Meiotic Behavior of 18 Species From Eight Families of Terrestrial Heteroptera

Authors: Souza, Hederson Vinicius De, Castanhole, Márcia Maria Urbanin, Gomes, Mariana Oliveira, Murakami, Aline Sumitani, Firmino, Tatiani Seni De Souza, et al.

Source: Journal of Insect Science, 14(149) : 1-8

Published By: Entomological Society of America

URL: https://doi.org/10.1093/jisesa/ieu011

RESEARCH

Meiotic Behavior of 18 Species From Eight Families of Terrestrial Heteroptera

Hederson Vinicius De Souza,[1] Márcia Maria Urbanin Castanhole, Mariana Oliveira Gomes, Aline Sumitani Murakami, Tatiani Seni De Souza Firmino, Priscila Samara Saran, Cecilia Artico Banho, Letícia Da Silva Monteiro, Jocielly Cristina Pereira Da Silva, and Mary Massumi Itoyama

Universidade Estadual Paulista (UNESP), Instituto de Biociências, Letras e Ciências Exatas, Departamento de Biologia, Laboratório de Citogenética e Molecular de Insetos, São José do Rio Preto, São Paulo, Brazil
[1]Corresponding author, e-mail: souzahv@gmail.com

Subject Editor: Igor Sharakhov

J. Insect Sci. 14(149): 2014; DOI: 10.1093/jisesa/ieu011

ABSTRACT. Insects of the suborder Heteroptera are known for their odor, for being pests, or for being disease carriers. To gain better insight into the cytogenetic characteristics of heteropterans, 18 species of terrestrial Heteroptera belonging to eight families were studied. The presence of heteropycnotic corpuscles during prophase I, terminal or interstitial chiasmas, telomeric associations between chromosomes, ring disposals of autosomes during metaphase, and late migrations of the sex chromosomes during anaphase were analyzed. These features showed identical patterns to other species of Heteroptera previously described in the literature. Another studied characteristic was chromosome complements. The male chromosome complements observed were $2n = 12$ chromosomes [$10A + XY$, *Galgupha sidae* (Amyot & Serville) (Corimelaenidae) and *Pachycoris torridus* (Scopoli) (Scutelleridae)]; $2n = 13$ [$10A + 2m + X0$, *Harmostes serratus* (Fabricius), *Harmostes apicatus* (Stål), *Jadera haematoloma* (Herrich-Schaeffer), *Jadera sanguinolenta* (Fabricius), *Jadera* sp. (Rhopalidae)], and *Neomegalotomus parvus* (Westwood) (Alydidae); $2n = 13$ [$12A + X0$, *Stenocoris furcifera* (Westwood) (Alydidae); $2n = 14$ [$12A + XY$, *Dictyla monotropidia* (Stål) (Tingidae)]; $2n = 19$ [$18A + X0$, *Acanonicus hahni* (Stål) (Coreidae)]; $2n = 21$ [$18A + 2m + X0$, *Acanthocephala* sp. (Dallas) (Coreidae)]; $2n = 27$ [$24A + 2m + X0$, *Anisoscelis foliacea marginella* (Dallas) (Coreidae)]; $2n = 18$ [$16A + XY$, *Oncopeltus fasciatus* (Dallas) (Lygaeidae)]; $2n = 17$ [$14A + X_1X_2Y$, *Oxycarenus hyalinipennis* (Costa) (Lygaeidae)]; $2n = 16$ [$12A + 2m + XY$, *Pachybrachius bilobatus* (Say) (Lygaeidae)]; $2n = 26$ [$24A + XY$, *Atopozelus opsinus* (Elkins) (Reduviidae)]; and $2n = 27$ [$24A + X_1X_2Y$, *Doldina carinulata* (Stål) (Reduviidae)]. The diversity of the cytogenetic characteristics of Heteroptera was reflected in the 18 studied species. Thus, this study extends the knowledge of these characteristics, such as the variations related to chromosome complements, sex chromosome systems, and meiotic behavior.

Key Words: chromosome, holocentric, cytogenetic, meiosis

Insects in the order Hemiptera are distributed worldwide and comprise the most diverse order, besides Endopterygota, with >90,000 extant species in approximately 140 families. Historically, Hemipterans were divided into two suborders: Heteroptera (bugs) and Homoptera (cicads, leafhoppers, aphids, whiteflies, and coccids; Gullan and Cranston 2008). Currently, the order Hemiptera is divided into three suborders: Auchenorrhyncha, Heteroptera, and Sternorrhyncha (Liang and Webb 2002).

Heteropterans possess a wide variety of feeding behaviors, but the majority rely exclusively on plant sap. There are also Heteroptera species that are predators of fungi and other arthropods, and other species are bloodsucking or necrophagous (Gullan and Cranston 2008).

Regarding cytogenetics, Heteroptera is characterized by holocentric chromosomes, in which the kinetochore structure is absent in meiotic cells (Motzko and Ruthmann 1984, Wolf 1996) and kinetic activity is restricted to the chromosome ends (Motzko and Ruthmann 1984, González-Garcia et al. 1996).

Insects of the family Alydidae are relatively small and are found in North America foliage and flowers (Froeschner 1988). The diploid chromosome number of this family is $2n = 13$ ($10A + 2m + X0$); Ueshima 1979). All species of Alydidae that have been cytogenetically described to date possess *m*-chromosomes and a single X chromosome during male meiosis (Da Cunha Marques 1945, Manna 1951, Ueshima 1979), except for *Akbaratus fasciatus* X_1X_2Y (Sands 1982).

Insects of the family Coreidae are widely distributed, although they are most abundant and grow the largest in tropical and subtropical regions. Most Coreidae have peculiar appearances and are of significant economic importance (Schuh and Slater 1995). The sex chromosome system prevalent in Coreidae is X0/XX (male/female), and a distinct cytogenetic feature in most species is the presence of a pair of *m*-chromosomes, which are achiasmatic, associate as a pseudobivalent in the center of the metaphase I plate and present prereductional division (Wilson 1905, Bressa et al. 2001). In the Coreidae species described thus far, the diploid number of chromosomes varies from 13 ($10A + 2m + X0$) to 28 ($24A + 2m + X_1X_20$), although most have 21 chromosomes (Ueshima 1979, Sands 1982, Manna 1984, Dey and Wangdi 1988, Satapathy and Patnaik 1989, Cattani and Papeschi 2004, Souza et al. 2007b).

The species of the family Corimelaenidae, treated by some authors as a subfamily of Cydnidae, represent approximately 200 species and nine genera distributed across the western hemisphere. They are small to medium sized, dark colored, and develop a convex scutellum that covers most of the forewing with a yellowish exochorion (Grazia et al. 1999). Cytogenetic data are scarce for this family, except for the family Cydnidae, which generally presents a diploid number ranging from 12 to 14 autosomes + XY (Mikolajski 1968, Ueshima 1979).

