Original Article

Cytogenetic and Genome Research

Cytogenet Genome Res 326803
DOI: 10.1159/000326803

Accepted: January 27, 2011
by M. Schmid
Published online: ■■■

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Cytogenetic Mapping of rRNAs and Histone H3 Genes in 14 Species of *Dichotomius* (Coleoptera, Scarabaeidae, Scarabaeinae) Beetles

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Key Words
Chromosomal evolution · Fluorescence in situ hybridization · Multigene family · Scarabaeidae

Abstract
Standard cytogenetic analyses and chromosomal mapping of the genes for 18S and 5S rRNAs and histone H3 were performed in 14 species of beetles of the genus *Dichotomius* (Coleoptera, Scarabaeidae, Scarabaeinae). Conserved karyotypes with 2n = 18 and biarmed chromosomes were observed in all species. Moreover, the presence of a large metacentric pair (pair 1) was characteristic in the studied species, evidencing a remarkable synapomorphy for this genus, which probably originated by an ancient fusion of 2 autosomes while the ancestral sex-chromosome pair remained conserved. FISH showed that the 5S rRNA and histone H3 genes are located in the proximal region of pair 2, with the 2 genes co-located. However, the major rDNA cluster probed by the 18S rRNA gene mapped to 1–3 bivalents, being exclusively autosomal, associated with sex elements, or both. In most species, the major rDNA cluster was observed in pair 3, and it was frequently (64.3%) located in the distal region regardless of the chromosome. The conserved number and position of the 5S rDNA/H3 histone cluster seems to be an ancient pattern shared by all of the studied species. In contrast, the major rDNA clusters apparently tolerate distinct patterns of diversification in the karyotypes of the species that could be associated with small inversions, ectopic recombination, and transposition. Moreover, we reinforced the association/co-localization between the 5S rRNA and histone H3 genes in this group contributing thus to the knowledge about the chromosomal organization and diversification patterns of multigene families in beetles and insects.
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The ribosomal RNA (rRNA) and histone multigene families in eukaryotic genomes are usually arrayed in clusters located on one or several chromosomes. The major ribosomal cluster (45S rDNA) is composed of a tandem array of transcribed units separated from each other by variable nontranscribed intergenic spacers. Each transcribed unit is formed by an external transcribed spacer followed by genes coding 28S, 5.8S, and 18S rRNAs; the genes are separated by internal transcribed spacers 1 and 2. The 5S rDNA cluster consists of highly conserved transcribed sequences of 120 bp separated from each other by a variable non-transcribed spacer [Long and Dawid,

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1424–8581/11/0000–0000$38.00/0

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1980]. The histone genes may be clustered in distinct chromosomal regions, and among invertebrates these genes are typically clustered as quartets (H2A, H2B, H3, and H4) or quintets (H2A, H2B, H3, and H4 plus H1), although scattered solitary genes were also reported [Lifton et al., 1977; Childs et al., 1981; Engel and Dodgson, 1981; Maxson et al., 1983].

The clustered organization of rRNA and histone genes makes them useful chromosomal markers that are easily detected and have helped to elucidate the karyotype variation and genomic organization in diverse groups of eukaryotes. Among animals, most studies have focused on the analysis of the chromosomal location of rDNAs, mostly 45S rDNA [e.g. Martínez-Navarro et al., 2004; Wang and Guo, 2004; Cabrero and Camacho, 2008; Pisano and Ghigliotti, 2009; Nguyen et al., 2010]. The 5S rRNA genes have been primarily mapped in fish [Martins and Galetti, 1999; Martins and Wasko, 2004; Pisano and Ghigliotti, 2009], and the histone genes have been mapped in a few species, for example in mammals, amphibians, fish, mollusks, and some insects [Graves et al., 1985; Tripputi et al., 1986; Turner et al., 1988; Hankeln el al., 1993; Pendás et al., 1994; Ranz et al., 2003; Zhang et al., 2007; Cabrero et al., 2009; Cabral-de-Mello et al., 2010a, 2011a]. These multigene families can be organized in separated clusters or can be associated, as has been described for example in arthropods, annelids, mollusks, and fish [Andrews et al., 1987; Drouin et al., 1987, 1992; Barzotti et al., 2000; Vitturi et al., 2002; Colomba et al., 2002; Eirín-López et al., 2004; Vitturi et al., 2004; Pisano and Ghigliotti, 2009; Cabral-de-Mello et al., 2010a, 2011a].

