with their brood kept at the laboratory (collected previously in 2003 and 2004 at the same site and kindly ceded by Dra. Ilka M. F. Soares) were studied. Additionally, three unparasitized colonies of *A. subterraneus brunneus* collected in 2007 from the same locality were analyzed.

The colonies were kept in the Insectarium of the Universidade Federal de Viçosa, under constant conditions of temperature and humidity (Della Lucia et al., 1993). The small colonies formed by the hosts or parasites were maintained in separate rooms to avoid any kind of exchange. At least 90 days were necessary before brood from each lineage being available for cytogenetic studies. This precaution was taken to make sure that the offspring originated from the host or the parasite.

2.2. Cytogenetic analysis

At least five individuals per colony were analyzed, with ten metaphases per individual on average. The metaphases were obtained from cerebral ganglia or testes of larvae after meconium elimination (Imai et al., 1988). The chromosomes were measured, and the karyotype was arranged based on the chromosome's arms ratio (r) following Levan et al. (1964). To assembly and measure the chromosomes, the software Corel ® Photopaint X3 and Image-Pro Plus ® were used.

Some individuals were submitted to the fluorochromes chromomycin A_3 (CMA_3) and 4'6-diamidino-2-phenylindole (DAPI) (Schweizer, 1980) and the C-banding technique (Sumner, 1972) with modifications (Barros et al., 2013a). Eight-10 individuals of each taxon were analyzed for each chromosomal banding technique. The metaphases were photographed using an Olympus BX60 microscope, linked to a Q Color 3 Olympus® image capture system. The filters WB (450–480 nm), and WU (330–385 nm) were used for analyzing CMA_3, and DAPI, respectively.

The fluorescence *in situ* hybridization (FISH) technique was carried out on the chromosomes of three individuals of *A. ameliae* to detect rDNA clusters by physical mapping following Pinkel et al. (1986). The 18S rDNA probe was obtained via polymerase chain reaction (PCR) amplification employing rDNA primers 18SF1 (5′–GTC ATA GCT TTG TCT CAA AGA–3′) and 18SR1.1 (5′–CGC AAA TGA AAC TTT AAT CT–3′) designed for the bee *Melipona quinquefasciata* Lepeletier, 1836 (Pereira, 2006). Genomic DNA of the ant

Table 1
Acromyrmex species cytogenetically studied. Chromosome number: diploid (2n), haploid (n); diploid chromosome morphology; sampling sites; references. Chromosome morphology was used according to literature published data.

Acromyrmex species	2n(n)	Diploid karyotypic formula	Locality	Reference
A. ambiguus (Emery, 1888)	38	2M + 6SM + 16ST+ 14A	F.D. Roosevelt PK. -Canelones, Uruguay	Goñi et al. (1983)
		2M + 6SM + 16ST+ 14A	Piriápolis-Maldonado, Uruguay	Goñi et al. (1983)
		14M + 12SM + 8ST + 4A	Ilha Comprida-SP, Brazil	Castro et al. (2020)
A. aspersus (Smith, 1858)	38	8m + 10sm + 16st + 4a	Viçosa-MG, Brazil	Teixeira et al. (2017)
A. balzani (Emery, 1890)	38	12m + 10sm + 14st + 2a	Viçosa-MG, Brazil	Barros et al. (2016)
		12m + 10sm + 14st + 2a	Paraopeba-MG, Brazil	Barros et al. (2016)
		12m + 10sm + 14st + 2a	Kourou, French Guiana	Aguiar et al. (2020)
A. coronatus (Fabricius, 1804)	38(19)	12m + 8sm + 16st + 2a (6m + 4sm + 8st + 1a)	São Tiago-MG, Brazil	Barros et al. (2016)
		12m + 8sm + 16st + 2a	Paraopeba-MG, Brazil	Barros et al. (2016)
A. crassispinus (Forel, 1909)	38	2M + 6SM + 16ST + 14A	Viçosa-MG, Brazil	Fadini & Pompolo (1996)
		12M + 20SM + 4ST + 2A	Ouro Preto-MG, Brazil	Castro et al. (2020)
A. disciger (Mayr, 1887)	38	10m + 12sm + 14st + 2a	Santos Dumont-MG, Brazil	Barros et al. (2016)
A. echinatior (Forel, 1899)	38	8m + 6sm + 14st + 10a	Barro Colorado, Panama	Barros et al. (2016)
A. heyeri (Forel, 1899)	38	2M + 6SM + 16ST+ 14A	Solís-Maldonado, Uruguay	Goñi et al. (1983)
		–	RS[a], Brazil	Santos-Colares et al. (1997)
A. hispidus Santschi, 1925	38	2M + 6SM + 16ST+ 14A	Solís-Maldonado, Uruguay	Goñi et al. (1983)
A. lundii (Guérin-Méneville, 1838)	38	10M + 14SM + 10ST + 4A	Dom Pedrito-RS, Brazil	Castro et al. (2020)
A. niger (Smith, 1858)	38	12m + 14sm + 10st + 2a	Viçosa-MG, Brazil	Barros et al. (2016)
A. nigrosetosus (Forel, 1908)	38	12M + 14SM + 10ST + 2A	Ouro Preto-MG, Brazil	Castro et al. (2020)
A. rugosus (Smith, 1858)	38	16m + 12sm + 8st + 2a	Florestal-MG, Brazil	Barros et al. (2016)
		16m + 12sm + 8st + 2a	Paraopeba-MG, Brazil	Barros et al. (2016)
		–	Marliéria-MG, Brazil	Castro et al. (2020)
A. subterraneus subterraneus (Forel, 1893)	38	2M + 6SM + 16ST + 14A	Viçosa-MG, Brazil	Fadini & Pompolo (1996)
		14M + 18SM + 4ST + 2A	Viçosa-MG, Brazil	Castro et al. (2020)
A. subterraneus molestans Santschi, 1925	38	2M + 6SM + 16ST + 14A	Viçosa-MG, Brazil	Fadini & Pompolo (1996)
		10m + 10sm + 16st + 2a	Viçosa-MG, Brazil	Teixeira et al. (2017)

MG: Minas Gerais state; SC: Santa Catarina state; RS: Rio Grande do Sul state.
[a] The sample site was assumed according to information available in the paper.

Camponotus rufipes (Fabricius, 1775) was used as a template in the PCR reactions. 18S rDNA probes were labeled maintaining the conditions for PCR amplification (Pereira, 2006) by an indirect method using digoxigenin-11-dUTP (Roche, Mannheim, Germany), and the FISH signals were detected with anti-digoxigenin-rhodamine (Roche Applied Science), following the manufacturer's protocol.

