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Research Article

Comparative Study of Spermatogenesis and Nucleolar Behavior in Testicular Lobes of *Euschistus heros* (Heteroptera: Pentatomidae)

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In some testicular lobes of the Pentatomidae there may be occurrence of atypical spermatogenesis or polymegaly, leading to the production of nonfertile sperm. The comparative analysis of spermatogenesis and nucleolar behavior in testicular lobes of *Euschistus heros* showed cells with polymegaly in lobes 4 and 6. Generally, when these lobes are present in the same individual, there is also the formation of atypical cells in the flanking lobe. Such characteristic was not seen in *E. heros*. However, differences regarding the concentration of heteropyknotic chromatin and silver-positive bodies in this lobe deserve attention. This study explored the literature and demonstrated the prevalence of some lobes in the formation of differentiated cells. It was also found in the literature that there is an association of the chromocenter with the nucleolus in several species of Pentatomidae, but in *E. heros* this association does not appear to occur.

1. Introduction

The presence of testes formed by a number of compartments referred to as "lobes" is a characteristic of the Heteroptera. In some species, one of these lobes is of the *harlequin* type that differs from the other lobes by showing spermatogonial cells with meiotic pairing, nonspecific association of the autosomal bivalents, anomalous arrangement of the chromosomes in the metaphase plate, anomalous chromosome segregation, and cell fusion, resulting in the production of spermatozoa with highly variable chromosome numbers. There are reports of this type of lobe in 15 genera in three Pentatomidae subfamilies (Discocephalinae, Edessinae, and Pentatominae) [1].

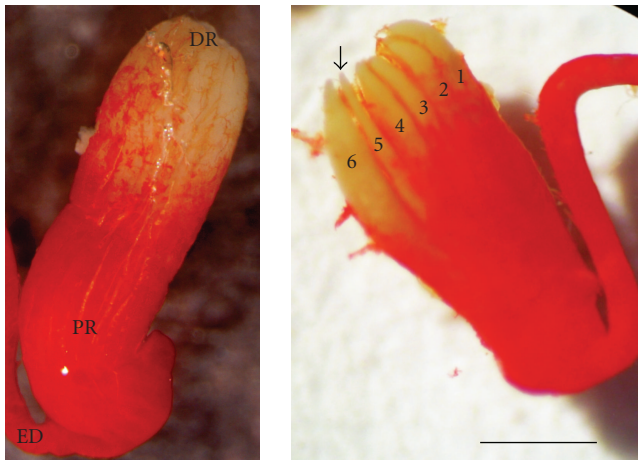
Other lobes may also be associated with the formation of nonfertile sperm; for example, in *Antiteuchus tripterus* (Pentatomidae), lobes 4 and 6 show cells with polymegaly and significant intralobular metabolic differences [2, 3].

The aspects regarding the number of testicular lobes and the formation of atypical sperm are little known and explored. This information is found scattered in the literature which makes it difficult to establish an evolutionary pattern among testicular lobes with regard to the formation of fertile and nonfertile sperm. For this reason, the data found in the literature are compiled in Table 1.

Recent studies have demonstrated the importance of examining the metabolic differences among species by the analysis of nucleolar bodies. It is known that the nucleolus or nucleolar bodies are related to the biosynthetic activity of the cell, so that the size and number of bodies depend on the functional characteristics of cells and may therefore reflect metabolic and functional differences [3–7].

Another aspect of nucleolar behavior in spermatogenesis is that, over time, several observations have suggested that the nucleolar granules or bodies which persist at the end of meiosis reorganize the nucleolus in early spermiogenesis and support protein synthesis in the process [8].

The study of metabolic differences between testicular lobes with analysis of nucleolar bodies has been proposed by Souza et al. [3], who found that the testicular lobes of *A. tripterus* showed significant differences in behavior and size of the nucleolar bodies, which may be due to



(a) (b)

Figure 1: Testis of *Euschistus heros* enclosed by red membrane, where the proximal region (PR) of the ejaculatory duct (ED) is more intense red than the Distal Region (DR) (a). Observe in (b) the presence of six elongated lobes of approximately the same length, with lobe 5 narrower than the others (arrow). Bar = 1 mm.

differences in the formation of sperm with a nonfertile function.

