# The tight genome size of ants: diversity and evolution under ancestral state reconstruction and base composition 

MARIANA NEVES MOURA ${ }^{1}$, DANON CLEMES CARDOSO ${ }^{1,2, \oplus}$ and MAYKON PASSOS CRISTIANO ${ }^{1,2, *, \odot}$<br>${ }^{1}$ Programa de Pós-graduação em Ecologia, Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa 36570-000, Minas Gerais, Brazil<br>${ }^{2}$ Departamento de Biodiversidade, Evolução e Meio Ambiente, Universidade Federal de Ouro Preto, Ouro Preto 35400-000, Minas Gerais, Brazil

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#### Abstract

The mechanisms and processes driving change and variation in the genome size (GS) are not well known, and only a small set of ant species has been studied. Ants are an ecologically successful insect group present in most distinct ecosystems worldwide. Considering their wide distribution and ecological plasticity in different environmental contexts, we aimed to expand GS estimation within Formicidae to examine distribution patterns and variation in GS and base composition and to reconstruct the ancestral state of this character in an attempt to elucidate the generalized pattern of genomic expansions. Genome size estimates were generated for 99 ant species, including new GS estimates for 91 species of ants, and the mean GS of Formicidae was found to be 0.38 pg . The AT/GC ratio was $62.40 / 37.60$. The phylogenetic reconstruction suggested an ancestral GS of 0.38 pg according to the Bayesian inference/Markov chain Monte Carlo method and 0.37 pg according to maximum likelihood and parsimony methods; significant differences in GS were observed between the subfamilies sampled. Our results suggest that the evolution of GS in Formicidae occurred through loss and accumulation of non-coding regions, mainly transposable elements, and occasionally by whole genome duplication. However, further studies are needed to verify whether these changes in DNA content are related to colonization processes, as suggested at the intraspecific level.


ADDITIONAL KEYWORDS: AT/GC ratio - character reconstruction - DNA content - evolution - phylogeny transposable elements - whole genome duplication.

## INTRODUCTION

Although genome size (GS) is a fundamental characteristic of an organism, the mechanisms and processes driving variation and evolution of this trait are poorly understood (Gregory, 2005; Bennett \& Leitch, 2011; Kang et al., 2014). Genome size in eukaryotes varies by > 200 000-fold (Gregory, 2001), without any apparent correlation with either the complexity of the organism or the number of genes (Petrov, 2001). This lack of correlation was originally called the 'C-value enigma' or the 'C-value paradox' (Thomas, 1971), but the current understanding of the eukaryotic genome does not support the idea that it

[^0]is a simple linear collection of genes, making the old interpretation of GS variation as 'paradoxical' obsolete (Gregory, 2001).

Genome size is also known as DNA content, amount of DNA or DNA C-value. This is widely studied in plants, and the leading reports on variation in GS between species, in addition to references on standardization of procedures for GS quantification, have concentrated mainly on these organisms (e.g. Bennett et al., 2003; Doležel \& Bartoš, 2005; Gregory, 2005; Doležel et al., 2007; Bennett \& Leitch, 2011, 2012; Vu et al., 2015; Clark et al., 2016; Hidalgo et al., 2017a, b; Pellicer et al., 2018). For instance, ‘The Plant DNA C-values Database' (http://data.kew.org/cvalues/) currently contains data for 8510 plant species, whereas the haploid DNA contents (C-values, in picograms) are currently available for only 6222 animal species (3793
vertebrates and 2429 invertebrates), with insects representing $21.6 \%$ of this total (Gregory, 2020).

Despite our expanded understanding of genomes through new sequencing technologies, the mechanisms and processes that drive changes in GS are still poorly understood, and it is still unclear why there is astonishing variation among organisms, especially between closely related species. Some studies have associated GS variation between closely related taxa with the number of chromosomes (Ardila-Garcia \& Gregory, 2009; Cardoso et al., 2012), although other studies have not shown a similar correlation (Gregory, 2001; Tavares et al., 2012). In addition, mutations, recombination and the accumulation or deletion of non-coding DNA have also been proposed as factors in GS variation and are considered to be the driving forces for species diversification (El-Shehawi \& Elseehy, 2017).

Generally, an increase in GS is, in many organisms, related to polyploidy events (Adams \& Wendel, 2005), the amount of heterochromatin (Lopes et al., 2009; Tavares et al., 2010; Cardoso et al., 2012), amplification of non-coding repetitive DNA (Kidwell, 2002; Vieira et al., 2002) and other repetitive genome sequences (Gregory \& Hebert, 1999; Petrov, 2001; Cardoso et al., 2018). Moreover, a positive correlation between GS and base composition, mainly the GC content, has been found within several groups of vertebrates (e.g. Vinogradov \& Borkin, 1993; Vinogradov, 1994, 1998), bacteria (Guo et al., 2009; Nishida, 2012; Zhang \& Gao, 2017) and in some monocot plants (Li \& Du, 2014). However, studies regarding base composition and the effect on GS variation in other groups are still lacking ( $\mathrm{Li} \& \mathrm{Du}, 2014$ ), as they are in invertebrates.

In a recent report, Alfsnes et al. (2017) analysed the patterns of GS variation among organisms with different levels of taxonomic relatedness in the two major arthropod groups: crustaceans (subphylum: Crustacea) and insects (class: Insecta), based on openly available data. They found that the main causes of expansion of GS are proliferation of noncoding elements and/or duplication events. However, for other groups of organisms, such as ants, these patterns are somewhat speculative; there are only two studies involving a large number of species that have attempted to elucidate GS variation in Formicidae and the mechanisms of genome evolution (Tsutsui et al., 2008; Ardila-Garcia et al., 2010). Given that these authors have had different goals, distinct protocols have been applied to estimate the GS values. Thus, in order to minimize errors in comparison, a standardized protocol was established to obtain an adequate suspension of nuclei for flow cytometry (FCM) analyses in ants (Moura et al., 2019; 2020). They also proposed that the variation in GS could be applied to population studies and that variations in GS among populations
are likely to be related to stress experienced during the colonization of new environments.

In this study, we first expand the GS database of the family Formicidae, specifically within subfamilies, to verify the amplitude of variation of this trait. Second, we establish a protocol for the determination of base composition through flow cytometry in ants to examine the patterns of distribution and variation of GS and base composition among taxa. Third, we correlate and provide a phylogenetic perspective on GS evolution in Formicidae by reconstructing ancestral character states.

## MATERIAL AND METHODS

## Colony sampling

Colonies of different ant species were collected during several field expeditions. The colonies were detected visually, both by observation of individuals and by identification of the nest entrance. A sample or the entire colony was collected as described by Cardoso et al. (2011), transported and kept in laboratory conditions until FCM analyses. In other cases, several individuals from the same colony were collected and transported to the laboratory for immediate use in experimental procedures. The samples were collected in a wide diversity of environments in the Brazilian states of Tocantins (TO), Bahia (BA), Minas Gerais (MG), Rio de Janeiro (RJ), Santa Catarina (SC) and Rio Grande do Sul (RS), and in total, individuals belonging to 174 colonies were collected (Supporting Information, Table $\mathrm{S} 1)$. Vouchers of each collected species were stored in absolute alcohol, assembled and sent for identification by Dr Rodrigo Feitosa, at the Universidade Federal do Paraná, and MSc. Júlio Chaul, at the Universidade Federal de Viçosa. Specimens that had not yet been identified at the species level were identified at the maximum hierarchical level of genus and have been described in the present study followed by 'sp.'. All vouchers have been deposited in the 'Cristiano and Cardoso Myrmecology Colletion' (CC-LGEP) of the Laboratório de Genética Evolutiva e de Populações, at the Universidade Federal de Ouro Preto.

## FLOW CYTOMETRY ANALYSES

## Estimation of total DNA content

The flow cytometry experiments were performed following the protocol established by Moura et al. (2020). Briefly, the nuclear DNA content of the target species was measured using Drosophila melanogaster (Meigen, 1830) as the internal standard $(1 \mathrm{C}=0.18 \mathrm{pg})$. Galbraith lysis buffer was used for the subfamilies Dolichoderinae, Formicinae, Myrmicinae and Pseudomyrmecinae; LB01 buffer was used for the
subfamilies Dorylinae, Ectatomminae and Ponerinae (see Moura et al., 2020). Heads of adult ant workers and the internal standard were cut with a scalpel blade and immersed in $100-300 \mu \mathrm{~L}$ of the buffer in a 1.5 mL microtube and ground to release the cell nuclei. Next, $600 \mu \mathrm{~L}$ of the buffer was added to the solution, and the solution was filtered through a $40 \mu \mathrm{~m}$ nylon mesh (Becton Dickinson) and stained with the addition of $6.5 \mu \mathrm{~L}$ of propidium iodide (PI) solution and $3.5 \mu \mathrm{~L}$ of RNAse. The samples were stored at $4{ }^{\circ} \mathrm{C}$ in the dark and analysed within 1 h after preparation.