In insects belonging to the order Coleoptera, knowledge about the chromosomal distribution of rRNA and histone genes is scarce. The location of the 45S rDNA has primarily been mapped in representatives of the families Carabidae, Cicindelidae, Scarabaeidae, and Chrysomelidae among others [Sánchez-Gea et al., 2000; Moura et al., 2003; Martínez-Navarro et al., 2004; Bione et al., 2005a; Almeida et al., 2010]. In the family Scarabaeidae, the 45S rDNA has been mapped in a few species (about 20), and the mapping of 5S rRNA and H3 histone genes has been restricted to only 1 species in the order Coleoptera, *Dichotomius geminatus* [Moura et al., 2003; Vitturi et al., 2003; Bione et al., 2005a, b; Silva et al., 2009; Cabral-de-Mello et al., 2010a, b; Oliveira et al., 2010].

Dichotomius (Coleoptera, Scarabaeidae, Scarabaeinae) is endemic to the Americas, and in Brazil, more than 80 species have been described. Knowledge of the karyotype organization in this genus is restricted to 6 species, revealing primarily the presence of 18 chromosomes and a large metacentric pair (pair 1) [Smith and Virkki, 1978; Vidal, 1984; Cabral-de-Mello et al., 2008; Silva et al., 2009]. The mapping of repeated multigene families, such as 45S rRNA, has been reported for 3 representatives, and the histone H3 and 5S rRNA genes have been mapped in only 1 species. The aim of this study was to advance the understanding of the chromosomal organization of rRNA and H3 histone genes in Scarabaeidae beetles and karyotype differentiation patterns in the genus *Dichotomius*. To this end, 14 species of *Dichotomius* were karyotyped, and their 5S and 18S rRNA and histone H3 genes were mapped. A conserved macro-chromosomal structure and stability for the location of 5S rRNA and H3 histone genes were revealed, whereas distinct degrees of variability for the 45S rDNA were observed. We also attempted to identify conserved karyological synapomorphy in this genus, and the data are discussed to elucidate the possible mechanisms involved in the diversification of karyotypes and multigene families.

Material and Methods

Adult male samples of 14 *Dichotomius* species were collected from distinct areas in Ceará, Mato Grosso, Minas Gerais, Paraná, Pernambuco, and São Paulo States, Brazil, using pitfall traps (table 1). The testes were dissected, fixed in Carnoy's solution (3:1 ethanol:acetic acid), and stored at –20 °C. Mitotic and meiotic chromosomes were obtained from fixed testicular cells, and the slides were prepared in 45% acetic acid by the squash technique. Coverslips were removed after the preparations were frozen by immersion in liquid nitrogen for a few seconds.

The FISH procedures were performed according to the adaptations proposed by Cabral-de-Mello et al. [2010a]. DNA probes for the 18S and 5S rRNA and histone H3 genes were obtained from cloned fragments of the genome of *D. geminatus*. The probes for 18S rDNA and histone H3 were labeled by nick translation using biotin-14-dATP (Invitrogen, San Diego, Calif., USA) and detected by avidin-FITC (fluorescein isothiocyanate) conjugate (Sigma-Aldrich, St Louis, Mo., USA). The 5S rDNA was labeled with digoxigenin-11-dUTP (Roche, Mannheim, Germany) by PCR and detected by anti-digoxigenin-rhodamine (Roche). Two-color FISH was performed for 18S/5S rDNA and 5S rDNA/histone H3. To map the 3 multigene families in the same cell, 2 FISH procedures were performed on the same slides. The probes for the 18S and 5S rRNA genes were hybridized first. After analysis and image captures, the slides were washed 3 times for 15 min each in 2× SSC at room temperature. In the second 2-color FISH round, the probes for the histone H3 and 5S rRNA genes were hybridized. The multigene family probes were also hybridized singly to eliminate any mistake in signal detection or interpretation errors. All of the preparations were counterstained with DAPI, and the coverslips were added after the application of Vectashield mounting medium (Vector, Burlingame, Calif., USA). For some images, the green signals of the 18S rRNA gene were pseudo-colored to pur-

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Table 1. Chromosomal location of 18S rDNA in 14 species of *Dichotomius.*