The analysis of nucleolar organizer regions (NORs) during prophase has shown a close association of the chromocenter with the nucleolar body [9, 10]. The chromocenter in Heteroptera is characterized by its heteropyknotic nature, where it can be composed of sex chromosomes or even by heterochromatic autosomes [9, 11, 12]. In general, the nucleolar body is disorganized during diakinesis [13], reorganizing itself only in the beginning of spermiogenesis, supporting the initiation of protein synthesis [8]. However, the relationship between chromatin heteropyknotic and nucleolar bodies during spermiogenesis has not been previously explored, according to the literature.

Thus, the objective of this study was to analyze spermatogenesis in each lobe of *Euschistus heros*, comparing it with nucleolar behavior throughout spermatogenesis and to analyze in detail the distribution pattern of testicular lobes, described in the literature, with regard to differentiated spermatogenesis.

2. Material and Methods

Fifteen adult males of *Euschistus heros* Fabricius, 1794 (Heteroptera, Pentatomidae, Pentatominae, Pentatomini) were collected on soybean plants (*Glycine max* (L.), in the city of Sao Jose do Rio Preto (20°47′13″ S, 49°21′38″ W), SP, Brazil. The insects were fixed in methanol:acetic acid (3 : 1), and their testicular lobes were separated and submitted to the squash technique with lacto-acetic orcein staining, which is chromosome specific.

To study nucleolar behavior during spermatogenesis, the slides were submitted to the silver impregnation technique [47, with modifications] to stain argyrophilic proteins, which are associated with rRNA and which can therefore localize the nucleolus or nucleolar bodies.

In the statistical analysis, measurements were taken of the diameter of 100 cells and their nuclei in the diffuse stage, that is, the stage after pachytene, which is characterized by its increased size and chromatin scattered throughout the nuclei, and of 100 cells nuclei in the stage of round spermatid in each lobe, randomly chosen. We used the program UTHSCSA Image Tool v.3.00 [48] and Minitab version 15.1 [49] for ANOVA (Tukey's comparison with 95% confidence interval) to compare the measurements of the cells between the testicular lobes. The best images were captured with a Zeiss microscope using the image analysis program AXIO VISION.

3. Results

3.1. Testis Morphology. The testes of *Euschistus heros* were enclosed by peritoneal sheath with red pigment, with the proximal region (PR) of the ejaculatory duct (ED) being more pigmented than the distal (DR) (Figure 1(a)). When the peritoneal sheath was removed in the distal region, we could observe the presence of 6 elongated lobes of approximately the same length, where lobe 5 was narrower than the others (Figure 1(b)).

3.2. Meiotic Behavior. The comparative analysis of meiotic cells of *Euschistus heros* stained with lacto-acetic orcein and silver impregnated showed that the behavior of cells in the six testicular lobes was quite similar, and therefore, the results are presented together.

During early prophase, a heteropyknotic body was observed at the periphery of the nucleus, and variation in cell diameter among the lobes was the only difference found. The diameter of the cells in lobes 1–3 (Figure 2(a)) was significantly smaller than that of lobe 5 cells (Figure 2(c)), which were smaller than cells in lobes 4 and 6 (Figure 2(b)). Due to these differences, an ANOVA test was performed, which showed that the lobes could be grouped by the size of the cells and their nuclei (Figures 2(a)–2(c)) into three different groups (group 1 = lobes 1, 2, and 3, group 2= lobe 5, group 3= lobe 4 and 6). Significant differences ($P < .0001$) were found between the groups, and the cells of group 1 (lobes 1–3) and their nuclei were smaller than those in group 2 (lobe 5) which, in turn, were smaller than those of group 3 (lobes 4 and 6) (Table 2).

During spermatogenesis, it was observed that the cells in diplotene/diakinesis showed chromosomes with chiasmata (Figure 2(d)). It was possible to determine in diakinesis and metaphase I the presence of a diploid number of $2n = 14$ (12A + XY) chromosomes (Figures 2(d), 2(e)). In anaphase/telophase I, it was observed that only autosomes undergo reductional segregation (Figures 2(f), 2(g)). During metaphase II, the autosomes were arranged in a ring with the sex chromosomes arranged inside (Figure 2(h)). During anaphase/telophase II, the sex chromosomes undergo equational division and it was possible to visualize lagging migration of the X chromosome (Figure 2(i)).

Table 1: All species of the family Pentatomidae in the literature where authors noted the number of testicular lobes and if they exhibited atypical meiosis and polymegaly. "No": characteristic not found; "—": information not found, "?": author not sure about the presence of the characteristics.