The probe $(TTAGG)_6$ was used in the physical mapping of telomeres by FISH. The telomeric probe was directly labeled with Cyanine-3 (Cy3) at the 5' end (Sigma, St. Louis, MO, USA). Telomeric probes were applied on the chromosomes of four individuals of *A. ameliae*, according to Pinkel et al. (1986), with modifications. Briefly, metaphasic chromosomes were denatured in 70% formamide/2 × SSC at 75 °C for 3 min; the probes were hybridized with chromosomes in 20 μL of hybridization mix (200 ng of the labeled probe, 2 × SSC, 50% formamide, and 10% dextran sulfate). The hybridization mix was heated for 10 min at 85 °C, and the slides covered with glass coverslip were incubated in a moist chamber at 37 °C overnight. Post-hybridization washes were carried out in 4 × SSC/Tween and then dehydrated in an alcohol series.

Finally, the slides submitted to FISH were mounted using the Antifade solution with DAPI (DAPI Fluorshield, Sigma Aldrich). The slides were analyzed under an Olympus BX53 epifluorescence microscope with an Olympus DP73F camera and analyzed using the CellSens Imaging software and filters WG (510-550 nm) for rhodamine and Cy3, and WU (330–385 nm) for DAPI.

The detection of active NORs was carried out on the chromosomes of six individuals of *A. ameliae* according to the Ag-NOR banding technique (Howell & Black, 1980).

3. Results

A total of 92 individuals including the parasite and its hosts were analyzed for their chromosomal numbers using conventional Giemsa staining (Table 2). The chromosome number observed for the host *A. subterraneus brunneus* was 2n = 38, n = 19 for females and males, respectively, in parasitized or non-parasitized nests. Similarly, the females of *A. subterraneus subterraneus* were found to have a chromosome number of 2n = 38 (Table 2, Fig. 1A–C). The social parasite *A. ameliae* presented a different chromosome number from its hosts: 2n = 36 and n = 18 (Fig. 1D–E) for females and males, respectively. The karyotypic formula of both hosts was 2n = 10m + 14sm + 12st + 2a and that of *A. ameliae* was 2n = 10m + 16sm + 8st + 2a, and n = 5m + 8sm + 4st + 1a (Tables S1–S3).

In both hosts and the parasite, GC-rich chromatin was detected in telomeric regions on the short arm of the largest subtelocentric chromosome pair (designated as the st1 pair by Barros et al., 2016; and herein) (Fig. 2). However, neither the parasite nor its hosts possessed AT-rich regions (Fig. 2).

The heterochromatin was detected on the chromosomes of the hosts and the social parasite in the following morphological types: metacentric (centromeric region), some subtelocentric, and acrocentric (short arms of both kinds). However, no significant difference in the distribution of heterochromatin between *A. ameliae* and its hosts was detected (Fig. 3). Furthermore, 18S rDNA clusters were detected in the terminal region of the st1 pair (Fig. 4A), whereas the telomeric sequence probe $(TTAGG)_6$ marked telomeres on all chromosomes of the parasite (Fig. 4B), although this motif was not detected in the intrachromosomal regions. Ag-NOR and FISH revealed the presence of a single NOR in the st1 pair of *A. ameliae*, which coincided with heterochromatic GC-rich regions (Fig. 5).

4. Discussion

4.1. Chromosome number, karyotypes, and banding patterns of the social parasite A. ameliae and its hosts

In all nine ant colonies examined, the social parasite *A. ameliae* possessed a different chromosome number (2n = 36) from its hosts

Table 2
Cytogenetics of the social parasite *Acromyrmex ameliae* and its hosts *Acromyrmex subterraneus subterraneus* and *Acromyrmex subterraneus brunneus*. Studied taxa, sample size: number of colonies and total of individuals analyzed with Giemsa staining, diploid (2n)/haploid (n) chromosome number and diploid (2n)/haploid (n) karyotype formulae.

Taxon	Colonies	Individuals	2n (n)	2n(n)
Acromyrmex ameliae De Souza, Soares, Della Lucia, 2007	9	49	36 (18)	10m + 16sm + 8st + 2a (5m + 8sm + 4st + 1a)
Acromyrmex subterraneus brunneus (Forel, 1912)	5[a]	25	38 (19)	10m + 14sm + 12st + 2a (5m + 7sm + 6st + 1a)
Acromyrmex subterraneus subterraneus (Forel, 1893)	3[b]	18	38	10m + 14sm + 12st + 2a

[a] One parasitized and the others were not parasitized.
[b] All of them parasitized.

(2n = 38) in sympatry. The chromosome number observed for *A. subterraneus subterraneus* is consistent with those reported by Fadini & Pompolo (1996) and Castro et al. (2020), with variations in karyotypic formula possibly due to differences in the measurements of the chromosomes in Castro et al. (2020), since Fadini & Pompolo (1996) probably did not perform any measurement for chromosome classification. The karyotype of *A. subterraneus brunneus* is described here for the first time.

Data from the present study indicated no evident cytogenetic distinction between the two hosts because they possessed similar karyotypes. However, their karyotypes do differ from that previously reported for another subspecies, *A. subterraneus molestans* Santschi, 1925. (Teixeira et al., 2017); they had fewer subtelocentric chromosomes than *A. subterraneus molestans*. These differences could be due to differential heterochromatin growth. The occurrence of heterochromatin in the short arms of chromosomes in the social parasite and its hosts suggests differential heterochromatin growth, as has previously been reported in other species of *Acromyrmex* (Barros et al., 2016) and the genus *Odontomachus* (Aguiar et al., 2020).

Morphological differences among the three subspecies of *A. subterraneus* are consistent with differences observed at the cytogenetic level. *A. subterraneus molestans* possess a curved inferior pronotal spine with a forward-facing tip. In contrast, *A. subterraneus subterraneus* and *A. subterraneus brunneus* possess straight inferior pronotal spines with a forward-facing tip. Furthermore, while the workers of *A. subterraneus molestans* are either light or dark brown in color, typically with darker coloration on the front of the head, the workers of its hosts, *A. subterraneus subterraneus* and *A. subterraneus brunneus* are distinguished by their light brown and dark brown to black colorations, respectively (Gonçalves, 1961; Forti et al., 2006). Differences in color tonalities can be observed in the same nest, which limits the subspecies distinction, according to Gonçalves (1961). Karyotype conservation between the two hosts suggests that *A. subterraneus subterraneus* is more closely related to *A. subterraneus brunneus* than to *A. subterraneus molestans*.

In *A. ameliae* and its hosts, GC-rich bands were detected on the short arms of the st1 chromosome pair, and this is consistent with the findings reported for other species of *Acromyrmex* (Barros et al., 2016; Teixeira et al., 2017). *A. ameliae* possessed 18S rDNA gene clusters in the st1 pair, coinciding with the GC-rich regions. A relationship between the CMA_3 region and NORs is common in different organisms (Symonová, 2019) and is corroborated in distant groups of ants, including leaf-cutting ants *Acromyrmex* spp. and *Atta* spp., based on FISH analysis (reviewed by Teixeira et al., 2021). Accordingly, it can be assumed that the heterochromatic GC-rich bands detected in the st1 chromosomes correspond to 18S rDNA clusters in the hosts. In addition, terminal location of rDNA seems to be a plesiomorphic trait in the genus *Acromyrmex*.