The family Lygaeidae consists of several species that have been cytogenetically analyzed, with chromosome numbers ranging from 14 to 30, and 14 chromosomes is the modal number for the family. Most species present *m*-chromosomes and the XY sex chromosome system, although the X_1X_2Y complement does occur, albeit less frequently (Ueshima 1979).

Among Heteroptera, the family Reduviidae is distinguished for consisting of insectivorous, phytophagous, and hematophagous insect species and is therefore of great importance for agricultural productivity and human health, because members of this family can transmit Chagas

disease during their feeding. Because they are larger in size than other bugs that act as predators, Reduviidae consume a higher number and also a broader spectrum of prey species (Panzer et al. 1992). The diploid chromosome number most commonly observed in Reduviidae is 24 ($22A + XY$), and the most common sex chromosome system is XY, although alternative chromosomal complements, such as X_1X_2Y, $X_1X_2X_3Y$, or $X_1X_2X_3X_4Y$, are also found in males. There are also reports of the presence of *m*-chromosomes (Ueshima 1979).

A notable cytogenetic aspect of the family Rhopalidae is that all species in this family that have been described in the literature have a chromosome complement of $2n = 13$ chromosomes, with 10 autosomes, two *m*-chromosomes, and one X sex chromosome (Ueshima 1979, Souza et al. 2009).

Scutelleridae comprise a small family of Heteroptera that used to be considered part of the Pentatomidae but now form a separate family. Insects in this family can vary in size from medium to large and are distributed worldwide, with 80 genera and 450 species. The most notable feature of these insects is their scutellum, which covers the entire abdomen, hides the wings, and gives them the appearance of a beetle. These insects feed on grasses, herbs, fruits, and flowers. The number of chromosomes in insects of this family varies from 12 to 14, with no *m*-chromosomes and the presence of the XY sex chromosome system (Papeschi and Bressa 2006).

Tingidae is a family composed of small insects (2–10 mm) with reticulated wing surfaces. These insects are often located on the abaxial surface of leaves, where they drink the sap and cause the yellowing and premature senescence of these leaves, resulting in great damage to plantations. Each individual completes its life cycle on the same plant and sometimes in the same general region of a plant. This family predominantly has six pairs of autosomes and the XY sex chromosome system; only one species with an X0 system has been reported (Nokkala and Nokkala 1984).

The suborder Heteroptera is extremely large, with >40,000 described species, but this number is most likely higher because many others species are yet to be identified and described. Cytogenetic information is scarce and highly variable between species of this suborder, even at the family level, where this information is predominantly available only for the families Coreidae and Pentatomidae. In other families of this suborder, variations related to the chromosome complements, the sex chromosome systems used and the meiotic behavior are poorly described. In this study, 18 Heteroptera species from eight different families were analyzed to broaden the cytogenetic information available for this suborder.

Materials and Methods

In this study were analyzed 18 species of terrestrial Heteroptera, belonging to eight families, as delineated in Table 1: Alydidae [*Neomegalotomus parvus* (Westwood) and *Stenocoris furcifera* (Westwood)], Coreidae [*Acanonicus hahni* (Stål), *Acanthocephala* sp., and *Anisoscelis foliacea marginella* (Dallas)], Corimelaenidae [*Galgupha sidae* (Amyot & Serville)], Lygaeidae [*Oncopeltus fasciatus* (Dallas), *Oxycarenus hyalinipennis* (Costa), and *Pachybrachius bilobatus* (Say)], Reduviidae [*Atopozelus opsimus* (Elkins) and *Doldina carinulata* (Stål)], Rhopalidae [*Harmostes serratus* (Fabricius), *Harmostes apicatus* (Stål), *Jadera haematoloma* (Herrich-Schaffer), *Jadera sanguinolenta* (Fabricius), and *Jadera* sp.], Scutelleridae [*Pachycoris torridus* (Scopoli)], and Tingidae [*Dictyla monotropidia* (Stål)]. The insects were fixed in methanol:acetic acid (3:1). Testicles from representative species for each family were extracted. Subsequently, the testicles of 10 adult males of each species collected in São José do Rio Preto (20° 47′32″ S, 49° 21′37″ W), São Paulo, Brazil, were placed on slides and stained with lacto-acetic orcein for cytogenetic analysis. Representative images were captured under a Zeiss Axio Scope A1 microscope using the Axio Vision LE (version 4.8) image analysis program.

Table 1. Classification and chromosomal complements of 18 species of Heteroptera

Family	Species	Chromosomal complement
Alydidae	*N. parvus*	13 ($10A + 2m + X0$)
	S. furcifera	13 ($12A + X0$)
Coreidae	*A. hahni*	19 ($18A + X0$)
	Acantocephala sp.	21 ($18A + 2m + X0$)
	An. foliacea marginella	27 ($24A + 2m + X0$)
Corimelaenidae	*G. sidae*	12 ($10A + XY$)
Lygaeidae	*O. fasciatus*	18 ($16A + XY$)
	Ox. hyalinipennis	17 ($14A + X_1X_2Y$)
	P. bilobatus	16 ($12A + 2m + XY$)
Reduviidae	*At. opsinus*	26 ($24A + XY$)
	D. carinulata	27 ($24A + X_1X_2Y$)
Rhopalidae	*H. apicatus*	13 ($10A + 2m + X0$)
	H. serratus	13 ($10A + 2m + X0$)
	J. haematoloma	13 ($10A + 2m + X0$)
	J. sanguinolenta	13 ($10A + 2m + X0$)
	Jadera sp.	13 ($10A + 2m + X0$)
Scutelleridae	*Pa. torridus*	12 ($10A + XY$)
Tingidae	*Di. monotropidia*	14 ($12A + XY$)

Results

Meiotic Behavior. Cytogenetic analysis of prophase I cells showed varied numbers of heteropyknotic corpuscles. Therefore, the number of corpuscles observed depends on the sex chromosome system belonging to each species. For example, in the species *N. parvus*, which has the X0 sex chromosome system, only a single, rounded corpuscle was observed, which was likely the X chromosome (Fig. 1a). One or two heteropyknotic corpuscles in XY chromosome systems were identified, which are likely to be associated or separated sex chromosomes. For example, in *O. fasciatus* (Fig. 1b) or *At. opsimus*, in which X and Y chromosomes are close, was found a single corpuscle with two distinct regions (Fig. 1c), but two distinct corpuscles was observed in *G. sidae* (Fig. 1d). *D. carinulata* had an X_1X_2Y sex chromosome complement, and it was observed with three well-separated heteropyknotic corpuscles (Fig. 1e). Heteropyknotic corpuscles were observed until the end of prophase I, as showed in *H. serratus* (Fig. 1f).

