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RESEARCH

# Cytogenetics Analysis and Testis Morphology of Aquatic Species of the Families Belostomatidae, Gelastocoridae, Gerridae, Notonectidae, and Veliidae (Heteroptera)

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**ABSTRACT.** The Heteroptera have holocentric chromosomes with kinetic activity restricted to the end of chromosomes. The first meiotic division is reductional for the autosomes and equational for the sexual. Only a few species of this suborder have been analyzed. In this study, we observed the morphologies of the testes of the Heteroptera species Belostoma anurum (Herrich-Schäffer, 1948), Belostoma micantulum (Stal, 1858), Gelastocoris angulatus (Melin, 1929), Gelastocoris flavus flavus (Guérin-Méneville, 1844), Rheumatobates crassifemur crassifemur (Esaki, 1926), Buenoa amnigenus (White, 1879), Buenoa unguis (Truxal, 1953), Martarega brasiliensis (Truxal, 1949), Martarega membranácea (White, 1879), Martarega uruguayensis (Berg, 1883), Rhagovelia tenuipes (Champion, 1898) and Rhagovelia zela (Drake, 1959). We found that the testes of these species can be round, round/spiral, or elongated/spiral. The size of the prophase I cells was found to vary, with the smallest ones being detected in *B. micantulum* and *Rha. zela,* the largest in *G. f. flavus*, and ones of intermediate size in *R. c. crassifemur* and *M. brasiliensis*. With respect to the chromosome complement, we verified the presence of $2n = \mathbf{16}$ (14A+XY, *B. micantulum* and *G. angulatus*), **21** (20A+X0, *R. c. crassifemur*), **23** (22A+X0, *Rha. zela* and *Rha. tenuipes*), **25** (24A+X0, *Bu. amnigenus* and *Bu. unguis;* 22A+2m+X0, *M. membranacea*), **27** (24A+2m+X0, *M. brasiliensis* and *M. uruguayensis*), **29** (26A+$X_1X_2Y$, *B. anurum*), and **35** (30A+$X_1X_2X_3X_4Y$, *G. f. flavus*). We found that the features of spermatogenesis in these species are similar to those of other previously described Heteroptera species, differing only in testicular morphology, chromosome number, and sex chromosome system.

**Key Words**: spermatogenesis, cytogenetics of Heteroptera, testicular lobe, meiosis, spermiogenesis

The aquatic and semiaquatic Heteroptera are widely distributed and inhabit all types of freshwater habitats. The aquatic insects belonging to the suborder Heteroptera have a direct life cycle (eggs, nymphs, and sexually mature adults). Because of their morphological peculiarities and behavioral traits, these predatory insects, regardless of the stage of development, are able to subdue and consume insects and small fish. Therefore, this group has an important relevance to the food chain, the transfer of nutrients in freshwater environments, and the population control of disease vectors (Schuh and Slater 1995, Vianna and Melo 2003).

In meiotic Heteroptera at leptotene–zygotene, the X chromosome is heteropycnotic and lies at the periphery of the nucleus. At zygotene and pachytene, the chromosomes are entangled. Pachytene is followed by a prominent diffuse stage observed in all wild specimens though not in inbred individuals. During this stage, autosomal bivalents do not decondense completely, and several heterochromatic regions can be observed; the X chromosome remains positively heteropycnotic and associated with a conspicuous nucleolus. Cell size increases, and the nucleus resembles an interphase-like state referred to as the diffuse stage after diplotene. At late diakinesis, the X chromosome becomes isopycnotic. At metaphase I, the X chromosome lies in the center of a ring formed by autosomal bivalents. At anaphase I, autosomal bivalents divide reductionally, whereas the X chromosome divides equationally. A second metaphase follows directly after telophase I with no delay. At metaphase II, the autosomes dispose at the equatorial plane, adopting a ring configuration with the X chromosome located in its center (Bressa et al. 2002). After telophase II, the formed daughter cells will target the process of spermiogenesis, the differentiation, and elongation of spermatids.

The sex chromosome systems described above include the simple XY/XX (74.7% of the species) and X0/XX (14.8%) with multiple chromosomes (originated by fragmentation of the X chromosome and, less frequently, the Y chromosome, $X_n0/X_nX_n$, $X_nY/X_nX_n$, and $XY_n/XX$) (10.3%) (Ueshima 1979; Manna 1984; Castanhole et al. 2008, 2010). There is also neo-XY (0.2%) (Chickering and Bacorn 1933, Schrader 1940, Jande 1959).

Species of the Belostomatidae family do not have the pair of m-chromosomes; the sex chromosome system can be XY, $X_nY$, or neo-XY, and the modal number of chromosomes for this family is $2n = 26$ chromosomes (Papeschi and Bressa 2006). For the Gelastocoridae family, only one species was analyzed cytogenetically: *Gelastocoris oculatus*, with $2n = 30A + X_1X_2X_3X_4Y$, without m-chromosomes (Ueshima 1979). The Gerridae family has the sex chromosome system X0, with a chromosome arrangement similar to a ring in meiotic metaphase and the absence of m-chromosomes. It has holocentric chromosomes, a prereductional division of autosomes and a postreductional division of sex chromosomes. The modal number of chromosomes for Gerridae is 21 (20A + X0) or 23 (22A + X0) (Ueshima 1979; Castanhole et al. 2008, 2010).

According to Ueshima (1979), only 12 species and 2 genera belonging to the Notonectidae family have been analyzed cytogenetically. The *Anisops* genera (Anisopinae) is characterized by the sex chromosomes system $X_1X_20$ and presents one pair of m-chromosomes; it is diploid, with 26 chromosomes for male and 28 for female. Species of the *Notonecta* genera (Notonectidae) have a sex chromosome system X0 or XY and $2n = 24$ (20A + 2m + XY) or 26 (22A + 2m + XY) chromosomes. The species of the Veliidae family, *Hebrovelia* sp., and *Microvelia reticulata* present $2n = 21$ (20A + X0) chromosomes

(Cobben 1968), and *Velia currens* (Poisson 1936) and *Velia* sp. (Ueshima 1979) present $2n = 25$ (24A + X0) chromosomes. Although all species have the sex chromosome system X0, XY was observed for *Microvelia douglasi*, though this result still has to be confirmed (Ueshima 1979).

The objective of this analysis was to describe the testicular morphology and number of lobes and to perform a detailed analysis of spermatogenesis (meiotic behavior and spermiogenesis) for species belonging to five families of aquatic Heteroptera.