Classification	No. lobes	Atypical meiosis	Polymegaly	References
Pentatomidae family				
Subfamily Asopinae				
Apateticus crocatus	7	No	No	[14]
Euthyrhynchus floridanus (Linnaeus)	6	No	No	[14]
Perillus bioculatus (Fabricius 1775) (as *Mineus bioculatus*, 1775)	7	No	No	[14, 15]
Podisus maculiventris (Say)	7	No	No	[14]
(as *P. modestus* (Dallas, 1851))				[15, 16]
(as *P. spinosus* (Dallas, 1851))				[15–19]
Stiretrus anchorago (Fabricius 1775)	7	No	No	[14, 16]
Subfamily Discocephalinae				
Tribe Discocephalini				
Antiteuchus tripterus (Fabricius,1787) (as *Mecistorhinus tripterus*)	6	5	4 and 6	[2, 20–22]
Dinocoris rufitarsus (Ruckes, 1958)	8	5	4 and 6	[21, 23]
Discocephalessa humilis (Herrich-Schaeffer, 1843) (as *Platycarenus notulatus* (Stal, 1862))	4	No	—	[20]
Platycarenus umbractulatus	7	No	—	In press
Tribe Ochlerini				
Alitocoris schraderi (Sailer, 1950)	5	5	4 degenerate	[21, 24]
Subfamily Edessinae				
Brachystethus rubromaculatus (Dallas, 1851)	4	4	—	[20]
Edessa bifida (Say)	5	No	2 and 4	[14]
E. meditabunda (Fabricius, 1794)	4	No	—	In press, [25]
Subfamily Pentatominae				
Tribe Aeliini				
Aelia americana	7	No	No	[14]
Tribe Carpocorini				
Carpocoris sp.	6	No	3 ?	[14]
Coenus delius (Say, 1832)	6	5	4 and 6	[14, 15, 17–19]
Cosmopepla bimaculata (Distant)	5	No	?	[14]
Euschistus euschistoides, (Vollenhoven, 1868) (as *E. ssilis* Uhler, 1871)	6	5	4 and 6	[14, 15, 19]
Euschistus heros (Fabricius, 1798)	6	No	4 and 6	Present work
E. ictericus (Linnaeus, 1763)	6	5	4 and 6	[14, 15]
E. inflatus	6	5	4 and 6	[14]
E. servus (Say, 1832)	6	5	4 and 6	[14, 15, 25, 26]
E. tristigmus (Say, 1832)	6	5 ?	4 and 6	[14, 15, 17, 18, 26]
E. variolarius (Palisot de Beauvois, 1805) (as *Pentatoma*)	6	5	4 and 6	[14, 15, 17, 18, 27–30]
Holcostethus limbolarius (Stål, 1872) (as *Peribalus*)	6	No	No	[14, 17, 18]
Mormidea quinqueluteum (Lichtenstien, 1796)	3	No	—	[1, 9]
Oebalus poecilus	4	No	—	[9]
O. (Fabricius, 1775) (as *Solubea pugnax*)	4	No	No	[14, 16, 26]
O. ypsilongriseus (De Geer, 1773)	4	No	—	[9]

Table 1: Continued.

Classification	No. lobes	Atypical meiosis	Polymegaly	References
Trichopepla semivittata (Say)	7	No	No	[14, 17, 18]
Tribe Chlorocorini				
Arvelius albopunctatus (De Geer, 1773)	6	4	3 and 5	[1, 14, 31]
Chlorocoris complanatus	7	5	4 and 6	In press
Loxa flavicollis (Drury, 1773) (as *L. florida* (Van Duzze))	7	5	4 and 6	[30, 32, 33]
L. viridis (Palisot de Beauvois, 1805) (as *L. picticornis* (Horvath, 1925))	7	5	4 and 6	[21, 32, 33]
Tribe Nezarini				
Chlorochroa uhleri (Stål)	6	No	3 and 5	[14]
Nezara viridula (Linnaeus, 1758)	6	4	3 and 5	[14, 34–39]
Rhytidolomia saucia (as *Chlorochroa saucia*) (Say, 1832)	6	No	3 and 5	[14, 40]
R. senilis (as *Chlorochroa senilis*) (Say, 1832)	6	3 and 5	No	[14, 40, 41]
Tribe Halyini				
Brochymena quadripustulata (Fabricius)	7	No	4 and 6	[14]
Tribe Pentatomini				
Acledra hilare (Say, 1832) (as Nezara hilaris)	6	No	No	[14, 15, 17–19, 34, 38]
Adevoplitus longicomis (Ruckes, 1958) (as *Pseudevoplitus longicomis*)	6	—	3 and 5	[21, 41]
Banasa calva (Say, 1832)	3	No	No	[14, 42–44]
B. dimidiata (Say, 1832)	3	No	No	[14, 43, 44]
Thyanta calceata (Say, 1832) [as *T. custator* (Fabricius, 1803)]	4	3 ?	No	[14, 31, 34]
T. casta	6	No	3 and 6	[14]
T. custator	4	3 ?	No	[14]
T. perditor (Fabricius, 1794)	3	No	—	[45]
Tribe Strachiini				
Murgantia histriônica	5	No	3 and 4	[14]
M. histriônica (var. *nigricans*)	5	No	3 and 4	[14]
Tribe Piezodorini				
Piezodorus guildinii (Westwood)	5 ?	No	No	[14, 46]