In the species *J. sanguinolenta* and *Jadera* sp., belonging to the same family (Rhopalidae), were found four heteropyknotic corpuscles: one more evident, one less evident, and two minor (Fig. 1g and h). Similarly, another exception was observed. In these species, the sex chromosome system was X0; in addition to having heteropycnotic sex chromosomes, they had highly condensed autosomes in early prophase I (Fig. 1h).

The chiasma was another characteristic observed in meiotic cells, which can be seen in mid-prophase I in species such as *N. parvus* (Fig. 1i) and *An. foliacea marginella* (Fig. 1j). It was observed as single or two, terminal or interstitial chiasma (Fig. 1k–m). Interstitial chiasma occurred at different distances along the entire chromosome and usually gave the chromosome a cross morphology (*N. parvus* and *A. hahni*; Fig. 1k and l). Chromosomes with two terminal chiasmata produced ring morphologies (*Ox. hyalinipennis*; Fig. 1m).

When chromosomes were condensed, they could be associated with telomeric regions (Fig. 2e). These associated chromosomes may form a group that remains until diplotene (Fig. 2a and b), when they begin to separate (Fig. 2c). This type of association was observed in *D. carinulata* (Fig. 2a–c) and *S. furcifera* (Fig. 2d). In other species, only some chromosomes are associated by their telomeres to the end of prophase, as observed in *G. sidae* (Fig. 2e and f). In other species, the different chromosomes do not unite.

It is possible to identify the chromosome with its homologs at the end of prophase I, including those associated by telomeric regions, as observed in *Jadera* sp. (Fig. 2g). The autosomal chromosomes were of similar size in all species, except in the species *G. sidae* (Fig. 2f), *Jadera* sp. (Fig. 2g and i), and *J. haematoloma* (Fig. 2h), which contain

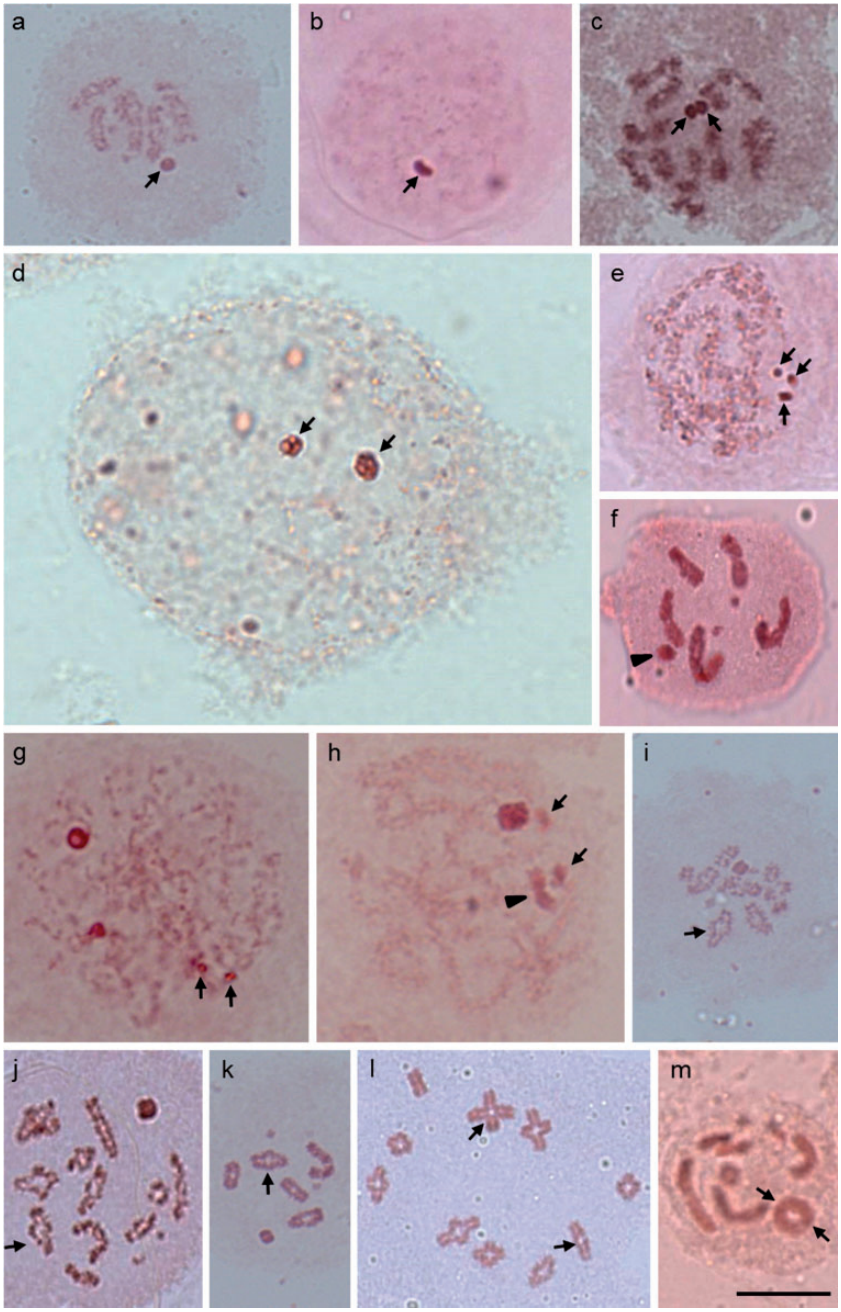

Fig. 1. Spermatogonial cells of *N. parvus* (a, i, k), *O. fasciatus* (b), *At. opsimus* (c), *G. sidae* (d), *D. carinulata* (e), *H. serratus* (f), *J. sanguinolenta* (g), *Jadera* sp. (h), *An. foliacea marginella* (j), *A. hahni* (l), and *Ox. hyalinipennis* (m) stained with lacto-acetic orcein. (a–e) Initial prophase showing an intensely stained, rounded heteropycnotic corpuscle (arrow in a), two closely associated corpuscles (arrow in b), less associated corpuscle (arrows in c), completely separate corpuscles (arrows in d), or three completely separate corpuscles (arrows in e); (f–j) the final stage of prophase, with persistent heteropycnotic corpuscles that are possibly the sex chromosomes (arrowhead in f and h). Other heteropycnotic chromosomal regions observed (arrows in g and h): (i–m) diplotene/diakinesis showing interstitial (arrows in i–l) or terminal (arrows in m) chiasmata. Bar: 10 µm.

one autosome that is much larger than the others. In addition, insects of the genus *Jadera* possess one pair of autosomes that do not associate with their telomeres (Fig. 2h).