Species	2n	Chromosomes										Collection site in Brazil
		1	2	3	4	5	6	7	8	X	y	
D. affinis (Felsche, 1910)	18			i								Botucatu, SP (22°53'S, 48°27'W)
D. bos[a] (Blanchard, 1843)	18			p								Botucatu, SP (22°53'S, 48°27'W) Saloá, PE (8°57'S, 36°43'W)
D. crinicollis[a] (Germar, 1824)	18			d						[b]		Carrancas, MG (21°28'S, 44°38'W)
D. depresicollis[a] (Harold, 1867)	18			i								Crato, CE (7°13'S, 39°24'W)
D. geminatus[a] (Arrow, 1913)	18			d	d							Igarassu, PE (07°48'S, 34°57'W) Maracaípe, PE (8°31'S, 35°01'W)
D. laevicollis[a] (Felsche, 1901)	18			d								Caruaru, PE (8°42'S, 35°15'W)
D. mórmon (Ljungh, 1799)	18						d		d	[b]		Botucatu, SP (22°53'S, 48°27'W)
D. aff *mundus* (Harold, 1869)	18	p										Botucatu, SP (22°53'S, 48°27'W)
D. nisus[a] (Olivier, 1789)	18									[b]	[b]	Botucatu, SP (22°53'S, 48°27'W) Igarassu, PE (07°48'S, 34°57'W) Terra Roxa, PR (24°09'S, 54°06'W)
D. semiaeneus (Germar, 1824)	18									[b]		Botucatu, SP (22°53'S, 48°27'W)
D. semisquamosus[a] (Curtis, 1845)	18			d						[b]		Caruaru, PE (8°42'S, 35°15'W) Caruaru, PE (08°22'S, 36°05'W) Igarassu, PE (07°48'S, 34°57'W) Saloá, PE (8°57'S, 36°43'W)
D. sericeus[a] (Harold, 1867)	18		d									Igarassu, PE (07°48'37''S, 34°57'25''W)
D. aff *sericeus* (Harold, 1867)	18			d								Caruaru, PE (08°22'S, 36°05'W)
D. sp. Hope, 1838	18			i								Barra do Garças, MT (15°55'S, 52°16'W)

[a] Species studied for H3 histone mapping. [b] Precise position of 45S rDNA in the sex chromosomes was not possible to determine.
p = Proximal, i = interstitial, d = distal in respect to the centromere; CE = Ceará, MG = Minas Gerais, MT = Mato Grosso, PE = Pernambuco, PR =Paraná, SP = São Paulo.

ple, and the chromosomal images were captured in grayscale. The images were captured using an Olympus BX61 microscope equipped with an Olympus DP71 digital camera. The brightness and contrast of the images were optimized using Adobe Photoshop CS2.

Results

All species studied showed similar karyotypes with the presence of 2n = 18 chromosomes, Xy_p sex-determining mechanism, biarmed autosomes and the X chromosome, whereas the y was punctiform. The presence of a large metacentric bivalent (pair 1), almost double the size of pair 2, was a remarkable characteristic of these 14 species (fig. 1a–i; 2a–h; 3a). The karyotypes of 8 of the 14 species are described here for the first time; moreover, other populations and distinct individuals from populations of previously analyzed species were also karyotyped, such as *D. bos*, *D. geminatus*, *D. laevicollis*, *D. nisus*, *D. semisquamosus*, and *D. sericeus*.

The mapping of the 3 multigene families (18S and 5S rRNA and histone H3) revealed distinct patterns of their chromosomal distribution (fig. 1a–i; 2a–h; 3a; table 1). The 5S rRNA gene was highly conserved in the proximal region of chromosome pair 2 (fig. 1a–f, h, i; 2a–h; 3a). Eight of the 14 species were randomly chosen for mapping of the histone H3 gene, and, similar to 5S rDNA, this sequence was located in the proximal region of chromosomal pair 2 and co-localized with the 5S rDNA (fig. 1h, i; 2a–h). Analyses of prophase chromosomes and interphase chromatin (fig. 2c, d) reinforced that the 2 genes were co-located/associated with each other. However, the 18S rDNA presented higher variability in the number and location of sites ranging from 1–5 sites per diploid genome and was located on autosomes, sex chromosomes, or both, being proximal, interstitial, or distal (see table 1). Although variability was observed for 18S rDNA, in 6 species this sequence was exclusively located on pair 3 (fig. 1b, c, e, f; 2a, h; table 1); in 3 species, it was additionally located on another chromosome: the X chromosome

