

"Much more than a neck": karyotype differentiation between *Dolichoderus attelaboides* (FABRICIUS, 1775) and *Dolichoderus decollatus* F. SMITH, 1858 (Hymenoptera: Formicidae) and karyotypic diversity of five other Neotropical species of *Dolichoderus* LUND, 1831

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Abstract

Dolichoderus LUND, 1831 is the most species-rich and morphologically diverse genus in the Dolichoderinae subfamily and comprises more than 150 described species. The ants in this genus are mainly arboreal and are mostly found in tropical humid forests. In Brazil, there are 35 *Dolichoderus* species grouped in nine species complexes. Some of these species, such as those in the *D. attelaboides* and *D. decollatus* complexes, are large in overall size and share several common morphological characters, which suggests a close phylogenetic relationship among them. In this study, classical and molecular cytogenetic techniques were used to investigate the karyotype differentiation between *Dolichoderus attelaboides* (FABRICIUS, 1775) (*D. attelaboides* complex) and *Dolichoderus decollatus* F. SMITH, 1858 (*D. decollatus* complex). Also, species in the following complexes were analyzed cytogenetically for the first time, namely *Dolichoderus lutosus* (F. SMITH, 1858), *Dolichoderus diversus* EMERY, 1894, and *Dolichoderus voraginosus* MACKAY, 1993 (*D. diversus* complex), *Dolichoderus bidens* (LINNAEUS, 1758) (*D. bidens* complex), and *Dolichoderus imitator* EMERY, 1894 (*D. imitator* complex). Our results revealed a high karyotype divergence between *D. attelaboides* and *D. decollatus*, indicating that chromosome rearrangements most likely had an important role in the diversification of these complexes. The chromosome numbers analyzed in this study ranged from $2n = 10$ to $2n = 58$ and placed Dolichoderinae as the third most karyotypically diverse group known within Formicidae. The differences in the location of DNA clusters between the two species in the *D. diversus* complex may have originated from pericentric inversions during karyotype diversification within this complex. Molecular phylogenetic analyses using fragments of the cytochrome oxidase I (COI) and long-wavelength rhodopsin (LW Rh) genes indicated that chromosomal rearrangements have played an important role in karyotype evolution and diversification in *Dolichoderus*, unlike other ant genera that exhibit highly conserved karyotypes. We conclude that the smaller and more numerous chromosomes arose as a result of successive events of fission.

Key words: Neotropical ants, cytogenetics, Dolichoderinae.

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Introduction

Dolichoderus LUND, 1831 is the most species rich and morphologically diverse genus in the Dolichoderinae subfamily. It comprises mostly arboreal ants found in tropical humid forests (SHATTUCK 1992, MACKAY 1993, CUEZZO 2003). The New World species, placed into 12 complexes,

are widely distributed from southern Canada to northeastern Argentina (MACKAY 1993). In Brazil, 35 species have been reported so far distributed in the *D. attelaboides*, *D. bidens*, *D. bispinosus*, *D. debilis*, *D. decollatus*, *D. diversus*, *D. imitator*, *D. laminatus*, and *D. rugosus* complexes

(MACKAY 1993, ORTIZ & FERNÁNDEZ 2011). Among these complexes, the *D. decollatus* and *D. attelaboides* complexes are noteworthy for the high morphological similarity among their species. However, the species within the *D. attelaboides* complex have an elongated vertex similar to a tubular neck. This vertex is the main character that allows distinguishing the species within the *D. attelaboides* complex from those species within the *D. decollatus* complex (MACKAY 1993).

According to MACKAY (1993), the Neotropical species of *Dolichoderus* belong to at least four distinct lineages that include 1) species of the former genus *Hypoclinea* MAYR, 1855 from the Neotropical region; 2) species of the former genus *Hypoclinea* from the Nearctic region and most species of the former genus *Monacis* ROGER, 1862; 3) species of the former genus *Monacis* of the complex *D. bispinosus*; and 4) species in the genus *Dolichoderus* sensu KEMPF (1972). Confirming previous evidence, SHATTUCK (1992) synonymized all the aforementioned genera based on morphological features present in both fossil and extant species. SHATTUCK (1992) suggested the monophyly of *Dolichoderus*, which was later corroborated by WARD & al. (2010) in a molecular phylogenetic study. The latter study included species within the former genera *Monacis*, *Hypoclinea*, and a species of *Dolichoderus* sensu KEMPF (1972). However, due to the limited number of *Dolichoderus* species included, the intrageneric relationships within this genus remained largely unresolved.