## Materials and Methods

In this study, we analyzed 12 species of aquatic Heteroptera belonging to five families: Belostomatidae (*Belostoma anurum* and *Belostoma micantulum*), Gelastocoridae (*Gelastocoris angulatus* and *Gelastocoris flavus flavus*), Gerridae (*Rheumatobates crassifemur crassifemur*), Notonectidae (*Buenoa amnigenus, Buenoa unguis, Martarega brasiliensis, Martarega membranacea*, and *Martarega uruguayensis*), and Veliidae (*Rhagovelia tenuipes* and *Rhagovelia zela*).

We analyzed 20 adult males of each species collected in the region of São José do Rio Preto city (20° 47′32″ S, 49° 21′37″ W), SP, Brazil. After collection, samples were fixed in methanol:acetic acid (3:1) and kept at 4°C. With the aid of a stereomicroscope LEICA, Germany 16, the testicles were extracted and analyzed with respect to their morphology, the peritoneal sheath, and the number of lobes. Thereafter, the testes were placed on slides, lacerated, and stained with orceína lacto-acética. Images were acquired using a microscope and the image analysis program AXIO VISION (Zeiss, Germany), version 4.8.

For the morphometric comparison of cells in early meiotic prophase, we analyzed a representative of each family: *B. micantulum* (Belostomatidae), *G. f. flavus* (Gelastocoridae), *R. c. crassifemur* (Gerridae), *M. brasiliensis* (Notonectidae), and *Rha. zela* (Veliidae). For each species, we analyzed 50 cells using the "measure" tool in AXIO Vision and 4.8 in $\mu m^2$ scale. An analysis of variance (ANOVA) test was used for statistical analysis using the program Minitab 16.1.0. The degree of reliability was considered to be highly significant at a value of $P \leq 0.05$.

## Results

**Testis Morphology.** The 12 species analyzed have testes surrounded by a transparent peritoneal sheath with variable morphology (round, round/spiral, or elongated/spiral). Species of the same family were found to be similar to one another, with the exception of the Notonectidae family in which the *Buenoa* genera have testes with round/spiral morphology, whereas the *Martarega* genera have elongated/spiral morphology.

The testes of the species of the Belostomatidae family (*B. anurum* and *B. micantulum*) are coiled in the distal region of the ejaculatory duct, forming a rounded structure (Fig. 1a) with five testicular lobes that are individualized in the proximal region of the ejaculatory duct (Fig. 1b). In Gelastocoridae (*G. angulatus* and *G. f. flavus*), the testicles are formed by two elongated lobes that are closely matched in the proximal region of the ejaculatory duct; in the distal region, they are separated and spiral with approximately five turns (Fig. 1c).

The testes of species *Bu. amnigenus* and *Bu. unguis* of the Notonectidae family also have a rounded morphology, but they are highly spiral (Fig. 1d). In *M. brasiliensis, M. membranacea*, and *M. uruguayensis*, the testes are elongated and spiral, even though these species are in the Notonectidae family (Fig. 1e). The number of turns is approximately three, and the testicles are formed of two lobes (Fig. 1e).

We observed rounded testes in the Gerridae (*R. c. crassifemur*, Fig. 1f) and Veliidae (*Rha. tenuipes* and *Rha. zela,* Fig. 1g and h, respectively) families. The species of the Gerridae family present two testicular lobes, both rounded and separated (Fig. 1f), and the species of the Veliidae family have a single lobe (Fig. 1g and h) (Table 1).

**Spermatogenesis.** The behavior of cells during meiotic prophase I varies according to species. The cells of *R. c. crassifemur* do not exhibit heteropycnotic corpuscles; in some cells, a small and elongated heteropycnotic region near the nucleolar corpuscle can be observed (Fig. 2a). In *G. f. flavus*, there are several small heteropycnotic corpuscles along the chromosome (Fig. 2b). In contrast, the cells of *B. micantulum* have a single large heteropycnotic corpuscle that is heavily stained and rounded (Fig. 2c), and the cells of *M. brasiliensis* have a corpuscle with no defined morphology (Fig. 2d).

During prophase I, it is possible to observe the interstitial chiasmata, which make the chromosomes cross-shaped, or terminals or double terminals chiasmata, which give the chromosomes a rounded morphology, as observed in *G. f. flavus* and *B. anurum* (Fig. 2e and f, respectively). At this stage, all species have chromosomes with a defined morphology. Some species, such as *M. uruguayensis*, also exhibit telomeric associations between the autosomes (Fig. 2g).

The diploid chromosome complement was verified with the presence of $2n = \mathbf{16}$ (14A+XY, *B. micantulum* and *G. angulatus*), **21** (20A+X0, *R. c. crassifemur*), **23** (22A+X0, *Rha. zela* and *Rha. tenuipes*), **25** (24A+X0, *Bu. amnigenus* and *Bu. unguis*; 22A+2m+X0, *M. membranacea*), **27** (24A+2m+X0, *M. brasiliensis* and *M. uruguayensis*), **29** (26A+$X_1X_2Y$, *B. anurum*) and **35** (30A+$X_1X_2X_3X_4Y$, *G. f. flavus*) (Fig. 3a–l) (Table 1).

At metaphase I, in the polar view, the arrangement of chromosomes is variable, particularly with respect to the sex chromosomes. The autosomes of all species are usually arranged in a ring. The location of the sex chromosomes depends on the particular system. With X0, the X chromosome is located outside of the ring formed by the autosomes (Fig. 3g). If the system is X0 with m-chromosomes, in most cases, the autosomes are arranged circularly with the m-chromosomes in the center and the X outside of the ring (Fig. 3i). With XY, the X is inside the ring formed by autosomes, and the Y is located with the autosomes in the ring (Fig. 3b). However, with $X_1X_2Y$, the X and the Y chromosomes are inside of the ring (Fig. 3a). With $X_1X_2X_3X_4Y$, the X chromosomes are in the center of the ring formed by the autosomes, and the Y is included in the ring with the autosomes (Fig. 3d).

During anaphase, we observed that the species exhibit regular migration of the chromosomes, with the exception of *M. brasiliensis, M. membranacea,* and *M. uruguayensis*. In these species, we observed chromosomes with late migration, both in the first and second division (Fig. 4a–d), and possibly the sex chromosome. In telophase I, we could see the m-chromosomes in the center of the ring formed by the autosomes, and the X was outside and migrated late (Fig. 4a).

