



Physical mapping of 18S rDNA and heterochromatin in species of family Lygaeidae (Hemiptera: Heteroptera)

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ABSTRACT. Analyses conducted using repetitive DNAs have contributed to better understanding the chromosome structure and evolution of several species of insects. There are few data on the organization, localization, and evolutionary behavior of repetitive DNA in the family Lygaeidae, especially in Brazilian species. To elucidate the physical mapping and evolutionary events that involve these sequences, we cytogenetically analyzed three species of Lygaeidae and found $2n$ (σ) = 18 (16 + XY) for *Oncopeltus femoralis*; $2n$ (σ) = 14 (12 + XY) for *Ochrinnus sagax*; and $2n$ (σ) = 12 (10 + XY) for *Lygaeus peruvianus*. Each species showed different quantities of heterochromatin, which also showed variation in their molecular composition by fluorochrome

staining. Amplification of the 18S rDNA generated a fragment of approximately 787 bp. The alignment of the consensus sequence with sequences from other species of Heteroptera deposited in the GenBank revealed a similarity of 98% with small differences. Fluorescent *in situ* hybridization with the 18S rDNA fragment revealed that this ribosomal gene was located in 1 autosomal pair at different positions in the three species. No cytogenetic data are available for these Brazilian species. The basal number and the possible chromosomal changes that occurred among the different species, as well as the evolution of these DNA sequences, are discussed.

Key words: Chromosome structure; Fluorescent *in situ* hybridization; Heterochromatin; Ribosomal genes

INTRODUCTION

The ribosomal DNA has been used in many molecular analyses in insects, especially those involving clarifications about phylogenetic relationships. In Hemiptera, these studies have revealed important findings compared to those obtained in other groups of insects (Caterino et al., 2000). Besides 18S rDNA, other classes of repetitive DNA with heterochromatic sequences have also been widely used as cytogenetic markers for comparisons across different groups of insects such as in Orthoptera (Cabral-de-Mello et al., 2011), Coleoptera (Almeida et al., 2010), Diptera (Rafael et al., 2006), and Heteroptera (Papeschi and Bressa, 2006; Bardella et al., 2010; Grozeva et al., 2010; Panzera et al., 2012). Some types of repetitive DNA can be chromosome-specific, such as those mostly found in *Drosophila melanogaster* (Bonaccorsi and Lohe, 1991) and those found on the sex chromosomes in species such as *Megoura* (Bizzaro et al., 1996).

Few studies have investigated the identification and location of repetitive DNA, particularly in Heteroptera. These studies revealed the distribution of 18S rDNA sites in autosomal chromosomes, such as those observed in *Pachylis argentinus* Berg, 1879, and *Nezara viridula* Linnaeus, 1758 (Papeschi et al., 2003), as well as in sex chromosomes such as those of the families Belostomatidae (Papeschi and Bressa, 2006), Cimicidae (Grozeva et al., 2010), and Reduviidae (Bardella et al., 2010). Although studies investigating the location of heterochromatin are more common in Heteroptera, most of them are restricted to conventional analyses such as C-banding technique (Papeschi and Bressa, 2006). Studies involving the molecular characterization of these repetitive DNAs in insects are scarce, compared to the great diversity of the group.

In Lygaeidae, cytogenetic studies shown a wide karyotypic diversity; however, most of them are restricted to conventional analysis. Thus far, chromosomal data are available for 13 subfamilies (Ueshima, 1979; Ueshima and Ashlock, 1980; Grozeva and Kuznetsova, 1993; Kaur et al., 2010), which suggest the existence of a chromosomal variation from $2n = 11$ in *Cryphula nitens* Barber 1955 (Ueshima, 1979) to $2n = 30$ in different species of the subfamily Cyminae. In addition, different sex chromosome systems, as well as the presence/absence of m-chromosomes, were observed (Grozeva and Kuznetsova, 1993).

Although the karyotype variability within the family Lygaeidae has been well documented, the distribution of rDNA and different classes of heterochromatin and the evolution-

ary events that occurred in this group are not well elucidated. Thus, in this study, we performed both conventional and molecular cytogenetic analyses of three species of the family Lygaeidae and determined the possible events that led to karyotype differentiation of this interesting group of insects.

MATERIAL AND METHODS

Samples and collection sites

Three species of family Lygaeidae [*Oncopeltus femoralis* Stål, 1874 (Figure 1a), *Ochrinnus sagax* Brailovsky, 1982 (Figure 1b), and *Lygaeus peruvianus* Brailovsky, 1978 (Figure 1c)] were collected from southern and southeastern Brazil (Table 1). Individuals from each species were identified and deposited at Universidade Federal do Pará (UFPA).