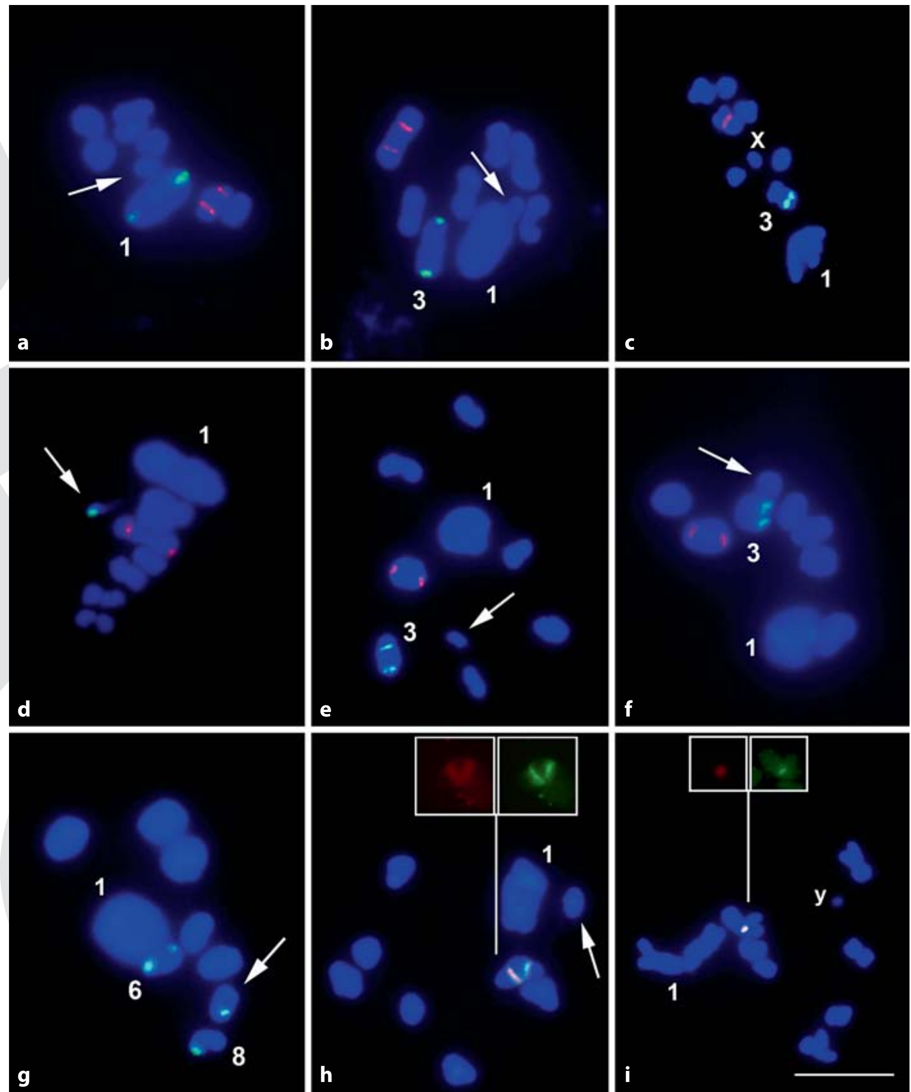

Fig. 1. Fluorescence in situ hybridization using 18S (green; **a–g**) and 5S rDNA (red; **a–f**, **h**, **i**) and the histone H3 gene (green; **h**, **i**) as probes in 8 representative species of *Dichotomius*. Metaphase I of **a** *D.* aff *mundus*, **b** *D.* aff *sericeus*, **d** *D. semiaeneus*, **e** *D. affinis*, **f** *Dichotomius* sp., **g** *D. mormon,* and **h** *D. crinicollis*. **c**, **i** Metaphase II of *D. depresicollis*. The arrows indicate the sex-chromosome bivalents, and the **inserts** in **h** and **i** show signals from separate probes for the 5S rRNA (red) and histone H3 (green) genes. Note the large metacentric pair indicated by the number 1. Other chromosomes are also indicated. Scale bar = 5 μm.

in *D. semisquamosus* (fig. 2f) and *D. crinicollis* (result not shown), and pair 4 in *D. geminatus* (fig. 2e). In *D. mormon*, *D.* aff *mundus*, *D. nisus,* and *D. semiaeneus* the 18S rDNA was observed in bivalents distinct from pair 3. It was located exclusively autosomal in *D.* aff *mundus* (pair 1) (fig. 1a) and *D. sericeus* (pair 2) (fig. 2b), associated with sex chromosomes in *D. nisus* (X and y) (fig. 2g) and *D. semiaeneus* (X) (fig. 1d), and mapped to both in *D. mormon* (pairs 6, 8 and X) that presented a polymorphic condition for pair 8 (fig. 1g).