The behavior of cells during spermiogenesis differs between families; however, some features are similar, for example, the morphology of early spermatids. All are rounded, but the location of the heteropycnotic material varies. For example, in Fig. 4e–h, it is only on one side of the cell, but in Gelastocoridae (Fig. 4k–m), the heteropycnotic material is rounded and heavily stained. In the other families, there are also round spermatids, but the chromatin material is homogeneously distributed and more strongly stained. Examples include the Gerridae families (Fig. 5a), Notonectidae (Fig. 5h), and Veliidae (Fig. 5n).

The spermatids may have an elliptical shape, as in Belostomatidae (Fig. 4g–i) and Gelastocoridae (Fig. 4p and q) or a rod shape, as in Gerridae (Fig. 5c–e), Notonectidae (Fig. 5i), and Veliidae (Fig. 5t–v). Between the rounded phases and the rod-shaped or elliptical phases, some families have large vesicles, as in Gelastocoridae (Fig. 4l–o) and Gerridae (Fig. 5b), whereas others have small vesicles, as in Veliidae (Fig. 5o–s). In species with large vesicles, the heteropycnotic material is located in the posterior region of the cell (Figs. 4m and 5b), whereas in cells with small vesicles, the heteropycnotic material is located in the anterior region. These spermatids initially have a sickle morphology (Fig. 5o–q), but during development, they become rounded (Fig. 5r and s) and later rod-shaped (Fig. 5t–v).

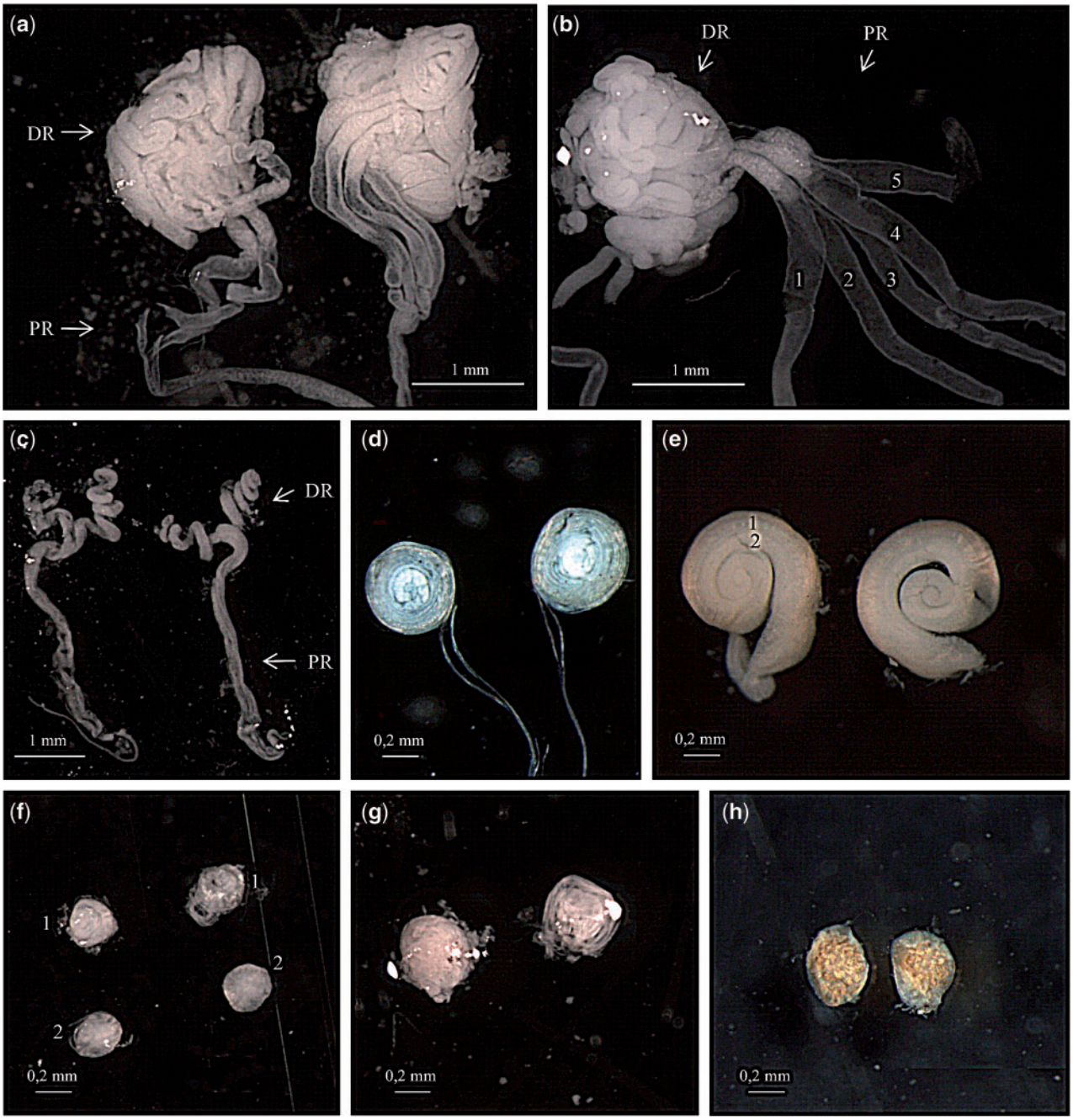


**Fig. 1.** Testes of *B. anurum* (a) and *B. micantulum* (b); the distal region (DR) is rounded and the proximal region (PR) elongated. Note the five testicular lobes in (b); in (c), testes of *G. f. flavus* consisting of two lobes that are elongated with a spiral distal extremity; in (d), testes of *Bu. unguis* characterized as rounded and highly spiral; in (e), testes of *M. membranacea* formed by two elongated and spiraling lobes; in (f), testes of *R. c. crassifemur*; in (g), testes of *Rha. tenuipes*; in (h), testes of *Rha. zela* rounded and formed by two (f) or one lobe (g, h). Bars: 0.2 mm and 1 mm.

In species that do not have vesicles (Belostomatidae and Notonectidae), the behavior of cells at this stage is similar. In Belostomatidae, spermatids are first rounded (Fig. 4e and f) and later resemble a teardrop (Fig. 4g and h) and then become elliptical (Fig. 4i,j). In Notonectidae, the spermatids are first rounded (Fig. 5h and k) posteriorly and then assume a rod shape (Fig. 5i).