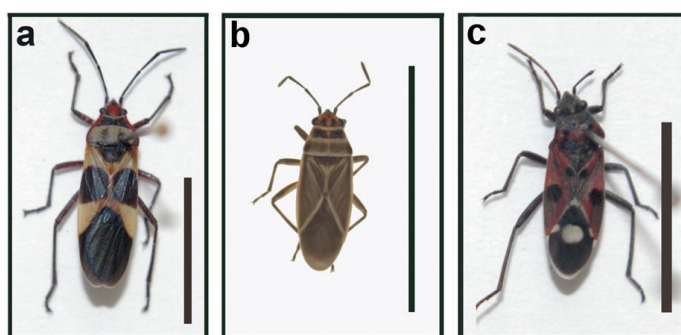


Figure 1. Lygaeidae species from Brazil southeast: **a.** *Oncopeltus femoralis*; **b.** *Lygaeus peruvianus*; and **c.** *Ochrinnus sagax*. Bar = 0,5 cm.

Table 1. Collection sites of Lygaeidae species.

Specie	Male	Female	Locality (City/State)	Geographic coordinates
<i>Oncopeltus femoralis</i>	17	12	Londrina/Paraná	23°21'47.06"S and 51°06'28.95"W
<i>Ochrinnus sagax</i>	4	8	Assis/São Paulo	22°28'39.80"S and 50°20'55.73"W
<i>Lygaeus peruvianus</i>	6	6	Londrina/Paraná	23°21'47.06"S and 51°06'28.95"W

Chromosome preparations and conventional staining

The gonads of adult specimens were dissected in physiological solution for insects (7.5 g NaCl, 2.38 g Na₂HPO₄, and 2.72 g KH₂PO₄ in 1 L distilled water). The testicles were treated with a hypotonic solution (tap water) for 3 min and fixed in methanol/acetic acid (3:1) for 30 min. Chromosome preparations were obtained by generating cellular suspension by maceration in 1 drop of 60% acetic acid; each gonad was previously treated with 45% acetic acid. These preparations were submitted to conventional staining with 3% Giemsa and chromosome banding techniques. Chromosome measurements were performed using the computer application MicroMeasure version 3.2 (Reeves and Tear, 2000).

Chromosome banding

The distribution of heterochromatin was analyzed by Giemsa C-banding after treatment with 0.2 M HCl, Ba(OH)₂, and 2X saline sodium citrate (SSC; Sumner, 1972). The GC- and AT-rich bands were detected using chromomycin A₃ (CMA₃) and 4'-6-diamino-2-phenylindole (DAPI), respectively. The slides were stained with 0.5 mg/mL CMA₃ for 1.5 h, washed in distilled water, and sequentially stained with 2 µg/mL DAPI for 30 min. Slides were mounted with a medium composed of glycerol/McIlvaine's buffer (pH 7.0; 1:1), plus 2.5 mM MgCl₂.

DNA isolation, polymerase chain reaction amplification, and genomic sequencing

In addition to karyotype studies, genomic DNA from a male of *Oncopeltus femoralis* was obtained from the muscle tissue sample. After the sample was digested with proteinase K for 3 h, phenol/Tris-HCl, pH 8.0 was added, followed by centrifugation and washing with phenol/Tris-HCl, pH 8.0 and chloroform-isoamyl alcohol. After an additional centrifugation, chloroform-isoamyl alcohol was added, and the DNA was precipitated with absolute and cold ethanol for 12 h at -20°C and eluted in Tris-EDTA (TE; 1:10) + RNase.

Unlabeled 18S rDNA probes were generated by polymerase chain reaction (PCR) by using universal arthropod primers: forward 5'-CCTGAGAAACGGCTACCACATC-3' and reverse 5'-GAGTCTCGTTTCGTTATCGGA-3' (Whiting, 2002). The fragment obtained was purified and sequenced using an automatic sequencer (Applied Biosystems® 3500xL Genetic Analyzers) equipped with 50 cm capillaries and POP6 polymer (Applied Biosystems®). The DNA sequence obtained was submitted to nucleotide BLAST (Zhang et al., 2000), in which homologous sequences of 18S rDNA from other species of Heteroptera were found, which, together with the sequence obtained from *Oncopeltus femoralis*, were submitted to nucleotide alignment by using *MEGA* version 5 (Tamura et al., 2011) and Multalin (Corpet, 1998) programs. Sequence data were deposited in the GenBank sequence database under accession No. JX110161.

