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Genome size in ants: retrospect and prospect

Mariana Neves Moura[1,2], Danon Clemes Cardoso[2,*], Brenda Carla Lima Baldez[3], Maykon Passos Cristiano[2,*]

[1] *Programa de Pós- Graduação em Ecologia, , Universidade Federal de Viçosa, CEP: 36.570–000, Viçosa, Minas Gerais, Brazil*
[2] *Departamento de Biodiversidade, Evolução e Meio Ambiente, Universidade Federal Ouro Preiato – UFOP, CEP: 35.400–000, Ouro Preto, Minas Gerais, Brazil*
[3] *Programa de Pós- Graduação em Ecologia de Biomas Tropicais, Universidade Federal de Ouro Preto, CEP: 35.400–000, Ouro Preto, Minas Gerais, Brazil*
*Corresponding authors: danon@ufop.edu.br; maykon@ufop.edu.br

Abstract. Genome size is very useful in studies regarding taxonomy, evolution, and reproductive biology in many animal groups, including insects. Herein, we assembled the information about genome size in ants, compiling the DNA content estimated so far, in order to evaluate the methods, the tissues and the internal standard applied to estimate the genomes size. All values were placed in a phylogenetic tree to put it in an evolutionary context and the means of the subfamilies were further compared statistically to investigate changes and trends in the variation across *taxa*. The compiled data resulted in 86 specimens of ants, comprising 69 different species. This number represents 0.52% of the total number of 13,369 ant species described, covering only 40 from 333 valid extant genera. The average Formicidae genome size was 0.36 pg (± 0.13). Most of the estimates were obtained through flow cytometry (83.5%), commonly using brain tissues, with *Drosophila melanogaster* as internal standard (76%). Differences in DNA content of ant species may be related to differences in the amount of heterochromatin and is not related with chromosome number. The evaluation of the genome size estimations currently available for ants has highlighted their scarcity. Such information would be valuable as independent data for the study of ant diversity and evolutionary biology. Further, we conclude that the standardization of the techniques used and a large–scale study on ant genome size are urgently required, given the importance of this insect group and the needs for the improvement in our knowledge on ant genome.

Keywords. C-value, DNA content, Genetic diversity, Genome, Evolution, Phylogeny.

INTRODUCTION

Ants comprise a monophyletic group with approximately 13,369 valid species distributed throughout the planet, with exception of extreme northern and southern latitudes (Bolton, 2018). They are one of the largest groups among insects in species diversity and biomass and together with some wasps and bees, are known as eusocial insects and comprise the order Hymenoptera (Hölldobler and Wilson, 1990; Ardila–Garcia et al., 2010).

They represent an important insect group to investigate the relationship between the genealogical lineages and the distribution patterns of species, due to their occurrence in different habitats of the most diverse ecosystems (Goodisman et al., 2008). Currently, the family Formicidae is divided into 17 extant and 3 extinct subfamilies, spanning 333 valid extant genera and 154 extinct genera (Bolton, 2018). The subfamily Myrmicinae is the largest and most diverse subfamily worldwide, covering about 47% of all ant species (Françoso and Brandão, 1993; Brandão, 1999).

Genome size, also named DNA content, DNA amount, or DNA C–value, has been described as a trait that 'uniquely lies at the intersection of phenotype and genotype', and the genome size of eukaryotes varies over five orders of magnitude, with a distribution skewed toward small values, around 2 picograms (pg) (Oliver et al., 2007). This variation does not seem to be correlated with the complexity of the organism or with the number of genes in eukaryotes, leading to what is called the "C–value paradox" (Moore, 1984; Gregory, 2001, 2005a; Eddy, 2012). It has been questioned, for example, why similar organisms with similar amounts of coding sequence have different amounts of DNA. While changes in gene sequences are often slow and gradual, changes in genome size can be rapid and abrupt as a consequence of chromosomal rearrangements or duplications (Alberts et al., 2007).

The main methods used to estimate the total nuclear genome size are image cytometry, flow cytometry (FCM), and complete genome sequencing (Gregory, 2005b). Image cytometry was the first method used to determine genome size estimates. Basically, it operates by statically imaging a large number of cells stained with specific chemicals or fluorochromes, using optical microscopy (Torresan et al., 1994; Basiji et al., 2007). In contrast, flow cytometry evaluates the relative fluorescence intensity of suspended nuclei, also stained with specific fluorochromes, and presents the data in a typical histogram with a higher peak relative to the nuclei in the G0/G1 phase of cell cycle, and a lower peak, relative to the nuclei in G2 phase (Price et al., 2000; Dolezel and Bartos, 2005). The complete genome sequencing method, on the other hand, provides the complete DNA sequence of the genome of an organism at a single time with the precise order of the nucleotides and an estimate of the genome size after its assembly (Klug et al., 2014). A fourth less common technique known as biochemical analysis (BCA) was used during the early studies of genome size. It includes 'the chemical extraction and quantification of DNA combined with cell counts to give an average DNA amount per nucleus or the reassociation kinetics, in which the DNA molecule was denatured and then the time taken for the strands to renature is used to calculate the amount of DNA (Gregory, 2005b). Among the methods, flow cytometry has been shown to be the least cost and time expensive technique when compared to other molecular tools and provides rapid generation of accurate results (Merkel et al., 1987; Doležel et al., 2007).