Although Belostomatidae and Notonectidae do not have vesicles at this stage, the elliptical spermatids have two small vesicles (Belostomatidae) (Fig. 4i) or a single large vesicle (Notonectidae) (Fig. 5l and m).

During the final elongation, all species retain their chromatin morphology (Figs. 4j, p, and q and 5f, g, and v), except the species *M. uruguayensis*, which is V-shaped in the posterior region of the tail (Fig. 5j).

**Morphometry of Cells in Prophase I.** We analyzed 50 cells in early meiotic prophase of *B. micantulum* (Belostomatidae), *G. f. flavus* (Gelastocoridae), *R. c. crassifemur* (Gerridae), *M. brasiliensis* (Notonectidae) and *Rha. zela* (Veliidae), and verified that the species *B. micantulum* and *Rha. zela* have the smallest cells with an average area of 396 and 596 $\mu m^2$, respectively (Table 2). *G. f. flavus* has the highest cell area and also the greatest variability in size (Fig. 6).

**Table 1. Characteristics of the testes and chromosome complement from the 12 species analyzed**

| Family | Species | Testis morphology | Lobe morphology | No. of lobes | 2*n* |
|---|---|---|---|---|---|
| Belostomatidae | *B. anurum* | Rounded in the distal region and elongated in the proximal region | Coiled in distal region and elongated in proximal region | 5 | 29 (26A + $X_1X_2Y$) |
| | *B. micantulum* | Rounded in the distal region and elongated in the proximal region | Coiled in distal region and elongated in proximal region | 5 | 16 (14A + XY) |
| Gelastocoridae | *G. angulatus* | Elongated and spiral at the distal extremity | Elongated and with lobes spiraling | 2 | 16 (14 + XY) |
| | *G. f. flavus* | Elongated and spiral at the distal extremity | Elongated and with lobes spiraling | 2 | 35 (30A + $X_1X_2X_3X_4Y$) |
| Gerridae | *R. c. crassifemur* | Round | Round | 2 | 21 (20A + X0) |
| Notonectidae | *Bu. amnigenus* | Round | Highly spiraling | 1 | 25 (24A + X0) |
| | *Bu. unguis* | Round | Highly spiraling | 1 | 25 (24A + X0) |
| | *M. brasiliensis* | Elongated/spiral | Spiraling | 2 | 27 (24A + 2m + X0) |
| | *M. membranacea* | Elongated/spiral | Spiraling | 2 | 25 (22A + 2m + X0) |
| | *M. uruguayensis* | Elongated/spiral | Spiraling | 2 | 27 (24A + 2m + X0) |
| Veliidae | *Rha. tenuipes* | Round | Round | 1 | 23 (22A + X0) |
| | *Rha. zela* | Round | Round | 1 | 23 (22A + X0) |

We observed cells of 2,154.37–27,154.04 $\mu m^2$ with an average of 10,641 $\mu m^2$ (Table 2). *R. c. crassifemur* and *M. brasiliensis* have intermediate cell sizes compared with *B. micantulum, G. f. flavus* and *R. zeal*, with an average of 3,800 and 3,651 $\mu m^2$, respectively (Table 2). *R. c. crassifemur* and *G. f. flavus* also presented very different cell sizes, varying from 1,165.64 to 10,500.48 $\mu m^2$. The statistical analysis (ANOVA test) performed using the program Minitab 16.1.0 showed that *B. micantulum* and *R. zeal* have similar cell sizes, as well as *R. c. crassifemur* and *M. brasiliensis* no significant differences between these species and between the two groups were observed. *G. f. flavus* it has the highest area was placed separately the other species, showing a significant difference in the area of cells in prophase I of this species, however, and the differences between the four other species also were significant by statistical analysis (Fig. 6).

## Discussion

The pigmentation of the peritoneal sheath that covers the testicles and testicular lobes has not been explored extensively in Heteroptera. The species analyzed in this study have a transparent peritoneal sheath. Other species described in the literature have a reddish sheath, as in *Hyalymenus* sp. and *Neomegalotomus pallescens* (Alydidae) (Souza et al. 2009); a yellowish sheath, as in *Zicca annulata* (Souza et al. 2007a); or colorless sheath, as in *Limnogonus aduncus* (Gerridae) (Castanhole et al. 2008). Considering our results and the few studies in the literature, there appears to be no pattern associated with sheath coloring. Within the Coreidae family, for example, there are reddish, yellowish, or colorless sheaths. Nonetheless, all aquatic Heteroptera described in the literature so far have transparent peritoneal sheaths; thus, it may be common within aquatic Heteroptera.

Common to all Heteroptera is that their testicles are formed of lobes that are elongated and always side by side. The number varies from three to seven (Souza et al. 2007a). In the Lygaeidae family, the number of lobes varies, with some species having two, four, six, or seven lobes. Testicles with seven lobes are considered to be the ancestral morphology (Grozeva and Kuznetsova 1992). *L. aduncus* (Gerridae, Castanhole et al. 2008) has two testicular lobes; *Mormidae v-luteum* (Pentatomidae, Souza et al. 2008) three lobes; *Oebalus poecilus, Oebalus ypisilongriseus* (Pentatomidae, Souza et al. 2008), *Z. annulata*, and *Chariesterus armatus* (Coreidae, Souza et al. 2007a) have four lobes; *Antitheuchus tripterus* (Pentatomidae, Souza et al. 2007b) six lobes; *Nysius californicus* (Lygaeidae, Souza et al. 2007c), *Anasa bellator*, *Athaumastus haematicus, Dallacoris obscura*, *Dallacoris pictus, Leptoglossus gonagra, Leptoglossus zonatus*, and *Sphictyrtus fasciatus* (Coreidae, Souza et al. 2007a) seven lobes.

The aquatic species analyzed in this study showed different testis morphology than the land species, that is, rounded in the distal region and elongated in the proximal for the Belostomatidae family, elongated and spiral at the distal extremity for Gelastocoridae, elongated and spiral or rounded for Notonectidae and rounded for Veliidae and Gerridae and the number of lobes was one, two, or five. In aquatic species, it was more common to observe testis morphology with rounded lobes arranged side by side. These data are important because when related to the phylogeny of the Heteroptera, it will be possible to propose an evolutionary hypothesis to explain the differences between terrestrial, aquatic, and semiaquatic species.