Fluorescence *in situ* hybridization

The 18S rDNA probe isolated using PCR was labeled with biotin-14-dATP by PCR and nick translation. Slides were dehydrated in alcohol series and washed in 15% formamide/0.2X SSC, pre-treated with DNase-free RNase (40 µg/mL in 2X SSC) at 37°C for 1 h and pepsin (0.005% in 10 mM HCl) at 37°C for 30 min, fixed with 4% fresh paraformaldehyde, dehydrated in an alcohol series, and air dried. The chromosomes were then denatured in 70% formamide/2X SSC at 70°C for 5 min. Subsequently, the slides were treated with 30 µL hybridization mixture containing 100 ng labeled probe (4 µL), 50% formamide (15 µL), 50% polyethylene glycol (6 µL), 20X SSC (3 µL), 100 ng calf thymus DNA (1 µL), and 10% SDS (1 µL). The material was denatured at 90°C for 10 min, and hybridization was performed overnight at 37°C in a humidified chamber. Post-hybridization washes were carried out at 42°C in 2X SSC, 20% formamide in 0.1X SSC, 0.1X SSC, and 4X SSC/0.2% Tween 20. The probe was detected using a solution of 5% bovine serum albumin (BSA) and fluorescein isothiocyanate (FITC)-conjugated avidin (50:0.5, v:v). The post-detection washes were performed in 4X SSC/0.2% Tween 20 at room temperature. Slides were mounted with 25 µL of a medium composed of 23 L 1,4-diazabicyclo[2.2.2]-octane (DABCO) solution [(2.3%), 20 mM Tris-HCl, pH 8.0, (2%), and glycerol (90%) in distilled water], 1 µL 2 µg/mL DAPI, and 1 µL 50 mM MgCl₂.

All the images were acquired using a Leica DM 4500 B microscope equipped with a DFC 300FX camera and Leica IM50 4.0 software and optimized for best contrast and brightness by using the iGrafx Image software.

RESULTS

Chromosome arrangements in the insect species

Oncopeltus femoralis

The chromosome number for all samples of *Oncopeltus femoralis* was $2n (\text{♂}) = 16 + XY$ (Figure 2). All specimens showed holokinetic chromosomes with homogeneous size among all chromosomes (Figure 2). It was possible to observe in diplotene/diakinesis the occurrence of up to 2 chiasmata in the longest bivalent (Figure 2a-c). The results of meiotic analyses in metaphase I showed a small difference in size between chromosomes X and Y (Figure 2d-f) and, at this stage, all cells showed the bivalents arranged in a ring and XY chromosomes positioned within the ring. Furthermore, the bivalents were confirmed to divide reductionally, whereas sex chromosomes divided equationally.

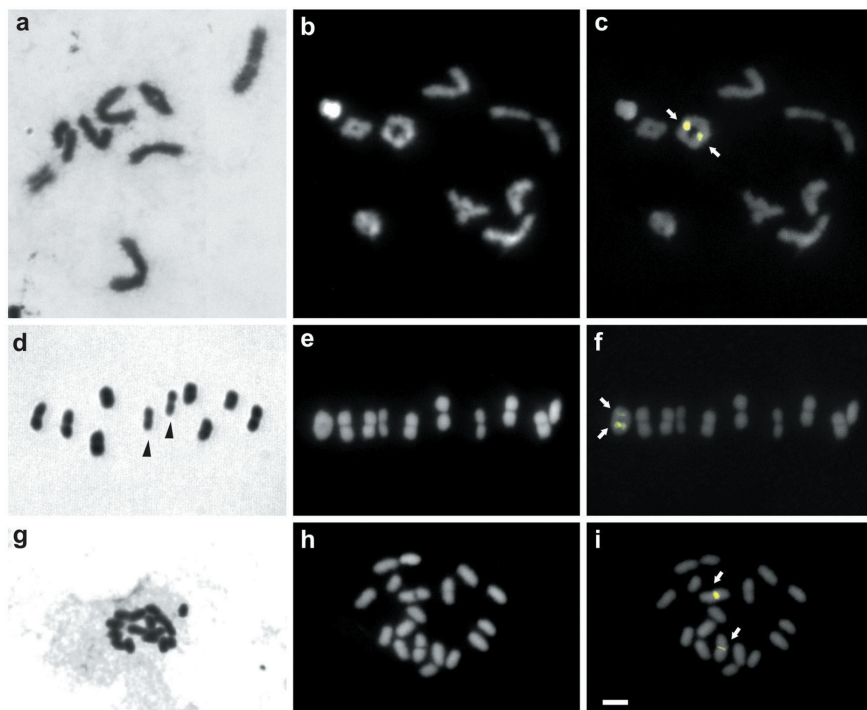


Figure 2. Meiotic and mitotic stages in males of *Oncopeltus femoralis* ($2n = 16+XY$) with Giemsa (**a. d. g.**), DAPI (**b. e. h.**), and CMA₃ (**c. f. i.**) staining. **a. b. c.** Diplotene; **d. e. f.** metaphase I; **g. h. i.** gonial mitotic metaphase. Arrows show the GC-rich blocks in one autosomal pair. Arrowheads show the sex chromosomes. Bars = 5 μm .