Table 2: Mean diameter of cells in diffuse stage and their respective nuclei and round spermatids of *Euschistus heros*, chosen randomly. The lateral bars indicate different groups with equality average. The values are given by Mean ± standard deviation (Mean ± SD). The unit utilized is micrometers (μm).

	Values (μm)		
Lobes	Cell	Nuclei	Spermatids
1	23.60 ± 0.20	16.68 ± 0.15	8.32 ± 0.06
2	23.51 ± 0.22	17.45 ± 0.15	8.72 ± 0.06
3	23.06 ± 0.24	15.79 ± 0.20	8.53 ± 0.05
4	26.08 ± 0.25	19.53 ± 0.17	8.64 ± 0.09
5	37.78 ± 0.43	27.70 ± 0.28	14.15 ± 0.10
6	42.72 ± 0.43	30.28 ± 0.29	13.27 ± 0.11
	$P < .0001$	$P < .0001$	$P < .0001$

When cells in prophase were silver impregnated, three round silver-positive bodies could be seen, two more impregnated with one bigger than the other, and the third lighter staining (Figures 2(j), 2(k)). It could also be observed that the lighter body may correspond to the heteropyknotic body revealed by the lacto-acetic orcein technique (Figures 2(a)–2(c)). In addition to the increased size of the cells in prophase of lobes 4 and 6, there were also several silver-positive bodies scattered throughout the nucleus (Figure 2(k)). With regard to metaphase I, two different behaviors were observed: lobes 1, 2, 3, 4, and 6 with impregnations in the cytoplasm and chromosomes (Figure 2(m)) and lobe 5 which showed negative silver impregnation (Figure 2(n)). During anaphase/telophase II, the positive silver impregnation behavior was similar for