Despite the high morphological variation and high diversity within *Dolichoderus*, with more than 150 species described worldwide (BOLTON 2014), little is known about their cytogenetics and phylogeny. The only karyotypes reported for this genus are those of five species in the Asia-Pacific region [Australia (CROZIER 1968, IMAI & al. 1977), Indonesia (IMAI & al. 1984), Japan (IMAI 1969), and Malaysia (IMAI & al. 1983, GOÑI & al. 1982)]. A wide range of chromosome numbers from $2n = 18$ in *Dolichoderus* sp. (GOÑI & al. 1982) to $2n = 30 - 33$ in *Dolichoderus thoracicus* (F. SMITH, 1860) (IMAI & al. 1983) has been described. This indicates that this genus has high karyotype diversity, a feature previously verified only in few genera within Formicidae such as in the ponerine genus *Neoponera* EMERY, 1901 with chromosome numbers ranging from $2n = 12$ in *Neoponera unidentata* (MAYR, 1862) to $2n = 64$ in *Neoponera verenae* FOREL, 1922 and in the myrmeciine genus *Myrmecia* FABRICIUS, 1804 with karyotypes varying from $2n = 2$ in *Myrmecia pilosula* F. SMITH, 1858 to $2n = 84$ in *Myrmecia brevinoda* FOREL, 1910 (see LORITE & PALOMEQUE 2010).

Cytogenetic studies have contributed significantly to the genetic characterization of Formicidae. Such studies have helped to distinguish species groups of controversial taxonomy (CROSLAND & CROZIER 1986, CROSLAND & al. 1988, MARIANO & al. 2006, SANTOS & al. 2010, MARIANO & al. 2012) and, importantly, cytogenetic studies have contributed to the knowledge of chromosome structure and chromosomal rearrangements involved in the karyotype evolution within this family (GOODISMAN & al. 2008, LORITE & PALOMEQUE 2010).

So far, karyotype information is available solely for a single Neotropical species within *Dolichoderus*, namely *Dolichoderus voraginosus* MACKAY, 1993 (AGUIAR & al. 2011). In the present study, we carried out cytogenetic and

molecular analyses on *D. attelaboides* (FABRICIUS, 1775), *D. decollatus* F. SMITH, 1858, *D. lutosus* (F. SMITH, 1858), *D. bidens* (LINNAEUS, 1758), *D. diversus* EMERY, 1894, *D. voraginosus*, and *D. imitator* EMERY, 1894. The recovered phylogeny was used as a framework to discuss our hypotheses on karyotype evolution for the aforementioned species. The results reported in this study will lend support to the discussion of karyotype evolution in *Dolichoderus*.

Material and methods

Sampling: Colonies of the seven species were collected in experimental fields at the Comissão Executiva do Plano da Lavoura Cacaueira-CEPLAC, Ilhéus, state of Bahia, Brazil ($14^{\circ} 45' S$, $39^{\circ} 13' W$). All sampling areas were within a 20-year-old cocoa grove shaded by exotic legume trees (*Erythrina fusca* and *E. poeppigiana*, Fabaceae) and fruit trees (*Artocarpus heterophyllus*, Moraceae, and *Musa* spp., Musaceae) (for description and information about the experimental cocoa fields, see DELABIE & al. 2007). The *Dolichoderus* ants included in this study were identified to the species level using the key included in the revision of the Neotropical species of the genus by MACKAY (1993). All the *Dolichoderus* we studied are true arboreal, except for *D. imitator* which is the only Neotropical species living on the ground, as far as we know. Both *D. attelaboides* and *D. decollatus* are large species which build in the vegetation broad nests made of dead and living leaves and organic material where a numerous population lives; *D. bidens* is also a rather large species that lives generally on shrubs where it forms polydomous nests made by dozens of similar structures protected by a cartoon wall in the back of living leaves (DELABIE & al. 1991); the populations of colonies of the other species, *D. diversus*, *D. lutosus* and *D. voraginosus*, are small, with less than 100 individuals, and are generally found in hollow rotten fruit and trunk cavities, as well as in the roots of epiphytic bromeliads. All of these ants tend sap-sucking Homoptera (DELABIE 2001). Voucher specimens of each colony were deposited at the Laboratório de Mirmecologia (CPDC Collection), CEPEC-CEPLAC, Ilhéus, Bahia, Brazil.

Conventional cytogenetic analysis: Mitotic metaphases were obtained from cerebral ganglia of prepupae following IMAI & al. (1988) and subsequently stained with Giemsa for the determination of chromosome number and morphology. Metaphases were photographed using a CX-41 microscope equipped with a digital camera C-7070 (Olympus, Tokyo, Japan). A minimum of five metaphases per individual was analyzed. The classification of the chromosomes followed LEVAN & al. (1964).

Fluorochrome staining: Chromomycin A3 [(CMA3) / 4', 6-diamidino-2-phenylindole (DAPI)] staining followed SCHWEIZER (1976). After three days, the slides were observed using an epifluorescence microscope (DMRA2, Leica Microsystems) and the images were captured using IM50 software (Leica Microsystems Imaging Solutions Ltd.).

Fluorescent in situ hybridization: Fluorescent in situ Hybridization (FISH) followed MOSCONE & al. (1996) with the modifications described by SANTOS & al. (2010). The ITS-1 probe was amplified from the 45S rDNA cluster using the primers 5'-TCAACACGGGACCCAGGCC-3' (forward-18S) and 5'-CGATGATCAAGTGTCTGCA-3' (reverse-5.8 S) described by PILGRIM & al. (2002).

Tab. 1: Taxa analyzed herein and respective GenBank accession numbers.