C/CMA₃/DAPI-banding showed 2 heterochromatic blocks, which were heteromorphic (Figure 2i). The distribution and heteromorphism of the heterochromatic blocks were confirmed in diplotene/diakinesis (Figure 2b and c), and their location in an autosomal bivalent was confirmed in metaphase I (Figure 2f). The analysis of spermatogonial cells in mitotic metaphase showed interstitial distribution of the heterochromatic block and CMA₃⁺/DAPI block in a large-sized chromosome pair and confirmed the telokinetic pairing (Figure 2f).

Ochrinnus sagax

Ochrinnus sagax showed $2n (\text{♂}) = 12 + XY$ (Figure 3), with a karyotype consisting of 6 large, 7 medium, and 1 small chromosome (Y), all holokinetic. It was possible to observe in diplotene/diakinesis the occurrence of 1 or 2 terminal chiasmata per bivalent; the sex chromosomes were achiasmatic and behaved as univalent (Figure 3a). Observing the pairing of autosomal chromosomes around chromosomes X and Y was possible in metaphase II (Figure 3d-f). The 2 longest autosomal chromosomes showed a nucleolar constriction in mitotic metaphase (Figure 3g-i).

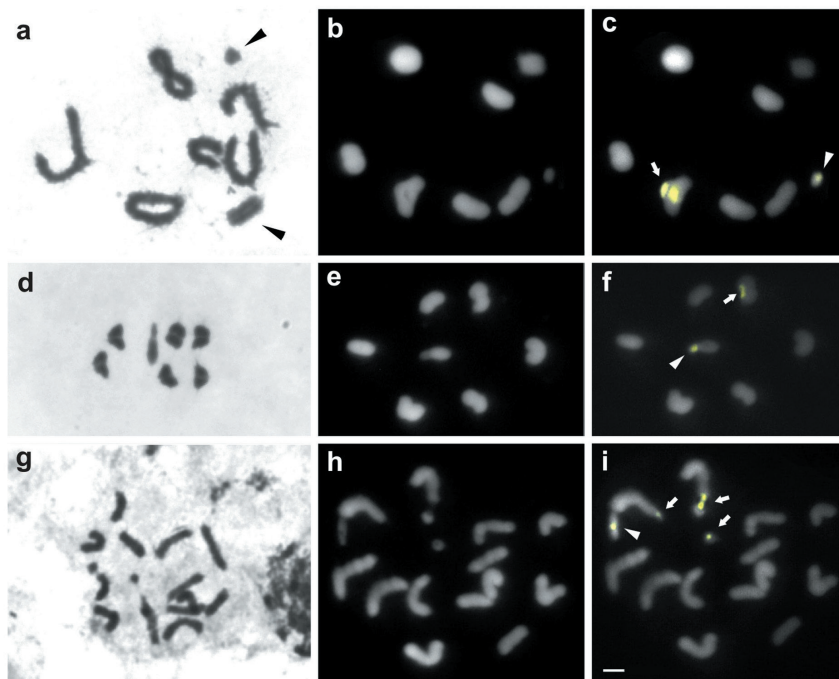


Figure 3. Male meiosis and mitosis in *Ochrinnus sagax* with Giemsa (a, d, g), DAPI (b, e, h), and CMA₃ (c, f, i) staining. a, b, c. Diplotene; d, e, f. metaphase II; g, h, i. mitotic metaphase. Arrowheads show the sex chromosomes. Arrows indicate the CMA₃⁺/DAPI blocks in the nucleolar constriction. Note the presence of these sites in an autosome pair and in the Y chromosome. Bars = 5 μ m.

The heterochromatic CMA₃⁺/DAPI blocks were evidenced in the larger autosomal pair, coincident with nucleolar constriction. Besides this block, the Y chromosome showed an interstitial CMA₃⁺/DAPI block (Figure 3i). The location of these blocks could also be con-

firmed by the analyses performed on the meiotic chromosomes in diplotene/diakinesis (Figure 3b and c) and metaphase II (Figure 3h and i).

Lygaeus peruvianus

All samples of *L. peruvianus* showed $2n$ (σ) = 10 + XY, with a karyotype consisting of 2 large, 9 medium, and 1 small chromosome, all holokinetic (Figure 4a-c). Analyses of meiocytes in metaphase I and II showed the arrangement in which the autosomal chromosomes formed a ring around the sex chromosomes. Chromosome pairings revealed the occurrence of the XY sex chromosome system, and the analysis of cells in mitosis enabled the determination of the difference in size between them (Figure 4d-i).