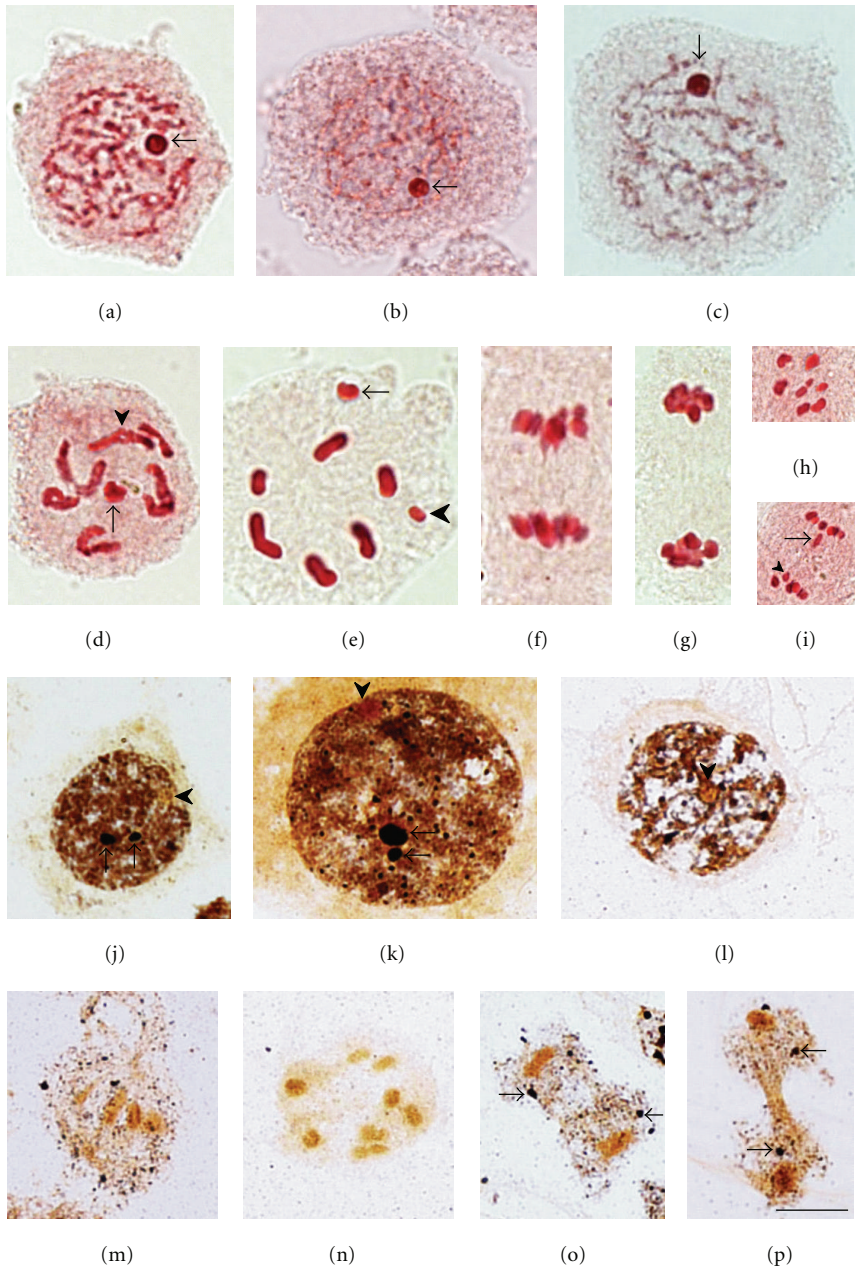


Figure 2: Meiotic cells stained with lacto-acetic orcein ((a)–(i)) and silver impregnated ((j)–(p)). ((a)–(c)) Early prophase I with heteropyknotic body in the periphery of the nucleus (arrows), with (a) belonging to lobes 1–3, (b) to 4 and 6 and (c) to 5; (d) diplotene/diakinesis showing a bivalent with interstitial chiasma (arrowhead) and heteropyknotic body (arrow); (e) final stage of diakinesis showing X and Y chromosome, respectively, arrow and arrowhead; ((f), (g)) anaphase/telophase I with regular segregation of chromosomes; (h) metaphase II showing the autosomes arranged in ring-shape and the sex chromosomes in the center; (i) anaphase/telophase II with lagging migration of the X chromosome (arrow) and the Y chromosome in the opposite part of the cell (arrowhead); ((j)–(l)) early prophases I showing two round silver-positive bodies (arrows) that are disorganized during the subsequent phases and one body less impregnated (arrowheads). Note that in prophase of lobes 4 and 6 (k), there are several silver-positive bodies scattered throughout the nucleus; (m), (n)) metaphase I with silver impregnation in the cytoplasm and chromosomes (m), differing from lobe 5, which has silver-negative metaphase (n); ((o), (p)) telophase II with nucleolar reorganization in both cell formations (arrows). Bar = 10 μm.

all lobes, that is, nucleolar reorganization in both cells in formation (Figures 2(o), 2(p)).

3.3. Behavior of Cells during Spermiogenesis. A comparative analysis of the cells during the spermiogenesis of *E. heros* stained with lacto-acetic orcein and silver impregnated showed that the behavior of cells in six testicular lobes was similar, and therefore, the results were presented together.

In the round spermatid, heteropyknotic staining was observed at the periphery and center of the nucleus, and