A notable chromosome rearrangement due to an inversion has previously been observed in *A. echinatior*, as evidenced by the GC-rich regions and 18S rDNA clusters when compared with other species of *Acromyrmex* (Barros et al., 2016; Teixeira et al., 2021), representing a derivative condition within the genus. Given that intrachromosomal rDNA clusters are present in *Am. striatus* (Teixeira et al., 2017), as well as in all species of *Atta* studied to date (Barros et al., 2015; Teixeira et al., 2017), and even in *Mycetomoellerius holmgreni* (Wheeler, 1925) (Barros et al., 2018), it is suggested that these intrachromosomal rDNA clusters represent a plesiomorphic character among leaf-cutting ants.

4.2. Evolutionary insights into the origin of the derived karyotype of *A. ameliae*

An important finding of the present study is that the number of chromosomes in the social parasite *A. ameliae* differs from that in its hosts in sympatry and in other species of *Acromyrmex* that have been cytogenetically studied (Table 1). To date, a total of 15 species (17 taxa) of *Acromyrmex* have been examined cytogenetically including data of the present study, representing 45% of the 33 valid species in this genus (Bolton, 2020).

Two main possibilities could explain the differences observed in the chromosome number and the structure of the social parasite *A. ameliae*. First, the karyotype of *A. ameliae* is possibly indicative of the conservation of a hypothetical ancestral condition characterized by a chromosome number of 2n = 36. However, this is considered unlikely, because this number differs from 2n = 38 that has been reported for all other species in the genus for which chromosome numbers have been determined. Moreover, the specialization of social parasites (De la Mora et al., 2020), which can be considered a derived condition, corroborates this possibility. Thus, it is unlikely that centric fission has increased the chromosome number from 2n = 36 to 38.

The second and more probable proposal is that the karyotype of *A. ameliae* (2n = 36) originated from a chromosomal rearrangement that reduced the diploid number from 38 to 36. Rearrangements involving chromosome number reduction have been a subject of extensive debate (Schubert & Lysak, 2011; Guerrero & Kirkpatrick, 2014). In this regard, fusion is defined by Guerrero & Kirkpatrick (2014) as a chromosomal rearrangement that brings together previously unlinked regions of the genome, thereby physically linking genes on different chromosomes. Most of these rearrangements are referred to as reciprocal translocations by Schubert & Lysak (2011), who argued that “genetic markers belonging to two ancestral genetic linkage groups segregate as a single linkage group in a derived species.”

Fusion can occur via different mechanisms, including end-to-end fusion, which involves the joining of two chromosomes at their telomeres, and Robertsonian translocation (also referred to as Robertsonian fusion), in which “two chromosomes break at the centromeres and switch arms.” In the latter case, there is a junction between chromosomes, with near-terminal centromeres producing a small chromosome that is usually lost. Furthermore, centric fusion occurs when two acrocentric chromosomes bind via their terminal centromeres to form a metacentric chromosome (Guerrero & Kirkpatrick, 2014).

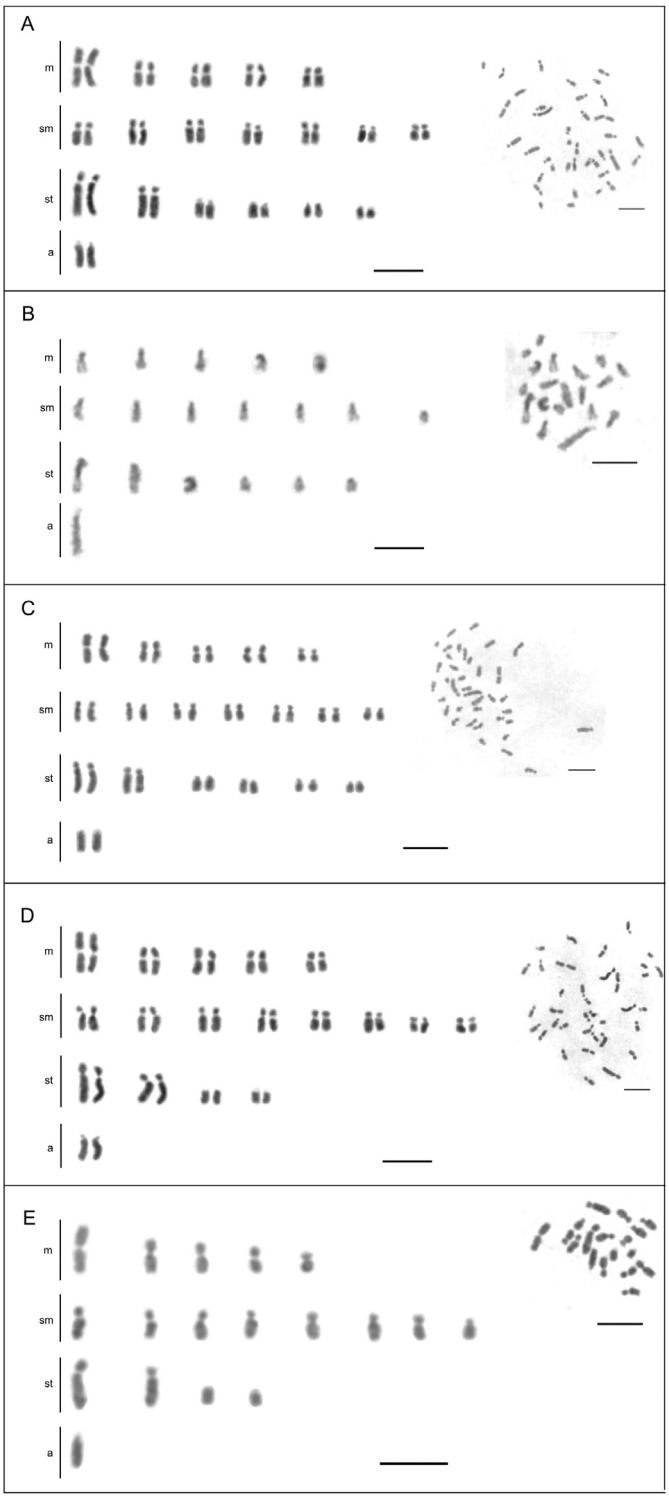

Fig. 1. Metaphases and respective karyotypes of the social parasite *A. ameliae* and its hosts *A. subterraneus brunneus* and *A. subterraneus subterraneus* stained with conventional Giemsa. (A) Female karyotype of *A. subterraneus brunneus*: 2n = 38. (B) Male karyotype of *A. subterraneus brunneus*: n = 19. (C) Female karyotype of *A. subterraneus subterraneus*: 2n = 38. (D) Female karyotype of *A. ameliae*: 2n = 36. (E) Male karyotype of *A. ameliae*: n = 18. Bar: 5 μm.