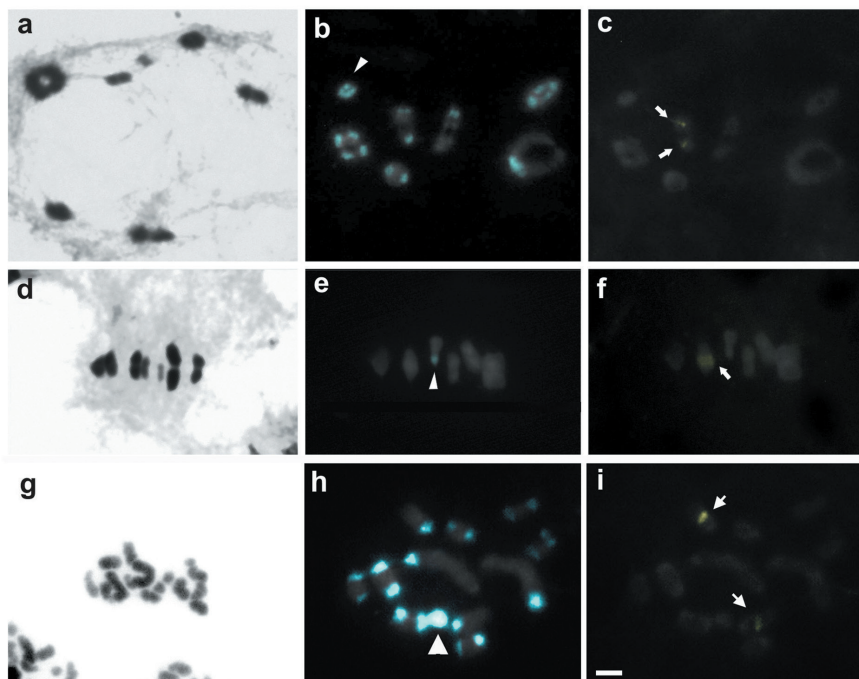


Figure 4. Meiotic and mitotic cells from *Lygaeus peruvianus* with Giemsa (a, d, g), DAPI (b, e, h), and CMA₃ (c, f, i) staining. a, b, c. Diplotene; d, e, f. metaphase II; g, h, i. mitotic metaphase. Arrowheads show the Y chromosome. Arrows indicate the CMA₃⁺/DAPI⁺ blocks. Note the presence of CMA₃⁰/DAPI⁺ terminal sites in all autosome pairs and in the Y chromosome. Bars = 5 μ m.

C-banding and CMA₃/DAPI staining revealed the localization of GC- and AT-rich blocks. In diplotene/diakinesis, a wide distribution of AT-rich terminal blocks in most bivalents were possible; however, in metaphase II, only the AT-rich block in Y chromosome was observed, probably due to the condensation of autosomes. It was possible to observe in a bivalent a GC-rich block in synteny with an AT-rich region (Figure 4a-f). The distribution of AT-rich blocks was also observed in mitotic cells, where the largest autosomal pair showed a DAPI⁺ marking placed in the terminal position (Figure 4h). In this stage, it was observed the

autosomal pair bearer of GC/AT-rich syntenic blocks. In addition, 7 chromosomes showed DAPI⁺ blocks in both terminal regions, and 3 chromosomes, including the X chromosomes, showed this sequence only in 1 terminal region. The Y chromosome showed DAPI⁺ marking throughout both in mitotic cells and in metaphase II (Figure 4h and i).

Sequencing and fluorescent *in situ* hybridization

Amplification of the 18S rDNA region of *Oncopeltus femoralis* with the specific primers generated a fragment of approximately 787 bp. The 719-bp consensus sequence of *Oncopeltus femoralis* generated through the alignment of the sequences consisted of 23.6% A, 23.9% C, 24.8% T, and 27.7% G bases (Figure 5a). The alignment of the consensus sequence with the 18S sequences from other species of Heteroptera deposited in the GenBank (Figure 5b) revealed a maximum similarity of 98% (Table 2) with small differences.