According to Gregory (2018), haploid DNA contents (C–values, in picograms - pg) are currently available for 6,222 species of animals (3,793 vertebrates and 2,429 invertebrates), with insects representing 21.6% of this total. Li and Heinz (2000) performed the first DNA content estimation of an ant by mean of biochemical analysis (BCA), to quantify the genome of *Solenopsis invicta* Buren, 1972. Subsequently, Johnston et al. (2004) also estimated the genome size of *S. invicta* but now using flow cytometry. Yet, in 2008, Tsutsui et al. (2008) carried out the first comprehensive study regarding the evolution of the genome size in ants, reporting genome size estimates for 40 species from nine subfamilies. This was the last inclusion of a large number of ant species estimates to the genome size database that was followed by the study of Ardila–Garcia et al. (2010), which added a further 29 species. These two studies raised different questions about genome size, being the first a study of genome size evolution in Formicidae and the second a study of correlation between genome size with parasitism and eusociality in the order Hymenoptera as a whole. It is important to note that they applied different methodologies in genome size estimation: in Tsutsui et al. (2008) the DNA content was estimated by using only flow cytometry, while Ardila–Garcia et al. (2010) also performed the FIAD method (Feulgen image analysis densitometry) to estimate the DNA content, and then compared the results from both techniques.

Later, others studies explored the DNA content of ants, however in some cases covering only one species through complete genome sequencing (*e.g.* Nygaard et al., 2011) or, in other cases, considering specifically an ant genus through flow cytometry. The genome size of the genus *Mycetophylax* Emery, 1913 (*sensu* Klingenberg and Brandão, 2009) was estimated by Cardoso et al. (2012) that explored the data placing them in a phylogenetic context, also correlating it with chromosome number of fungus–growing ants; and Aguiar et al. (2016) that evaluated three *Camponotus* Mayr, 1861 species, exploring their correlation with the karyotype of the studied species.

Despite the importance of genome size, little is known about the ecological and evolutionary consequences of DNA amount in ants. Yet, the biological sig-

nificance and evolution of the genome size diversity in other groups has received much more attention over the last decades (Dufresne and Jeffery, 2011; Alfsnes et al., 2017; Pellicer et al., 2018). The diversity of genome size in plants has been shown to correlate with several phenotypic features of cells and ultimately the organisms. For instance, plant species with larger genomes are adapted to xeric and higher elevation environments (*e.g.* Bottini et al., 2000). Here, we evaluate the available information about the genome size of ants, assembling the DNA content estimated so far, in order to provide insights into the distribution, evolution and possible consequences of ant genome size diversity. We have also investigated and verified the needs of a re–evaluation in the genome size data (DNA C–value) for ants, as well the technique used in the estimation of the DNA content in respect of methodological issues such as: the internal standard and tissues used in the analysis. The basic information about ant genomes analyzed here may improve our knowledge about the evolution and diversification regarding this diverse group of insects and may help as a baseline and guidance for future studies about ant genome biology.

MATERIALS AND METHODS

To evaluate the knowledge about nuclear DNA content on ants, we compiled the haploid genome size estimates for ants and other insect groups from the Animal Genome Size Database (Gregory, 2018) and from the literature by searching in the publication databases Scopus® and ISI Web Science Knowledge™, by using the terms: "genome size", "DNA amount", "C–value" and "ants". Based on the seven manuscripts found on ant genome size, we evaluated the method used to measure genome size, the type of tissue and the internal standard used to obtain the total content of DNA.

To examine the genome size variation over Formicidae subfamilies we compiled the estimates in a Table of all the values available in the literature, expressed in picograms of DNA (pg) and mega base pairs (Mbp). Then we manually placed them in the phylogenetic tree proposed by Moreau and Bell (2013) by collapsing branches with equal names (same Operational Units - OTUs) and separating the subfamilies by color. General linear models were built to check for differences between the averaged genome sizes of the sampled subfamilies. The differences in genome size average for each subfamily were assessed by variance analysis of the GLM. When the *p*-value of ANOVA was significant ($p < 0.05$), a contrast analysis at 5% level was then performed to determine which mean was different. All the statistical analysis was performed in R v2.15.1 software (R Core Team, 2013) and GLM was submitted to residual analysis to evaluate adequacy of normal error distribution (Crawley, 2013).