As previously mentioned, the sex chromosomes are usually heteropycnotic by the end of prophase I, making their identification relatively easy. Similarly, a pair of *m*-chromosomes could also be identified at this stage (Fig. 2j).

In early metaphase I, the autosomes begin to arrange in a circle (polar view), whereas the sex chromosomes and *m*-chromosomes are organized differently. The location of all chromosomes depends on the sex chromosome system of each species and whether they have *m*-chromosomes. Therefore, if a species presented an X0 sex chromosome system but had no *m*-chromosomes, the X chromosome was found outside of the ring formed by the autosomes as in *A. hahni* (Fig. 2k). Conversely, if a species possessed an X0 sex chromosome system and had *m*-chromosomes, the X chromosome was observed outside the ring formed by the autosomes but the *m*-chromosomes were found inside the ring, forming a pseudobivalent (Fig. 2l, *Jadera* sp.).

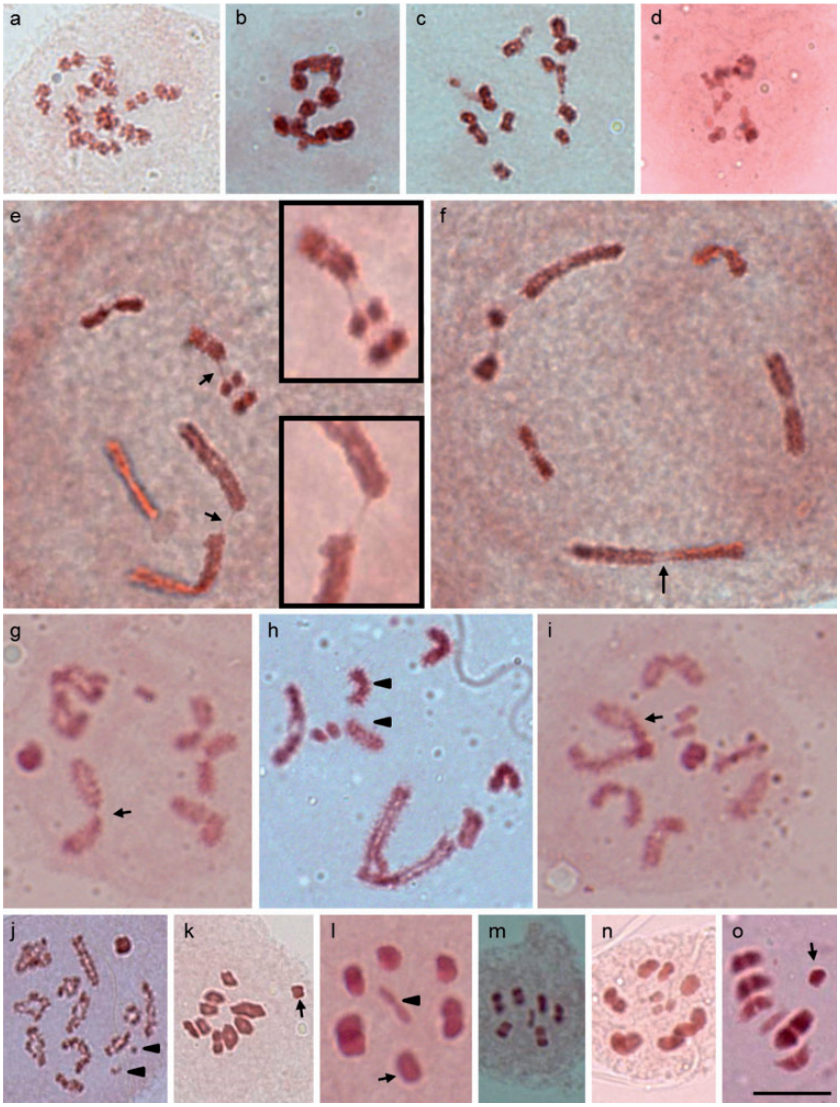

Fig. 2. Spermatogonial cells of *D. carinulata* (a–c), *S. furcifera* (d), *G. sidae* (e, f), *Jadera* sp. (g, i, l), *J. haematoloma* (h, o), *An. foliacea marginella* (j), *A. hahni* (k), *Di. monotripidia* (m), and *Ox. hyalinipennis* (n) stained with lacto-acetic orcein. (a–d) Chromosomes associating at their telomeres; they appear Z-shaped in (b); (e–f) telomeric association of a few chromosomes (arrows in e and inset); (g) association of telomeres between autosomes (arrow); (h) autosomes associated at nontelomeric regions (arrowheads). Notice the differentiated size of autosomes (f–i, arrows); (j) presence of *m*-chromosomes (arrowheads); (k) the circular arrangement of autosomes and a sex chromosome out of the ring (arrow shows the X chromosome); (l) autosomes arranged in a circle, with the X chromosome inside the ring (arrow) and the *m*-chromosomes in the center of the ring (arrowhead); (m, n) sex chromosomes in the center of the autosomal ring, X and Y (m) and X_1X_2Y (n); and (o) metaphase in a side view, with the heteropycnotic sex chromosome (arrow). Bar: 10 μm.

When species possessed XY sex chromosome systems, both sex chromosomes remained at the center of the ring (Fig. 2m, *Di. monotripidia*). Additionally, all the species that presented with X_1X_2Y sex chromosome systems had their sex chromosomes located inside the autosomal ring as in *Ox. hyalinipennis* (Fig. 2n).

The homologous chromosomes migrate to the central region of the cell (side view) then begin to separate from each other, and in some species the sex chromosomes were separated from the other chromosomes (Fig. 2o, *J. haematoloma*).

Another feature in the analyzed species was the lagging chromosome of the sex chromosomes during anaphase I and II. In the species that possess the X0 sex chromosome system, the late migration was observed in anaphase I when the two cells were being formed. Because these chromosomes in Heteroptera are divided equally in the first division, each of the two daughter cells receives a sex chromosome after the separation of sister chromatids. Therefore, in the second division, only one cell will receive the sex chromosome (Fig. 3a, *A. hahni*). This sex chromosome was observed as a heteropyknotic body until the end of anaphase I (Fig. 3b, *J. haematoloma*), and in some species (Fig. 3c, *A. hahni*), even into telophase II. However, *G. sidae* displayed a different pattern because the sex chromosome appears heteropyknotic and a "filament" of chromatin migrated later (Fig. 3d).