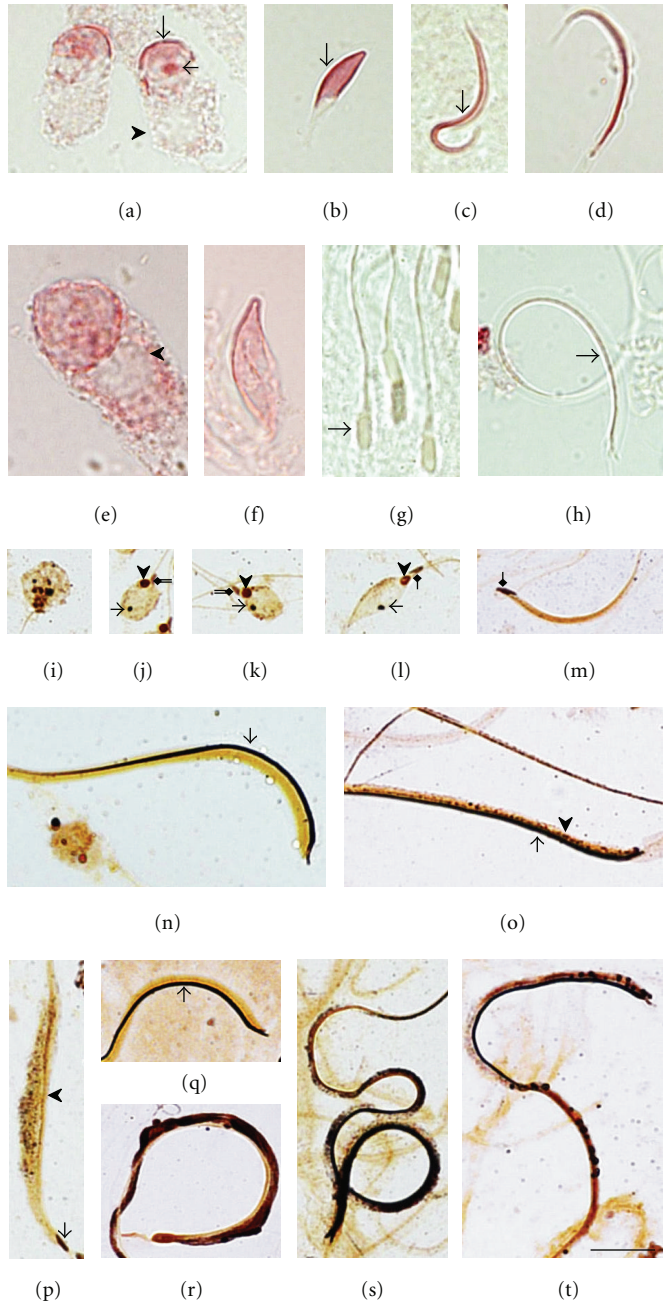


Figure 3: Spermiogenesis cells stained with lacto-acetic orcein ((a)–(h)) and silver impregnated ((i)–(t)). (a)–(d) Development of spermatids in lobes 1–3 and 5. (a) Round spermatids showing heteropyknotic material at the periphery and center of the nucleus (arrows) and a vesicle in the posterior region (arrowhead); (b) spermatid in elongation with heteropyknotic material at the periphery of the nucleus (arrow); (c) in a following stage, the heteropyknotic chromatin is visualized on only one side (arrow); (d) in a later stage, it becomes indistinguishable; (e)–(h) spermatids of lobes 4 and 6; (e) round spermatids showing a vesicle in the posterior region (arrowhead); (f) spermatid in elongation; (g) spermatid at the later stage of development with chromatin stained weakly and in the posterior region with a protuberance (arrow); (h) sperm showing a thin heteropyknotic chromatin on one side of the head (arrow); ((i)–(m)) round spermatid with several silver-positive bodies; ((j)–(l)) during the subsequent phases were observed three silver-positive bodies: one rod-shape and lighter close to region of tail formation ((j), smaller arrow), one round intensely stained (arrowhead), both in the posterior region of the spermatid and another smaller than others (bigger arrow) that was visualized in the middle region of the spermatid ((k), (l)); (m) spermatid in development with only one silver-positive body in the posterior region of the nucleus (arrow); (n) spermatozoon of lobes 1–3 with continuous silver-positive staining in the posterior region of the head (arrow) and in lobe 5 (o) a continuous positive silver impregnation (arrow) and several small bodies in all the nucleus (arrowhead). ((p)–(t)) Spermiogenesis of lobes 4 and 6; (p) spermatid in development showing strong silver-positive body in the posterior region of the head (arrow), several small silver-positive bodies in the middle region and a line-shaped silver staining from middle to anterior region (arrowhead); ((q)–(t)) developed spermatids showing four silver-positive behaviors: (q) continuous and linear (arrow); (r) large and amorphous; (s) linear and throughout the extent of the head and several round and small silver-positive bodies and (t) with the same previous characteristics, but the silver-positive bodies are larger and smaller in number. Bar= 10 μm.