RESULTS AND DISCUSSION

Overview: number of estimates, methods, tissues and internal standards used

The compiled data resulted in 86 specimens of ants whose genome size had been estimated, comprising 69 different species (Table 1). This number represents 0.52% of the total number of 13,369 ant species accepted until now, covering only 40 genera from 333 accepted (Bolton, 2018). From 17 existing subfamilies, we only found estimates for nine, with Myrmicinae having the largest number of species evaluated (32 spp.) (Figure 1). The number of estimates may reflect the richness of this subfamily that is the most diverse within Formicidae. Yet, Formicinae and Dolichoderinae together bear 20 spp. with DNA content estimates available. These three subfamilies represent 65% of DNA content estimates on ants.

The two main methods used to estimate DNA content in ants were FCM and FIAD. A third method, biochemical analysis (BCA), was used in a pioneering work from Li and Heinz (2000) in order to estimate the genome size sole for *Solenopsis invicta*. It is important to mention that *S. invicta* has the genome size estimates by all three methods listed above and different values were obtained in each estimate: 0.60 pg by BCA (Li and Heinz, 2000), 0.47 pg by FIAD (Ardila-Garcia et al., 2010) and 0.77 pg by flow cytometry (Johnston et al., 2004). Such huge variation in genome sizes may be explained by the occurrence of different ploidy levels in *S. invicta* or even outcomes due the different techniques employed in the studies. Cytogenetical evidence suggests that there may be different levels of ploidy in *S. invicta*.

All genome sizes are estimated by mean of comparison with nuclei of reference standard, whose genome size is known that is called the "internal standard". In the genome size estimation *Drosophila melanogaster* Meigen, 1830 (0.18 pg), *Scaptotrigona xantotricha* Moure, 1950 (0.43 pg) and *Tenebrio molitor* Linnaeus, 1758 (0.52 pg) are the internal standards most commonly used considering Hymenoptera as a whole. Most of the estimates were obtained using *D. melanogaster* as internal standard (76%), while FCM was the most common method used (83.5%). Generally, brain tissue is used to estimate nuclear genome size, but cells (hemocytes) obtained through hemolymph smears have also been tested (Ardi-

Table 1. Overview of the genome size data available in literature for Formicidae species.