The Heteroptera have holocentric chromosomes (Ueshima 1979, Souza et al. 2007a, Costa et al. 2008, Castanhole et al. 2010). In leptotene–zygotene, the X chromosome is heteropycnotic and located on the periphery of the nucleus; in zygotene and pachytene, the chromosomes are tangled. At the end of diakinesis, the X chromosome becomes isopycnotic (Bressa et al. 2002). The species analyzed in this study also exhibited these characteristics, indicating that these behaviors are common for Heteroptera. According to the literature, however, the sex chromosome is not always heteropycnotic; in *Belostoma dentatum*, bivalent autosomes are continually condensed, whereas both sex chromosomes are decondensed and negatively heteropycnotic during diakinesis (Papeschi and Bidau 1985).

During prophase I, the species analyzed in this study showed interstitial or terminal chiasmata, single or double. This behavior has also been observed in Coreidae (Souza et al. 2007a) and should be common to most species.

During metaphase, the autosomes in the analyzed species always form a ring. The placement of the other chromosomes (X, Y, and m-chromosomes) varies: if the species has the sex chromosome system X0, the X is out of the ring, similar to other species of Coreidae (Souza et al. 2007a); when the system is X0 with m-chromosomes, most of the time, the X is out of the ring and the m-chromosomes are in the center of the ring, as in species of Coreidae (Souza et al. 2007a).

When the system is XY or $X_1X_2X_3X_4Y$, the X chromosome is inside the ring and the Y is together with the autosomes forming the ring. In contrast, other species such as Pentatomidae place the two sex chromosomes inside the ring formed by autosomes (Souza et al. 2008), and in the $X_1X_2Y$ system, the sex chromosomes are also inside the ring.

The number of autosomes varies in Heteroptera from 4 (Belostomatidae) to 80 (Miridae); these numbers are not typical for the suborder (Ueshima 1979). It is difficult to say what the ideal chromosome number is for the whole suborder because all eight of the largest infraordens within Heteroptera have not been studied in depth cytologically (Grozeva and Nokkala 1996).

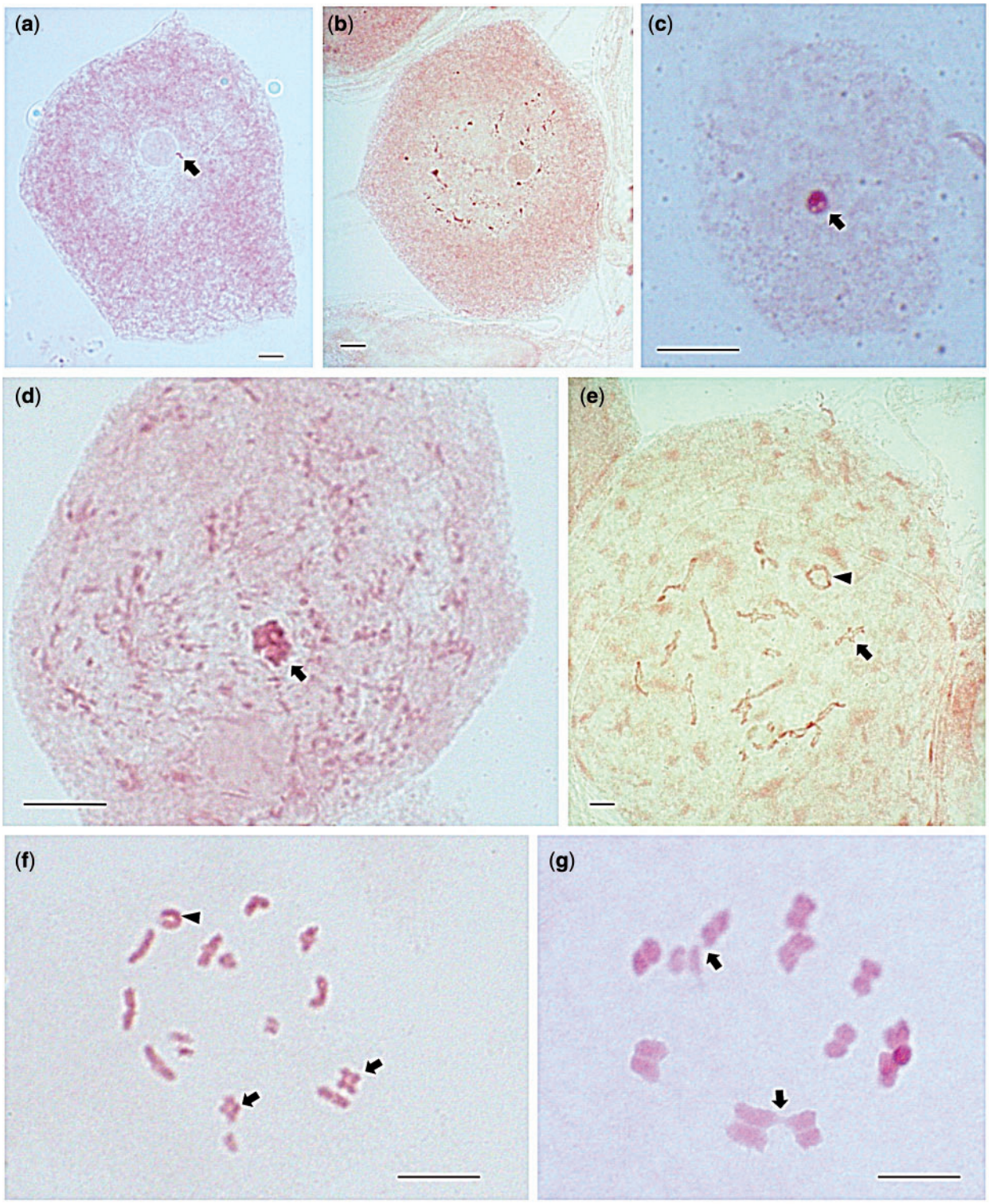


**Fig. 2.** Cells of the seminiferous tubules of *R. c. crassifemur* (a), *G. f. flavus* (b, e), *B. micantulum* (c), *M. brasiliensis* (d), *B. anurum* (f), and *M. uruguayensis* (g) stained with lacto-acetic orcein. (a) Prophase I with a small heteropycnotic corpuscle near the nucleolus (arrow); (b) prophase I with several small heteropycnotic corpuscles; (c) prophase I with heteropycnotic corpuscle (arrow); (d) prophase I with a large corpuscle heteropycnotic, rounded with no defined morphology (arrow); (e, f) diplotene/diakinesis with interstitial chiasmata that give the chromosome a cross-shaped morphology (arrow) or double terminals chiasmata that give the chromosomes a rounded morphology (arrowhead); (g) diplotene/diakinesis with telomeric associations between the autosomes (arrow). Bars: 10 μm.