Species	COI	LWR	Reference
<i>Dolichoderus attelaboides</i>	KU187246	KU232982	present study
<i>Dolichoderus decollatus</i>	KU187247	KU232983	present study
<i>Dolichoderus bidens</i>	KU187249	KU232988	present study
<i>Dolichoderus debilis</i>	KU187250	KU232986	present study
<i>Dolichoderus voraginosus</i>	KU187251	KU232992	present study
<i>Dolichoderus diversus</i>	KU187252	KU232991	present study
<i>Dolichoderus lutosus</i>	KU187254	KU232990	present study
<i>Dolichoderus quadridenticulatus</i>	KU187255	KU232989	present study
<i>Dolichoderus imitator</i>	KU187257	KU232984	present study
<i>Azteca beltii</i> (voucher z256)	JQ867689	JQ868413	PRINGLE & al. (2012)
<i>Azteca ovaticeps</i> (voucher B224)	JQ867544	JQ868268	PRINGLE & al. (2012)
<i>Forelius pruinosus</i> (voucher: CASENT0106039)	HQ207390	EF013574	LUCKY (2011)
<i>Leptomyrmex unicolor</i> (voucher: CASENT0127091)	HQ207384	HQ207452	LUCKY (2011)
<i>Leptomyrmex wiburdi</i> (voucher: CASENT0127059)	HQ207388	HQ207457	LUCKY (2011)

Tab. 2: Chromosome number, karyotype formula, CMA₃⁺ / DAPI⁻ bands, and ribosomal sites in the *Dolichoderus* species studied herein.

Species	2N	Karyotype formula	Differential fluorochrome staining (CMA ₃ ⁺ / DAPI ⁻ bands)	FISH (ITS)
<i>Dolichoderus lutosus</i>	10	2k = 4 M + 6 SM	Centromeric bands in all chromosomes and an interstitial band in the chromosome pair 2	Interstitial ribosomal cluster in the chromosome pair 2
<i>Dolichoderus bidens</i>	18	2k = 6 M + 12 SM	Centromeric bands in all chromosomes and an interstitial band in the chromosome pair 1	Interstitial ribosomal cluster in the chromosome pair 1
<i>Dolichoderus voraginosus</i>	20	2k = 14 M + 6 SM	Centromeric bands in all chromosomes. The chromosome pair 1 lacks the telomeric band.	Telomeric ribosomal cluster in the chromosome pair 1
<i>Dolichoderus diversus</i>	22	2k = 10 M + 12 SM	Centromeric bands in all chromosomes and an interstitial band in the chromosome pair 1	Interstitial ribosomal cluster in the chromosome pair 1
<i>Dolichoderus imitator</i>	38	2k = 6 M + 28 SM + 4 A	Interstitial bands in the chromosome pair 1	Interstitial ribosomal cluster in the chromosome pair 1
<i>Dolichoderus decollatus</i>	38	2k = 6 M + 32 SM	Interstitial bands in the chromosome pair 2	Interstitial ribosomal cluster in the chromosome pair 2
<i>Dolichoderus attelaboides</i>	58	2k = 2 M + 50 SM + 6 A	Terminal band in the long arm of the chromosome pair 2	Ribosomal cluster in the long arm of the chromosome pair 2

Genomic DNA extraction and gene amplifications:

Genomic DNA was extracted from one leg of a single specimen of each species included in this study using the DNeasy™ Tissue Kit (Qiagen Inc., Valencia, CA) and followed the manufacturer's instructions. For the molecular phylogenetic analysis two different gene regions were amplified. The first was a mitochondrial region (cytochrome oxidase subunit I gene – COI) and the second a nuclear region (long wavelength rhodopsin – LWR) using primers LCO1490 / HCO2198 for COI (FOLMER & al. 1994) and LR143F / LR639ER (WARD & DOWNIE 2005) respectively. DNA amplification was carried out in 25µL volume reactions: 12.45 µL ultra-pure water, 2.5 µL 10X buffer, 3.0 µL

25 mM MgCl₂, 2.5 µL 100 mM dNTP, 1.25 µL of each primer (20 mM), 3 µL of DNA, and 0.3 µL Taq DNA polymerase (Promega). The gene amplification consisted of an initial step at 94 °C for 1 min followed by 39 cycles (denaturation at 94 °C for 1 min, annealing at 52 °C (for both genes) for 1 min, and extension at 72 °C for 1 min) and a final extension step at 72 °C for 10 min. Amplifications were performed using an Eppendorf® Mastercycler thermocycler.

Phylogenetic analysis: Bayesian analysis was performed using the program MrBayes 3.1.2 (HUELSENBECK & RONQUIST 2001). Data from both genes were concatenated in a matrix with a total of 1,135 base pairs. The best nucleo-

tion substitution model was determined for each codon partitioned gene region using the Akaike information criterion implemented in the program jModelTest 2.1.4 (DAR- RIBA & al. 2012). The best fit models inferred for COI and LW Rh were GTR + I + G and HKY + I, respectively. The Bayesian analyses were performed with two simultaneous sets of four chains using the default values for search MrBayes 3.1.2. Searches were made in two simultaneous and independent runs of the Markov Chain Monte Carlo (MCMC) and for 10 million generations with trees sampled every 1,000 generations. Convergence of the two MCMC independent runs and burnin were accessed in Tracer 1.6 (RAMBAUT & al. 2014). Trees were edited in FigTree v1.4.2 (RAMBAUT 2009). DNA sequences used as outgroups were obtained from GenBank (Tab. 1).