The analysis was performed on a FACSCalibur (Becton Dickinson) cytometer equipped with a laser source ( 488 nm ) at the Universidade Federal de Ouro Preto, and histograms were obtained with CELL QUEST software. For each sample, $\geq 10000$ nuclei were analysed for their relative fluorescence intensity. Three independent replications (i.e. three individuals per colony) were conducted, and histograms with a coefficient of variation $>5 \%$ were rejected, in which case a new specimen was measured. Histograms were analysed using FLOWING v.2.5.1 software (http:// www.flowingsoftware.com). The GS of each specimen was calculated using the 1 C -value of $D$. melanogaster, and the values were obtained according to equation from the study by Doležel \& Bartoš (2005). A general average was obtained per species sampled.

Additional GS data for 79 specimens belonging to 67 ant species were extracted from the Animal Genome Size Database (Gregory, 2020) of previously published studies (Li \& Heinz, 2000; Johnston et al., 2004; Sirviö et al., 2006; Tsutsui et al., 2008; ArdilaGarcia et al., 2010; Cardoso et al., 2012; Aguiar et al., 2016) and two GS values for the outgroup species, Apis mellifera (Linnaeus, 1748) and Chalybion californicum (Saussure, 1867).

## Determination of genomic AT and GC base composition

The mean AT/GC ratio was calculated for some species following the protocol established by Schwencke et al. (1998) for plants. Initially, the total nuclear content of these species was determined according to the procedure described above. An additional sample was treated with 4,6-diamidino-2-phenylindole (DAPI) to stain AT-rich regions of the genome specifically. Drosophila melanogaster was also used as an internal standard in these estimates because its base composition has already been determined (AT $=59 \%$, GC $=41 \%$; Danilevskaya et al., 1991; Adams et al., 2000; Ahuja \& Neale, 2005; Soares, 2012).

The analysis was performed on a FACSCanto II (Becton Dickinson) cytometer equipped with an ultraviolet lamp (388 nm) at the Universidade Federal
de Juiz de Fora, and $\geq 10000$ nuclei were analysed for each sample. Three independent replications were conducted, and histograms with a coefficient of variation $>5 \%$ were rejected. Histograms were analysed as aforementioned. The AT composition of the target species was determined using the following formula described by Godelle et al. (1993): $\mathrm{AT}_{\text {sample }}(\%)=\mathrm{AT}_{\text {internal standard }}(\%) \times\left(R_{\mathrm{DAPI}} / R_{\mathrm{PI}}{ }^{1 / 3}\right.$, where $R_{\mathrm{PI}}$ is the ratio of sample fluorescence intensity relative to the standard using the PI fluorochrome, and $R_{\text {DAPI }}$ is the DAPI ratio. The composition of GC bases was determined as follows: GC (\%) = $100 \%$ - AT (\%), as suggested by Bogunic et al. (2003).

## PHYLOGENETIC ANALYSIS

## Taxon sampling and phylogenetic analyses

A total of 83 Formicidae species were used in the phylogenetic analysis, including one taxon from Amblyoponinae, eight taxa from Dolichoderinae, two from Dorylinae, four from Ectatomminae, eight from Formicinae, 45 from Myrmicinae, ten from Ponerinae and four from Pseudomyrmecinae. Three species were included as outgroups: Apis mellifera (Apidae), Chalybion californicum (Sphecidae) and Mischocyttarus flavitarsis (Saussure, 1854) (Vespidae). All molecular operational taxonomic units were obtained from GenBank (Supporting Information, Table S2) and, owing to the best coverage of species with GS estimation, the genes long-wavelength rhodopsin ( $L W-R h$ ) and wingless ( $w g$ ) were chosen for the analysis. Subfamilies and species that did not have estimates of GS were not included in the phylogenetic analysis.

The $L W-R h$ and $w g$ nuclear genes were aligned separately using the Muscle algorithm (Edgar, 2004) provided in MEGA v.7.0 (Kumar et al., 2016). The intron of the $L W-R h$ gene was excluded from the alignment, and the aligned sequences of both genes were concatenated manually for further analyses. To select the substitution model of DNA evolution that best fitted each potential partition under Akaike's information criterion (AIC) and the Bayesian information criterion (BIC), we used the software PartitionFinder 2 (Lanfear et al., 2014, 2017). The models of evolution estimated for each gene codon position are presented in the Supporting Information (Table S3). Considering the estimated parameters, Bayesian analysis was conducted for phylogenetic inference using MRBAYES v.3.2.6 (Ronquist et al., 2012). Trees were searched with two independent runs, with four Markov chains each (one cold and three heated). Each chain was run for 50 million generations and sampled every 5000 generations. Convergence of the cold chains was analysed using the program TRACER
v.1.6 (Drummond \& Rambaut, 2007), and a traditional burn-in on the first $25 \%$ of the trees was performed before using the remaining topologies to build a final majority rule consensus tree with its respective branch lengths, which was viewed using FigTree v.1.3 (Rambaut, 2008).

## Reconstruction of ancestral genome size

All 1C-values estimated in this study and the 1C-values extracted from the Animal Genome Size Database (Gregory, 2020) were plotted on the phylogenetic tree. To estimate the ancestral GS throughout the phylogeny, three different reconstruction methods were used: maximum parsimony (MP) analysis in MESQUITE v.3.04 (Maddison \& Maddison, 2011); the maximum likelihood (ML) reconstruction method implemented in StableTraits (Elliot, 2014); and a Bayesian inference (BI) via Markov chain Monte Carlo (MCMC) in BayEsTraits v.3.0 (Pagel et al., 2017) with the 'continuous random walk' model. Initially, we also verified whether the GS evolved according to a Brownian motion model of evolution throughout the phylogeny in BaYESTRAITS; the ancestral genome was calculated assuming a Brownian motion model along the phylogeny and using a model with correction of the parameters $\delta, \kappa$ and $\lambda$ in the phylogeny, as described by Pagel et al. (1997, 2004).

## STATISTICAL ANALYSES

To analyse the GS variation in Formicidae subfamilies, the mean GS per subfamily was calculated and plotted in the phylogenetic tree generated in this study, with collapsed branches. General linear models (GLMs) were built to test for differences between the average GSs of the sampled subfamilies. Differences in GS averages for each subfamily were assessed by variance analysis of the GLM. When the $P$-value of the ANOVA was significant ( $P<0.05$ ), a contrast analysis at the $5 \%$ level was performed to determine the average difference between groups. The statistical analysis was performed in $R$ v.2.15.1 software ( $R$ Core Team, 2013), and the GLM was submitted to residual analysis to evaluate the adequacy of the error distribution (Crawley, 2013).

## RESULTS

## GENOME SIZE ESTIMATIONS

In this study, we present new GS estimates for 99 ant species (Table 1), including new genome size estimates for 91 species of ants. To calculate the means and percentages, 79 estimates from the literature, corresponding to 67 species, were also included. The
number of estimates was thus increased by > 100\% and now represents almost $1 \%$ out of a total of 13750 valid species (AntWeb, 2020). Of the 337 accepted genera (AntWeb, 2020) there are now 56 estimates for GS, with Acromyrmex Mayr, 1865 being the genus with the largest number of species estimated (13 in total). From 17 existing Formicidae subfamilies, there are nine estimates, with Myrmicinae having the largest number of measurements (100) and species (90), followed by Formicinae, with a total of 22 estimates corresponding to 20 species, and Dolichoderinae, with 18 estimates corresponding to 16 species.

The mean GS of the family Formicidae is 0.38 pg . The lowest 1C-values are found in Dolichoderus mariae Forel, 1885, Dorymyrmex bureni (Trager, 1988) and Paratrechina longicornis (Latreille, 1802), with 0.18 pg, and the highest value was found in Apterostigma sp. 3, with 0.81 pg . Overall, the estimated 1C-values varied between subfamilies, ranging from 0.18 to 0.61 pg in Dolichoderinae (average 0.29 pg ), from 0.22 to 0.37 pg in Dorylinae (average 0.30 pg ), from 0.32 to 0.71 pg in Ectatomminae (average 0.45 pg ), from 0.18 to 0.39 pg in Formicinae (average 0.31 pg ), from 0.21 to 0.81 pg in Myrmicinae (average 0.39 pg ), from 0.25 to 0.63 pg in Ponerinae (average 0.47 pg ) and from 0.29 to 0.41 pg in Pseudomyrmecinae (average 0.37 pg ). The Amblyoponinae subfamily contained only two values, with a average GS of 0.36 pg , and Myrmeciinae only one value, 0.28 pg . From the total of 179 values estimated, $84 \%$ ranged from 0.25 to 0.50 pg (Table 1).