Some of these types of rearrangements can be discarded when the karyotype from *A. ameliae* is analyzed. Centric fusion, for example, is considered unlikely, because there are no large distinct chromosomal metacentric pairs, that are characteristic features of this type of fusion in the karyotype of *A. ameliae* when compared with other species of *Acromyrmex*. Non-reciprocal translocation, also referred to as unbalanced segregation from reciprocal translocation by Schubert & Lysak (2011), is equally unlikely, because it involves the elimination of chromatin portions, and this is usually lethal.

Data on the pattern of telomeres, which consist of tandemly repeated $(TTAGG)_6$ motifs, are available for 38 species in the family Formicidae (reviewed by Kuznetsova et al., 2019; Castro et al., 2020). The presence of the pentanucleotide repeat $(TTAGG)_6$ was observed at the telomeres of all chromosomes of the social parasite. The signal intensity was accentuated in metaphases with different condensation degrees. Although telomeric repeats are usually located at the terminal regions of the chromosomes, interstitial telomeric sequences can also be detected and are typically considered to be relicts of the tandem fusion of ancestral chromosomes (Lin & Yan, 2008; Olsson et al., 2018). Remnants of telomeres, as a result of end-to-end chromosome fusion, were not detected in the intrachromosomal regions of *A. ameliae*. Additionally, the absence of a distinctive chromosome pair would appear to indicate that telomere fusion is unlikely to have contributed to the reduction in chromosome number observed in this social parasite.

Taking the cytogenetic data into consideration, we suggest that a chromosomal breakage occurred between two non-homologous subtelocentric chromosomes and the bonding of the long arms resulted in an additional submetacentric chromosomal pair. This rearrangement corresponds to Robertsonian translocation (Fig. 6), in which the heterochromatic small short arms of the subtelocentric pairs are lost, and thus, there is a loss of telomeres and heterochromatic sequences. This hypothesis explains the reduction in chromosome number from 38 to 36, the absence of a distinctive rearranged chromosome, as well the absence of interstitial telomeric sequences in *A. ameliae* karyotype.

There are reports of the natural occurrence of chromosome reduction in other species of ants, including *Pheidole nodus* Smith, 1874 (Imai & Kubota, 1975), *Myrmecia croslandi* Taylor, 1991 (Imai & Taylor, 1989), and *C. rufipes* (Mariano et al., 2001; Aguiar et al., 2017). Among fungus-farming ants fusions seems to have occurred during the chromosomal evolution of *Mycetomoellerius* (reviewed by Barros et al., 2018) and in the clade *Mycetophylax conformis* and *Mycetophylax morschi* (Cardoso et al., 2014). Although centric fission appears to be the most common mechanism associated with ant evolution, other types of rearrangement have also been observed (Imai et al., 1994).

Since heterozygotes (i.e., those with 2n = 37 chromosomes) were not detected among the *A. ameliae* examined, this suggests the fixation of the chromosomal rearrangement. The hypothesis that a small population is essential for rapid speciation is accepted among evolutionary biologists (Bush, 1975; King, 1993). Considering that the population size of social parasites is lower than that of their host species (Buschinger, 2009; De la Mora et al., 2020), they are likely more vulnerable to having isolated geographic lineages (De la Mora et al., 2020) especially if dispersal is limited, favoring speciation. Interestingly, the nuptial flight of *A. ameliae* under laboratory conditions has been reported, in which both males and females fly off together and thus, there is no prevention of inbreeding in this specific case (De Souza et al., 2011). Stochastic processes within the restricted population of *A. ameliae* may have helped in the fixation of chromosomal rearrangement or even contributed to speciation.

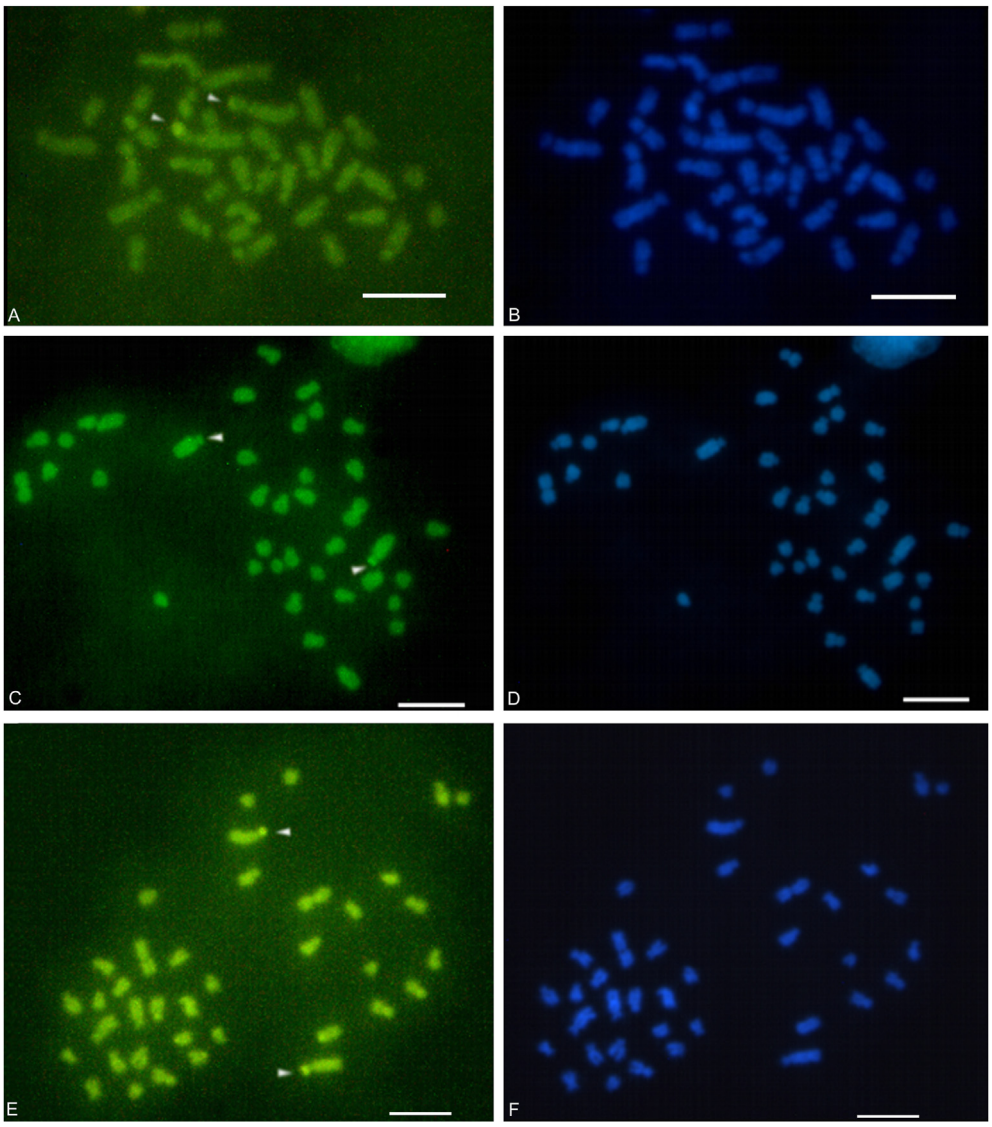

Fig. 2. Metaphases of the social parasite and its hosts after fluorochrome staining with CMA_3 and DAPI, respectively. (A–B) Parasite *A. ameliae* 2n = 36. (C–D) *A. subterraneus subterraneus* 2n = 38. (E–F) *A. subterraneus brunneus* 2n = 38. Arrows indicate GC-rich regions (CMA_3^{+}) in terminal region on the short arms of the largest subtelocentric chromosome pair. Bar: 5 μm.