Results

Our analyses revealed substantial variation in chromosome number and morphology in the karyotypes of the species studied (Fig. 1, Tab. 2). Chromosome number ranged from $2n = 10$ in *Dolichoderus lutosus* to $2n = 58$ in *D. attelaboides*, the latter being the largest chromosome number observed within the Dolichoderinae to date. The karyotypes analyzed herein were predominantly submetacentric, except for *D. voraginosus* ($2n = 20$), whose karyotype consisted of mostly metacentric chromosomes.

CMA_3^+ / DAPI-bands were located in the pericentromeric regions of chromosomes in *Dolichoderus lutosus*, *D. diversus*, *D. voraginosus*, and *D. bidens* (Fig. 2: A1, A2, A3, and A4, respectively). In *D. attelaboides* and *D. decollatus*, the CMA_3^+ / DAPI band was located on the second chromosome pair. In *D. attelaboides*, the band extends along the entire length of the short chromosome arms and part of the long arms. FISH results revealed that there are only two ribosomal DNA clusters and that the clusters are located on homologous chromosomes in the studied species. The ribosomal DNA clusters were detected on pair 1 in *D. voraginosus*, *D. diversus*, *D. bidens*, and *D. imitator* (Fig. 2: B2, B3, B4, and B7, respectively) and on pair 2 in *D. lutosus*, *D. attelaboides*, and *D. decollatus* (Fig. 2: B1, B5, B6). Ribosomal sites were variable in size and location. Small terminal bands were detected on chromosome pair 1 in *D. voraginosus*. Interstitial bands were detected in *D. lutosus*, *D. bidens*, *D. diversus*, *D. decollatus*, and *D. imitator*. Coincidentally with the CMA_3^+ / DAPI results, *D. attelaboides* showed larger bands encompassing the entire extension of the short arm and part of the long arm on both chromosomes of pair 2.

The *Dolichoderus* species included in this study were recovered as a clade with strong support (PP = 1) in the molecular phylogeny (Fig. 3). These species were separated into three major clades. The first clade clustered *Dolichoderus attelaboides* and *D. decollatus* together with strong support (PP = 1). The second clade comprised *D. bidens* plus *D. quadridenticulatus* (PP = 1) and *D. diversus* plus *D. voraginosus*, (PP = 1), *D. lutosus* (PP = 0.94), and *D. debilis* (PP = 1). The third clade comprised only *D. imitator* (PP = 0.98).

Discussion

The high chromosome number variation, considering the data reported herein and in LORITE & PALOMEQUE (2010), makes *Dolichoderus* the most cytogenetically diverse genus