Subfamily	Species	1C-value (pg)	1C-value (Mbp)	Method	Cell type	Standard	References
Amplyoponinae	*Amblyopone pallipes (Haldeman, 1844)**	0.34	332.52	FCM	BR	DM	Tsutsui et al., 2008
	*Amblyopone pallipes (Haldeman, 1844)**	0.37	361.86	FCM	BR	DM	Ardila-Garcia et al., 2010
Dolichoderinae	*Dolichoderus mariae* (Forel, 1885)	0.18	176.04	FCM	BR	DM	Ardila-Garcia et al., 2010
	Dolichoderus taschenbergi (Mayr, 1866)	0.23	224.94	FCM	BR	DM	Ardila-Garcia et al., 2010
	Dorymyrmex bicolor Wheeler, 1906	0.25	244.5	FCM	BR	DM	Tsutsui et al., 2008
	Dorymyrmex bureni (Trager, 1988)	0.18	176.04	FIAD	HE	TM	Ardila-Garcia et al., 2010
	Forelius pruinosus (Roger, 1863)	0.22	215.16	FIAD	HE	TM	Ardila-Garcia et al., 2010
	Linepithema humile (Mayr, 1868)	0.26	254.28	FCM	BR	DM	Tsutsui et al., 2008
	Linepithema humile (Mayr, 1868)	0.26	250.8	Genome sequencing	NS	NS	Smith et al., 2011
	Liometopum occidentale Emery, 1895	0.29	283.62	FCM	BR	DM	Tsutsui et al., 2008
	Tapinoma sessile (Say, 1836)	0.37	361.86	FCM	BR	DM	Ardila-Garcia et al., 2010
	Tapinoma sessile (Say, 1836) *A*	0.38	371.64	FCM	BR	DM	Tsutsui et al., 2008
	Tapinoma sessile (Say, 1836) *B*	0.61	596.58	FCM	BR	DM	Tsutsui et al., 2008
Dorylinae	*Cerapachys edentata*	0.22	215.16	FCM	BR	DM	Tsutsui et al., 2008
	Eciton burchelli (Westwood, 1842)	0.27	264.06	FCM	BR	DM	Tsutsui et al., 2008
	Labidus coecus (Latreille, 1802)	0.37	361.86	FCM	BR	DM	Tsutsui et al., 2008
Ectatomminae	*Ectatomma tuberculatum* (Olivier, 1792)	0.71	694.38	FCM	BR	DM	Tsutsui et al., 2008
Formicinae	*Camponotus castaneus* (Latreille, 1802)	0.31	303.18	FCM	BR	DM	Tsutsui et al., 2008
	Camponotus crassus Mayr, 1862	0.29	283.62	FCM	BR	SX	Aguiar et al., 2016
	Camponotus floridanus (Buckley, 1866)	0.23	224.94	FIAD	HE	TM	Ardila-Garcia et al., 2010
	Camponotus floridanus (Buckley, 1866)	0.245	240	Genome sequencing	NS	NS	Bonasio et al., 2010
	Camponotus pennsylvanicus (De Geer, 1773)	0.33	322.74	FCM	BR	DM	Tsutsui et al., 2008
	Camponotus renggeri Emery, 1894	0.29	283.62	FCM	BR	SX	Aguiar et al., 2016
	Camponotus rufipes (Fabricius, 1775)	0.29	283.62	FCM	BR	SX	Aguiar et al., 2016
	Formica pallidifulva Wheeler, 1913	0.39	381.42	FCM	BR	DM	Tsutsui et al., 2008
	Lasius (Acanthomyops) latipes (Walsh, 1863)	0.27	264.06	FCM	BR	DM	Ardila-Garcia et al., 2010
	Lasius alienus (Foerster, 1850)	0.31	303.18	FCM	BR	DM	Tsutsui et al., 2008
	Lasius minutus Emery, 1893	0.23	224.94	FCM	BR	DM	Ardila-Garcia et al., 2010
	Paratrechina longicornis (Latreille, 1802)	0.18	176.04	FIAD	HE	TM	Ardila-Garcia et al., 2010
	Prenolepis imparis (Say, 1836)	0.30	293.4	FCM	BR	DM	Tsutsui et al., 2008
Myrmeciinae	*Myrmecia varians* Mayr, 1876	0.28	273.84	FCM	BR	DM	Tsutsui et al., 2008
Myrmicinae	*Acromyrmex echinatior* (Forel, 1899)	0.36	335	FCM	BR	CRBC	Sïrvio et al., 2006
	Acromyrmex echinatior (Forel, 1899)	0.32	313	Genome sequencing	NS	NS	Nygaard et al., 2011
	Aphaenogaster rudis (texana group N16) Enzmann, 1947	0.43	420.54	FCM	BR	DM	Ardila-Garcia et al., 2010
	Aphaenogaster rudis (texana group N17) Enzmann, 1947	0.46	449.88	FCM	BR	DM	Ardila-Garcia et al., 2010
	Aphaenogaster rudis (texana group N22b) Enzmann, 1947	0.44	430.32	FCM	BR	DM	Ardila-Garcia et al., 2010
	Aphaenogaster fulva Roger, 1863	0.42	410.76	FCM	BR	DM	Ardila-Garcia et al., 2010
	Aphaenogaster treatae Forel, 1886	0.50	489	FCM	BR	DM	Ardila-Garcia et al., 2010
	Apterostigma dentigerum Wheeler, 1925	0.65	635.7	FCM	BR	DM	Tsutsui et al., 2008
	Atta cephalotes (Linnaeus, 1758)	0.31	303.18	FCM	BR	DM	Tsutsui et al., 2008
	Atta cephalotes (Linnaeus, 1758)	0.30	290	Genome sequencing	NS	NS	Suen et al., 2011
	Atta colombica Guérin-Méneville, 1844	0.31	303.18	FCM	BR	DM	Tsutsui et al., 2008
	Atta texana (Buckley, 1860)	0.27	264.06	FCM	BR	DM	Ardila-Garcia et al., 2010