Spermiogenesis was also analyzed in all 18 species. Vesicles were observed next to the nucleus in the rounded morphology of all early spermatids in the early stages of spermiogenesis (Fig. 3e and f), except in the species *J. haematoloma* and *Ox. hyalinipennis*, in which vesicles persisted along the entire stretch of the spermatid. *J. haematoloma* presented one large and several smaller vesicles during the elliptical stage (Fig. 3j). Only larger vesicles are observed in a more developed stage of the spermatid (Fig. 3k). *Ox. hyalinipennis* spermatids contained several small vesicles during the differentiation stage (Fig. 3l).

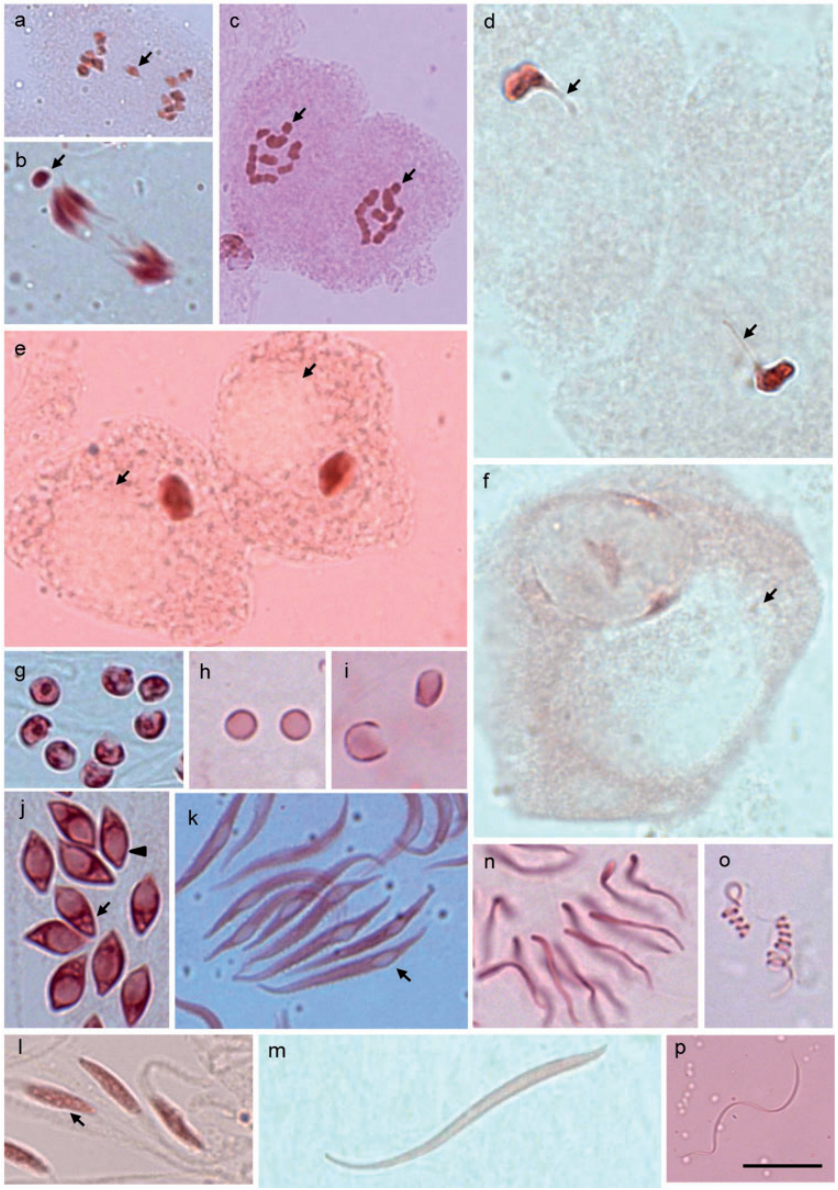

Fig. 3. Spermatogonial cells of *A. hahni* (a, c), *J. haematoloma* (b, g, j, k, p), *G. sidae* (d, f, m), *P. bilobatus* (e), *Ox. hyalinipennis* (l), and *O. fasciatus* (h, i, n, o) stained with lacto-acetic orcein. (a, b) Anaphase I with lagging chromosome of the sex chromosome (a, arrow). Note that this chromosome remains heteropycnotic until the end of anaphase (b, arrow); (c) telophase II with a regular division, showing the heteropycnotic corpuscles (arrows); (d) material chromatin with late migration (arrows); (e, f) round spermatid with heteropycnotic material and a large vesicle (arrows); (g) round spermatid with chromatin around the nuclear envelope and inside the nucleus; (h, i) round spermatids with chromatin evenly distributed throughout the matrix and around the nuclear envelope; (j) elliptical spermatids presenting a larger vesicle (arrowhead) and several smaller vesicles (arrow); (k) spermatids being elongated with a single vesicle (arrow); (l) several small vesicles during differentiation (arrow); (m–p) spermatid in elongation, with coiled tail (n,o); and small head and long tail (p). Bar: 10 μm.

In early spermatids, chromatin was either distributed uniformly throughout the nucleus (Fig. 3h and i) or located near the nuclear envelope and inside the nucleus (Fig. 3g). The elongation of the spermatid was generally similar in all species. Spermatids had a small head and long tail and were straight (Fig. 3m and p), with few exceptions, such as *O. fasciatus*, which had spermatids with spiral tails (Fig. 3n and o). It was also observed that the cells in *G. sidae* were much larger than in other examined species (Fig. 3d).

Chromosome Complement. The chromosome complements of meiotic cells were examined (Fig. 4a–r). Although all species of the family Alydidae have the same chromosome complement ($2n = 13$), *N. parvus* (Fig. 4a) has *m*-chromosomes and *S. furcifera* does not possess *m*-chromosomes (Fig. 4b). Nearly all analyzed species of the family Coreidae possessed the X0 sex chromosome system, with 18 autosomes (except for *An. foliacea marginella*, which possessed 24; Fig. 4e) and *m*-chromosomes, as seen in *Acantocephala* (Fig. 4d), but not in *A. hahni* (Fig. 4c).

The family Rhopalidae was the only family examined in which all the species have the same chromosome complement of $2n = 13$ chromosomes ($10A + 2m + X0$; Fig. 4l–p). *G. sidae* (Corimelanidae, Fig. 4f) and *Pa. torridus* (Scutellerridae, Fig. 4i) have the same chromosome complement, despite belonging to different families ($10A + XY$). *Di. monotropidia* (Tingidae, Fig. 4r) exhibited a chromosome complement of $2n = 14$ ($12A + XY$). Regarding the Reduviidae family, two X chromosomes were observed in the species *D. carinulata*, which possessed a chromosome complement of $2n = 27$ ($24A + X_1X_2Y$; Fig. 4k).