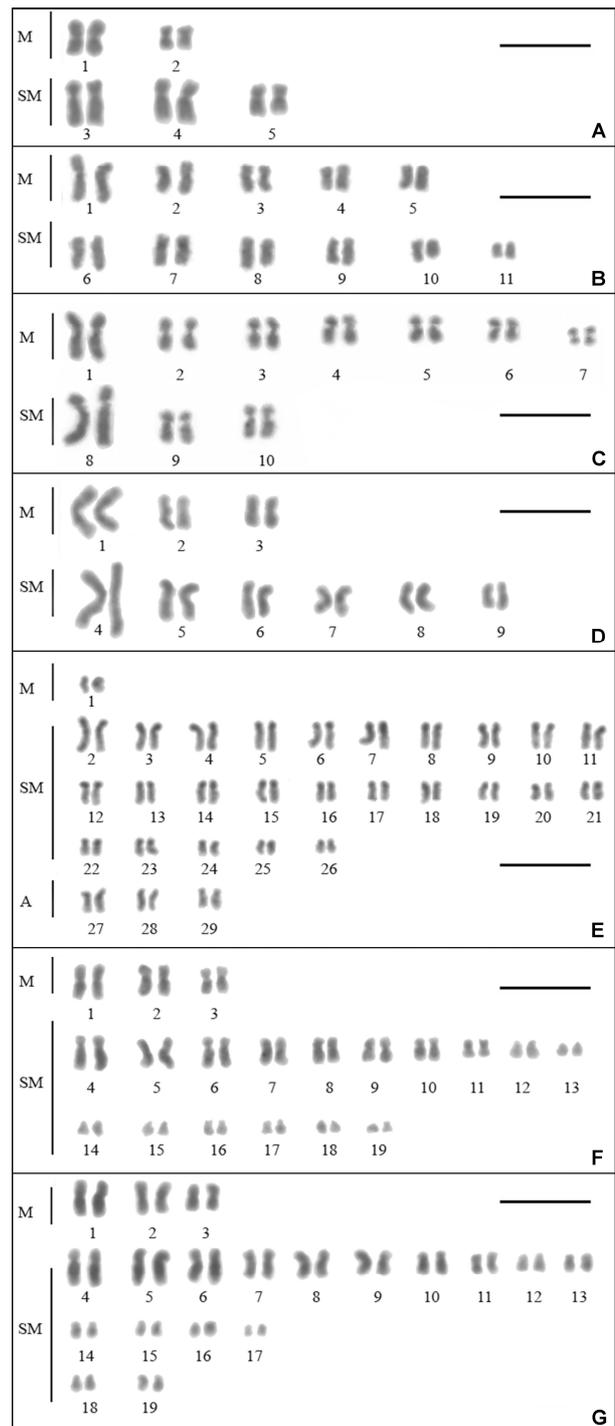
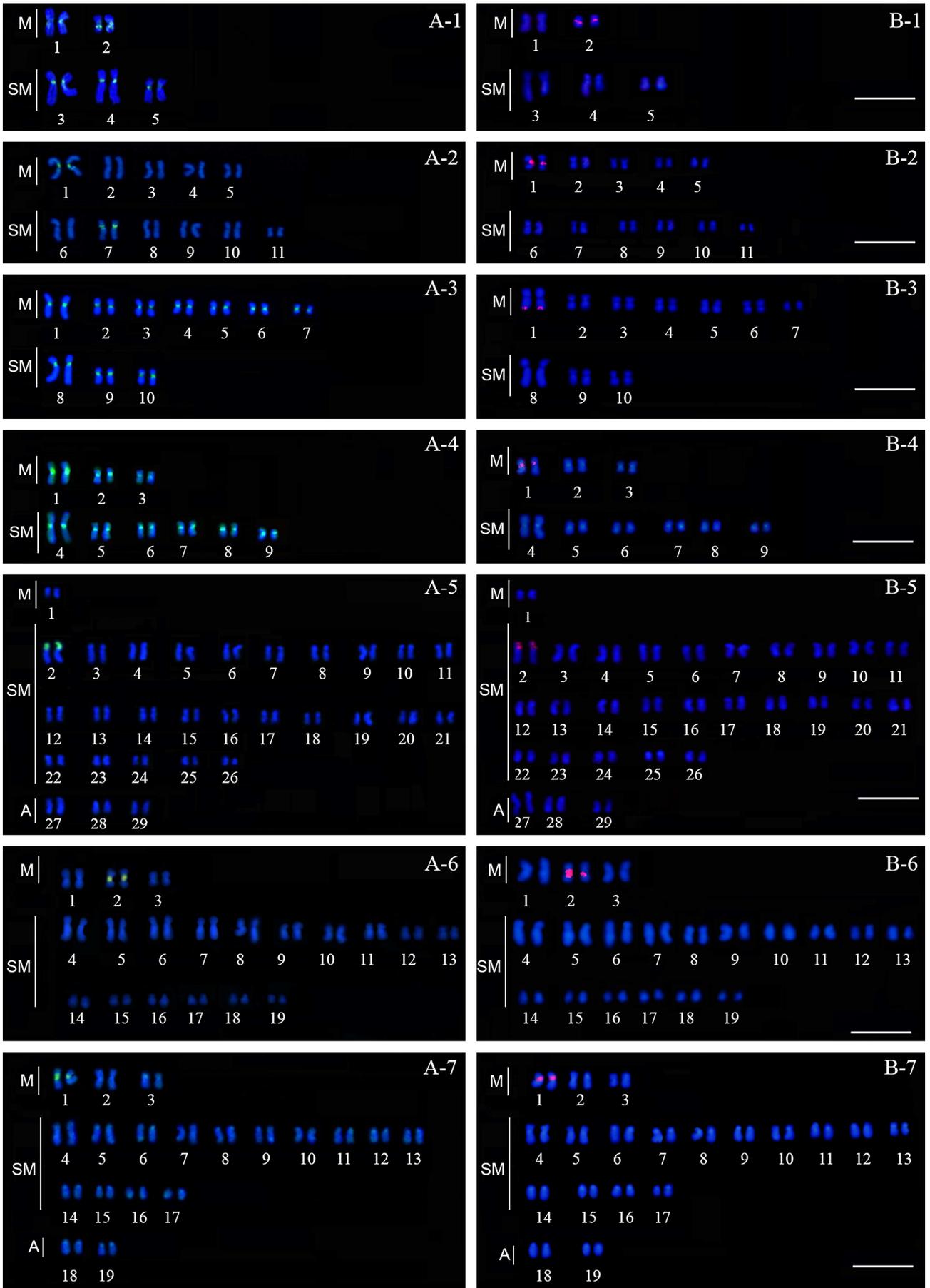


Fig. 1: Karyograms of *Dolichoderus lutosus* (A), *D. diversus* (B), *D. voraginosus* (C), *D. bidens* (D), *D. attelaboides* (E), *D. decollatus* (F), and *D. imitator* (G). Bar = 10 μ M.

Fig. 2: Karyograms showing CMA_3^+ / DAPI-bands (yellow-greenish regions on the chromosomes) and ribosomal cluster sites (reddish regions) in *Dolichoderus lutosus* (A-1 / B-1), *D. diversus* (A-2 / B-2), *D. voraginosus* (A-3 / B-3), *D. bidens* (A-4 / B-4), *D. attelaboides* (A-5 / B-5), *D. decollatus* (A-6 / B-6), and *D. imitator* (A-7 / B-7). Bar = 10 μ M.

across the 14 genera already studied within the Dolichoderinae (Tab. 3) (LORITE & PALOMEQUE 2010, CARDOSO & al. 2012).