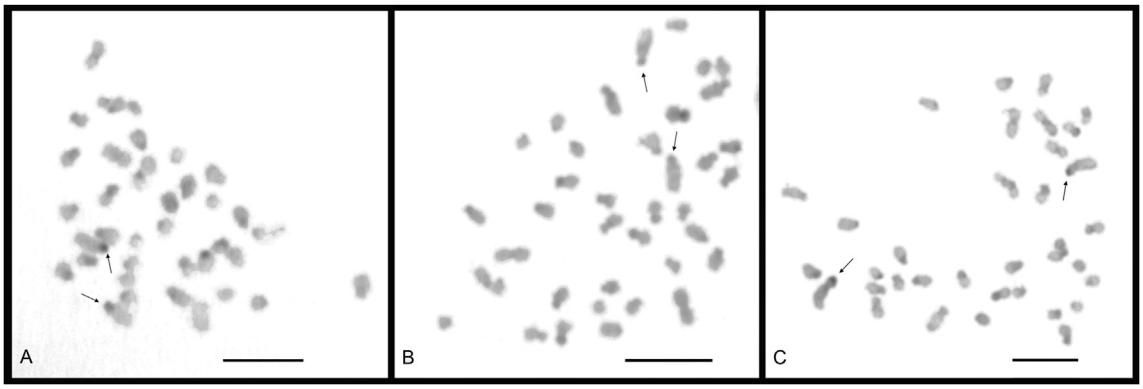

Fig. 3. Heterochromatic regions on metaphasic chromosomes of social parasite and its hosts. (A) Host *A. subterraneus brunneus*. (B) Host *A. subterraneus subterraneus*. (C) Social parasite *A. ameliae*. The arrows indicate heterochromatic blocks in the terminal region on the short arms of the largest subtelocentric chromosome pair. Bar: 5 μm.

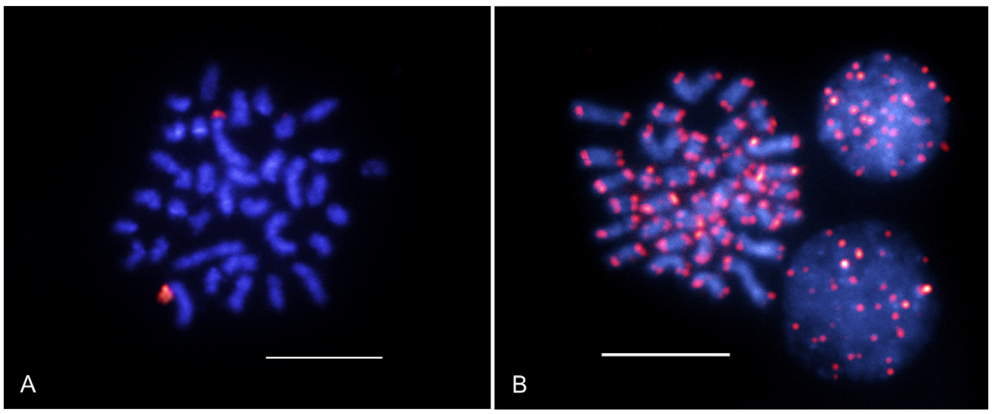

Fig. 4. Physical mapping by fluorescence *in situ* hybridization on the chromosomes of the parasite *Acromyrmex ameliae* (2n = 36). (A) Ribosomal clusters using the probe 18S rDNA. (B) Telomeres with the probe $(TTAGG)_6$. Red blocks indicate markings for the respective probe. Bar: 5 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

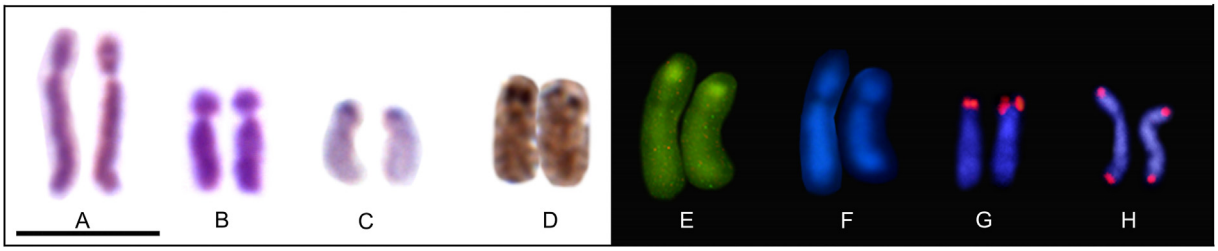

Fig. 5. Largest subtelocentric chromosome pair (st 1 pair) of the social parasite *Acromyrmex ameliae*. (A–B) Chromosomes with different degrees of condensation. (C) Heterochromatic blocks. (D) Active nucleolus organizing regions. (E) GC and (F) AT-rich regions. (G) 18S rDNA ribosomal clusters. (H) Telomeric clusters $(TTAGG)_6$. Bar: 5 μm.

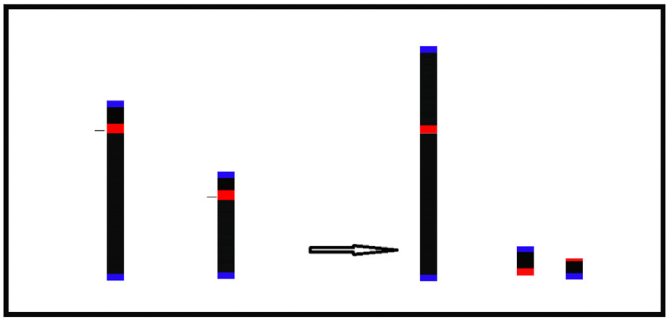

Fig. 6. Schematic Robertsonian translocation rearrangement leading to chromosome number reduction on the parasite *Acromyrmex ameliae.* The rearrangement should involve chromosome breaks indicated by the black traces on non-homologous subtelocentric chromosomes followed by an incorrect bonding resulting in a submetacentric chromosome. This kind of rearrangement results in loss of part of chromosome. Red rectangle: centromeric heterochromatin, blue rectangle: telomeres, thin black bars indicate chromosomal breaks. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4.3. A comparison of karyotypes among A. ameliae and other Acromyrmex spp.