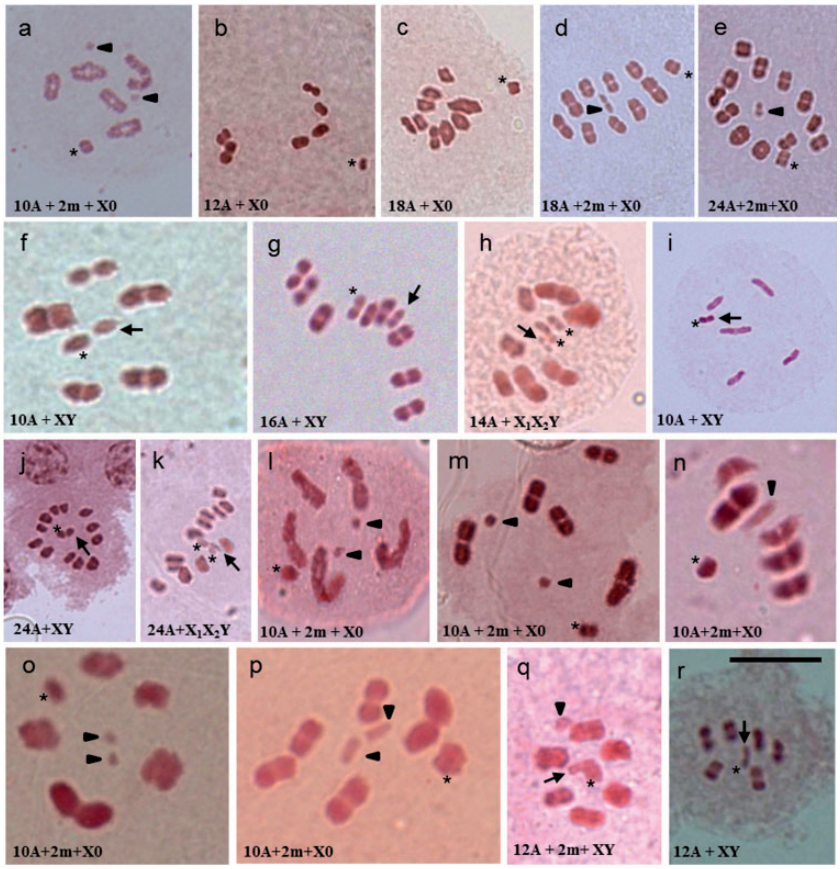

Fig. 4. Metaphase cells of *N. parvus* (a), *S.* (*Oryzocoris*) *furcifera* (b), *A. hahni* (c), *Acantocephala* sp. (d), *An. foliacea marginella* (e), *G. sidae* (f), *O. fasciatus* (g), *Ox. hyalinipennis* (h), *Pa. torridus* (i), *At. opsinus* (j), *D. carinulata* (k), *H. apicatus* (l), *H. serratus (m)*, *J. haematoloma* (n), *J. sanguinolenta* (o), *Jadera* sp. (p), *P. bilobatus* (q), and *Di. monotropidia* (r) stained with lacto-acetic orcein. Asterisks indicate the X chromosomes, arrows show the Y, and the arrowhead indicates *m*-chromosomes. Bar: 10 μm.

This species differed from *At. opsinus* (Reduviidae) by presenting a chromosome complement of $2n = 26$ (24A + XY). The highest diversity of chromosome complements was observed in the species of the family Lygaeidae: $2n = 18$ ($16A + XY$; *O. fasciatus*, Fig. 4g), $2n = 17$ ($14A + X_1X_2Y$; *Ox. hyalinipennis*, Fig. 4h), and $2n = 16$ ($12A + 2m + XY$; *P. bilobatus*, Fig. 4q; Table 1).

Discussion

The ancestral number of chromosomes in heteropterans is an evolutionary characteristic that is difficult to establish because of their holokinetic characteristics. Due to this peculiarity, it is possible to occur fragmentations or fucions (Ueshima and Ashlock 1980, Jacobs 2004). In the species analyzed in this article, the only family in which all species have the same chromosome complement was Rhopalidae, $2n = 13$ ($10A + 2m + X0$). The Alydidae species had the same number of chromosomes, $2n = 13$, but the distribution of chromosomes was different, with some possessing a $10A + 2m + X0$ complement and others a $12A + X0$ chromosome complement. Other families (Coreidae, Lygaeidae, and Reduviidae) for which were analyzed more than one species had chromosome complements that differed between species of the same family. Among the three families investigated, only in Coreidae did all species have the same sex chromosome system (X0), whereas Lygaeidae and Reduviidae presented X_1X_2Y and XY, and Corimelaenidae, Scutelleridae, and Tingidae had the XY system. Even the presence or absence of *m*-chromosomes depends on the family; Alydidae, Coreidae, and Lygaeidae contained some species with and some without these chromosomes, demonstrating the karyotypic diversity in families of Heteroptera. However, the analysis of species conducted in this work and the analyses previously described in the literature did not reveal a clear pattern for the number of chromosomes for each family, whereas the sex chromosome system appears to be more common in every family. Based on the number of chromosomes (13), the X0 sex chromosome system, and the presence of *m*-chromosomes, this study suggests that the families Alydidae and Rhopalidae are evolutionarily close. Similarly, Corimelaenidae and Scutelleridae demonstrate similar chromosome complements, identical sex chromosome systems, and contain no *m*-chromosomes.

Previously published data (Ueshima 1979, Souza et al. 2009), which observed that all species of Rhopalidae had 13 chromosomes, with 10 autosomes, two *m*-chromosomes, and one sex chromosome, were consistent with our data, and we can thus reaffirm that the species in this family have conserved chromosome complements. According to these studies, most species of the family Alydidae also possess the same chromosome complement, with the exception of approximately 23% of the species that have 14 or 17 chromosomes. Our analysis of Alydidae species is consistent with these results, and it can be suggested that these families are evolutionarily close. It was also verified that *S. furcifera* is the only species belonging to the family Alydidae that has no *m*-chromosomes between the analyzed species.

Species that belong to the family Coreidae have extremely variable chromosome numbers, ranging from 13 to 28 chromosomes. Most species in this family, however, have 21 chromosomes (Ueshima 1979; Sands 1982; Manna 1984; Dey and Wangdi 1988; Satapathy and Patnaik 1989; Cattani and Papeschi 2004; Souza et al. 2007b, 2009). Our results confirm these data because different chromosome complements were verified in the three analyzed species of Coreidae. Another

characteristic previously observed for the species of this family is that most have *m*-chromosomes and the X0 sex chromosome system, a characteristic that is also observed in the species examined in this work, except for *A. hahni*, which had no *m*-chromosomes. Because most species analyzed have *m*-chromosomes and an X0 sex chromosome system, it can be inferred that the families Alydidae, Coreidae, and Rhopalidae are evolutionarily close.