Subfamily	Species	1C-value (pg)	1C-value (Mbp)	Method	Cell type	Standard	References
	Crematogaster hespera Buren, 1968*	0.28	273.84	FCM	BR	DM	Tsutsui et al., 2008
	Eurhopalothrix procera (Emery, 1897)	0.39	381.42	FCM	BR	DM	Tsutsui et al., 2008
	Messor andrei (Mayr, 1886)*	0.26	254.28	FCM	BR	DM	Tsutsui et al., 2008
	Monomorium viridum Brown, 1943	0.50	489	FIAD	HE	TM	Ardila-Garcia et al., 2010
	Mycetophylax conformis (Mayr, 1884)	0.32	312.96	FCM	BR	SX	Cardoso et al., 2012
	Mycetophylax morschi (Emery, 1888)	0.32	312.96	FCM	BR	SX	Cardoso et al., 2012
	Mycetophylax simplex (Emery, 1888)	0.39	381.42	FCM	BR	SX	Cardoso et al., 2012
	Myrmecina americana Emery, 1895 A	0.26	254.28	FCM	BR	DM	Tsutsui et al., 2008
	Myrmecina americana Emery, 1895 B	0.31	303.18	FCM	BR	DM	Tsutsui et al., 2008
	Pheidole dentata Mayr, 1886	0.24	234.72	FIAD	HE	TM	Ardila-Garcia et al., 2010
	Pheidole floridana Emery, 1895	0.21	205.38	FIAD	HE	TM	Ardila-Garcia et al., 2010
	Pheidole hyatti Emery, 1895	0.33	322.74	FCM	BR	DM	Tsutsui et al., 2008
	Pogonomyrmex badius (Latreille, 1802)	0.27	264.06	FCM	BR	DM	Tsutsui et al., 2008
	Pogonomyrmex barbatus (Smith, 1858)	0.24	235	Genome sequencing	NS	NS	Smith et al., 2011
	Pogonomyrmex californicus (Buckley, 1867)	0.25	244.5	FCM	BR	DM	Tsutsui et al., 2008
	Pogonomyrmex coarctatus Mayr, 1868	0.29	283.62	FCM	BR	DM	Tsutsui et al., 2008
	Pyramica rostrata (Emery, 1895)	0.28	273.84	FCM	BR	DM	Tsutsui et al., 2008
	Sericomyrmex amabilis Wheeler, 1925	0.45	440.1	FCM	BR	DM	Tsutsui et al., 2008
	Solenopsis invicta Buren, 1972	0.62	606.36	BCA	BR	NS	Li and Heinz 2000
	Solenopsis invicta Buren, 1972	0.77	753.06	FCM	BR	DM	Johnston et al., 2004
	Solenopsis invicta Buren, 1972	0.47	459.66	FIAD	HE	TM	Ardila-Garcia et al., 2010
	Solenopsis invicta Buren, 1972	0.49	482	Genome sequencing	NS	NS	Wurm et al., 2011
	Solenopsis molesta Emery, 1895	0.38	371.64	FCM	BR	DM	Ardila-Garcia et al., 2010
	Solenopsis xyloni McCook, 1880	0.48	469.44	FCM	BR	DM	Tsutsui et al., 2008
	Temnothorax ambiguus (Emery, 1895)	0.31	303.18	FCM	BR	DM	Ardila-Garcia et al., 2010
	Temnothorax texanus (Wheeler, 1903)	0.32	312.96	FCM	BR	DM	Ardila-Garcia et al., 2010
	Tetramorium caespitum	0.26	254.28	FCM	BR	DM	Tsutsui et al., 2008
	Tetramorium caespitum (Linnaeus, 1758)	0.27	264.06	FCM	BR	DM	Ardila-Garcia et al., 2010
	Trachymyrmex septentrionalis (McCook, 1881)	0.25	244.5	FIAD	HE	TM	Ardila-Garcia et al., 2010
Ponerinae	*Dinoponera australis* Emery, 1901	0.57	557.46	FCM	BR	DM	Tsutsui et al., 2008
	Harpegnathos saltator Jerdon, 1851	0.34	330	Genome sequencing	NS	NS	Bonasio et al., 2010
	Odontomachus bauri Emery, 1892	0.49	479.22	FCM	BR	DM	Tsutsui et al., 2008
	Odontomachus brunneus (Patton, 1894)	0.33	322.74	FIAD	HE	TM	Ardila-Garcia et al., 2010
	Odontomachus brunneus (Patton, 1894)	0.44	430.32	FCM	BR	DM	Tsutsui et al., 2008
	Odontomachus Cephalotes Smith, 1863	0.43	420.54	FCM	BR	DM	Tsutsui et al., 2008
	Odontomachus chelifer (Latreille, 1802)	0.54	528.12	FCM	BR	DM	Tsutsui et al., 2008
	Odontomachus clarus Wheeler, 1915	0.42	410.76	FCM	BR	DM	Tsutsui et al., 2008
	Odontomachus haematodus (Linnaeus, 1758)	0.51	498.78	FCM	BR	DM	Tsutsui et al., 2008
	Ponera pennsylvanica Buckley, 1866	0.55	537.9	FCM	BR	DM	Ardila-Garcia et al., 2010
	Ponera pennsylvanica Buckley, 1866	0.60	586.8	FCM	BR	DM	Tsutsui et al., 2008
Pseudomyrmicinae	*Pseudomyrmex ejectus* (Smith, 1858)	0.29	283.62	FIAD	HE	TM	Ardila-Garcia et al., 2010
	Pseudomyrmex gracilis (Fabricius, 1804)	0.35	342.3	FCM, FIAD	BR, HE	DM, TM	Ardila-Garcia et al., 2010
	Pseudomyrmex gracilis (Fabricius, 1804)	0.40	391.2	FCM	BR	DM	Tsutsui et al., 2008

Method: FCM = Flow cytometry, FIAD = Feulgen image analysis densitometry; Cell type: BR = Brain tissue, HE = Haemocyte; Standard: DM = *Drosophila melanogaster*, CRBC = Chicken Red Blood Cells, SX = *Scaptotrigona xantotricha*, TM = *Tenebrio molitor*, NS = not specified. *Valid names: *Stigmatomma pallipes* (Haldeman, 1844); *Crematogaster laeviuscula* Mayr, 1870; Veromessor andrei (Mayr, 1886), respectively.