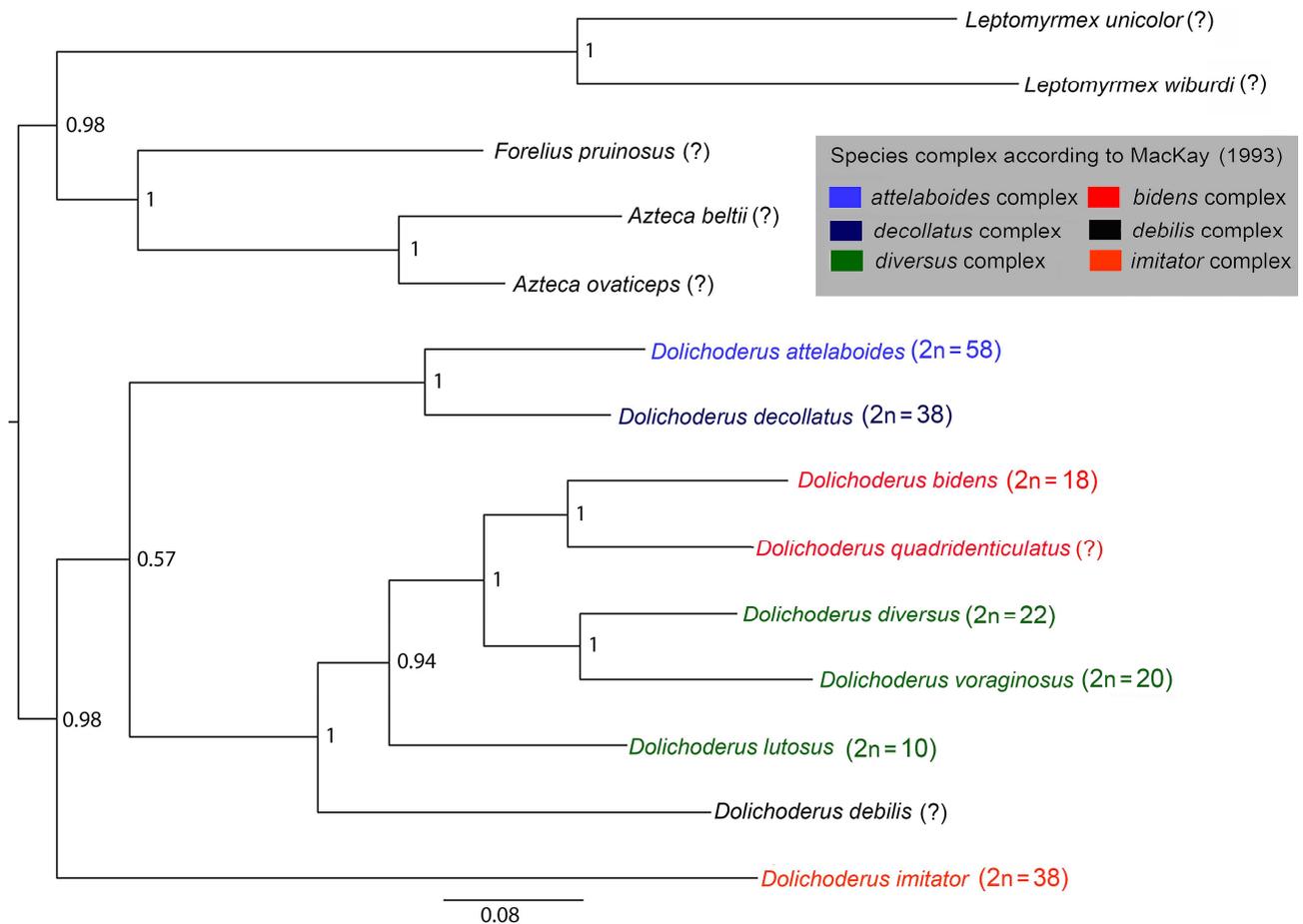


Fig. 3: Phylogram of *Dolichoderus* based on Bayesian analysis of a concatenated matrix including partial sequences of the Cytochrome Oxidase I and Long-Wavelength Rhodopsin genes. Numbers in the nodes represent Bayesian posterior probabilities. DNA sequences for the genera *Azteca*, *Forelius*, and *Leptomyrme* were obtained from GenBank.