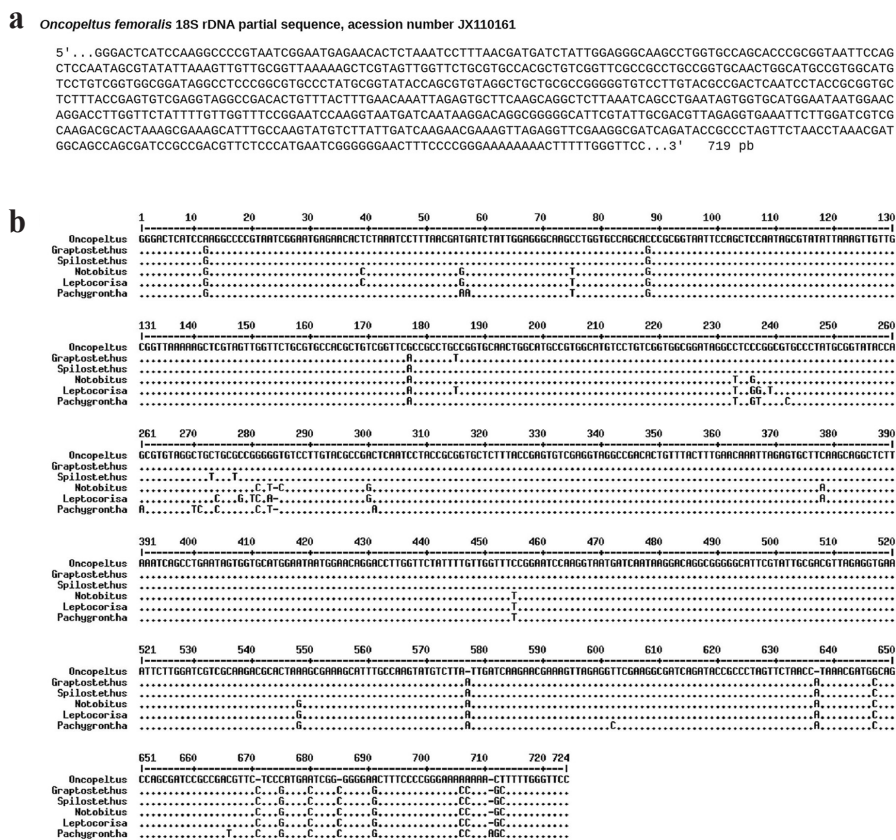


Figure 5. a. Partial sequence of 18S rDNA gene of *Oncopeltus femoralis* (GenBank accession No. JX110161); **b.** alignment of partial sequence of 18S gene of *O. femoralis*, with 18S sequences from other species of Heteroptera obtained from the GenBank (*Graptostethus quadrisignatus* accession AY627325, *Spilostethus hospes* accession AY627319, *Notobitus meleagris* accession AY627321, *Leptocoris* sp accession AY627320, *Pachygrontha antennata* accession AY324852) showing highly conserved regions among the sequences analyzed. Dots indicate sequence identity and letters denote absence of homology.

Table 2. Percent sequence identity of 18S rDNA gene of *Oncopeltus femoralis* with others Heteroptera species.

Species	1	2	3	4	5	6
1. <i>Oncopeltus femoralis</i>	-					
2. <i>Graptostethus quadrisignatus</i>	0.982	-				
3. <i>Spilostethus hospes</i>	0.980	0.996	-			
4. <i>Notobitus meleagris</i>	0.966	0.982	0.980	-		
5. <i>Leptocoris</i> sp	0.958	0.977	0.973	0.989	-	
6. <i>Pachygrontha antennata</i>	0.957	0.973	0.972	0.979	0.974	-

The fluorescence *in situ* hybridization performed using the isolated 18S rDNA fragment revealed sites in different positions among the 3 species. *Oncopeltus femoralis* showed an 18S rDNA site in an autosomal bivalent, observed in pachytene and metaphase II (Figure 6a and b). *Ochrinnus sagax* showed this sequence in the largest autosomal bivalent (Figure 6c), whereas, in *Lygaeus peruvianus*, the isolated sequence was found in the interstitial region of an autosomal pair (Figure 6d).

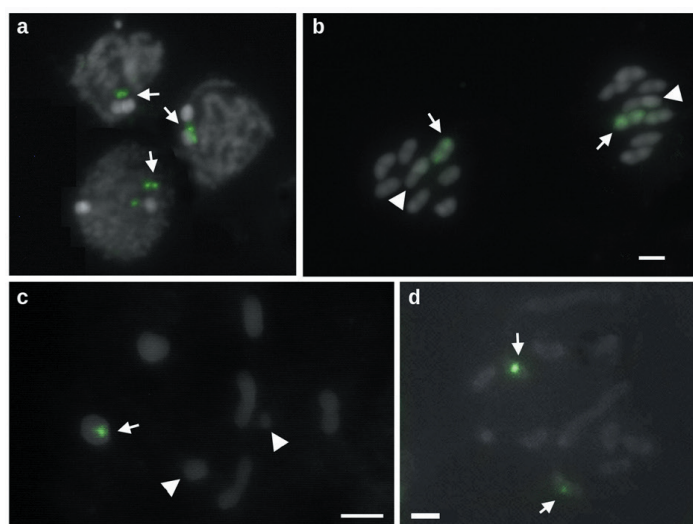


Figure 6. Location of rDNA 18S sites by fluorescent *in situ* hybridization at Lygaeidae species: **a.** interphasic nucleus and pachytene of *Oncopeltus femoralis*; **b.** metaphase II of *O. femoralis*; **c.** diplotene/diakinesis of *Ochrinnus sagax*; **d.** mitotic metaphase of *Lygaeus peruvianus*. Arrows indicate the ribosomal sites in one autosomal pair in each species studied. Arrowheads show the Y chromosome. Bars = 5 μ m.