The base composition values in Myrmicinae oscillated from AT $=59.17 \%$ in Pheidole sp. 2 to $64.82 \%$ in Atta sexdens (Linnaeus, 1758), and the mean AT was $62.14 \%$. Similar values were found in Ectatomminae and Pseudomyrmecinae, with AT of $60.22 \%$ and $60.44 \%$, respectively. It was not possible to compare the AT and GC means of the subfamilies because only Myrmicinae was represented by more than one species. The highest AT values, in Atta sexdens (Linnaeus, 1758) (64.82\%) and Acromyrmex nigrosetosus (Forel, 1908) ( $63.75 \%$ ), were not correlated with larger GSs because the former had a GS of 0.33 pg and the latter 0.35 pg , and the highest GS value was found in Apterostigma sp. 3, with 0.81 pg and $\mathrm{AT}=62.18 \%$. The GS values (in picograms) and base composition (AT and GC percentages) estimated for the species in the present study are summarized in Table 2.

The simultaneous analyses of target species and D. melanogaster (internal standard) suspensions of nuclei provided histograms with fluorescence peaks corresponding to the mean DNA content of the $\mathrm{G}_{0} /$ $\mathrm{G}_{1}$ and $\mathrm{G}_{2}$ nuclei of both organisms, stained with PI (Fig. 1A-C) and, for some species, DAPI (Fig. 1D-F). The $G_{0} / G_{1}$ peaks of all specimens included in this study could be discriminated clearly, and their coefficients of variation were always $<5 \%$, which is considered
Table 1. Genome sizes estimated for all studied specimens, the mean calculated for each species (in picograms and megabase pairs), standard deviation and applied methodology

| Species | Subfamily | Mean <br> 1 C -value (pg) | SD | Mean <br> 1C-value (Mbp) | References | Method | Cell type | Standard sp. |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Acromyrmex ambiguus (Emery, 1888) | Myrmicinae | 0.33 | 0.01 | 322.74 | This study | FCM | BR | DM |
| Acromyrmex balzani (Emery, 1890) | Myrmicinae | 0.37 | 0.00 | 361.86 | This study | FCM | BR | DM |
| Acromyrmex coronatus (Fabricius, 1804) | Myrmicinae | 0.34 | 0.00 | 332.52 | This study | FCM | BR | DM |
| Acromyrmex crassispinus (Forel, 1909) | Myrmicinae | 0.34 | 0.01 | 332.52 | This study | FCM | BR | DM |
| Acromyrmex disciger (Mayr, 1887) | Myrmicinae | 0.33 | 0.00 | 322.74 | This study | FCM | BR | DM |
| Acromyrmex echinatior (Forel, 1899) | Myrmicinae | 0.36 | - | 335 | Sirviö et al. (2006) | FCM | BR | CRBC |
| Acromyrmex niger (Smith, 1858) | Myrmicinae | 0.36 | 0.00 | 352.08 | This study | FCM | BR | DM |
| Acromyrmex nigrosetosus (Forel, 1908) | Myrmicinae | 0.35 | 0.00 | 342.3 | This study | FCM | BR | DM |
| Acromyrmex rugosus (Smith, 1858) | Myrmicinae | 0.35 | 0.00 | 342.3 | This study | FCM | BR | DM |
| Acromyrmex subterraneus brunneus (Forel, 1912) | Myrmicinae | 0.34 | 0.00 | 332.52 | This study | FCM | BR | DM |
| Acromyrmex subterraneus molestans <br> Sntschi, 1925 | Myrmicinae | 0.34 | 0.01 | 332.52 | This study | FCM | BR | DM |
| Acromyrmex subterraneus subterraneus (Forel, 1893) | Myrmicinae | 0.35 | 0.01 | 342.3 | This study | FCM | BR | DM |
| Amoimyrmex striatus (Roger, 1863) | Myrmicinae | 0.35 | 0.00 | 342.3 | This study | FCM | BR | DM |
| Stigmatomma pallipes (Haldeman, 1844) | Amblyoponinae | 0.34 | - | 335.5 | Tsutsui et al., (2008) | FCM | BR | DM |
| Stigmatomma pallipes | Amblyoponinae | 0.37 | - | 361.86 | Ardila-Garcia et al., (2010) | FCM | BR | DM |
| Aphaenogaster fulva (Roger, 1863) | Myrmicinae | 0.42 | - | 410.76 | Ardila-Garcia et al., (2010) | FCM | BR | DM |
| Aphaenogaster Mayr, 1853 (rudis-texana group N16) | Myrmicinae | 0.43 | - | 420.54 | Ardila-Garcia et al., (2010) | FCM | BR | DM |
| Aphaenogaster (rudis-texana group N17) | Myrmicinae | 0.46 | - | 449.88 | Ardila-Garcia et al., (2010) | FCM | BR | DM |
| Aphaenogaster (rudis-texana group N22b) | Myrmicinae | 0.44 | - | 430.32 | Ardila-Garcia et al., (2010) | FCM | BR | DM |
| Aphaenogaster treatae Forel, 1886 | Myrmicinae | 0.50 | - | 489 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Apterostigma dentigerum Wheeler, 1925 | Myrmicinae | 0.65 | - | 636.4 | Tsutsui et al. (2008) | FCM | BR | DM |
| Apterostigma Mayr, 1865 sp. 1 | Myrmicinae | 0.74 | 0.00 | 723.72 | This study | FCM | BR | DM |
| Apterostigma sp. 2 | Myrmicinae | 0.69 | 0.01 | 674.82 | This study | FCM | BR | DM |
| Apterostigma sp. 3 | Myrmicinae | 0.81 | 0.01 | 792.18 | This study | FCM | BR | DM |

Table 1. Continued

| Species | Subfamily | Mean <br> 1C-value (pg) | SD | Mean <br> 1C-value (Mbp) | References | Method | Cell type | Standard sp. |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Apterostigma sp. 4 | Myrmicinae | 0.63 | 0.00 | 616.14 | This study | FCM | BR | DM |
| Atta cephalotes (Linnaeus, 1758) | Myrmicinae | 0.31 | - | 300.1 | Tsutsui et al. (2008) | FCM | BR | DM |
| Atta columbica Guérin-Méneville, 1844 | Myrmicinae | 0.31 | - | 298.8 | Tsutsui et al. (2008) | FCM | BR | DM |
| Atta laevigata (Smith, 1858) | Myrmicinae | 0.33 | 0.01 | 322.74 | This study | FCM | BR | DM |
| Atta robusta Borgmeier, 1939 | Myrmicinae | 0.34 | 0.00 | 332.52 | This study | FCM | BR | DM |
| Atta sexdens rubropilosa (Linnaeus, 1758) | Myrmicinae | 0.33 | 0.01 | 322.74 | This study | FCM | BR | DM |
| Atta texana (Buckley, 1860) | Myrmicinae | 0.27 | - | 264.06 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Azteca chartifex spiriti Forel, 1912 | Dolichoderinae | 0.36 | 0.00 | 352.08 | This study | FCM | BR | DM |
| Azteca muelleri Emery, 1893 | Dolichoderinae | 0.37 | 0.00 | 361.86 | This study | FCM | BR | DM |
| Brachymyrmex coactus Mayr, 1887 | Formicinae | 0.39 | 0.01 | 381.42 | This study | FCM | BR | DM |
| Brachymyrmex Mayr, 1868 sp. 1 | Formicinae | 0.34 | 0.00 | 332.52 | This study | FCM | BR | DM |
| Brachymyrmex sp. 2 | Formicinae | 0.36 | 0.01 | 352.08 | This study | FCM | BR | DM |
| Brachymyrmex sp. 3 | Formicinae | 0.35 | 0.00 | 342.3 | This study | FCM | BR | DM |
| Brachymyrmex sp. 4 | Formicinae | 0.33 | 0.00 | 322.74 | This study | FCM | BR | DM |
| Camponotus blandus (Smith, 1858) | Formicinae | 0.29 | 0.01 | 283.62 | This study | FCM | BR | DM |
| Camponotus bonariensis Mayr, 1868 | Formicinae | 0.36 | 0.01 | 352.08 | This study | FCM | BR | DM |
| Camponotus castaneus (Latreille, 1802) | Formicinae | 0.31 | - | 304.2 | Tsutsui et al. (2008) | FCM | BR | DM |
| Camponotus crassus Mayr, 1862 | Formicinae | 0.29 | - | 286 | $\begin{aligned} & \text { Aguiar et al. } \\ & (2016) \end{aligned}$ | FCM | BR | SX |
| Camponotus crassus | Formicinae | 0.29 | 0.01 | 283.62 | This study | FCM | BR | DM |
| Camponotus floridanus (Buckley, 1866) | Formicinae | 0.23 | - | 224.94 | Ardila-Garcia et al. (2010) | FIA | HE | TM |
| Camponotus pennsylvanicus (De Geer, 1773) | Formicinae | 0.33 | - | 322.8 | Tsutsui et al. (2008) | FCM | BR | DM |
| Camponotus renggeri Emery, 1894 | Formicinae | 0.29 | - | 286 | $\begin{aligned} & \text { Aguiar et al. } \\ & (2016) \end{aligned}$ | FCM | BR | SX |
| Camponotus renggeri | Formicinae | 0.32 | 0.01 | 312.96 | This study | FCM | BR | DM |
| Camponotus rufipes (Fabricius, 1775) | Formicinae | 0.29 | - | 286 | $\begin{aligned} & \text { Aguiar et al. } \\ & (2016) \end{aligned}$ | FCM | BR | SX |
| Camponotus Mayr, 1861 sp. | Formicinae | 0.31 | 0.01 | 303.18 | This study | FCM | BR | DM |
| Cephalotes atratus (Linnaeus, 1758) | Myrmicinae | 0.40 | 0.02 | 391.2 | This study | FCM | BR | DM |
| Cephalotes depressus (Klug, 1824) | Myrmicinae | 0.53 | 0.01 | 518.34 | This study | FCM | BR | DM |
| Cephalotes pusillus (Klug, 1824) | Myrmicinae | 0.38 | 0.01 | 371.64 | This study | FCM | BR | DM |