Most insects examined in the family Lygaeidae present *m*-chromosomes and the XY sex chromosome system (Ueshima 1979, Souza et al. 2007c). According to Ueshima (1979), *O. fasciatus* has chromosome complement $14A + XY$; however, the species analyzed in this study had chromosome complement $16A + XY$. *Ox. hyalinipennis* and *P. bilobatus* showed the same chromosome complement described by Ueshima (1979). Therefore, more specimens of *O. fasciatus* belonging to other geographical locales should be examined to verify whether this is an intrapopulation difference.

The chromosome complements of species of the family Reduviidae, Scutelleridae, and Tingidae described in this study are consistent with those described in the literature. Because of the small number of species analyzed from the family Corimelaenidae, further studies will be required to conclusively predict the pattern of chromosome complements in this family.

Information on the evolution of these chromosomes is scarce in the existing literature because of the lack of distinct centromere morphology and the ability to longitudinally differentiate chromosomes to detect structural variation. For these reasons, chromosomal rearrangements, such as inversions and reciprocal translocations, are rarely reported in these organisms, further preventing specific conclusions. Therefore, a larger number of species should be evaluated using other techniques to better understand the evolution of chromosomes in these species.

Although *m*-chromosomes present differentiated behaviors, their functions and origin have not yet been established. Our analyses showed that the species *N. parvus* possess $10A + 2m + X0$, whereas *S. furcifera* were $12A + X0$. However, *P. bilobatus* (Lygaeidae) can also be used as an example, which has $12A + 2m + XY$ chromosome complements, and the modal chromosome number for this family is $14A + XY$. These characteristics must be explored by other approaches to establish the function and origin of the *m*-chromosome.

The presence of heteropycnotic corpuscles during prophase I, terminal or interstitial chiasmas, telomeric associations between chromosomes, ring disposal of autosomes during metaphase, late migration of the sex chromosome during anaphase, a reductional first meiotic division and an equational second meiotic division for autosomes, and the sex chromosomes of the opposite sex, does not produce a distinguishing feature to identify species because all the species analyzed in this study exhibit the same pattern as other species previously described in the literature (Ueshima 1979; Papeschi and Bidau 1985; Bressa et al. 2002; Souza et al. 2007b, 2009; Costa et al. 2008; Castanhole et al. 2010; Souza and Itoyama 2010, 2011). The larger chromosomes observed in *G. sidae* and *Jadera* sp. may have originated recently, as they are present in karyotypes with the lowest number of chromosomes.

The location of sex chromosomes during metaphase I is related to specific families or to the presence or absence of *m*-chromosomes. Hence, e.g., if a species is X0, the X chromosome lies outside the ring formed by the autosomes. However, if the species has *m*-chromosomes, the X chromosome still lies outside the ring and the *m*-chromosome lies inside the ring. When species are XY or X_1X_2Y, all sex chromosomes remain at the center of the ring formed by the autosomes. These features have also been observed by Ueshima (1979) and Souza et al. (2007a,b,c, 2008; Souza and Itoyama 2010, 2011).

Information regarding spermiogenesis in Heteroptera remains extremely rare. It is known that early spermatids, which are round, contain vesicles of varying sizes and uncertain function, with larger vesicles present in some species (Castanhole et al. 2010). During elongation, the spermatid may attain an elliptical morphology, as observed in terrestrial Heteroptera (Souza and Itoyama 2011), or a rod-like shape, usually observed in aquatic species (Castanhole et al. 2008, 2010). The species investigated in this study had all the features described earlier, indicating that these features are similar between species.

The Heteropteras, therefore, are suitable insects for the analysis of meiosis because they have many characteristics that can be examined (presence or absence of *m*-chromosomes, different sex chromosome systems, different chromosomal complements) and still more questions (Which of these characteristics are ancestral? What is the content of vesicles of spermatids?) that continue to intrigue us and need further research to be answered. Thus, this study extends the knowledge of these characteristics and collaborates to solve those questions.

Acknowledgments

This work was financially supported by São Paulo Research Foundation (FAPESP), National Council for Scientific and Technological Development (CNPq), and Foundation for the Development of the State University of São Paulo (FUNDUNESP). Luiz Antônio Alves Costa, University of Rio de Janeiro, José Antônio Marin Fernandes, Federal University of Pará, and Helcio Gil Santana of the Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, assisted in the identification of insects.

References Cited

Bressa, M. J., A. G. Papeschi, and M. L. Larramendy. 2001. Meiotic studies in *Largaeus alboornatus* Blanchard (Heteroptera, Lygaeidae: Lygaeinae). Caryologia 55: 15–19.

Bressa, M. J., E. Fumagalli, S. Ituarte, M. V. Frassa, and M. L. Larramendy. 2002. Meiotic studies in *Dysdercus* Guérin Méneville 1831 (Heteroptera: Pyrrhocoridae). II. Evidence on variations of the diffuse stage between wild and laboratory-inbred populations of *Dysdercus chaquency* Freiberg, 1948. Hereditas 137: 125–131.

Castanhole, M. M., L. L. Pereira, H. V. Souza, H. E. Bicudo, L. A. Costa, and M. M. Itoyama. 2008. Heteropicnotic chromatin and nucleolar activity in meiosis and spermiogenesis of *Limnogonus aduncus* (Heteroptera, Gerridae): a stained nucleolar organizing region that can serve as a model for studying chromosome behavior. Genet. Mol. Res. 7: 1398–1407.

Castanhole, M. M., L. L. Pereira, H. V. Souza, and M. M. Itoyama. 2010. Spermatogenesis and karyotypes of three species of water striders (Gerridae, Heteroptera). Genet. Mol. Res. 9: 1343–1356.

Cattani, M. V., and A. G. Papeschi. 2004. Nucleolus organizing regions and semi-persistent nucleolus during meiosis in *Spartocera fusca* (Thunberg) (Coreidae, Heteroptera). Hereditas 140: 105–111.

Costa, L. C., M. T. V. Azeredo-Oliveira, and E. Tartarotti. 2008. Spermatogenesis and nucleolar activity in *Triatoma klugi* (Triatomine, Heteroptera). Genet. Mol. Biol. 31: 438.

Da Cunha Marques, A. X. 1945. Cariologia comparada de alguns Hemípteros Heterópteros (Pentatomideos e coreideos). Memorias e Estudos do Museu Zoologico da Universidade de Coimbra, 163: 1–116.

Dey, S. K., and T. Wangdi. 1998. Chromosome number and sex chromosome system in forty-four species of Heteroptera. Chromosome Inf. Serv. 45: 5–8.