DISCUSSION

All species analyzed in this study showed cytogenetic features common to the sub-family Lygaeinae, as the presence of holokinetic chromosomes (common in Heteroptera) was confirmed by meiotic and mitotic behavior and the presence of a XX/XY sex chromosome system (Grozeva and Kuznetsova, 1993). Furthermore, m-chromosomes can be found in many species of the family; however, these chromosomes were not observed in the samples of this study, as well as in the species of Lygaeinae studied to date (Ueshima, 1979; Ueshima and

Ashlock, 1980; Grozeva and Kuznetsova, 1993).

In the specimens of the family Lygaeidae analyzed herein, a change in the diploid number was observed, which ranged from $2n$ (σ) = 18 in *Oncopeltus femoralis* to $2n$ (σ) = 14 in *Ochrimnus sagax*, while *Lygaeus peruvianus* showed $2n$ (σ) = 12. Besides the variation in the diploid number, in *O. femoralis* and *O. sagax*, chromosome numbers decreased homogeneously in size, i.e., there was not a great difference in size between the chromosome pairs (Figures 2 and 3). The same was not true for *L. peruvianus*, where the first chromosome pair had a different size compared to that of the other chromosomes (Figure 4). Another interesting finding was the difference in size of sex chromosomes in the 3 species. The X and Y chromosomes in *O. femoralis* are of similar size, while, in *O. sagax*, the X chromosome is considerably longer than the Y chromosome, as observed in *L. peruvianus*. This increase/reduction in diploid number as well as the difference in size between chromosomes might be related to agmatoploidy/simploidy events, previously found in other groups of Heteroptera (Bressa et al., 2009; Bardella et al., 2010). This variation in the diploid number between specimens was also found in different species of the family Lygaeidae. The greatest variation in diploid number occurred in the subfamilies Rhyparochrominae ($2n = 11$ to 20) and Cyminae ($2n = 16$ to 30), although there are reports of such events in most of the other subfamilies (Ueshima and Ashlock, 1980; Grozeva and Kuznetsova, 1993).

With regard to heterochromatin, the distribution in heteropterans ranges from absence to presence of terminal heterochromatic blocks in most chromosomes, which, in some species, were characterized as AT-rich (Bressa et al., 2005; Papeschi and Bressa, 2007). In many cases, only 1 GC-rich heterochromatic block (interstitial or terminal) is recognized, which is often associated with the nucleolus organizer region (NOR; Camacho et al., 1985; Papeschi et al., 2003; Grozeva et al., 2004). Reports of interstitial heterochromatic blocks scattered throughout most autosomes are scarce and restricted to the family Curidae (*Holhymenia rubiginosa* and *Spartocera batatas*) (Fabricius, 1798) (Franco et al., 2006; Bressa et al., 2008). In the family Lygaeidae, studies on the heterochromatic location are limited to the genus *Dieuches* (Eyles, 1973), in which the 3 species analyzed showed AT-rich heterochromatic blocks already described in Heteroptera (Kaur et al., 2010).

Among all species studied, *Oncopeltus femoralis* had the lowest amount of heterochromatin on only 1 chromosome pair (the largest of the complement) with interstitial CMA₃⁺/DAPI⁺, which also showed size heteromorphism (Figures 2i and 7). This heteromorphism has already been reported in other species of Heteroptera, including *Holhymenia rubiginosa* (Bressa et al., 2008). In *Ochrimnus sagax*, although the CMA₃⁺/DAPI⁺ blocks have also been observed on the first pair of autosomal chromosomes, they were different from those found in *Oncopeltus femoralis*, since their positioning was coincident with the nucleolar constriction and in a region closer to the telomere. Furthermore, this species showed an interstitial CMA₃⁺/DAPI⁺ block in the Y chromosome (Figures 3i and 7).

Even though both species showed differences in the location of GC-rich repetitive DNA, they did not show any AT-rich blocks. In contrast, in *L. peruvianus*, although 2 autosomal chromosomes showed interstitial blocks (Figures 4i and 7), large DAPI⁺ heterochromatic terminal blocks were observed on all autosomes and on the X chromosome, while the Y chromosome appeared entirely DAPI⁺ (Figures 4h and 7). The large amount of DAPI⁺ heterochromatin in *L. peruvianus* can be explained by the equilocal distribution (Heitz, 1957).