Table 1. Continued

| Species | Subfamily | Mean 1C-value (pg) | SD | Mean <br> 1C-value (Mbp) | References | Method | Cell type | Standard sp. |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Ooceraea australis (Forel, 1900) | Dorylinae | 0.22 | - | 210.7 | Tsutsui et al. (2008) | FCM | BR | DM |
| Crematogaster goeldii Forel, 1903 | Myrmicinae | 0.41 | 0.02 | 400.98 | This study | FCM | BR | DM |
| Crematogaster hespera Buren, 1968 | Myrmicinae | 0.28 | - | 275.9 | Tsutsui et al. (2008) | FCM | BR | DM |
| Crematogaster Lund, 1831 sp. 2 | Myrmicinae | 0.40 | 0.01 | 391.2 | This study | FCM | BR | DM |
| Crematogaster nigropilosa Mayr, 1870 | Myrmicinae | 0.39 | 0.00 | 381.42 | This study | FCM | BR | DM |
| Crematogaster torosa Mayr, 1870 | Myrmicinae | 0.38 | 0.01 | 371.64 | This study | FCM | BR | DM |
| Cyphomyrmex Mayr, 1862 sp. 1 | Myrmicinae | 0.42 | 0.01 | 410.76 | This study | FCM | BR | DM |
| Cyphomyrmex sp. 2 | Myrmicinae | 0.28 | 0.02 | 273.84 | This study | FCM | BR | DM |
| Cyphomyrmex sp. 3 | Myrmicinae | 0.32 | 0.00 | 312.96 | This study | FCM | BR | DM |
| Cyphomyrmex pr. minutus Mayr, 1862 | Myrmicinae | 0.33 | 0.00 | 322.74 | This study | FCM | BR | DM |
| Cyphomyrmex transversus Emery, 1894 | Myrmicinae | 0.50 | 0.01 | 489 | This study | FCM | BR | DM |
| Dinoponera australis Emery, 1901 | Ponerinae | 0.57 | - | 554.7 | Tsutsui et al. (2008) | FCM | BR | DM |
| Dolichoderus bispinosus (Olivier, 1792) | Dolichoderinae | 0.27 | 0.00 | 264.06 | This study | FCM | BR | DM |
| Dolichoderus mariae Forel, 1885 | Dolichoderinae | 0.18 | - | 176.04 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Dolichoderus taschenbergi (Mayr, 1866) | Dolichoderinae | 0.23 | - | 224.94 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Dormyrmex bicolor Wheeler, 1906 | Dolichoderinae | 0.25 | - | 249.0 | Tsutsui et al. (2008) | FCM | BR | DM |
| Dorymyrmex brunneus Forel, 1908 | Dolichoderinae | 0.26 | 0.01 | 254.28 | This study | FCM | BR | DM |
| Dorymyrmex bureni (Trager, 1988) | Dolichoderinae | 0.18 | - | 176.04 | Ardila-Garcia et al. (2010) | FIA | HE | TM |
| Dorymyrmex Mayr, 1866 sp. 1 | Dolichoderinae | 0.23 | 0.01 | 224.94 | This study | FCM | BR | DM |
| Dorymyrmex sp. 2 | Dolichoderinae | 0.27 | 0.00 | 264.06 | This study | FCM | BR | DM |
| Eciton burchellii (Westwood, 1842) | Dorylinae | 0.27 | - | 263.9 | Tsutsui et al. (2008) | FCM | BR | DM |
| Eciton burchellii | Dorylinae | 0.29 | 0.00 | 283.62 | This study | FCM | BR | DM |
| Ectatomma brunneum Smith, 1858 | Ectatomminae | 0.38 | 0.01 | 371.64 | This study | FCM | BR | DM |
| Ectatomma edentatum Roger, 1863 | Ectatomminae | 0.36 | 0.00 | 352.08 | This study | FCM | BR | DM |
| Ectatomma tuberculatum (Olivier, 1792) | Ectatomminae | 0.71 | - | 690.4 | Tsutsui et al. (2008) | FCM | BR | DM |
| Eurhopalothrix procera (Emery, 1897) | Myrmicinae | 0.39 | - | 377.2 | $\begin{aligned} & \text { Tsutsui et al. } \\ & (2008) \end{aligned}$ | FCM | BR | DM |
| Forelius pruinosus (Roger, 1863) | Dolichoderinae | 0.22 | - | 215.16 | Ardila-Garcia et al. (2010) | FIA | HE | TM |

Table 1. Continued

| Species | Subfamily | Mean <br> 1C-value (pg) | SD | Mean <br> 1C-value (Mbp) | References | Method | Cell type | Standard sp. |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Formica pallidefulva Latreille, 1802 | Formicinae | 0.39 | - | 385.1 | Tsutsui et al. (2008) | FCM | BR | DM |
| Gnamptogenys Roger, 1863 sp. | Ectatomminae | 0.32 | 0.02 | 312.96 | This study | FCM | BR | DM |
| Gnamptogenys striatula Mayr, 1884 | Ectatomminae | 0.50 | 0.01 | 489 | This study | FCM | BR | DM |
| Hylomyrma reitteri (Mayr, 1887) | Myrmicinae | 0.45 | 0.01 | 440.1 | This study | FCM | BR | DM |
| Hypoponera Santschi, 1938 sp. 1 | Ponerinae | 0.40 | 0.01 | 391.2 | This study | FCM | BR | DM |
| Hypoponera sp. 2 | Ponerinae | 0.50 | 0.00 | 489 | This study | FCM | BR | DM |
| Hypoponera sp. 3 | Ponerinae | 0.39 | 0.01 | 381.42 | This study | FCM | BR | DM |
| Hypoponera sp. 4 | Ponerinae | 0.47 | 0.01 | 459.66 | This study | FCM | BR | DM |
| Hypoponera sp. 5 | Ponerinae | 0.36 | 0.02 | 352.08 | This study | FCM | BR | DM |
| Labidus coecus (Latreille, 1802) | Dorylinae | 0.37 | - | 365.8 | Tsutsui et al. (2008) | FCM | BR | DM |
| Lasius latipes (Walsh, 1863) | Formicinae | 0.27 | - | 264.06 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Lasius alienus (Foerster, 1850) | Formicinae | 0.31 | - | 307.7 | Tsutsui et al. (2008) | FCM | BR | DM |
| Lasius minutus Emery, 1893 | Formicinae | 0.23 | - | 224.94 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Linepithema humile (Mayr, 1868) | Dolichoderinae | 0.26 | - | 250.8 | Tsutsui et al. (2008) | FCM | BR | DM |
| Linepithema micans (Forel, 1908) | Dolichoderinae | 0.29 | 0.01 | 283.62 | This study | FCM | BR | DM |
| Liometopum occidentale Emery, 1895 | Dolichoderinae | 0.29 | - | 282.0 | Tsutsui et al. (2008) | FCM | BR | DM |
| Megalomyrmex incisus Smith, 1947 | Myrmicinae | 0.46 | 0.05 | 449.88 | This study | FCM | BR | DM |
| Veromessor andrei (Mayr, 1886) | Myrmicinae | 0.26 | - | 253.5 | Tsutsui et al. (2008) | FCM | BR | DM |
| Monomorium viridum Brown, 1943 | Myrmicinae | 0.50 | - | 489 | Ardila-Garcia et al. (2010) | FIA | HE | TM |
| Mycetarotes parallelus (Emery, 1906) | Myrmicinae | 0.38 | 0.01 | 371.64 | This study | FCM | BR | DM |
| Mycetarotes sp. Emery, 1913 | Myrmicinae | 0.48 | 0.01 | 469.44 | This study | FCM | BR | DM |
| Mycetophylax conformis (Mayr, 1884) | Myrmicinae | 0.31 | 0.01 | 303.18 | This study | FCM | BR | DM |
| Mycetophylax conformis | Myrmicinae | 0.32 | - | 312.96 | $\begin{aligned} & \text { Cardoso et al. } \\ & \quad(2012) \end{aligned}$ | FCM | BR | ST |
| Mycetophylax morschi (Emery, 1888) | Myrmicinae | 0.34 | 0.01 | 332.52 | This study | FCM | BR | DM |
| Mycetophylax morschi | Myrmicinae | 0.32 | - | 312.96 | $\begin{aligned} & \text { Cardoso et al. } \\ & \quad(2012) \end{aligned}$ | FCM | BR | ST |
| Mycetophylax simplex (Emery, 1888) | Myrmicinae | 0.41 | 0.01 | 400.98 | This study | FCM | BR | DM |