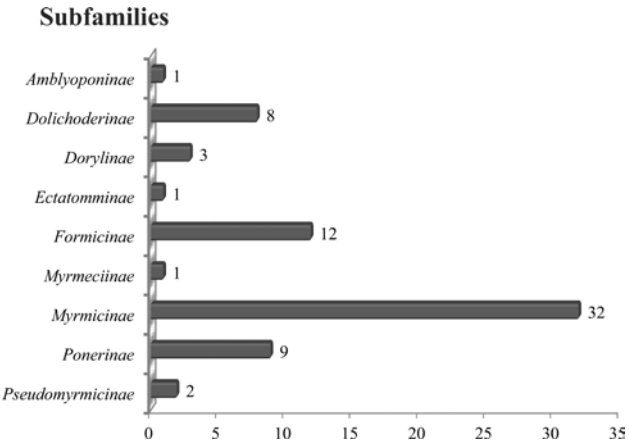

Figure 1. Distribution of the number of species across Formicidae subfamilies with published genome size estimates. The list of species is presented in Table 1.

la-Garcia et al., 2010). Considering *S. xantotricha*, this internal standard was started to be used in studies comprised stingless bees, and after with ants by the same research group (Tavares et al. 2010, Cardoso et al. 2012, Aguiar et al. 2016). Since no genome size histograms are available in either Ardila-Garcia et al. (2010) or Tsutsui et al. (2008), it is impossible to compare the usefulness of one or another internal standard considering the other two studies (Cardoso et al. 2012 and Aguiar et al. 2016) used *S. xantotricha*. In studies with plants, the choice of an appropriate internal standard considers the genome size magnitude of standard and studied group, mainly to avoid superposition of picks.

Concerning the methods employed in genome size estimation, the study from Ardila-Garcia et al. (2010) is the only one that multiple species in the same work had the genome measured by two methods. They evaluated by FIAD and FCM the genome size on *Odontomachus brunneus*, *Pseudomyrmex gracilis*, and *Solenopsis invicta* and showed that the estimates using the first method tended to be smaller. The authors argue that the values from both techniques do not differ statistically. However, it is difficult to say that this difference is solely due to the technique itself, since both the tissue and the internal standard used during the analysis were different.

The nuclear DNA content of some ants has also been measured using a fourth method, which utilized complete genome sequencing techniques in species such as *Acromyrmex echinatior* (Forel, 1899) (Nygaard et al., 2011), *Atta cephalotes* (Linnaeus, 1758) (Suen et al., 2011), *Camponotus floridanus* (Buckley, 1866) (Bonasio et al., 2010), *Harpegnathos saltator* Jerdon, 1851 (Bonasio et al., 2010), *Linepithema humile* (Mayr, 1868) (Smith et al., 2011), *Pogonomyrmex barbatus* (Smith, 1858) (Smith et al., 2011) and *Solenopsis invicta* (Wurm et al., 2011) (Table 1). The genome size of *Ac. echinatior* was 313 Mbp (or 0.32 pg considering 1 pg = 978 Mbp; (Doležel et al., 2003)) obtained with complete genome sequencing (Nygaard et al., 2011) and 335 Mbp (0.36 pg) by FCM (Sïrvio et al., 2006). This difference can be attributed to the loss of repetitive regions and some chromosomal regions, such as telomeres, through genome sequencing techniques (Gregory, 2005b). The same was observed in *A. cephalotes*, whose genome size estimated by complete genome sequencing was 290 Mbp (approximately 0.30 pg) (Suen et al., 2011) and by FCM was 303.18 Mbp (approximately 0.31 pg) (Tsutsui et al., 2008). The differences were greater in *S. invicta*, whose genome size was obtained with all four different techniques (BCA, FIAD, FCM, and Genome Sequencing): 606 Mbp (0.62 pg) (Li and Heinz, 2000) by BCA, 459 Mbp (0.47 pg) (Ardila-Garcia et al., 2010) by FIAD, 753 Mbp (0.77 pg) (Johnston et al., 2004) by FCM and 482 Mbp (0.49 pg) (Wurm et al., 2011) by genome sequencing. Values obtained with FIAD and genome sequencing are more similar. So, considering the loss of certain repetitive regions of DNA by the complete genome sequencing and the difficulties in using other techniques such as BCA and FIAD (mainly due to the low number of repetitions available to estimate de DNA amount) the use of FCM has proven to be the most efficient methodology to obtain accurately the total DNA content.

Genome size evolution

The reported DNA C–value of insects range from 0.07 pg (*Clunio tsushimensis* Tokunaga, 1933 – Diptera) to 16.93 pg (*Podisma pedestris* Linnaeus, 1758 – Orthoptera) and out of 1344 estimates found, 1224 (91%) were comprised of values between 0.07 to 2.00 pg (Gregory, 2018). From 27 orders of insects, 24 currently have estimates of genome size, with Diptera accounting for the largest number of measurements (386 specimens, 29% of the total), followed by Coleoptera (278 specimens, 21% of the total) and Hymenoptera (240 specimens, 18% of the total). The average genome size for the Formicidae (Hymenoptera) was 0.36 pg (± 0.13), with values ranging from 0.18 pg (the smallest value, found in Dolichoderinae and in Formicinae) to 0.77 pg in *S. invicta* (Myrmicinae) (Table 1; Figure 2), being always less than 1 pg. This is in accordance with the pattern already observed for others eukaryotes that most of the distribution of genome size is skewed towards smaller values (Oliver et al., 2007), since it is evident that the number of species declines as the genome doubles in size.