Genus	2n		Number of species karyotyped	Number of species per genus
	Lowest Number	Highest Number		
<i>Anonychomyrma</i>	16	16	2	26
<i>Azteca</i>	28	28	1	84
<i>Bothriomyrme</i>	22	22	2	23
<i>Doleromyrma</i> *	12	14	2	2
<i>Dolichoderus</i> **	10	58	11	132
<i>Dorymyrme</i>	18	26	5	60
<i>Forelius</i>	32	32	1	18
<i>Iridomyrme</i> ***	16	48	15	79
<i>Leptomyrme</i>	24	24	1	27
<i>Linepithema</i>	16	16	2	20
<i>Ochetellus</i>	28	28	1	7
<i>Philidris</i>	16	16	1	9
<i>Tapinoma</i>	10	18	7	69
<i>Technomyrme</i> ****	28	30	5	93

Tab. 3: Variation on the chromosome number for 14 of the 28 described genera within *Dolichoderinae* (three of the genera listed by LORITE & PALOMEQUE (2010) were synonymized and herein we also included the genus *Azteca*). Data from LORITE & PALOMEQUE (2010) and CARDOSO & al. (2012) (for the genus *Azteca*). * Only morpho-species; ** including species analyzed in this paper; *** only four species described, 11 are morphospecies (all have 2n = 18); **** four, out of the five species listed here, are morphospecies.

It is noteworthy that, even though *Iridomyrme* MAYR, 1862 is the most cytogenetically studied genus in this sub-family (LORITE & PALOMEQUE 2010), only three distinct karyotypes have been reported for this genus, with chromosome number ranging from 2n = 14 in *Iridomyrme* sp. to 2n = 48 in *Iridomyrme anceps* (ROGER, 1863) (IMAI & al. 1977, IMAI & al. 1984).

The significant karyotype diversity found among *Dolichoderus* species might be explained by an earlier origin for this group compared with the other genera within *Dolichoderinae*. The chromosome variation reported herein also corroborates the hypothesis that older ant groups tend

to have more diversified karyotypes as has been reported for some poneroid species since they have had more time to evolve and diversify (MARIANO & al. 2012, LORITE & PALOMEQUE 2010, BRADY & al. 2006, MOREAU & al. 2006). According to MOREAU & BELL (2013), their molecular phylogeny suggests that *Dolichoderus* emerged approximately 60 million years ago and is in a more basal position in regards to most genera in Dolichoderinae such as *Iridomyrmex* MAYR (~ 20 M.A.), *Tapinoma* FOERSTER, 1850 (~ 30 M.A.), and *Linepithema* MAYR, 1866 (~ 15 M.A.). The most recent common ancestor of *Dolichoderus* is older than other related genera in the subfamily Dolichoderinae, and this more ancestral condition was first suggested by BROWN (1973) and recently has been corroborated by the molecular data of WARD & al. (2010).

The significant karyotype variation found in Neotropical *Dolichoderus* is similar to what has been reported for other Neotropical genera such as *Gnamptogenys* ROGER, 1863, *Pseudomyrmex* LUND, 1831, and *Neoponera* EMERY, 1901 (MARIANO & al. 2012). Moreover, our data place Dolichoderinae as the third most karyotypically diverse group known in Formicidae, following the subfamilies Ponerinae and Myrmicinae (LORITE & PALOMEQUE 2010). Before the present study, the number of species with known karyotypes (about 50 species), along with the previously reported karyotype diversity, indicated that Dolichoderinae showed little variation in chromosome number (LORITE & PALOMEQUE 2010). However, it should be emphasized that previous studies were restricted to species of the Indomalayan and Australian regions where, within this subfamily, apparently there is a lower intrageneric karyotype diversity (CROZIER 1968, IMAI 1969, IMAI & al. 1977, GOÑI & al. 1982, IMAI & al. 1983, IMAI & al. 1984).

Our molecular analysis reveals some important aspects regarding species complexes within *Dolichoderus*. Despite being phylogenetically closely related (Fig. 3; also see Appendix S1 and Appendix S2, as digital supplementary material to this article, at the journal's web pages), *D. attelaboides* and *D. decollatus* have distinct karyotypes regarding number, morphology, and chromosomal location of the rDNA cluster. Our data suggest that karyotype changes may have had an important role in the diversification of the species within the *D. attelaboides* and *D. decollatus* complexes similar to what has been reported for other Formicidae taxa (IMAI & al. 1994, MARIANO & al. 2006, SANTOS & al. 2010). Among the chromosome rearrangements observed in ants, centric fission seems to be more common and therefore most likely constitutes the main mechanism in shaping chromosome evolution. According to the Minimum Interaction Theory (IMAI & al. 1988), such mechanism of chromosome change would be favoured as it reduces genetic risks of deleterious reciprocal translocations by producing smaller and more numerous chromosomes. However, there are other potential alternative explanations for our results as well. It has been hypothesized that selection on social insects would strongly favor high overall rates of recombination allowing for the genetic diversification of offspring, for example, by an increase in the number of chromosomes (SHERMAN 1979, SEGER 1983) or an increase in the intra-chromosomal recombination rate (WILFERT & al. 2007). Thus, selection on recombination could indirectly select for increased chromosome number and genetic diversity is known to increase the resistance

to parasites or stabilize the division of labor (SHERMAN 1979). Also, genetic drift could account for a rapid change in chromosome number following a reduction in population size. However, no reduction in chromosome number variation has been observed in ant species with a wider geographic range, thus suggesting that drift can not be considered as the main mechanism of chromosome number evolution (ROSS & al. 2014). These authors caution, though, that there are not conclusive analyses and the role of historical population in the current distribution should also be taken into account. Regarding the establishment of new chromosome variants, meiotic drive, the nonrandom segregation of chromosomes during female meiosis, has been considered the factor with the strongest potential to influence their fixation in mammals (PARDO-MANUEL DE VILLENNA & SAPIENZA 2001).