The comparison of the diploid karyotypic formula of *A. ameliae* with its hosts showed the occurrence of an extra submetacentric chromosome pair. On the other hand, two pairs of subtelocentric chromosomes were lacking (Fig. 1). These data point to the relationship between the parasite and its hosts due the absence of two subtelocentric pairs can explain the chromosome reduction. The Robertsonian translocation between two subtelocentric chromosomes should have originated a submetacentric chromosome and the lack of a distinctive chromosome of large size compared to the most preeminent observed (e.g. the first metacentric and the first and second subtelocentric) suggests loss of genetic material after the rearrangement.

However, the chromosome markers used in the present study did not enable us to identify which chromosomes have been involved in the rearrangement, and thus, based on the currently available data, it was not yet possible to determine the phylogenetic relationship between the karyotype of the parasite and its hosts. Nevertheless, several karyotype dissimilarities are observed between *A. ameliae* and other *Acromyrmex* spp., that may suggest a less closely relationship between them since cytogenetic data may reflect phylogenetic history.

With respect to *A. echinatior*, which is distributed mainly in Central America, the number and size of acrocentric chromosomes in this species is quite different from that of other *Acromyrmex* species. *Acromyrmex balzani* (Emery, 1890) occurs sympatrically with *A. ameliae*; however, the marked difference between the size of the st1 pair and the first metacentric pair is exclusive to *A. balzani* (Barros et al., 2016; Aguiar et al., 2020). Other *Acromyrmex* species show notable differences in the karyotypic formula in relation to parasite, with larger number of metacentric, submetacentric subtelocentric and/or acrocentric chromosomes (Table 1). Differential heterochromatic growth on short arms of the chromosomes contributes to the variations of karyotypic formulas among species (Barros et al., 2016).

With respect to GC-rich regions in the chromosomes of *Acromyrmex* species, *A. balzan*i, *A. echinatior*, *Acromyrmex niger* (Smith, 1858), and *A. rugosus* show distinct patterns from those of *A. ameliae*, among which, *A. balzan*i and *A. echinatior* are characterized by differences in the short arm and interstitial region,

respectively; the latter species has additional markings on the telomeric region (Barros et al., 2016). In *A. niger*, additional heterochromatic GC-rich bands are observed in the st1 pair and the second–highest pair (st2). The constitution of these additional GC bands in *A. niger* and *A. rugosus* is yet to be determined and may not be important in phylogenetic studies. Additionally, the intensity of other bands in *A. rugosus* is weaker than that of bands in *A. niger* and could indicate different constitutions. Multiple GC-rich bands colocalized with heterochromatic regions are observed in different genera of fungus-growing ants including the leaf-cutting *Am. striatus* (Barros et al., 2010, 2013b, 2018; Cristiano et al., 2013), which need to be investigated in future studies to understand if they have a common ancestor.

In summary, some species of *Acromyrmex* (e.g., *A. balzani* and *A. echinatior*) may not have a closely relationship with the social parasite due to notable variations in their karyotypes. However, we were not able to point the possible origin of the parasite because the hosts do not share unique characteristics with the parasite, which could exclude the others. Further molecular cytogenetic studies and molecular phylogeny can contribute to the understanding of the speciation mechanisms in social parasites and their hosts.

5. Conclusions

Based on the findings of this study and other currently available data, we can infer that the karyotype of the social parasite is a derivation of the main karyotype 2n = 38 and can be considered the plesiomorphic condition for the genus *Acromyrmex*. Comparative analyses of the cytogenetic data obtained for *A. ameliae*, its hosts, and other species of *Acromyrmex* indicate that the probable mechanism underlying this chromosomal variation involves a Robertsonian translocation between two non-homologous subtelocentric chromosome pairs, resulting in a submetacentric chromosome pair in *A. ameliae*.

Although the distinct karyotype of *A. ameliae* (n = 18), the chromosome number in *Acromyrmex* is so far very conservative, however analyses of the karyotypes of parasite species can yield novel insights with regards to the evolution of *Acromyrmex*. The findings of our research reinforce the value of cytogenetics as such information can be used to corroborate the differences identified in morphological and reproductive studies of *A. ameliae* in relation to its hosts (De Souza et al., 2007; Soares et al., 2010).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful to V & M Florestal do Brazil SA for fieldwork support, Terezinha Della Lucia for the use of Insectarium for analyses and suggestions on the manuscript, Ilka M. F. Soares for providing ant colonies, Riviane Rodrigues da Hora for assistance during the experiments, Manoel J. Ferreira for field and laboratory support, and Alice S. Pereira for technical assistance. We also thank Dr. Silvia G. Pompolo for encouragement and valuable support. We acknowledge Laboratório de Sistemática Molecular Beagle at Universidade Federal de Viçosa for the use of equipment. This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) and Programa de Auxílio ao Pesquisador – PAPESQ/UNIFAP/2019.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcz.2021.06.012.

References

Aguiar, H.J.A.C., Barros, L.A.C., Alves, D.R., Mariano, C.S.F., Delabie, J.H.C., Pompolo, S.G., 2017. Cytogenetic studies on populations of *Camponotus rufipes* (Fabricius, 1775) and *Camponotus renggeri* Emery, 1894 (Formicidae: Formicinae). PloS One 12 (5), e0177702. https://doi.org/10.1371/journal.pone.0177702.

Aguiar, H.J.A.C., Barros, L.A.C., Silveira, L.I., Petitclerc, F., Etienne, S., Orivel, J., 2020. Cytogenetic data for sixteen ant species from North-eastern Amazonia with phylogenetic insights into three subfamilies. Comp. Cytogenet. 14 (1), 43–60. https://doi.org/10.3897/CompCytogen.v14i1.46692.

Barros, L.A.C., Aguiar, H.J.A.C., Mariano, C.S.F., Delabie, J.H.C., Pompolo, S.G., 2010. Cytogenetic characterization of the lower-Attine *Mycocepurus goeldii (Formicidae:* Myrmicinae: Attini). Sociobiology 56 (1), 57–66.

Barros, L.A.C., Mariano, C.S.F., Pompolo, S.G., 2013a. Cytogenetic studies of five taxa of the tribe Attini (Formicidae: Myrmicinae). Caryologia 66 (1), 59–64. https://doi.org/10.1080/00087114.2013.780443.

Barros, L.A.C., Aguiar, H.J.A.C., Mariano, C.S.F., Delabie, J.H.C., Pompolo, S.G., 2013b. Cytogenetic characterization of the ant *Trachymyrmex fuscus* Emery, 1934 (Formicidae: Attini) with the description of a chromosomal polymorphism. Annales de la Société Entomologique de France 49 (4), 367–373.

Barros, L.A.C., Teixeira, G.A., Aguiar, H.J.A.C., Mariano, C.S.F., Delabie, J.H.C., Pompolo, S.G., 2014. Banding patterns of three leafcutter ant species of the genus *Atta* (Formicidae: Myrmicinae) and chromosomal inferences. Fla. Entomol. 97, 1694–1701. https://doi.org/10.1653/024.097.0444.