Concerning autosomes, most of the 18S rDNA sites (18 out of 28 sites, corresponding to 64.3%) were located in distal regions, 6 (21.4%) were interstitial, and 4 (14.3%) were proximal but not far from the centromere (table 1). Interestingly, all distal sites were located in the short chromosomal arm, whereas the interstitial sites were located in the short arm in *D. affinis* and *Dichotomius* sp. and in the long arm in *D. depressicollis*. For the sex chromosomes, it was not possible to determinate the precise position of the 18S rDNA due to their condensation and small size. At least 15 cells (including distinct mitotic and meiotic cycle phases) per individual and 3 individuals per species of each population were analyzed by FISH to determine the chromosomal location of the DNA markers assayed. Figure 3a summarizes all data obtained, and figure 3b shows chromosome pair 3 in metaphases I and II and spermatogonial metaphases, indicating the precise location of the 18S rDNA.

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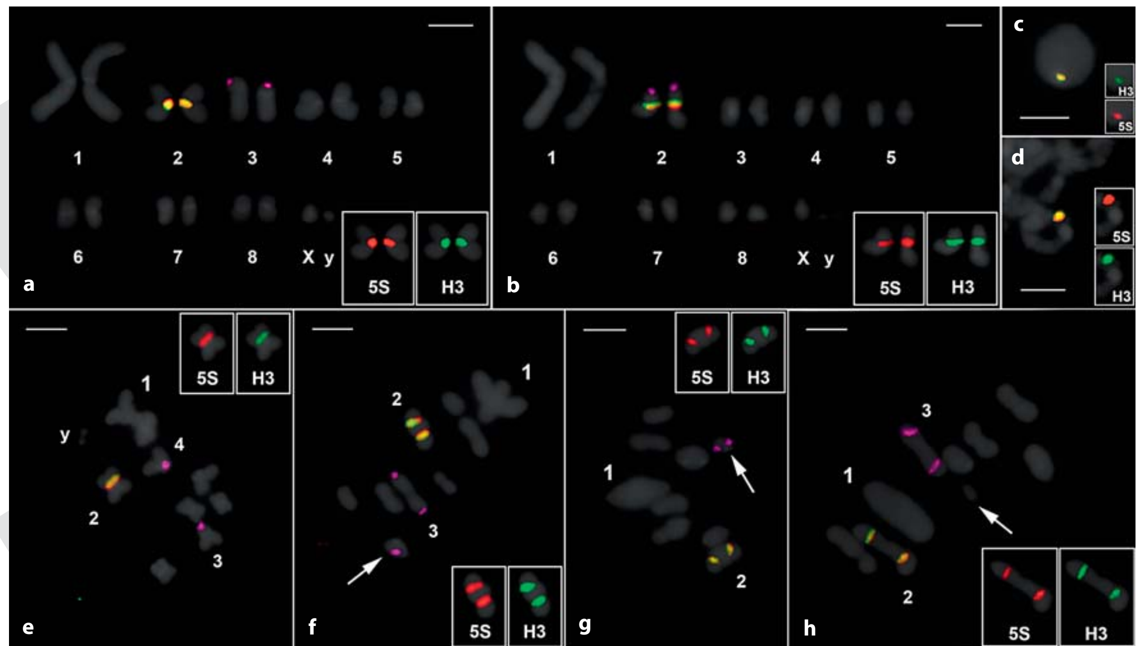

Fig. 2. Chromosomal mapping of the 18S rRNA (purple), 5S rRNA (red), and histone H3 (green) multigene families in 6 species of *Dichotomius*. Karyotypes from **a** *D. laevicollis* and **b** *D. sericeus*, **c** interphase nucleus from *D. bos*, **d** partial pachytene from *D. geminatus*, **e** metaphase II from *D. geminatus*, **f** metaphase I chromosomes from *D. semisquamosus*, **g** *D. nisus,* and **h** *D. bos* are shown. The arrows indicate the sex bivalents, and the **inserts** show the chromosomes labeled with separate probes for the 5S rRNA (red) and histone H3 (green) genes. Note that the 5S rRNA and histone H3 gene sites overlap in all cells, including interphase nuclei (**c**), less condensed chromosomes in an initial meiotic pachytene (**d**) and the large metacentric pair indicated by the number 1. Other chromosomes are also indicated. Scale bars = 5 μm.