Most karyotyped species in this genus, including the species in the present study, fall within the range of chromosome numbers from $2n = 18$ in *Dolichoderus* sp. (GOÑI & al. 1982) and *D. bidens* to $2n = 30 - 33$ in *Dolichoderus thoracicus* (F. SMITH, 1860) (see IMAI & al. 1983). Chromosome numbers within this range are also found in the outgroup, $2n = 24$ for *Leptomymex* (IMAI & al. 1977) and *Azteca* (CARDOSO & al. 2012). Given the present analysis, it is reasonable to assume that the higher chromosome numbers found within *Dolichoderus* most likely represent an ancestral state.

In *Dolichoderus attelaboides*, the decreased number of metacentric chromosomes could have resulted from successive events of centric fission and pericentric inversion. The differences in the location of the rDNA cluster between *D. attelaboides* and *D. decollatus* may have originated from pericentric inversions. The differences may even have resulted from a centric fission in an ancestral karyotype involving a chromosome pair similar to the second pair in *D. decollatus*. In this latter case, the event must have been followed by in tandem growth of the heterochromatin in the short arm, which resulted in a submetacentric morphology in *D. attelaboides*. Alternatively, the decrease observed in the number of metacentric chromosomes could have been due solely to fission. The short arm of the second pair in *D. attelaboides* could have been generated by rDNA amplification before an event of centric fission based on the fact that the size of the NOR region in this species is larger than that observed in other *Dolichoderus* species. This considerable karyotype divergence between species of the *D. attelaboides* and *D. decollatus* complexes adds new characters that strengthen their classification into distinct groups as proposed by MACKAY (1993). Considering the likely proximity between these two complexes, their differentiation might have resulted from chromosome rearrangements.

Species within the *D. diversus* complex clustered together with species of the *D. bidens* complex in our molecular analysis (Fig. 3), suggesting that the former is not a monophyletic group. A high karyotype diversity was observed among the species analysed within the *diversus* complex (*Dolichoderus lutosus*, *D. diversus*, and *D. voraginosus*) with chromosome numbers ranging from $2n = 10$ to $2n = 22$. For instance, *D. diversus* and *D. voraginosus* have approximately twice as many chromosomes as *D. lutosus*. Within this group, *D. diversus* and *D. voraginosus* have more similar karyotypes in all aspects, thus reinforcing their

close relationship as seen in our phylogenetic analyses, in which they are clustered together with strong support (PP = 1).

The pattern of numerical and structural variation observed in the clade containing the *D. diversus* + *bidens* + *debilis* complexes also suggests karyotype evolution by centric fission followed by in tandem growth of heterochromatin, according to the model of karyotype evolution of ants proposed by IMAI & al. (1988). Besides centric fissions, pericentric inversions seem to have contributed to the karyotypic diversification in this group, since the locations of the rDNA clusters differ among species. Despite the karyotype and molecular diversity revealed by this study, species within the *D. diversus* complex share several characteristics such as behavior (lack of aggressiveness), nest architecture (opportunistic nesting), and morphology (small to medium body size and alitrunk shape). On the other hand, *Dolichoderus bidens* (*D. bidens* complex) showed a karyotype very close to those of *D. diversus* and *D. voraginosus*, and the location of its rDNA cluster is similar to *D. diversus*, which is corroborated by our molecular analysis that placed them in the same clade. *Dolichoderus bidens*, together with *D. lutosus* and *D. diversus*, used to belong to the former genus *Hypoclinea* and was placed in the *bidens* complex in the most recent revision of the Neotropical species of *Dolichoderus* (MACKAY 1993). However, *D. bidens* shows differences in behavior (aggressive when the nest is disturbed) and nesting habits (polydomous carton nests), which could justify the placement of this species in a different group.

The basal (i.e., earliest branching) position of *Dolichoderus imitator* (*D. imitator* complex) in our phylogenetic tree corroborates its placement into a separate species group such as proposed by MACKAY (1993). *Dolichoderus imitator* is the single species in the *D. imitator* complex (MACKAY 1993) and has terricolous habits in contrast with the other Neotropical species of the genus, which are all arboreal. The karyotype of this species is similar to that of *D. decollatus* (*D. decollatus* complex).

The cytogenetic and molecular data were essential to better elucidate the relationship between the *D. attelaboides* and *D. decollatus* complexes. The karyotype differences reported herein show that there is more than simply a neck separating these two complexes. Moreover, the karyotype characterization also revealed marked genetic differences among species within the *D. diversus* complex and allowed us to propose an explanation for karyotype evolution of this group and the other species analyzed in this study. This first cytogenetic study of Neotropical *Dolichoderus* shows that this genus also has high karyotype and genetic diversity along with its large morphological diversity. Additional cytogenetic and molecular studies, including other species, should also contribute to a better understanding of mechanisms involved in the karyotype evolution of this genus.

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