Table 1. Continued

| Species | Subfamily | Mean 1C-value (pg) | SD | Mean <br> 1C-value (Mbp) | References | Method | Cell type | Standard sp. |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Mycetophylax simplex | Myrmicinae | 0.39 | - | 381.42 | Cardoso et al. (2012) | FCM | BR | ST |
| Mycetomoellerius urichii (Forel, 1893) | Myrmicinae | 0.47 | 0.01 | 459.66 | This study | FCM | BR | DM |
| Mycetomoellerius holmgreni (Wheeler, 1925) | Myrmicinae | 0.33 | 0.01 | 322.74 | This study | FCM | BR | DM |
| Mycetomoellerius iheringi (Emery, 1888) | Myrmicinae | 0.40 | 0.01 | 391.2 | This study | FCM | BR | DM |
| Mycocepurus goeldii (Forel, 1893) | Myrmicinae | 0.42 | 0.00 | 410.76 | This study | FCM | BR | DM |
| Myrmecia varians Mayr, 1876 | Myrmeciinae | 0.28 | - | 269.5 | Tsutsui et al. (2008) | FCM | BR | DM |
| Myrmecina americana Emery, 1895 (A) | Myrmicinae | 0.26 | - | 250.7 | Tsutsui et al. (2008) | FCM | BR | DM |
| Myrmecina americana (B) | Myrmicinae | 0.31 | - | 302.9 | Tsutsui et al. <br> (2008) | FCM | BR | DM |
| Myrmicocrypta Smith, 1860 sp. 1 (JSC - 048 _Sosa Calvo) | Myrmicinae | 0.48 | 0.01 | 469.44 | This study | FCM | BR | DM |
| Myrmicocrypta sp. 2 | Myrmicinae | 0.39 | 0.00 | 381.42 | This study | FCM | BR | DM |
| Neocerapachys Borowiec, 2016 sp. | Dorylinae | 0.36 | 0.00 | 352.08 | This study | FCM | BR | DM |
| Neoponera marginata (Roger, 1861) | Ponerinae | 0.63 | 0.01 | 616.14 | This study | FCM | BR | DM |
| Odontomachus bauri Emery, 1892 | Ponerinae | 0.49 | - | 477.3 | Tsutsui et al. (2008) | FCM | BR | DM |
| Odontomachus bauri | Ponerinae | 0.38 | 0.01 | 371.64 | This study | FCM | BR | DM |
| Odontomachus brunneus (Patton, 1894) | Ponerinae | 0.33 | - | 322.74 | Ardila-Garcia et al. (2010) | FIA | HE | TM |
| Odontomachus brunneus | Ponerinae | 0.44 | - | 429.8 | Tsutsui et al. <br> (2008) | FCM | BR | DM |
| Odontomachus cephalotes Smith, 1863 | Ponerinae | 0.43 | - | 425.0 | Tsutsui et al. (2008) | FCM | BR | DM |
| Odontomachus chelifer (Latreille, 1802) | Ponerinae | 0.54 | - | 523.2 | $\begin{aligned} & \text { Tsutsui et al. } \\ & \quad(2008) \end{aligned}$ | FCM | BR | DM |
| Odontomachus clarus Roger, 1861 | Ponerinae | 0.42 | - | 414.0 | Tsutsui et al. (2008) | FCM | BR | DM |
| Odontomachus haematodus (Linnaeus, 1758) | Ponerinae | 0.51 | - | 496.5 | $\begin{aligned} & \text { Tsutsui et al. } \\ & (2008) \end{aligned}$ | FCM | BR | DM |
| Odontomachus meinerti Forel, 1905 | Ponerinae | 0.55 | 0.00 | 537.9 | This study | FCM | BR | DM |
| Pachycondyla estriata Smith, 1858 | Ponerinae | 0.51 | 0.01 | 498.78 | This study | FCM | BR | DM |
| Paratrechina longicornis (Latreille, 1802) | Formicinae | 0.18 | - | 176.04 | Ardila-Garcia et al. (2010) | FIA | HE | TM |
| Pheidole dentata Mayr, 1886 | Myrmicinae | 0.24 | - | 234.72 | Ardila-Garcia et al. (2010) | FIA | HE | TM |

Table 1. Continued

| Species | Subfamily | Mean <br> 1C-value (pg) | SD | Mean <br> 1C-value (Mbp) | References | Method | Cell type | Standard sp. |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Pheidole floridana Emery, 1895 | Myrmicinae | 0.21 | - | 205.38 | Ardila-Garcia et al. (2010) | FIA | HE | TM |
| Pheidole hyatti Emery, 1895 | Myrmicinae | 0.33 | - | 326.7 | Tsutsui et al. (2008) | FCM | BR | DM |
| Pheidole oxyops Forel, 1908 | Myrmicinae | 0.37 | 0.00 | 361.86 | This study | FCM | BR | DM |
| Pheidole radoszkowskii Mayr, 1884 | Myrmicinae | 0.28 | 0.02 | 273.84 | This study | FCM | BR | DM |
| Pheidole Westwood, 1839 sp. 2 | Myrmicinae | 0.37 | 0.01 | 361.86 | This study | FCM | BR | DM |
| Pheidole sp. 3 | Myrmicinae | 0.36 | 0.00 | 352.08 | This study | FCM | BR | DM |
| Platythyrea punctata (Smith, 1858) | Ponerinae | 0.25 | 0.01 | 244.5 | This study | FCM | BR | DM |
| Pogonomyrmex badius (Latreille, 1802) | Myrmicinae | 0.27 | - | 262.8 | Tsutsui et al. (2008) | FCM | BR | DM |
| Pogonomyrmex californicus (Buckley, 1867) | Myrmicinae | 0.25 | - | 249.5 | Tsutsui et al. (2008) | FCM | BR | DM |
| Pogonomyrmex coarctatus Mayr, 1868 | Myrmicinae | 0.29 | - | 282.9 | Tsutsui et al. (2008) | FCM | BR | DM |
| Pogonomyrmex naegelii Emery, 1878 | Myrmicinae | 0.36 | 0.02 | 352.08 | This study | FCM | BR | DM |
| Ponera pennsylvanica Buckley, 1866 | Ponerinae | 0.55 | - | 537.9 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Ponera pennsylvanica | Ponerinae | 0.60 | - | 591.9 | Tsutsui et al. (2008) | FCM | BR | DM |
| Prenolepis imparis (Say, 1836) | Formicinae | 0.30 | - | 296.2 | Tsutsui et al. (2008) | FCM | BR | DM |
| Pseudomyrmex ejectus (Smith, 1858) | Pseudomyrmecinae | 0.29 | - | 283.62 | Ardila-Garcia et al. (2010) | FIA | HE | TM |
| Pseudomyrmex gracilis (Fabricius, 1804) | Pseudomyrmecinae | 0.35 | - | 342.3 | Ardila-Garcia et al. (2010) | FCM. FIA | BR. HE | DM. TM |
| Pseudomyrmex gracilis | Pseudomyrmecinae | 0.40 | - | 387.0 | Tsutsui et al. (2008) | FCM | BR | DM |
| Pseudomyrmex gracilis | Pseudomyrmecinae | 0.41 | 0.01 | 400.98 | This study | FCM | BR | DM |
| Pseudomyrmex schuppi (Forel, 1901) | Pseudomyrmecinae | 0.38 | 0.02 | 371.64 | This study | FCM | BR | DM |
| Pseudomyrmex termitarius (Smith, 1855) | Pseudomyrmecinae | 0.39 | 0.01 | 381.42 | This study | FCM | BR | DM |
| Strumigenys rostrata Emery, 1895 | Myrmicinae | 0.28 | - | 278.6 | $\begin{aligned} & \text { Tsutsui et al. } \\ & (2008) \end{aligned}$ | FCM | BR | DM |
| Sericomyrmex amabilis Wheeler, 1925 | Myrmicinae | 0.45 | - | 440.7 | Tsutsui et al. (2008) | FCM | BR | DM |
| Sericomyrmex Mayr, 1865 sp. 1 | Myrmicinae | 0.42 | 0.03 | 410.76 | This study | FCM | BR | DM |
| Sericomyrmex sp. 2 | Myrmicinae | 0.42 | 0.01 | 410.76 | This study | FCM | BR | DM |