Discussion

A similar macro-chromosomal structure was observed in the 14 species analyzed here, including distinct populations. The presence of 2n = 18 and the biarmed chromosome pair 1, which is distinct in size, was previously reported for other populations of *D. bos*, *D. geminatus*, *D. laevicollis*, *D. nisus*, *D. semisquamosus*, and *D. sericeus* [Silva et al., 2009; Cabral-de-Mello et al., 2010a] and was observed in 8 additional species studied here for the first time. These results indicate a karyotype stability in the genus at both intra- and interspecific levels, at least with regard to the macro-chromosomal structure. Bearing in mind that 2n = 20 is considered ancient for Coleoptera and Scarabaeidae [Smith and Virkki, 1978; Yadav and Pillai, 1979], the presence of the diploid number 2n = 18 and a large metacentric pair (pair 1) in *Dichotomius* could have arisen from fusion of 2 autosomes, while the ancestral sex system remained conserved. This fusion likely occurred before the diversification of *Dichotomius* species; thus, chromosome 1 represents a marker and a remarkable synapomorphy of this genus. However, this chromosomal fusion remains to be elucidated by more refined techniques like hybridization of telomeric sequences that can reveal interstitial telomeric sites in chromosomes involved in chromosomal rearrangements, such as Robertsonian fusions.

Recently, Cabral-de-Mello et al. [2011b] hybridized a C_0t-1 DNA (a pool of highly and moderately repetitive sequences) fraction obtained from the genome of *D. geminatus* against the chromosomes of 5 other *Dichotomius* species, revealing the occurrence of conspicuous and conserved terminal/sub-terminal signals in all autosomes (being probably the functional telomeres or telomere-associated DNA), but interstitial sites in pair 1 (probably generated by chromosomal fusion) were not observed. However, the authors did not exclude the possibility that pair 1 originated via fusion and proposed that the absence of interstitial signals with the C_0t-1 DNA probe could be correlated to a rapid modification of terminal sequences after the fusion, leading to the failure of interstitial C_0t-1 DNA site detection in FISH experiments.

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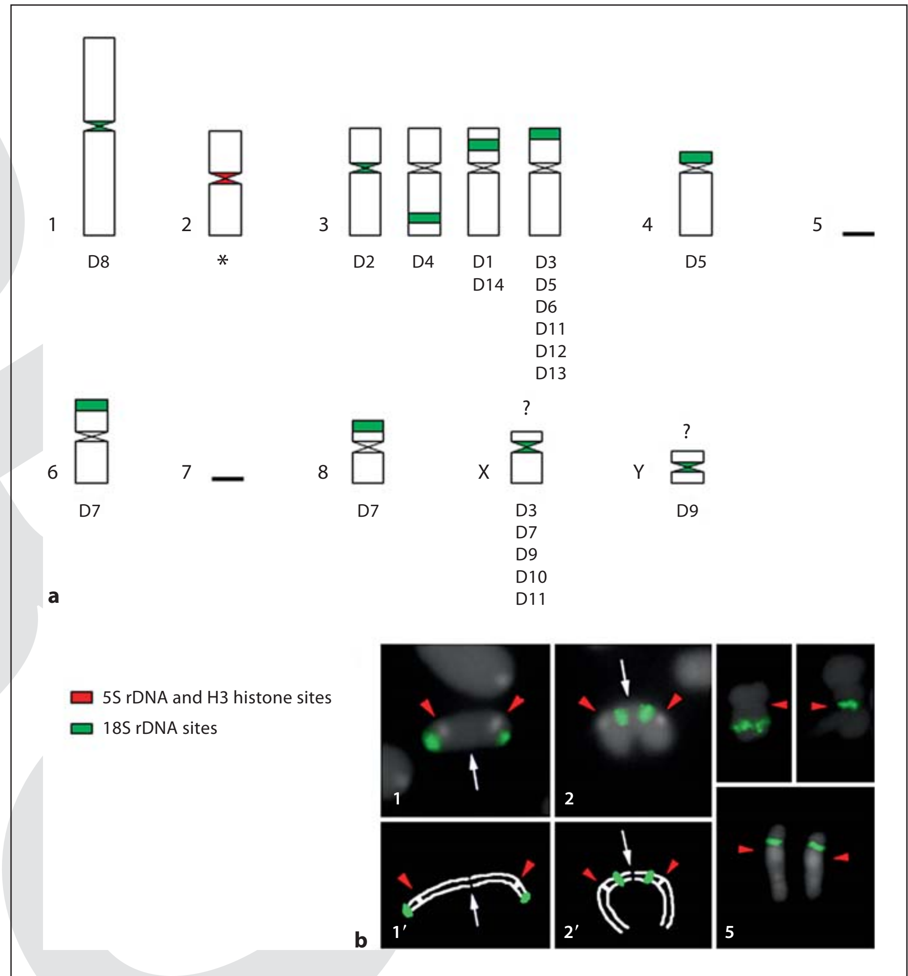