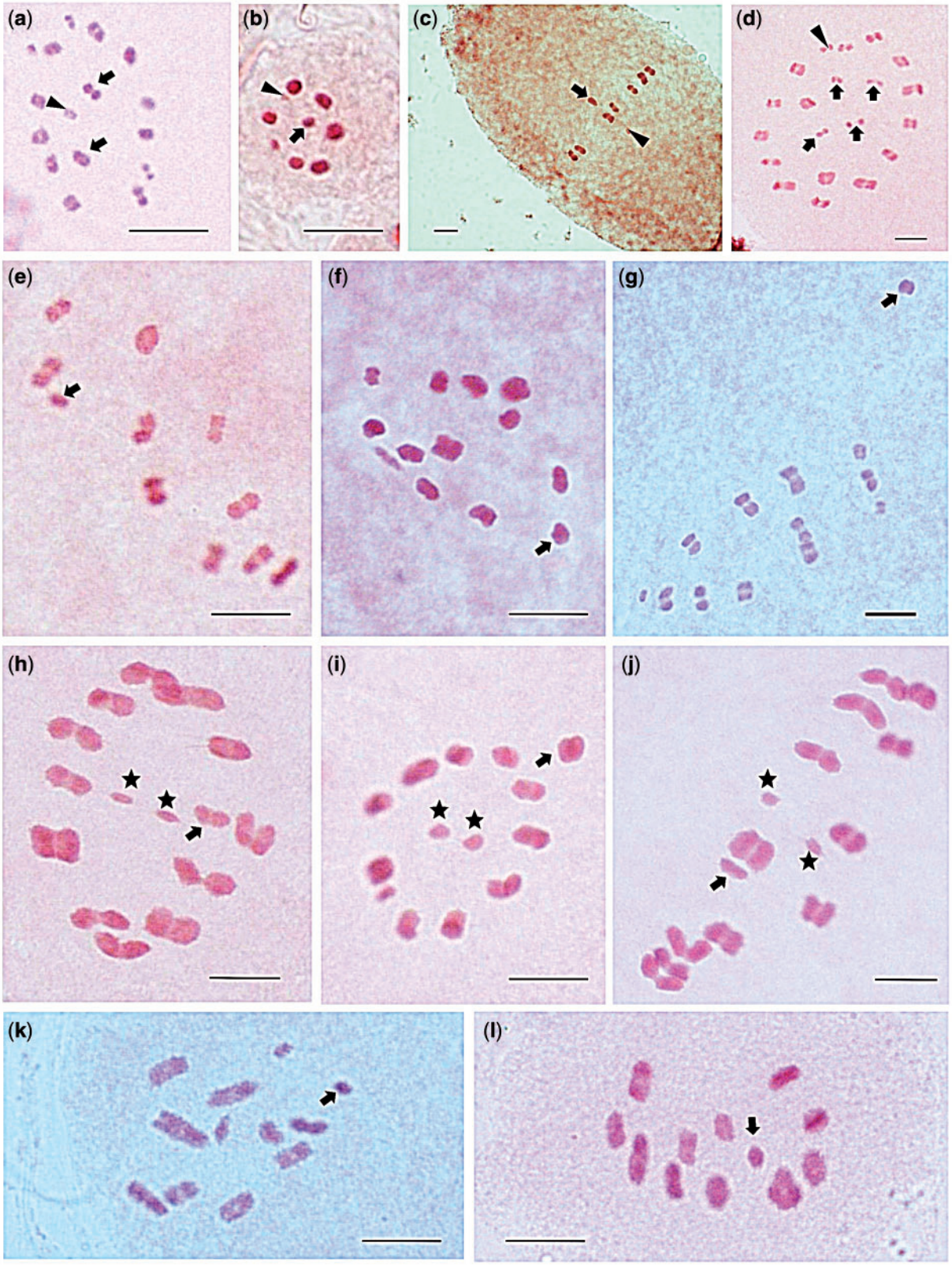


**Fig. 3.** Cells of the seminiferous tubules in metaphase I stained with lacto-acetic orcein, showing chromosome complements of (a) *B. anurum,* $2n = 29$ ($26A+X_1X_2Y$); (b) *B. micantulum,* $2n = 16$ ($14A+XY$); (c) *G. angulatus,* $2n = 16$ ($14A+XY$); (d) *G. f. flavus,* $2n = 35$ ($30A+X_1X_2X_3X_4Y$); (e) *R. c. crassifemur,* 21 ($20A+X0$); (f) *Bu. amnigenus,* $2n = 25$ ($24A+X0$); (g) *Bu. unguis,* $2n = 25$ ($24A+X0$); (h) *M. brasiliensis,* $2n = 27$ ($24A+2m+X0$); (i) *M. membranacea,* $2n = 25$ ($22A+2m+X0$); (j) *M. uruguayensis,* $2n = 27$ ($24A+2m+X0$); (k) *Rha. tenuipes,* $2n = 23$ ($22A+X0$); (l) *Rha. zela,* $2n = 23$ ($22A+X0$). Arrows, X chromosomes; arrowhead, Y chromosomes; and asterisk, m-chromosomes. Bars: 10 μm.