Table 1. Continued

| Species | Subfamily | Mean <br> 1C-value (pg) | SD | Mean <br> 1C-value (Mbp) | References | Method | Cell type | Standard sp. |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Sericomyrmex sp. 3 | Myrmicinae | 0.42 | 0.01 | 410.76 | This study | FCM | BR | DM |
| Sericomyrmex sp. 4 | Myrmicinae | 0.39 | 0.00 | 381.42 | This study | FCM | BR | DM |
| Solenopsis invicta Buren, 1972 | Myrmicinae | 0.62 | - | 606.36 | Li and Heinz (2000) | BCA | BR | NS |
| Solenopsis invicta | Myrmicinae | 0.77 | - | 753.06 | Johnston et al. (2004) | FCM | BR | DM |
| Solenopsis invicta | Myrmicinae | 0.48 | - | 469.44 | Ardila-Garcia et al. (2010) | FIA | HE | TM |
| Solenopsis molesta (Say, 1836) | Myrmicinae | 0.38 | - | 371.64 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Solenopsis saevissima (Smith, 1855) | Myrmicinae | 0.50 | 0.01 | 489 | This study | FCM | BR | DM |
| Solenopsis Westwood, 1840 sp. 1 | Myrmicinae | 0.45 | 0.01 | 440.1 | This study | FCM | BR | DM |
| Solenopsis sp. 2 | Myrmicinae | 0.39 | 0.00 | 381.42 | This study | FCM | BR | DM |
| Solenopsis sp. 3 | Myrmicinae | 0.41 | 0.01 | 400.98 | This study | FCM | BR | DM |
| Solenopsis xyloni McCook, 1880 | Myrmicinae | 0.48 | - | 472.3 | Tsutsui et al. (2008) | FCM | BR | DM |
| Strumigenys denticulata Mayr, 1887 | Myrmicinae | 0.32 | 0.00 | 312.96 | This study | FCM | BR | DM |
| Strumygenys Smith, 1860 (ufv 06) sp. 1 | Myrmicinae | 0.32 | 0.00 | 312.96 | This study | FCM | BR | DM |
| Tapinoma melanocephalum (Fabricius, 1793) | Dolichoderinae | 0.28 | 0.01 | 273.84 | This study | FCM | BR | DM |
| Tapinoma sessile (Say, 1836) | Dolichoderinae | 0.37 | - | 361.86 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Tapinoma sessile $A$ | Dolichoderinae | 0.38 | - | 374.4 | Tsutsui et al. (2008) | FCM | BR | DM |
| Tapinoma sessile B | Dolichoderinae | 0.61 | - | 593.1 | Tsutsui et al. (2008) | FCM | BR | DM |
| Temnothorax ambiguus (Emery, 1895) | Myrmicinae | 0.31 | - | 303.18 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Temnothorax texanus (Wheeler, 1903) | Myrmicinae | 0.32 | - | 312.96 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Tetramorium caespitum (Linnaeus, 1758) | Myrmicinae | 0.26 | - | 256.4 | Tsutsui et al. (2008) | FCM | BR | DM |
| Tetramorium caespitum | Myrmicinae | 0.27 | - | 264.06 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Trachymyrmex septentrionalis (McCook, 1881) | Myrmicinae | 0.25 | - | 244.5 | Ardila-Garcia et al. (2010) | FIA | HE | TM |
| Wasmannia auropunctata (Roger, 1863) | Myrmicinae | 0.36 | 0.01 | 352.08 | This study | FCM | BR | DM |
| Wasmannia lutzi Forel, 1908 | Myrmicinae | 0.37 | 0.00 | 361.86 | This study | FCM | BR | DM |

Table 1. Continued

| Species | Subfamily | Mean <br> 1C-value (pg) | SD | Mean <br> 1C-value (Mbp) | References | Method | Cell type | Standard sp. |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Wasmannia sp. Forel, 1893 | Myrmicinae | 0.38 | 0.00 | 371.64 | This study | FCM | BR | DM |
| Apis mellifera (Linnaeus, 1748) | Apidae - outgroup | 0.24 | - | 234.72 | Ardila-Garcia et al. (2010) | FCM | BR | DM |
| Chalybion californicum (Saussure, 1867) | Sphecidae outgroup | 0.54 | - | 528.12 | Ardila-Garcia et al. (2010) | FCM | BR | DM |

appropriate for GS determination using FCM (Cardoso et al., 2012). Six representative histograms, three for GS and three for base composition, are shown in Figure 1.

## PhyLOGENETIC ANALYSIS

An alignment length of 871 bp was obtained for the $L W-R h$ and $w g$ nuclear regions from 83 sequences of Formicidae plus three outgroup species, which includes 500 variable sites ( $57.4 \%$ ). The Bayesian consensus phylogenetic tree based on the $L W$ $R h$ and $w g$ genes is show in Figure 2. Formicidae is recovered as monophyletic with a high value of posterior probability (PP) [node 1 ( n 1 ), $\mathrm{PP}=1$ ], suggesting that the tree is adequate for further analysis. Subfamily Amblyoponinae, represented by only one species, is recovered as a sister group to all the other ants, as is Ponerinae, with all the species grouped into one clade [node $2(\mathrm{n} 2), \mathrm{PP}=1$ ]. Node 3 ( n 3 ), which has a $\mathrm{PP}=0.93$, comprises the remaining subfamilies sampled, with Dorylinae [node $4(\mathrm{n} 4), \mathrm{PP}=1$ ] as the sister group to the others. Node 5 (n5), with a high posterior probability value ( $\mathrm{PP}=0.95$ ), is divided into two clades: the first clade [node 6 ( n 6 ), $\mathrm{PP}=0.69$ ] containing the subfamilies Formicidae [node $7(\mathrm{n} 7), \mathrm{PP}=0.97$ ], Ectatomminae [node 8 (n8), PP = 1] and Pseudomyrmicinae [node 9 (n9), $\mathrm{PP}=1$ ]; and the second clade [node 10 ( n 10 ), $\mathrm{PP}=0.71$ ] containing the other two subfamilies, Dolichoderinae [node 11 ( n 11 ), $\mathrm{PP}=1$ ] and Myrmicinae [node 12 (n12), PP = 1]. Within Myrmicinae, a last monophyletic clade stands out [node 13 ( n 13 ), $\mathrm{PP}=1$ ], which is composed of the restricted group of fungus-farming ants.

## RECONSTRUCTION OF ANCESTRAL GENOME SIZE

In the present study, we verified whether GS evolved according to a Brownian motion model of evolution along the phylogeny. The test revealed that it was indeed the case according to the available data ( $P>0.05$ ). This could be verified by computing the phylogeny correction parameters, $\delta, \kappa$ and $\lambda$, because the values found were close to one, which is consistent with the constantvariance model (sometimes called Brownian motion) and is a correct representation of the data (Pagel et al., 2017). However, to confirm this result and leave no possible bias in the reconstruction analysis of the ancestral genome, the values found with ML and BI were both calculated assuming a Brownian motion model in the phylogeny and with correction of the parameters, which showed no differences between the results generated.

In addition, the GS values of the ancestor nodes were calculated using three different methods, also resulting in no significant differences among