Barros, L.A.C., Aguiar, H.J.A.C., Teixeira, G.A., Mariano, C.S.F., Teixeira, M.C., Delabie, J.H.C., Pompolo, S.G., 2015. Cytogenetic data on the threatened leafcutter ant *Atta robusta* borgmeier, 1939 (Formicidae: Myrmicinae: attini). C. R. Biol. 338 (10), 660–665. https://doi.org/10.1016/j.crvi.2015.07.006.

Barros, L.A.C., Aguiar, H.J.A.C., Mariano, C.S.F., Andrade-Souza, V., Costa, M.A., Delabie, J.H., Pompolo, S.G., 2016. Cytogenetic data on six leafcutter ants of the genus *Acromyrmex* Mayr, 1865 (Hymenoptera, Formicidae, Myrmicinae): insights into chromosome evolution and taxonomic implications. Comp. Cytogenet. 10 (2), 229–243. https://doi.org/10.3897/CompCytogen.v10i2.7612.

Barros, L.A.C., Teixeira, G.A., Aguiar, H.J.A.C., Lopes, D.M., Pompolo, S.G., 2018. Cytogenetic studies in *Trachymyrmex holmgreni* Wheeler, 1925 (Formicidae: Myrmicinae) by conventional and molecular methods. Sociobiology 65 (2), 185–190. https://doi.org/10.13102/sociobiology.v65i2.2214.

Bekkevold, D., Boomsma, J.J., 2000. Evolutionary transition to a semelparous life history in the socially parasitic ant *Acromyrmex insinuator*. J. Evol. Biol. 13, 615–623. https://doi.org/10.1046/j.1420-9101.2000.00201.x.

Bolton, B., 2020. An online catalog of the ants of the world. Available from. https://antcat.org. (Accessed 10 April 2020).

Buschinger, A., 1986. Evolution of social parasitism in ants. Trends Ecol. Evol. 1, 155–160. https://doi.org/10.1016/0169-5347(86)90044-3.

Buschinger, A., 1990. Sympatric speciation and radiative evolution of socially parasitic ants – heretic hypotheses and their factual background. Zool. Syst. Evol. 28, 241–260. https://doi.org/10.1111/j.1439-0469.1990.tb00379.x.

Buschinger, A., 2009. Social parasitism among ants: a review (Hymenoptera: Formicidae). Myrmecol. News 12, 219–235.

Bush, G.L., 1975. Modes of animal speciation. Annu. Rev. Ecol. Evol. Syst. 6 (1), 339–364. https://doi.org/10.1146/annurev.es.06.110175.002011.

Cardoso, D.C., Pompolo, S.G, Cristiano, M.P, Mara, G.T., 2014. The role of fusion in ant chromosome evolution: insights from cytogenetic analysis using a molecular phylogenetic approach in the genus *Mycetophylax*. PLOS ONE 9 (4), e87473. https://doi.org/10.1371/journal.pone.0087473.

Castro, C.P.M., Cardoso, D.C., Micolino, R., Cristiano, M.P., 2020. Comparative FISH-mapping of TTAGG telomeric sequences to the chromosomes of leafcutter ants (Formicidae, Myrmicinae): is the insect canonical sequence conserved? Comp. Cytogenet. 14 (3), 369–385. https://doi.org/10.3897/CompCytogen.v14i3.52726.

Cristiano, M.P., Cardoso, D.C., Fernandes-Salomão, T.M., 2013. Cytogenetic and molecular analyses reveal a divergence between *Acromyrmex striatus* (Roger, 1863) and other congeneric species: taxonomic implications. PloS One 8 (3), e59784. https://doi.org/10.1371/journal.pone.0059784.

Cristiano, M.P., Cardoso, D.C., Sandoval-Gómez, V.H., Simões-Gomes, F.C., 2020. *Amoimyrmex* Cristiano, Cardoso & Sandoval, gen. nov. (Hymenoptera: Formicidae): a new genus of leaf-cutting ants revealed by multilocus molecular phylogenetic and morphological analyses. Austral Entomol. 59 (4), 643–676. https://doi.org/10.1111/aen.12493.

Della Lucia, T.M.C., Vilela, E.F., Anjos, N., Moreira, D.D.O., 1993. Criação de formigas cortadeiras em laboratório. In: Della Lucia, T.M.C. (Ed.), As Formigas Cortadeiras. Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, pp. 151–162.

De la Mora, A., Sankovitz, M., Purcell, J., 2020. Ants (Hymenoptera: Formicidae) as host and intruder: recent advances and future directions in the study of exploitative strategies. Myrmecol. News 3, 53–71.

De Souza, D.J., Soares, I.M.C., Della Lucia, T.M.C., 2007. *Acromyrmex ameliae* sp. n. (Hymenoptera: Formicidae): a new social parasite of leaf-cutting ants in Brazil. Insect Sci. 14, 251–257. https://doi.org/10.1111/j.1744-7917.2007.00151.x.

De Souza, D.J., Ribeiro, M.M.R., Mello, A., Lino-Neto, J., Dângelo, R.C., Della-Lucia, T.M.C., 2011. A laboratory observation of nuptial flight and mating behaviour of the parasite ant *Acromyrmex ameliae* (Hymenoptera: Formicidae). Ital. J. Zool. 78 (3), 405–408. https://doi.org/10.1080/11250003.2010.490567.

Fadini, M.A.M., Pompolo, S.G., 1996. Cytogenetics of some ant species of the tribe Attini (Hymenoptera, Formicidae) from the region of Viçosa, MG. Braz. J. Genet. 19, 53–55.

Feitosa, R.M., Hora, R.R., Delabie, J.H.C., Valenzuela, J., Fresneau, D., 2008. A new social parasite in the ant genus *Ectatomma* F. Smith (Hymenoptera, Formicidae, Ectatomminae). Zootaxa 1713, 47–52. https://doi.org/10.5281/zenodo.180970.

Forti, L.C., Andrade, M.L., Andrade, A.P.P., Lopes, J.F., Ramos, V.M., 2006. Bionomics and identification of *Acromyrmex* (hymenoptera: Formicidae) through an illustrated key. Sociobiology 48 (1), 135–153.

Gonçalves, C.R., 1961. O gênero *Acromyrmex* no Brasil (Hym. Formicidae). Stud. Entomol. 4, 113–180.

Goñi, G., Zolessi, L.C., Imai, H.T., 1983. Karyotype of thirteen ant species from Uruguay (Hymenoptera - Formicidae). Caryologia 36, 363–371. https://doi.org/10.1080/00087114.1983.10797677.

Guerrero, R.F., Kirkpatrick, M., 2014. Local adaptation and the evolution of chromosome fusions. Evolution 68 (10), 2747–2756. https://doi.org/10.1111/evo.12481.

Howell, W.M., Black, D.A., 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36, 1014–1015. https://doi.org/10.1007/BF01953855.

Hölldobler, B., Wilson, E.O., 1990. The Ants, first ed. Harvard University Press, USA.

Hora, R.R., Doums, C., Poteaux, C., Fénéron, R., Valenzuela, J., Heize, J., Fresneau, D., 2005. Small queens in the ant *Ectatomma tuberculatum*: a new case of social parasitism. Behav. Ecol. Sociobiol. 59, 285–292. https://doi.org/10.1007/s00265-005-0040-9.