As can be seen in Figure 2 the variation of genome size among species of a subfamily is similar to the varia-

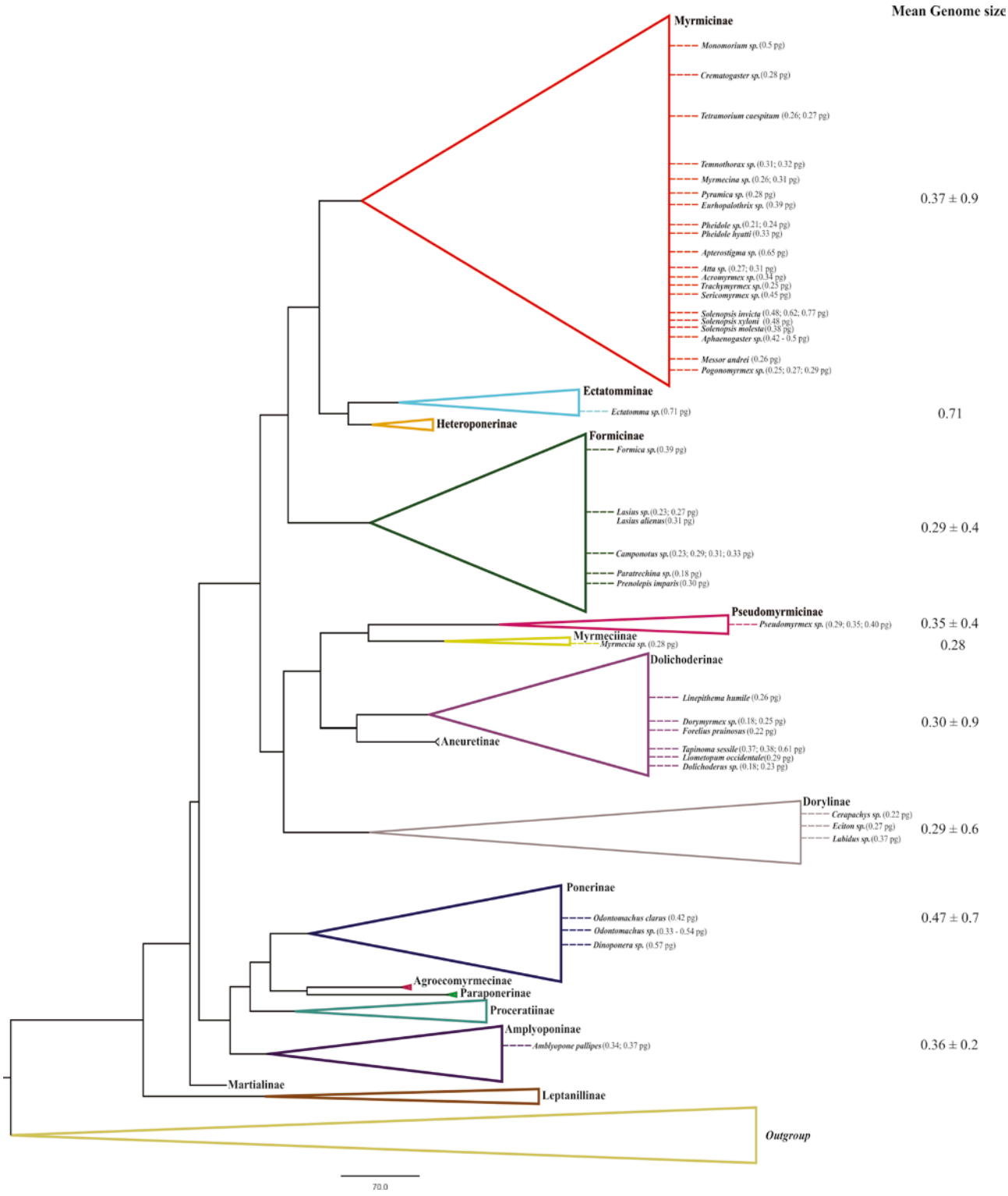

Figure 2. Phylogeny of the extant Formicidae. Phylogenetic tree redrawn from Moreau and Bell (2013). The figure highlights the subfamilies containing species with estimated genome size. Aside of each terminal on the tree the genome size is shown in picograms (pg) of DNA and also the mean genome size per Formicidae subfamilies.