Froeschner, R. C. 1988. Family Alydidae Amyot and Serville, 1843, pp. 4–11. *In* **T. J. Henry and R. C. Froeschner** (eds.), Catalog of the Heteroptera, or true bugs, of Canada and the Continental United States. E. J. Brill, London, UK.

González-Garcia, J. M., C. Antonio, J. A. Suja, and J. S. Rufas. 1996. Meiosis in holocentric chromosomes: kinetic activity is randomly restricted to the chromatid ends of sex univalents in *Graphosoma italicum* (Heteroptera). Chromosome Res. 4: 124–132.

Grazia, J., N. D. F. Fortes, and L. A. Campos. 1999. Pentatomoidea, pp. 101–111. In **C. A. Joly and C. E. M. Bicudo** (eds.), Biodiversidade do Estado de São Paulo, Brasil: Síntese do conhecimento ao final do século XX, vol. 5: Invertebrados terrestres. São Paulo: FAPESP.

Gullan, P. J., and O. S. Cranston. 2008. Os insetos: um resumo de entomologia, 3rd ed. Roca, São Paulo, Brazil.

Jacobs, D. H. 2004. The evolution of a neo-XY1Y2 sex chromosome system by autosome sex chromosome fusion in *Dundocoris nodulicarius* Jacobs (Heteroptera: Aradidae: Carventinae). Chromosome Res. 12: 175–191.

Liang, A. P., and M. D. Webb. 2002. New taxa and revisionary notes in Rhinaulacini spittlebugs from southern Asia (Homoptera: Cercopidae). J. Nat. History 36: 729–756.

Manna, G. K. 1951. A study of chromosomes during meiosis in forty-three species of Indian Heteroptera. Proc. Zool. Soc. Bengal 4: 1–116.

Manna, G. K. 1984. Chromosomes in evolution in Heteroptera, pp. 189–225. *In* **A. K. Sharma** (ed.), Chromosomes in evolution of eukaryotic groups. CRC Press, Boca Raton, FL.

Mikolajski, M. 1968. Chromosome numbers in five species of Pentatomidae Reut (Hemiptera - Heteroptera). Experientia 24: 1062–1063.

Motzko, D., and A. Ruthmann. 1984. Spindle membranes in mitosis and meiosis of the heteropteran insect *Dysdercus intermedius*. A study of the interrelationship of spindle architecture and the kinetic organization of chromosomes. Eur. J. Cell Biol. 33: 205–216.

Nokkala, S., and C. Nokkala. 1984. The occurrence of the X0 sex chromosome system in *Dictyonata tricornis* (Schr.) (Tingidae, Hemiptera) and its significance for concepts of sex chromosome system evolution in Heteroptera. Hereditas 100: 299–301.

Panzera, F., F. Alvarez, J. Sanchez-Rufas, R. Perez, J. A. Suja, E. Scovortzoff, J. P. Dujardin, E. Estramil, and R. C. Salvatillar. 1992. Heterochromatin polymorphism in holocentric chromosomes of *Triatoma infestans* (Hemiptera: Reduviidae). Genome 35: 1068–1074.

Papeschi, A. G., and C. J. Bidau. 1985. Chromosome complement and male meiosis in four species of *Belastoma Latreille* (Heteroptera-Belostomatidae). Rev. Bras. Genet. 2: 249–261.

Papeschi, A. G., and M. J. Bressa. 2006. Evolutionary cytogenetics in Heteroptera. J. Biol. Res. 5: 3–21.

Sands, V. E. 1982. Cytological studies of the Coreidae and Alydidae (Hemiptera: Heteroptera). II. Karyological changes exemplified by Malaysian genera. Caryologia 35: 333–345.

Satapathy, S. N., and S. C. Patnaik. 1989. Chromosome numbers in forty-one species of Indian Heteroptera. Chromosome Inf. Serv. 47: 3–5.

Schuh, T. T., and J. A. Slater. 1995. The bugs of the world (Hemiptera: Heteroptera): classification and natural history, Cornell University Press, Ithaca, New York.

Souza, H. V., and M. M. Itoyama. 2010. Comparative study of spermatogenesis and nucleolar behavior in testicular lobes of *Euschistus heros* (Heteroptera: Pentatomidae). Psyche: J. Entomol 2010: 10.

Souza, H. V., and M. M. Itoyama. 2011. Comparative analysis of heteropyknotic chromatin and silver-stained material in *Platycarenus umbractulatus* (Pentatomidae: Discocephalinae). Inv. Reprod. Devel. 55: 65–72.

Souza, H. V., H. E. M. C. Bicudo, L. A. A. Costa, and M. M. Itoyama. 2007a. A study of meiosis and spermatogenesis in different testicular lobes of *Antiteuchus tripterus* (Heteroptera: Pentatomidae). Eur. J. Entomol. 104: 353–262.

Souza, H. V., R. L. M. Arakaki, L. N. Dias, A. S. Murakami, L. A. A. Costa, H. E. M. C. Bicudo, and M. M. Itoyama. 2007b. Cytogenetical aspects of testicular cells in economically important species of Coreidae family (Heteroptera). Cytologia 72: 49–56.

Souza, H. V., H. E. M. C. Bicudo, and M. M. Itoyama. 2007c. Study of chromosomal and nucleolar aspects in testes of *Nysius californicus* (Heteroptera: Lygaeidae). Genet. Mol. Res. 6: 33–40.

Souza, H. V., M. M. U. Castanhole, H. E. M. C. Bicudo, L. A. A. Costa, and M. M. Itoyama. 2008. Morphological patterns of the heteropycnotic chromatin and nucleolar material in meiosis and spermiogenesis of some Pentatomidae (Heteroptera). Genet. Mol. Biol. 31: 686–691.

Souza, H. V., F. B. Souza, S. R. C. Maruyama, M. M. U. Castanhole, and M. M. Itoyama. 2009. Meiosis, spermatogenesis and nucleolar behavior in the seminiferous tubules of Alydidae, Coreidae and Rhopalidae (Heteroptera) species. Genet. Mol. Res. 8: 1383–1396.

Ueshima, N. 1979. Animal cytogenetics. Insecta 6. Hemiptera: Heteroptera. Gebruder Borntraeger, Berlin, Germany.

Ueshima, N., and P. D. Ashlock. 1980. Cytotaxonomy of the Lygaeidae (Hemiptera: Heteroptera). Univ. Kans. Sci. Bull. 51: 717–801.

Wilson, E. B. 1905. The chromosomes in relation to the determination of sex in insects. Science 22: 500–502.

Wolf, K. W. 1996. Acetilation of α-tubulin in male meiotic spindles of *Pyrrhocoris apterus*, an insect with holocentric chromosomes. Protoplasma 191: 148–157.

Received 4 November 2012; accepted 18 March 2013.