Imai, H.T., Kubota, M., 1975. Chromosome polymorphism in the ant, *Pheidole nodus*. Chromosoma 51, 391–399. https://doi.org/10.1007/BF00326325.

Imai, H., Taylor, R.W., Crosland, M.W., Crozier, R.H., 1988. Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis. Jpn. J. Genet. 63, 159–185. https://doi.org/10.1266/jjg.63.159.

Imai, H.T., Taylor, R.W., 1989. Chromosomal polymorphisms involving telomere fusion, centric inactivation and centromere shift in the ant *Myrmecia (pilosula)* n=1. Chromosoma 98, 456–460. https://doi.org/10.1007/BF00292792.

Imai, H.T., Taylor, R.W., Crozier, R.H., 1994. Experimental bases for the minimum interaction theory. Chromosome evolution in ants of the *Myrmecia pilosula* species complex (Hymenoptera: Formicidae: Myrmeciinae). Jpn. J. Genet. 69, 137–182. https://doi.org/10.1266/jjg.69.137.

King, M., 1993. Species Evolution: the Role of Chromosome Change. Cambridge University Press King.

Kuznetsova, V., Grozeva, S., Gokhman, V., 2019. Telomere structure in insects: a review. J. Zool. Syst. Evol. Res. 58 (1), 127–158. https://doi.org/10.1111/jzs.12332.

Levan, A., Fredga, K., Sandberg, A.A., 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52, 201–220. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x.

Lin, K.W., Yan, J., 2008. Ending in the middle: current knowledge of interstitial telomeric sequences. Mutat. Res. 658, 95–110. https://doi.org/10.1016/j.mrrev.2007.08.006.

Mariano, C.S.F., Pompolo, S.G., Delabie, J.H.C., Campos, L.A.O., 2001. Estudos cariotípicos de algumas espécies neotropicais de *Camponotus* Mayr (Hymenoptera: Formicidae). Rev. Bras. Entomol. 45, 267–274.

Olsson, M., Wapstra, E., Friesen, C.R., 2018. Evolutionary ecology of telomeres: a review. Ann. N. Y. Acad. Sci. 1422 (1), 5–28. https://doi.org/10.1111/nyas.13443.

Pereira, J.O.P., 2006. Diversidade genética da abelha sem ferrão *Melipona quinquefasciata* baseada no sequenciamento das regiões ITS1 parcial e 18S do DNA ribossômico nuclear. Thesis, Universidade Federal do Ceará, Brazil.

Pereira, T.T.P., Reis, A.C.C.C., Cardoso, D.C., Cristiano, M.P., 2018. Molecular phylogenetic reconstruction and localization of the (TTAGG)n telomeric repeats in the chromosomes of *Acromyrmex striatus* (Roger, 1863) suggests a lower ancestral karyotype for leafcutter ants (Hymenoptera). Comp. Cytogenet. 12, 13–26. https://doi.org/10.3897/CompCytogen.v12i1.21799.

Pinkel, D., Straume, T., Gray, J.W., 1986. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc. Natl. Acad. Sci. U.S.A. 83, 2934–2938. https://doi.org/10.1073/pnas.83.9.2934.

Rabeling, C., Messer, S., Lacau, S., Nascimento, I.C., Bacci, M., Delabie, J.H.C., 2019. *Acromyrmex fowleri*: a new inquiline social parasite species of leaf-cutting ants from South America, with a discussion of social parasite biogeography in the Neotropical region. Insectes Soc. 66 (3), 435–451. https://doi.org/10.1007/s00040-019-00705-z.

Santos-Colares, M.C., Viégas, J., Roth, M.M., Loeck, A.E., 1997. Preparation of mitotic chromosomes of leaf-cutting ants from the genera *Atta* and *Acromyrmex*. Braz. J. Genet. 20, 25–27. https://doi.org/10.1590/S0100-84551997000100005.

Schubert, I., Lysak, M.A., 2011. Interpretation of karyotype evolution should consider chromosome structural constraints. Trends Genet. 27 (6), 207–216. https://doi.org/10.1016/j.tig.2011.03.004.

Schweizer, D., 1980. Simultaneous fluorescent staining of R bands and specific heterocromatic regions (DA/DAPI-bands) in human chromosomes. Cytogenet. Cell Genet. 27, 190–193. https://doi.org/10.1159/000131482.

Smith, J.A., Tierney, S.M., Park, Y.C., Fuller, S., Schwarz, M.P., 2007. Origins of social parasitism: the importance of divergence ages in phylogenetic studies. Mol. Phylogenet. Evol. 43, 1131–1137. https://doi.org/10.1016/j.ympev.2006.12.028.

Soares, I.M., Della Lucia, T., Pereira, A.S., Serrão, J.E., Ribeiro, M.M., De Souza, D.J., 2010. Comparative reproductive biology of the social parasite *Acromyrmex ameliae* de Souza, Soares & Della Lucia and of its host *Acromyrmex subterraneus subterraneus* Forel (Hymenoptera: Formicidae). Neotrop. Entomol. 39 (5), 714–719. https://doi.org/10.1590/S1519-566X2010000500007.

Sumner, A.T., 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. 83, 438–442. https://doi.org/10.1016/0014-4827(72)90558-7.

Sumner, S., Aanen, D.K., Delabie, J., Boomsma, J.J., 2004. The evolution of social parasitism in *Acromyrmex* leaf-cutting ants: a test of Emery' rule. Insectes Soc. 51, 37–42. https://doi.org/10.1007/s00040-003-0723-z.

Stuart, R.J., 2002. The behavioural ecology of parasites. In: Lewis, E.E. (Ed.), The Behavioural Ecology of Social Parasitism in Ants. University of Florida, USA, pp. 315–336.

Symonová, R., 2019. Integrative rDNAomics - importance of the oldest repetitive fraction of the eukaryote genome. Genes 10 (5), 345–360. https://doi.org/10.3390/genes10050345.

Teixeira, G.A., Barros, L.A.C., Aguiar, H.J.A.C., Pompolo, S.G., 2017. Comparative physical mapping of 18S rDNA in the karyotypes of six leafcutter ant species of the genera *Atta* and *Acromyrmex* (Formicidae: Myrmicinae). Genetica 145, 351–357. https://doi.org/10.1007/s10709-017-9970-1.

Teixeira, G.A., Aguiar, H.J.A.C., Petitclerc, F., Orivel, J., Lopes, D.M., Barros, L.A.C., 2021. Evolutionary insights into the genomic organization of major ribosomal DNA in ant chromosomes. Insect Mol. Biol. 30 (3), 340–354. https://doi.org/10.1111/imb.12699.

Tinaut, A., Ruano, F., 1999. Parasitismo social. Bol. Soc. Entomol. Aragonesa 26, 727–740.

Wilson, E.O., 1971. The Insect Societies. Harvard Univ. Press, Cambridge.