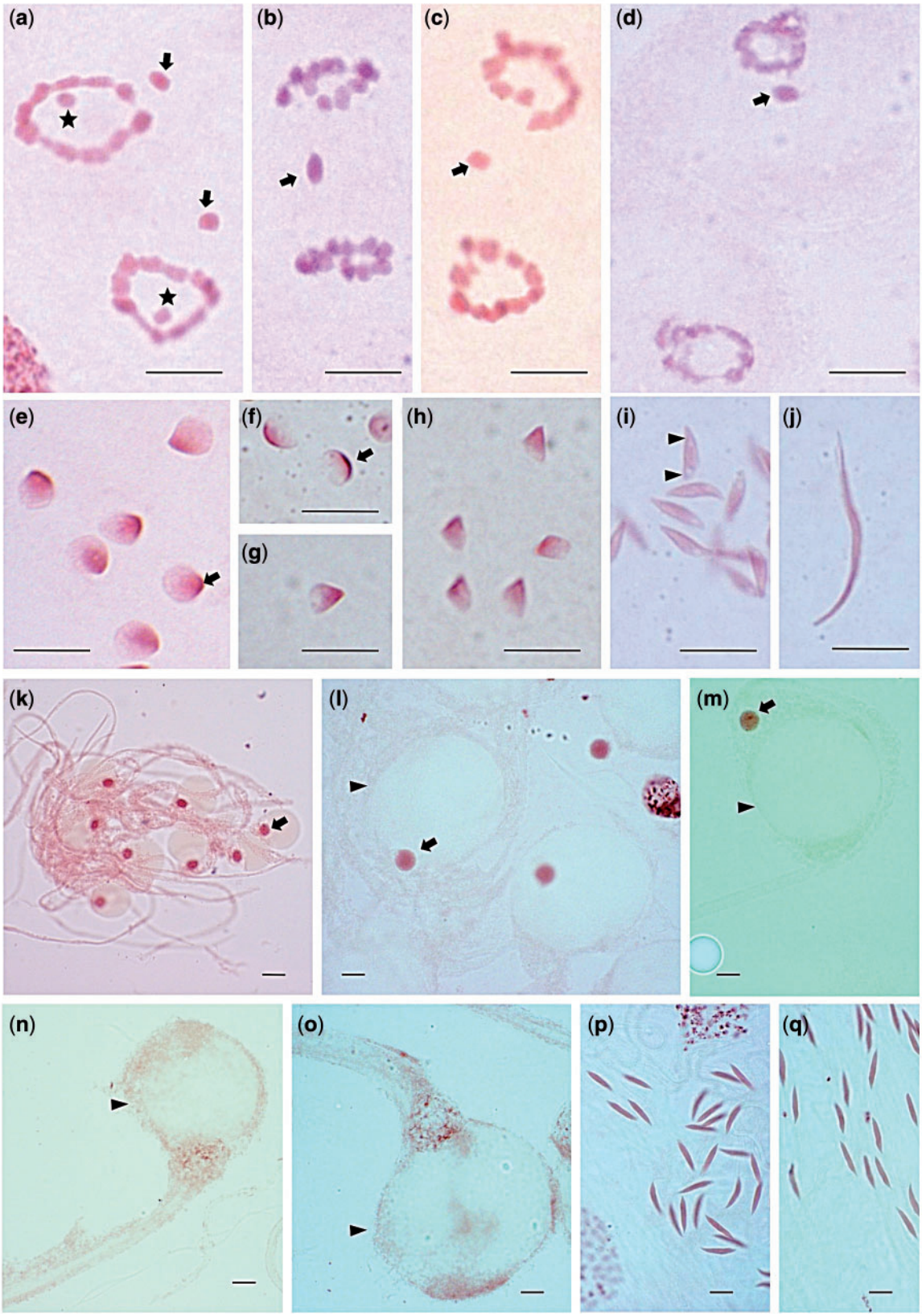


**Fig. 4.** Cells of the seminiferous tubules of *M. membranacea* (a, c), *M. brasiliensis* (b, d), *B. anurum* (e), *B. micantulum* (f–j), *G. f. flavus* (k, n, o), and *G. angulatus* (l, m, p, q) stained with lacto-acetic orcein. (a) anaphase I/telophase I chromosome with late migration of the X sex chromosome (arrow) and the m-chromosomes in the center of the ring (asterisk); (b, c) anaphase II/telophase II with late migration of the X chromosome (arrows); (d) telophase II with the X chromosome only in one of the cells (arrow); (e, f) round spermatids with the heteropycnotic material on one side of the spermatid (arrows); (g, h) spermatids in a teardrop shape; (i) elliptical spermatids with small vesicles (arrowheads); (j) spermatid in final elongation; (k–m) round spermatids with heteropycnotic material evident (arrows) and a large vesicle (arrowheads); (n, o) elongating spermatids with a large vesicle in the anterior region (arrowheads); (p, q) elliptical spermatids. Bars: 10 μm.