tion found between subfamilies. Significant differences in genome size were observed between the subfamilies sampled (ANOVA, *p-value* < 0.01). Through contrast analysis, most of the subfamilies grouped statistically (group average = 0.34 pg, *p-value* > 0.05) except for Ponerinae, whose average was different from the others (average = 0.47, *p-value* < 0.01). The subfamilies Ectatomminae (*Ectatomma tuberculatum* (Olivier, 1792), 0.71 pg) and Myrmeciinae (*Myrmecia varians* Mayr, 1876, 0.28 pg) were not considered in the analysis because only one value for each was available, so it was not possible to calculate a mean for the comparison test (Figure 2). Differences in the genome size were also observed between genera within the sampled subfamilies and mainly between species of the same genus, as observed in *Atta* Fabricius, 1804 spp. (*e.g. Atta cephalotes* = 0.31 pg and *Atta texana* (Buckley, 1860) = 0.27 pg), *Camponotus* spp. (*e.g. Camponotus floridanus* = 0.23 pg and *Camponotus pennsylvanicus* (De Geer, 1773) = 0.33 pg) and *Odontomachus* spp. (*e.g. Odontomachus brunneus* (Patton, 1894) = 0.33 pg and *Odontomachus chelifer* (Latreille, 1802) = 0.54 pg) (Table 1, Figure 2). These differences in genome size among closely related species have been associated in several studies with the amount of heterochromatin in the chromosomes (Lopes et al., 2009; Tavares et al., 2010; Cardoso et al., 2012), transposable elements (Kidwell, 2002; Vieira et al., 2002) and other repetitive genome sequences (Gregory and Hebert, 1999; Petrov, 2001). In some species, as *Ectatomma tuberculatum* and *Apterostigma dentigerum* Wheeler, 1925 the differences in genome size was correlated with whole genome duplication events given the large genome size of this both species when compared with the others of Formicidae (0.71 pg and 0.65 pg, respectively) (Tsutsui et al., 2008).

The correlation between genome size and chromosome number has been reported in some studies for ants, for example, Cardoso et al. (2012) within fungus–growing ants. In their study, they found a relationship between these two characteristics being *Sericomyrmex amabilis* Wheeler, 1925 the species with the highest number of chromosomes and also the largest genome size and other two species with the lowest number of chromosomes also had the smallest genome size. Correlation between chromosome and genome size has been reported for some insects. For instance, Ardila–Garcia and Gregory (2009) also found this positive correlation among species of damselflies, but not in dragonflies (Insecta: Odonata). Lack of correlation between genome size and chromosome number has been shown in the highly eusocial stingless bees of Meliponini tribe (Hymenoptera: Apidae) (Tavares et al., 2012). Yet, body size was correlated with genome size among dragonflies and damselflies (Ardila–Garcia and Gregory, 2009), but not among stingless bees (Tavares et al., 2010) or ants (Tsutsui et al., 2008). These contradictory observations remain the issue whether genome size is shaped by neutral or natural selection.

It has been proven that changes in genome size are related to the addition and deletion of heterochromatin and that species with low amounts of heterochromatin also have lower DNA content per haploid nucleus, likewise the reverse is also true (Tavares et al., 2017). Although conclusion remarks still unlike due the limited availability of data and sampling representing more genera and species, important question could be addressed when more data became available. Considering the assembled data e evidences from other social insects, as bees, we propose that the differences in DNA content among ant species may also be related to the different amount of heterochromatin in the chromosomes. Nevertheless, we emphasize that this can only be confirmed after a detailed study of chromosomal structure and chromosome counts across genera and subfamilies.

CONCLUSIONS AND PERSPECTIVES

The compilation of the genome size data currently available in the literature for ants has highlighted the scarcity of estimates for this hyper–diverse family (with only 0.52% of known species having been estimated). Little is known about the methodologies employed and the lack of standardization of the works makes it problematic to compare the different estimates (Ardila–Garcia et al., 2010; Doležel and Greilhuber, 2010), especially regarding the buffer to isolate the nuclei, tissue and internal standard used. Also, the mechanisms involved in the evolution of the genome in ants are still unknown, especially those related to the total amount of heterochromatin in chromosomes and their relationship with genome size; the whole–genome duplication events, which could explain the large variation of the genome of some species, such as *Ectatoma tuberculatum* and *Apterostigma dentigerum* (Tsutsui et al., 2008); and polyploidy events as in *Solenopsis invicta* males (Glancey et al., 1976; Lorite and Palomeque, 2010). Our analysis highlight the importance and accuracy of the use of FCM to estimate the genome size of species and the possibility of obtaining robust results, since a large number of nuclei (10.000 or more per sample) are analyzed to determine the DNA content. Therefore, the standardization of the techniques used and a large–scale study of the ant genome size are urgently required, given the ecological and economic importance of this group contributing to

our knowledge on ant evolution by using another genetic diversity and independent dataset.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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