Fig. 3. a Ideograms showing the distribution of 18S rDNA (green) and 5S rDNA/H3 histone (red). D1: *Dichotomius affinis*, D2: *D. bos*, D3: *D. crinicollis*, D4: *D. depressicollis*, D5: *D. geminatus*, D6: *D. laevicollis*, D7: *D. mormon*, D8: *D.* aff *mundus*, D9: *D. nisus*, D10: *D. semiaeneus*, D11: *D. semisquamosus*, D12: *D. sericeus*, D13: *D.* aff *sericeus*, D14: *Dichotomius* sp. The black lines indicate chromosomes that harbor neither 45S/5S rDNA nor the histone cluster in any of the species. **b** Chromosome 3 selected from metaphase I (1, 1', 2, 2'), metaphase II (3, 4) and spermatogonial metaphase (5) showing in detail the position of the 18S rDNA: (1) distally in the short arm, (2, 5) interstitially in the short arm, (3) interstitially in the long arm, and (4) proximally. (1) *D.* aff *sericeus*, (2) *D. affinis*, (3) *D. depressicollis*, (4) *D. bos*, (5) *Dichotomius* sp. 1' and 2' represent a graphical structure of the bivalent 3 in metaphase I of 1 and 2, respectively. Red arrowheads indicate the centromere and white arrows the positions of chiasmata.

The presence of the Xy system is common in *Dichotomius*, occurring in all of the species and being characterized by the Xy_p configuration, and it is considered ancestral and frequently observed in Scarabaeidae and Coleoptera [Smith and Virkki, 1978; Yadav and Pillai, 1979; Dutrillaux and Dutrillaux 2009]. In contrast, in *D. sericeus*, a distinct sex mechanism, Xy_r (rod-shaped), was reported by Silva et al. [2009]; however, the *D. sericeus* individuals analyzed here presented an Xy_p sex mechanism, indicating the occurrence of polymorphisms related to the meiotic behavior of the sex chromosomes, which could represent an initial step of further sex chromosome differentiation. The Xy_r sex mechanism is rare in Coleoptera, and it has only been reported in approximately 10 species of Scarabaeidae [Smith and Virkki, 1978; Yadav et al., 1979].

Another remarkable characteristic observed in all species is the presence of 5S rDNA in pair 2, revealing an intense conservation of this cluster in the genus. It is impossible to determine if this characteristic is ancient in the family Scarabaeidae and order Coleoptera or if it represents a synapomorphy for *Dichotomius*; thus, the analysis of additional species of this group is necessary. In other insects, a distinct scenario has been described for 5S rDNA organization, although until now, the results are incipient. For example, in grasshoppers single and multiple 5S rDNA sites have been reported [Cabrero et al., 2003; Loreto et al., 2008; Teruel et al., 2010; Cabral-de-Mello et al., 2011a]. These variable results indicate that distinct evolutionary trends are driving the chromosomal organization of 5S rDNA in the genomes of insects.

In addition to the conservation of 5S rDNA in pair 2, in 8 of the analyzed species (table 1) the histone H3 gene was also conserved and co-located on the same chromosome. An association between these 2 genes was previously reported in 2 crustaceans [Andrews et al., 1987;

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Barzotti et al., 2000], 1 mollusk [Eirín-López el al., 2004], 4 Proscopiidae grasshoppers [Cabral-de-Mello et al., 2011a], and in the beetle *D. geminatus* [Cabral-de-Mello et al., 2010a]. Besides the association of histone genes and 5S rDNA reported in Coleoptera, Roehrdanz et al. [2010] recently identified interspersion of 45S rDNA and the histone quintet clusters. The number of clusters and specific chromosomal position of the histone H3 gene is apparently highly conserved among animals [e.g. Hankeln el al., 1993; Pendás et al., 1994; Cabrero et al., 2009; Cabral-de-Mello et al., 2011a], and this was also observed for the *Dichotomius* species analyzed here, indicating that the chromosomal location of the histone gene clusters has been well-conserved over a long period of evolution. In grasshoppers for example, a conservation of the chromosomal location of histone clusters was recently described for Acrididae, with an absence of changes for the 60 million years since the origin of the group [Cabrero et al., 2009], and also for Proscopiidae [Cabral-de-Mello et al., 2011a], a more ancient grasshopper family. According to Cabrero et al. [2009], the high conservation at the level of chromosomal location in grasshoppers suggests strong purifying selection operating on this trait, causing most chromosomal mutations to be intolerable. This idea could be also applicable to *Dichotomius* genomes, and bearing this hypothesis in mind, we can propose that the immobility of the 5S rDNA in the genome of *Dichotomius* beetles is a result of its association with histone genes, leading to the same evolutionary patterns for the 2 sequences at the chromosomal level. The association or co-localization at the molecular level for these multigene families needs to be investigated further, although the possible association of these sequences could be reinforced here by the observation of interphase chromatin and early meiotic cells (fig. 2c, d), in which the chromosomes are much less condensed than in metaphase.