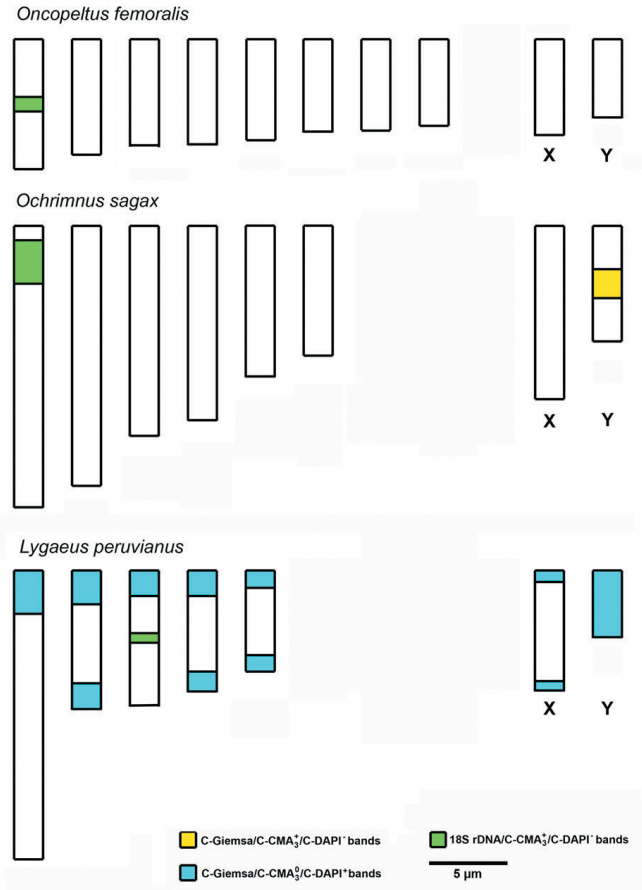


Figure 7. Idiograms and chromosomal physical mapping of Lygaeidae species studied.

The isolation, characterization, and localization of 18S rDNA sequences also proved to be interesting in the cytogenetic differentiation of species of Lygaeidae. Comparison of sequences from the GenBank by using the BLAST tool revealed that the 18S rDNA sequence obtained showed a similarity of 98% to those of other species of the family (Table 2). Small differences could be observed through the alignments, but this similarity confirms the nature of the isolated sequence. Almeida et al. (2010) analyzed the 18S rDNA isolated sequence of *Omophoita octoguttata* (Fabricius, 1875) (Coleoptera) and also found these differences in these kinds of repetitive DNA. Those authors attributed such differences to the result of transition and transversion base changes. Analysis of the alignments of the 18S rDNA isolated in this study suggested that both base changes occurred with the same frequency. Thus, these changes could maintain the homogeneity of DNA, thereby preserving the structure and function of the ribosome. However, the molecular process of concerted evolution might also be responsible for the changes in this sequence, suggesting that this family of repetitive DNA did not evolve independently; this has been proposed for the evolution of ribosomal genes in eukaryotic organisms (Elder and Turner, 1995; Liao, 1999).

The fluorescent *in situ* hybridization with the isolated 18S rDNA showed different

location patterns among species of Lygaeidae. *Oncopeltus femoralis* showed an 18S rDNA interstitial site in an autosomal bivalent; *Ochrinnus sagax* showed this sequence in the largest autosomal bivalent in the interstitial terminal region, whereas, in *Lygaeus peruvianus*, the rDNA cistrons were found in the interstitial region of an autosomal pair. In Heteroptera, studies focusing on the identification and localization of 18S rDNA on chromosomes are restricted to a few species of some families and the results have shown that the 18S rRNA gene might be located in both the autosomes and the sex chromosomes (Papeschi et al., 2003; Bardella et al., 2010; Grozeva et al., 2010; Poggio et al., 2011; Panzera et al., 2012). However, most of the studies revealed that these cistrons at terminal position of the chromosomes were different from those found in the species analyzed in this study.

The isolated 18S rDNA sequence, along with the localization and identification of the base composition of heterochromatic blocks, constituted an important tool for the physical mapping of such chromosomes and differentiation among species. In many organisms, the GC-rich sites have been associated with the location of NORs (da Rosa et al., 2009; Bardella et al., 2010). In the 3 species of Lygaeidae, the 18S rDNA site varied with regard to the chromosomal location and was co-located with the CMA_3^+ heterochromatic blocks in all the 3 species. Moreover, the hybridization of the probe revealed that the CMA_3^+ block present on the Y chromosome of *Ochrinnus sagax* did not show any 18S rDNA sequences, and that this block corresponded to another type of GC-rich heterochromatin (Figure 7).

Our data showed a great diversity in the distribution of heterochromatic blocks and 18S rDNA in the individuals of the family Lygaeidae; similar findings have been reported in Coreidae (Bressa et al., 2005) and Triatominae (Bardella et al., 2010). The differences in the sequence of 18S rDNA can be associated with some transitions/transversions of bases among other species of Heteroptera, and events of concerted evolution might have been involved in the evolution of this sequence. Considering the proposition made by Ueshima (1979) that the karyotype $2n = 14 (12 + XY)$ is the most common for the subfamily Lygaeinae, we can suggest that *Ochrinnus sagax* presents a plesiomorphic karyotype similar to that found in *Oncopeltus femoralis* and *L. peruvianus*; the latter would be species with derived karyotypes resulting from simploidy and agmatoploidy events, respectively. Due to the lack of chromosomal and phylogenetic studies in the subfamily Lygaeinae and the little knowledge of the biogeographical distribution of these species, identifying the ancestral karyotype of this group is not possible.

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