a vesicle was seen in the posterior region (Figure 3(a)); in the elongated spermatid, the heteropyknotic material could be visualized at the periphery of the nucleus (Figure 3(b)). In a following stage of the development of the spermatid, the heteropyknotic chromatin could be observed on only one side (Figure 3(c)) while in a later stage becomes indistinguishable (Figure 3(d)). Despite the behavior of the spermatids in early development being similar among all six lobes, it was observed, in regard to the diameter of round spermatids, that the lobes could be grouped into two, by the size of cells (group 1= 1–3 and lobe 5, group 2= lobes 4, and 6). The cells of group 1 (lobes 1-3 and 5, Figure 3(a)) were significantly smaller than those in group 2 (lobes 4 and 6, Figure 3(e)) ($P < .0001$) (results summarized in Table 2). The same development pattern of cells in spermiogenesis was observed of lobes 4 and 6 in relation to the other lobes; however, the heteropyknotic chromatin in early spermatids was less evident (Figures 3(e), 3(f)). In the developed spermatid, it is inconspicuous, where a protuberance appears in the posterior region of the nucleus (Figure 3(g)) and fine chromatin along the head of the sperm in formation (Figure 3(h)).

With the use of silver impregnation in the cells of spermiogenesis, round spermatids were observed with several round silver-positive bodies (Figure 3(i)), which moved to the posterior region of the nucleus, near the tail in formation (Figure 3(i)) and during the development of the spermatid (Figure 3(j)–3(l)), three silver-positive bodies were observed: one rod-shape and lighter located at the beginning of the formation of the tail, and two round ones of different sizes and intensities of impregnation. The largest and least impregnated was located in the posterior region of the spermatid's nucleus, close to the rod-shaped body, and the smaller and most impregnated was close to the anterior region of the spermatid's head (Figure 3(j)). With the development of the spermatid, apparently, these bodies remain in the same locations (Figure 3(j)–3(l)). During later stage of development there was only one silver-positive body in the posterior region of the nucleus (Figure 3(m)).

Differences were seen among the lobes in the pattern of silver impregnation in the developed spermatid. In lobes 1–3, this spermatid showed only a continuous silver impregnation in the posterior region of the head (Figure 3(n)), while in lobe 5, besides showing this continuous impregnation in the same region, also contained several small silver-positive bodies in the entire nucleus (Figure 3(o)). The developed spermatids in lobes 4 and 6 exhibited intense and elongated silver impregnation in the posterior region of the head, several small silver-positive dots in the middle region, and a line of silver staining from the middle region up to the anterior region of the head (Figure 3(p)). In the elongated spermatid, four different behaviors were noted in regard to distribution of silver impregnation: continuous and linear (Figure 3(q)); large and amorphous (Figure 3(r)); one linear region in the entire extent of head and several small dots, distributed throughout the head (Figure 3(s)); one linear region in the entire extent of head, several small dots, and larger round stained bodies distributed throughout the head (Figure 3(t)).

4. Discussion

The testes of Pentatomidae are divided by connective tissue into subunits called "lobes". The most common number of lobes is seven, although there are variations among tribes and species [1, 50]. Based on the literature review regarding the number of lobes (Table 1), it could be seen that among the six species of the subfamily Asopinae, only one (*Euthyrhynchus floridanus*) has six testicular lobes, while the others have seven. In the other subfamilies, the distribution of lobes was found to be heterogeneous, ranging from four to eight lobes in the Discocephalinae, four to five in the Edessinae, and three to seven in the Pentatominae. At this moment, it is not possible to establish a direct relationship between the number of lobes and subfamily. Unfortunately, despite that the Heteroptera are composed of many species, very few have been studied in regard to this aspect. The subfamily Pentatominae, the most studied, showed wide variation with relation to the number of lobes. Therefore, a larger number of species should be analyzed to determine the ancestral number of lobes as well as to understand this diversity in the number of lobes.

Another feature found in species of the family Pentatomidae is the presence of a different lobe called *harlequin*. Schrader [21, 23, 32] was surprised that the forces of evolution have persisted with the *harlequin* lobes, a structure that produces heteropolyploid sperm not used in fertilization. They suggested that this sperm should provide additional nutrients, especially nucleoproteins for the development of eggs.

Atypical meiosis is another unusual feature that can be observed in the *harlequin* lobes. Table 1 shows the prevalence of these lobes in the subfamilies Discocephalinae, Edessinae, and Pentatominae. Among the six species of the subfamily Asopinae, no distinguishing characteristic was mentioned. However, there are no data in the literature with regard to the number of lobes and formation of atypical cells for the other subfamilies (Cyrtocorinae, Phyllocephalinae, Podopinae, and Serbaninae).

It could also be observed from the analysis of 50 species in Table 1 that 19 (38.0%) have atypical meiosis, of which 14 (73.7%) correspond to lobe 5, three (15.8%) to lobe 4, and three (15.8%) to lobe 3. It was found only one occurrence involving the lobes 3 and 5 in the same individual.