In contrast to the extensive conservation of the 5S rDNA/histone H3 clusters, more variable patterns were observed for the number and chromosomal positions of the 45S rDNA clusters. Variations were observed at the intra- and interspecific levels for the species studied here. A similar variability for number and position of rDNA clusters has also been reported in other Coleoptera [reviewed by Schneider et al., 2007], and for *Dichotomius* the plausible explanation (other than small chromosomal rearrangements) for the variations reported in number and position is the occurrence of amplification/dispersion of 45S rDNA copies in this genus. Nevertheless, using silver nitrate staining, the presence of one chromosome responsible for nucleolar organization was found to be the most common pattern in Coleoptera [reviewed by Schneider et al., 2007], and it is possible to speculate that the genomes of the coleopteran species have tolerated selection for the activation of rDNA clusters on only one autosomal pair.

Although the presence of 45S rDNA in pair 1 (resulting from fusion) was observed in *D.* aff *mundus*, it seems that the rDNA sites in this chromosome could result from a recent transposition occurring after the fusion. The same idea concerning transposition is capable of explaining the presence of 45S rDNA in the sex chromosomes not to be a product of chromosomal rearrangements. Interestingly, the presence of rDNA sites mapped by FISH in the y chromosome as observed in *D. nisus* has not often been reported for Coleoptera; in Scarabaeidae, it was described in a species with a derived sex system (neo-XY) that had undergone chromosomal rearrangements which changed the position of the rDNA from autosomes to the sex bivalent [Arcanjo et al., 2009]. In Coleoptera as a whole, repositioning of rDNA clusters to sex chromosomes has been well documented, for example in tiger beetles (Cicindelidae) of the genus *Cicindela*, as a result of translocations between autosomes and sex chromosomes and fusion or fissions between X chromosomes [Galián et al., 2007].

The 45S rDNA observed in pair 3 of different species of *Dichotomius* presented variability for its specific position being proximal, interstitial in the short or long arm, and distal in the short arm. The apparent mechanism responsible for its variation may be associated with small chromosomal rearrangements (such as inversions) that do not modify the chromosomal morphology. In other groups, various mechanisms have been proposed to explain the amplification/spreading and location of 45S rDNA sites at the intra- and interspecific levels: (a) mobility of rDNA associated with transposable elements [Raskina et al., 2008], (b) equilocal dispersion of heterochromatin and rDNA [Pedersen and Linde-Laursen, 1994], and (c) chromosomal rearrangements. Similar to what we have proposed here in *Dichotomius*, in other insect such as grasshoppers, butterflies, and moths structural chromosomal rearrangements, ectopic recombination, and transposition have been postulated to drive the diversification of 45S rDNA in both number and position [Cabrero and Camacho, 2008; Nguyen et al., 2010]. The variation in 45S rDNA observed in *Dichotomius* could be favored by its usual distal location (64.3% of autosomal sites) that are more dynamic chromosomal regions, which facilitates transposition events and leads to the dispersion of these segments in the genome [Schweizer and

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Loidl, 1987]. Similar ideas were proposed in fish genomes for 45S rDNA in relation to its variation as well as for the conservation of 5S rDNA, which is interstitially located and seems to be protected from dispersion mechanisms [Martins and Wasko, 2004].

In summary, our results demonstrate that the chromosomal conservation observed for the macro-chromosomal structure in the genus *Dichotomius* is not reflected at the microgenomic level, at least concerning 45S rDNA. The other 2 multigene families mapped (5S rRNA and H3 histone genes) were well conserved in both their number and location, evidencing ancient characteristics of the genus, likewise the diploid number 2n = 18 and a large metacentric pair (pair 1). Further studies should focus on the specific mechanism associated with the dispersion of the 45S rDNA and the conservation of the 5S rDNA/H3 histone sequences, and this genus provides interesting material for this purpose. Moreover, we reinforced the possible co-location/association of 5S rRNA and H3 histone genes in Scarabaeidae by analyzing less condensed chromosomes and chromatin, and contributed to the knowledge regarding the chromosomal organization and diversification patterns of multigene families in beetles and insects as a whole.

Acknowledgments

This study was supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors are grateful to Fernando Augusto Barbosa Silva for the taxonomic identification of the specimens analyzed in this study.

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