Table 2. Percentage of AT and GC bases of the species with genome size estimated in the present study

| Species | Subfamily | Mean 1C-value | AT (\%) | GC (\%) |
| :--- | :--- | :--- | :--- | :--- |
| Ectatomma brunneum | Ectatomminae | 0.38 | 60.22 | 39.78 |
| Acromyrmex nigrosetosus | Myrmicinae | 0.35 | 63.75 | 36.25 |
| Acromyrmex rugosus | Myrmicinae | 0.35 | 63.23 | 36.77 |
| Acromyrmex subterraneus brunneus | Myrmicinae | 0.34 | 63.38 | 36.62 |
| Acromyrmex subterraneus subterraneus | Myrmicinae | 0.35 | 63.72 | 36.28 |
| Apterostigma sp. 3 | Myrmicinae | 0.81 | 62.18 | 37.82 |
| Atta sexdens | Myrmicinae | 0.33 | 64.82 | 35.18 |
| Cephalotes pusillus | Myrmicinae | 0.38 | 62.59 | 37.41 |
| Cyphomyrmex transversus | Myrmicinae | 0.50 | 62.03 | 37.97 |
| Megalomyrmex incisus | Myrmicinae | 0.46 | 61.27 | 38.73 |
| Mycetarotes sp. | Myrmicinae | 0.48 | 61.54 | 38.46 |
| Mycetarotes paralellus | Myrmicinae | 0.38 | 61.80 | 38.20 |
| Myrmicocrypta sp. 1 | Myrmicinae | 0.48 | 61.45 | 38.55 |
| Pheidole sp. | Myrmicinae | 0.37 | 59.17 | 40.83 |
| Mycetomoellerius holmgreni | Myrmicinae | 0.33 | 62.58 | 37.42 |
| Mycetomoellerius ihering | Myrmicinae | 0.40 | 62.14 | 37.86 |
| Pseudomyrmex termitarius | Pseudomyrmecinae | 0.39 | 60.44 | 39.56 |
| Mean |  | 0.42 | 62.14 | 37.86 |

them. The ancestral Formicidae (n1; Fig. 2) GS reconstructed was 0.37 pg using MP, 0.37 pg using ML ( $0.20-0.55,95 \%$ highest posterior density) and $0.38 \pm 0.05 \mathrm{pg}$ using BI. The values obtained with the three methods showed only marginal variation among them, as observed, for example, for node 5 ( $\mathrm{MP}=0.37, \mathrm{ML}=0.36$ and $\mathrm{MCMC}=0.35 \pm 0.04$ ), node $8(\mathrm{MP}=0.45, \mathrm{ML}=0.45$ and $\mathrm{MCMC}=0.44 \pm$ 0.05 ) and node $9(\mathrm{MP}=0.37, \mathrm{ML}=0.37$ and MCMC $=0.37 \pm 0.05)$. The only node that presented a marginally higher variation was node 13 , showing a lower value with $\mathrm{ML}(\mathrm{ML}=0.34 \mathrm{pg} ; 0.24-0.43,95 \%$ highest posterior density) than with MP $(0.40 \mathrm{pg})$ and BI $(0.40 \pm 0.05 \mathrm{pg})$, but the value found with the other two methods was within the confidence interval of the first.

## STATISTICAL ANALYSES

Significant differences in GS were observed between the subfamilies sampled (ANOVA, $P<0.01$ ). Through contrast analysis, the subfamilies Dolichoderinae, Dorylinae, Formicinae, Amblyoponinae and Pseudomyrmecinae grouped statistically (group average $=0.31 \mathrm{pg}, P>0.05$ ) as did the subfamilies Myrmicinae and Ectatomminae (group average $=0.39 \mathrm{pg}, P>0.05$ ) (Fig. 3). Only the mean of Ponerinae differed from all others (average $=0.47$, $P<0.01$ ). Myrmeciinae ( 0.28 pg ) was not considered in the analysis because only one GS value was available.

## DISCUSSION

Ants were confirmed to have tiny genomes even after adding approximately 100 new estimates. The mean GS values for the subfamilies of Formicidae were similar to those reported by Tsutsui et al. (2008), but the amplitude of variation was higher in the present study, which covered a larger number of species. In general, all subfamilies sampled with a larger number of species (normally more than five) presented a greater range of GS variation, with Myrmicinae being the most prominent ( $0.21-0.81 \mathrm{pg}$ ).

The GS values reported here for some species that have previously been estimated with flow cytometry were found to be close. For example, the published value of Eciton burchellii (Westwood, 1842) was 0.27 pg by Tsutsui et al. (2008) and 0.29 pg in the present study, for Camponotus renggeri Emery, 1894 it was 0.29 pg reported by Aguiar et al. (2016) and 0.32 pg in the present study, and for Camponotus crassus Mayr, 1862 the value found by Aguiar et al. (2016) and in the present study was 0.29 pg .

This amplitude of variation within a subfamily mainly reflected the large variation in GS found between genera and, in a few cases, between species of the same genus. This is the opposite of what was found in the order Lepidoptera (Gregory \& Hebert, 2003), where more variation was observed between subfamilies than within a subfamily. It is also the opposite of what was suggested by Tsutsui et al. (2008) for ants, probably owing to the number of species


Figure 1. Fluorescence intensity histograms obtained from three different species, with Drosophila melanogaster as internal standard, stained with propidium iodide (PI; A-C) or 4,6-diamidino-2-phenylindole (DAPI; D-F). The $x$-axis corresponds to the scale of fluorescence intensity, and the $y$-axis represents the number of nuclei with that fluorescence intensity.
sampled, and might account for the difference in the mean GS value found for the Ectatomminae subfamily, which was 0.71 pg according to Tsutsui et al. (2008)
and 0.45 pg in the present study. The explanation might be that the only species addressed by Tsutsui et al. (2008) showed a putative whole nuclear genome


Figure 2. Bayesian consensus tree resulting from the $L W-R h$ and $W g$ gene alignments ( 871 bp ). Coloured dots on the branches indicate the values of posterior probability (PP): green dots represent values between 1.00 and 0.95 , yellow dots between 0.94 and 0.90 , and red dots $\leq 0.89$. The nodes are indicated with numbers. Values above and below the branches represent the ancestral genome size (GS; 1C-values, in picograms) at particular nodes: in blue is the value generated by the maximum likelihood (ML) [asterisks are related to confidence interval (CI) values shown in Supporting Information, Table S4]; orange is the value generated by maximum parsimony (MP); and black, given below the branches, is the value generated by Bayesian inference (BI). Genome size data (1C-values) were obtained in the present work (pink dots) or taken from the literature (grey dots).
duplication, and this was not the pattern for the other species of the genus; this was confirmed by the GS estimate of two other species in the present study that exhibited approximately half of the estimated value found by Tsutsui et al. (2008) ( 0.38 pg in Ectatomma brunneum Smith, 1858 and 0.36 pg in Ectatomma edentatum Roger, 1863; Table 1).

Our results suggest that genomic expansion through whole genome duplication occurred in the Apterostigma Mayr, 1865 lineage, as was
also suggested by Tsutsui et al. (2008), based on Apterostigma dentigerum Wheeler, 1925 ( 0.65 pg ). Two morphospecies, Apterostigma sp. 2 and sp. 4, presented GS values similar to the already published estimates, 0.69 and 0.63 pg , respectively. Nonetheless, Apterostigma sp. 1 and sp. 3 presented higher values, 0.74 and 0.81 pg , respectively. All these values were almost double those estimated for other species of the subfamily Myrmicinae, especially within the group of fungus-farming ants, such as Mycocepurus goeldii


Figure 3. Mean genome size (in picograms and megabase pairs) estimated for Formicidae subfamilies. The phylogenetic tree generated in the present study was redrawn, with collapsed branches corresponding to species of the same subfamily.
(Forel, 1893), which had a GS of 0.42 pg , and the genus Cyphomyrmex Mayr, 1862, which had an average GS of 0.37 pg .
Tsutsui et al. (2008) suggested that genome expansion by whole genome duplication in Apterostigma and Ectatomma Smith, 1858 would have occurred in the ancestor of each genus, potentially 80-90 Mya for Ectatomma and more recently for Apterostigma. However, this might be inconsistent considering the estimated GS values for the two other species studied here, indicating that the whole genome duplication might have occurred at the species level within Ectatomma. However, this seems likely for Apterostigma spp., because all five species for which GS has been estimated presented apparently duplicated values ( $\sim 0.70 \mathrm{pg}$ ). The results found in the present study also corroborated those of Cardoso et al. (2012), who suggested that there was no evidence of whole genome duplication in the Neoattina genera, such as Mycetophylax spp. (mean GS $=0.35 \mathrm{pg}$ ), Cyphomyrmex spp. (mean GS $=0.37 \mathrm{pg}$ ), Mycetomoellerius spp . (mean GS $=0.36 \mathrm{pg}$ ), Sericomyrmex spp . (mean GS $=0.42 \mathrm{pg}$ ), Acromyrmex spp. (mean GS $=0.34 \mathrm{pg}$ ) and Atta spp. (mean GS $=0.32 \mathrm{pg}$ ), suggesting that this phenomenon was related only to the Paleoattina clade, which includes Apterostigma spp.

We measured base composition by flow cytometry for the first time in Formicidae species. The mean values obtained for both the family ( $\mathrm{AT}=62.14 \%$ and GC $=37.86 \%$ ) and the subfamilies of Myrmicinae ( $\mathrm{AT}=62.38 \%$ and $\mathrm{GC}=37.62 \%$ ), Ectatomminae (AT $=60.22 \%$ and $\mathrm{GC}=39.78 \%$ ) and Pseudomyrmecinae ( $\mathrm{AT}=60.44 \%$ and $\mathrm{GC}=39.56 \%$ ) are similar to the values reported for bees [Scaptotrigona xantotricha Moure, 1950, AT $=61.32 \%$ and $\mathrm{GC}=38.68 \%$; Trigona hyalinata (Lepeletier, 1836), AT $=62.40 \%$ and $\mathrm{GC}=37.60 \%$; Partamona rustica Pedro \& Camargo, $2003 \mathrm{AT}=62.82 \%$ and $\mathrm{GC}=37.18 \%$; Soares, 2012]. Lorite \& Palomeque (2010) suggested that the large GS values found in Ectatomma and Apterostigma were related to the difference in the amount of heterochromatin, because the number of chromosomes of Ectatomma tuberculatum (Olivier, 1792) was $n=18$ (Barros et al., 2008) and that of a species of the genus Apterostigma was $n=10-12$ (Murakami et al., 1998), which is not considered high for a chromosome complement. However, this theory is not supported by the analysis of the AT/GC ratio of the species in Table 2, where no correlation between GS and total amount of AT or GC was observed.