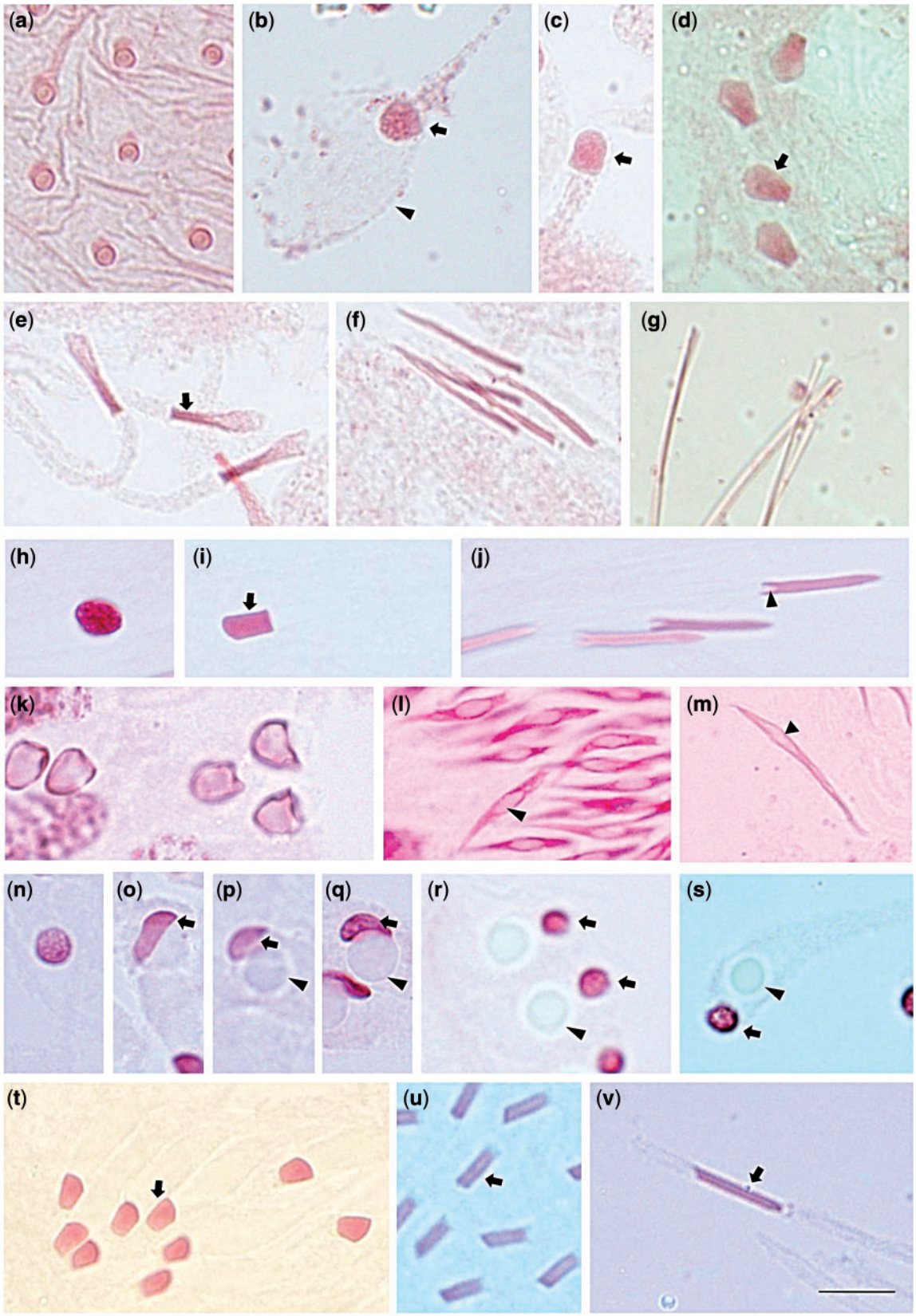


**Fig. 5.** Cells of the seminiferous tubules of *R. c. crassifemur* (a–g), *M. uruguayensis* (h–j), *Bu. unguis* (k–m), *Rha. zela* (n, o, p, q, t, v), and *Rha. tenuipes* (r, s, u) stained with lacto-acetic orcein. (a) round spermatids; (b) elongating spermatids with the heteropycnotic material in the posterior region (arrow) and a large vesicle in the anterior region (arrowhead); (c–e) elongating spermatids with the rod-shaped heteropycnotic material (arrows); (f, g) elongating spermatids; (h) round spermatids; (i) elongating spermatids with the rod-shaped heteropycnotic material (arrow); (j) elongating spermatids with the posterior region of the tail v-shaped (arrowhead); (k) spermatids with an irregular morphology and heteropycnotic material distributed along the periphery; (l, m) elongating spermatids with a small vesicle inside (arrowheads); (n) round spermatids; (o–q) round spermatids with sickle-shaped heteropycnotic material (arrows) and a small vesicle (arrowheads); (r, s) elongating spermatids with the heteropycnotic material in the anterior region (arrows) and a vesicle in the posterior region (arrowheads); (t–v) elongating spermatids with rod-shaped heteropycnotic material (arrows). Bar: 10 μm.

**Table 2. Averages and standard deviations obtained from the morphometric analysis of 50 cells in prophase I of the five species of aquatic Heteroptera**

| Species | *N* | Average ($\mu m^2$) | Standard deviation |
|---|---|---|---|
| *B. micantulum* | 50 | 396 | 126 |
| *G. f. flavus* | 50 | 10,641 | 6,333 |
| *R. c. crassifemur* | 50 | 3,800 | 2,427 |
| *M. brasiliensis* | 50 | 3,651 | 483 |
| *Rha. zela* | 50 | 596 | 275 |

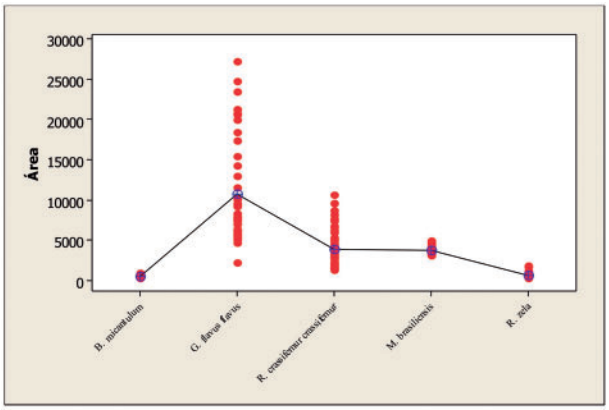


**Fig. 6.** Distribution of cell area ($\mu m^2$) in prophase I of the five species of Heteroptera analyzed.

A larger complement of chromosomes is thought to arise from a smaller complement through the duplication or fragmentation of a pair of autosomes (Ueshima and Ashlock 1980). According to Jacobs (2004), because Heteroptera have holocentric chromosomes, fragmentation is the most likely origin for an increase in chromosome number as it generates segments that can regularly migrate to the poles during anaphase and persist for many cell generations. Chromosome fusion is also theoretically easier in organisms with holocentric chromosomes.

In the Belostomatidae family, *B. micantulum* has $2n = 16$ and *B. anurum* has $2n = 29$; in the Gelastocoridae family, *G. angulatus* is $2n = 16$ and *G. f. flavus* $2n = 35$; in the Notonectidae family, *Bu. amnigenus, Bu. unguis,* and *M. membranacea* have $2n = 25$; and *M. brasiliensis* and *M. uruguayensis*, $2n = 27$. The species with more chromosomes must have originated from species with less through fragmentation of the autosomes and/or sex chromosomes according to the logic mentioned above.

There is currently little information on the evolution of these chromosomes. Because of the lack of a morphologically differentiated centromere and longitudinal chromosome differentiation, it is difficult to detect structural variation. Thus, chromosomal rearrangements, such as inversions and reciprocal translocations, are rarely reported in these organisms. A greater number of species should be evaluated with numerous techniques to better understand this group that is so understudied.

During the analysis, we observed that the cells in prophase I of aquatic species were much larger than those of terrestrial species described in the literature, thus, we analyzed cells of one representative of each family in prophase I. The species *G. f. flavus*, which is a semi-aquatic species that lives in aquatic or terrestrial environments, showed the greatest difference in cell area. *B. micantulum*, which is also semi-aquatic, presented a cell area in prophase I that was similar to *Rha. zela,* which lives on the water surface; thus, we cannot conclude that the environment determines cell area. *R. c. crassifemur,* which also lives on the surface, was similar to *M. brasiliensis*, which lives underwater. The environment probably does not affect cell area, but more research will be necessary to confirm this finding. The underlying reasons for the large differences in cell area between species would be interesting for understanding the evolution of these species and should be the subject of future work.

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