In addition to this atypical meiosis, the production of different-size sperm from the normal meiotic process also presents an evolutionary mystery. Although many species of insects have sperm showing polymegaly, which vary only in size and have a normal chromosome complement, all forms of sperm morphology may not be equally involved in fertilization [51]. The capacity of fertilization in sperm of different morphologies has not been demonstrated. At least for the large sperm, a nutrition argument similar to proposed to *harlequins* sperm [21] could be advantageous to the formation of multiple sperm morphology.

In Table 1, polymegaly was described in 25 (50.0%) species, of which 14 (56.0%) correspond to the lobes 4 and 6 simultaneously, that is, both lobes showed cells with polymegaly in the same individual, and only one (4.0%)

case not involving simultaneity in the formation of cells with polymegaly was found for lobes 3 and 4. These simultaneous events involving lobes 2 and 4, 3 and 4, 3 and 5 and 3 and 6 occurred in 1 (4.0%), 2 (8.0%), 5 (20.0%), and 1 (4.0%) cases, respectively.

When analyzing these two characteristics, the formation of atypical cells and polymegaly, it could be seen in Table 1, that when there are two lobes in the same species showing polymegaly, these lobes usually flank another lobe, as occurs in the species *Antiteuchus tripterus*. Especially for lobes 4 and 6, in which 12 simultaneous events were observed, only one did not show lobe 5 (flanking lobe) with the formation of atypical cells. This exception corresponds to species *Euschistus heros,* analyzed in this study. Although there was no formation of atypical cells in lobe 5, differences were found in the diameter of cells in prophase and in the concentration of silver impregnation, mainly in the sperm, suggesting the necessity to explore the true function of the sperm in this lobe, using other approaches.

Table 1 indicates that the minimum number of lobes found for the family Pentatomidae is three, as occurs in the species *Mormidae quinqueluteum* [1] and *Banasa calva* [43]. When a species possessed lobes forming differentiated cells (atypical or showing polymegaly), there was also the presence of at least three lobes demonstrating the production of fertile sperm, as in species *A. tripterus* [2] which has six lobes, three lobes responsible for producing typical sperm and the other three producing differentiated cells. Therefore, it could be inferred from the data obtained to date, that three is the minimum number of lobes needed to produce fertile sperm. Another feature that could be shown among these analyzed species described in Table 1, was that lobe 1 invariably produces fertile sperm, that is, with meiosis and production of typical sperm, while the only case of production of differentiated cells involving lobe 2 occurred in the species *Edessa bifida* [14].

When formation of differentiated cells occurred, this was related to lobes 4 to 6. This may suggest a preadaptation to the formation of nonfertile sperm in these lobes. However, with the data obtained so far, it has not been possible to characterize a distribution pattern of evolution of nonfertile sperm between the subfamilies or tribes.

The species *E. heros* showed cells in the diffuse stage in lobes 4 and 6, significantly larger than in other lobes, and therefore considered polymegaly. Morphologically, lobe 5 was narrow, in contrast to that in the species *A. tripterus* studied by Souza et al. [2]; lobes 4 and 6 produced cells with polymegaly, but lobe 4 was the narrowest in this species, while the lobe 5 (*harlequin* lobe) was disproportionately larger, forming a slightly twisted testis.

The differences found in regard to heteropyknotic material and silver impregnation in the lobes of *E. heros* showed that polymegaly is accompanied by differences in the behavior of these structures, especially when comparing spermiogenesis. Intensive silver impregnation and no condensed chromatin in spermatids and sperm may reflect intense metabolic activity and suggest a change in function in the sperm of these lobes.

Regarding cytogenetics, in Heteroptera, there is generally a close association of the chromocenter with the nucleolus in prophase I. Souza et al. [9] found an unusual morphology of the nucleolar body, called mushroom, which supports the association of the chromocenter, probably the more stained portion (the cap) with the nucleolus (the stem). The species of this study showed a heteropyknotic body when stained with lacto-acetic orcein, and when silver impregnated showed two darker bodies which were probably the nucleolar bodies, and a lighter one which was probably the chromocenter. It is interesting to note that the chromocenter may not be associated directly with the nucleolus in *E. heros*, diverging from other species of the Pentatomidae that show this association [9].

Thus, this paper provides insights into evolutionary process of the formation of nonfertile sperm, once this field lacks a more accurate exploration of such theme by different approaches.

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