The Bayesian consensus phylogenetic tree based on the $L W-R h$ and $w g$ genes recovered Formicidae
as a monophyletic family, with a high value of PP ( $\mathrm{n} 1, \mathrm{PP}=1$ ). The relationships between subfamilies and species were also consistent with those found in other studies, such as the work by Moreau et al. (2006, 2013), Ward et al. (2015) and Branstetter et al. (2017), although they comprised only a subset of species and subfamilies. Several genera were also recovered as monophyletic: Aphaenogaster Mayr, 1853, Apterostigma, Camponotus Mayr, 1861, Atta Fabricius, 1804, Cephalotes Latreille, 1802, Crematogaster Lund, 1831, Ectatomma, Gnamptogenys Roger, 1863, Lasius Fabricius, 1804, Linepithema Mayr, 1866, Mycetophylax Emery, 1913 (sensu Klingenberg \& Brandão, 2009), Odontomachus Latreille, 1804, Pheidole Westwood, 1839, Pogonomyrmex Mayr, 1868, Pseudomyrmex Lund, 1831, Solenopsis Westwood, 1840 and Acromyrmex, except for Acromyrmex striatus (Roger, 1863), which emerged as a sister group to the other leafcutter ants, as demonstrated by Cristiano et al. (2013). However, several studies have already demonstrated paraphyletism in the genera Aphaenogaster (Brady et al., 2006; Moreau, 2006, 2013), Camponotus (Brady et al., 2000, 2006; Moreau et al., 2013) and Odontomachus (Moreau et al., 2013), and the results obtained in this work might be a bias of the number of species sampled.

The reconstructed ancestral GS for Formicidae was 0.38 pg according to the BI method and 0.37 pg according to the ML and parsimony methods. This value was congruent with the overall mean GS for the family, also reflecting the distribution of the data (see Fig. 2). Despite this relatively small ancestral genome ( $<1 \mathrm{pg}$ ), smaller genomes than this can be found along the phylogenetic tree, but the change in values does not follow the evolution of the subfamilies. This means that the evolution of the genome was not linear, being smaller in the more basal branches, such as Amblyoponinae, and higher in the most derived ones, such as Dolichoderinae, Ectatomminae and Myrmicinae. In contrast, genomic expansions and retractions occur in all subfamilies.

The reconstructed ancestral GSs of all the subfamilies (n2-n13; Fig. 2) had similar values to those of Formicidae ( $0.37 / 0.38 \mathrm{pg}$ ), being higher only in Ectatomminae (n8: 0.45 pg with ML and MP; 0.44 pg with BI ) and the clade of fungus-farming ants ( $\mathrm{n} 13: 0.40 \mathrm{pg}$ with BI and MP; 0.34 pg with ML). This highlighted an interesting pattern: five of the nine sampled subfamilies (Dolichoderinae, Dorylinae, Formicinae, Myrmicinae and Ponerinae) presented at one extreme of GS variation values that were exactly half the value of the ancestral genome (i.e. 0.18 pg ) and, at the other extreme, values that were essentially twice the others ( $>0.60 \mathrm{pg}$ ), indicating gain or loss of an amount of DNA for genera in the
subfamilies sampled. In contrast, Ectatomminae presented values close to the ancestral genome ( 0.36 pg in Ectatomma edentatum), intermediate values ( 0.50 pg in Gnamptogenys striatula Mayr, 1884) and duplicate values ( 0.71 pg in Ectatomma tuberculatum), indicating an increase in GS only in the subfamily. Amblyoponinae and Pseudomyrmicinae presented values close to the ancestral genome, except for Pseudomyrmex ejectus (Smith, 1858), which had the smallest GS ( 0.29 pg ). However, the GS was estimated with a different methodology, with image cytometry using a blood smear and Tenebrio molitor Linnaeus, 1758 as an internal standard.

When considering only the species collected and estimated in the present study, the pattern observed was even more homogeneous. Of the 99 estimated values, $91 \%$ were between 0.25 and 0.50 pg , with only one value being lower than the minimum limit ( 0.23 pg in Dorymyrmex sp. 1); only three values were intermediate between 0.50 and 0.60 pg (Pachycondyla striata Smith, $1858=0.51 \mathrm{pg}$, Cephalotes depressus (Klug, 1824) $=0.53 \mathrm{pg}$ and Odontomachus meinerti Forel, $1905=0.55 \mathrm{pg}$ ); and five values were $>0.60 \mathrm{pg}$, for the species of Apterostigma previously mentioned and Neoponera marginata (Roger, 1861) ( 0.63 pg ).

Increasing and decreasing GSs are usually related to chromosomal alterations that can be numerical (e.g. euploidy and/or aneuploidy) or structural (e.g. deletion and duplication) (Moura et al., 2018). These changes are considered key factors in the evolution of genomes in plants (e.g. Campos et al., 2011; LepersAndrzejewski et al., 2011; Szadkowski et al., 2011). However, these types of modifications, in addition to changing the GS, are usually associated with deleterious phenotypic effects (Gregory, 2005) and are not considered the main mechanism effecting GS changes in animal species. Instead, for some animal species it has already been demonstrated that GS is strongly related to the abundance of transposable elements (TEs) and, for humans, it has been shown that nearly $45 \%$ of the genome is composed of TEs and their inactive remnants (International Human Genome Sequencing Consortium, 2001; Gregory, 2005).

Moura et al. (2020) suggested that the differences in GS found between populations of the same species of ants could be related to the stress of colonization of new environments and that the increase in GS might be correlated with the accumulation of TEs. Considering all the values discussed above in relationship to the ancestral GS of Formicidae and the base composition rate of the species shown on Table 2, some processes have a greater effect on the evolution of the genome for the family, such as the accumulation of non-coding elements and whole genome duplication events.

Some authors have suggested that the movement and accumulation of TEs have exerted a strong influence on the evolution of their hosts and that the accumulation of these elements in the genome is a gradual process (Brookfield, 2005; Feschotte, 2008; Alfsnes et al., 2017). For instance, the accumulation of TEs explains the difference in GS of many species and genera in relationship to the ancestral genome of Formicidae and of the subfamilies themselves, especially those having a GS $>0.38$ but $<0.60 \mathrm{pg}$, where the process of whole genome duplication would fit better. In addition, it also explains the decrease in GS in relationship to the ancestral genome, suggesting that the loss of an amount of DNA occurs in these noncoding regions, because no function or trait was lost in species with smaller GS (e.g. $<0.25 \mathrm{pg}$ ).

## Conclusion

The results obtained in this study improve the knowledge concerning the GS of Formicidae, the base composition of some species, and patterns of GS evolution through phylogeny from an ancestral genome. Flow cytometry procedures have been established for determination of genomic AT/GC ratios, a tool that has been little used in insects. The flow cytometry data reported here also contribute to the understanding of GS diversity and range of variation in Formicidae, knowledge hitherto skewed given the number of species analysed in earlier studies. The results of our study suggest that the evolution of GS in Formicidae was attributable to the loss and accumulation of noncoding regions, mainly TEs, and, in some specific cases, by whole genome duplication. However, the processes underlying these genome enlargements and retractions need further analysis, mainly through species diversification studies, to verify whether these changes in DNA content are related to the colonization process of the species, as suggested at the intraspecific level (Moura et al., 2020). Furthermore, the genome base composition needs to be estimated in a greater number of species to verify the range of variation of AT/GC ratios in the subfamilies and, importantly, to understand whether the composition differs in those species with a smaller GS, especially those with estimated GS $<0.25 \mathrm{pg}$. Nevertheless, this work will serve as a guide for future whole genome sequencing projects, in which all the limits of variation in the genome size of species can be covered.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:
Table S1. Species sampled during field expeditions, with data on subfamily, localities and geographical coordinates. Table S2. Sequences used in the phylogenetic analysis, with their accession numbers in GenBank.
Table S3. Models of evolution estimated for each gene and codon position with PartitionFinder 2. The models listed were used for each data partition in a Bayesian analysis.
Table S4. Confidence interval (CI) of the values generated by the maximum likelihood method in StableTraits.


[^0]:    *Corresponding author. E-mail: maykonpcristiano@gmail.com

