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Luis Lenin Vicente Pereira

Análise dos aspectos ultraestruturais da espermatogênese de Heteroptera

São José do Rio Preto
2017

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Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Biociências, área de concentração em Genética, junto ao Programa de Pós-Graduação em Biociências, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de São José do Rio Preto.

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“A ignorância afirma ou nega veementemente; a ciência duvida. ’’

François Marie Arouet (Voltaire)

RESUMO

A subordem Heteroptera possui sete infraordens com aproximadamente 80 famílias. A maioria ocorre em todos os continentes (exceto Antártica) e algumas ilhas. Além dos Heteroptera terrestres, há os aquáticos e semi-aquáticos que são amplamente distribuídos, e surpreendem por sua capacidade de habitar uma extraordinária variedade de ecossistemas, sendo encontrados em habitats de água doce e marinho, e variada faixa de altitude entre 0 e 4.700 m. Estudos sobre aspectos ultraestruturais da espermatogênese e, especificamente, a estrutura do espermatozoide em Heteroptera ainda são escassos, por este motivo o objetivo do presente estudo foi o de analisá-los, por meio de cortes semifinos corados com azul de toluidina ou impregnados por íons prata, e cortes ultra-finos analisados em microscopia eletrônica de transmissão, utilizando testículos de machos adultos das famílias Belostomatidae, Gelastocoridae, Gerridae, Mesoveliidae, Notonectidae e Veliidae. Após a análise ultraestrutural da espermatogênese foi possível determinar que o padrão flagelar do axonema é de 9+9+2 para todas as espécies analisadas sendo, portanto, o padrão para essa subordem, as mitocôndrias durante a espermatogênese assumem diferentes morfologias, sendo que inicialmente as mitocôndrias se unem formando o complexo mitocondrial e, posteriormente, se divide em dois derivados mitocondriais que estão posicionados bilateralmente em relação ao axonema. Os derivados mitocondriais apresentaram tamanhos diferentes para as espécies *B. amnigenus* (Notonectidae) e *R. c. crassifemur* (Gerridae) e para as demais espécies o tamanho foi semelhante. As células germinativas possuem em seu citoplasma o acúmulo de um material denominado corpo cromatóide estando

localizado próximo ao núcleo. Com relação ao comportamento nucleolar da espécie *Martarega brasiliensis* foi observado de um a quatro corpúsculos nucleolares em células de Prófase I comprovando uma grande atividade sintética das células nessa fase da divisão celular. Células em Metáfase I apresentaram regiões organizadoras nucleolares na região telomérica de um dos autossomos. Ainda, nessa espécie, foi possível observar, em Anáfase I, vários corpúsculos nucleolares persistindo até a fase de Telófase I. Todas as ultraestruturas descritas nas espécies analisadas foram semelhantes às descritas na literatura para Heteroptera, corroborando as características sinapomórficas dessa subordem sendo elas: a) a presença de duas pontes que ligam o material intertubular do axonema flagelar às cisternas achatadas que aderem aos lados internos dos derivados mitocondriais; b) padrão flagelar do axonema de 9+9+2 e c) ausência de corpos acessórios.

Palavras-chave: Axonema. Acrossomo. Complexo mitocondrial. Derivado mitocondrial. Mitocôndrias.

ABSTRACT

The suborder Heteroptera has seven infraorders with approximately 80 families. Most occur in all continents, except Antarctica and some islands. In addition to terrestrial Heteroptera, there are also widely distributed aquatic and semi-aquatic species. This suborder have adapted to live in an extraordinary variety of ecosystems as freshwater and marine habitats and at altitudes ranging from 0 m to 4,700 m. The research concerning the ultrastructural aspects of spermatogenesis is a large and growing field of study, however, in the case of Heteroptera, research is still scarce. For this reason, the aim of this study was to analyze the ultrastructures and spermatogenesis through semi-thin sections stained with toluidine blue or silver ions (Ag-NOR) and ultrathin sections examined in transmission electron microscopy, using testes of adult males ofthe following families: Belostomatidae, Gelastocoridae, Gerridae, Mesoveliidae, Notonectidae and Veliidae. After ultrastructural analysis of spermatogenesis, it was possible to determine that the flagellar pattern of the axoneme is 9+9+2 for all species, being therefore, the pattern for this suborder. As spermatogenesis progresses, the mitochondria begins to cluster and concentrate on only one side of the cell. Then, the mitochondria combine to form a single mitochondrial complex, which subsequently divides into two mitochondrial derivatives. They are positioned on opposite sides of the axoneme. The mitochondrial derivatives presented different sizes for the species B. amnigenus (Notonectidae) and R. c. crassifemur (Gerridae) and for the other species the size was similar. The germ cells have in their cytoplasm the accumulation of a material denominated the chromatoid body, being located near the nucleus. Regarding the nucleolar behavior, M. brasiliensis showed nucleus in prophase I composed by the nucleolus and nucleolar corpuscles that varied from one to four, emphasizing that this insect has great synthetic activity during meiosis. The analysis of cells in metaphase I, showed that M. brasiliensis presents nucleolar organizing region (NOR) in at least one autosome. Furthermore, was not observed the phenomenon of nucleolar persistence. All the ultrastructures described in the analyzed species were similar to those described in

the literature for Heteroptera, corroborating the synapomorphic characteristics of this suborder, being them: a) two opposite bridges in the axoneme connect the flattened cisterns adherent to the internal side of each mitochondrial derivative to the intertubular material; b) flagellar pattern of the axoneme of 9+9+2; c) accessory bodies are absent all along the flagellum.

Keywords: Axoneme. Acrosome. Mitochondrial complex. Mitochondrial derivative. Mitochondria.

SUMÁRIO

I. INTRODUÇÃO

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1. Características Gerais dos Heteroptera Aquáticos

Hemiptera é o quinto maior grupo de insetos, com aproximadamente 82.000 espécies descritas (CRYAN; URBAN, 2012). É composto por quatro subordens, sendo elas Auchenorryncha, Coleorryncha, Sternorryncha e Heteroptera (FORERO, 2008), constituindo um dos grupos mais numerosos entre os hemimetábolos com sua monofilia sendo reconhecida com base em estruturas bucais específicas da mandíbula e da maxila (FORERO, 2008).

A subordem Heteroptera possui sete infraordens com aproximadamente 80 famílias. Nenhum outro grupo de insetos possui tamanha diversidade com relação ao habitat, como os Heteroptera, pois ocorre em todos os continentes (exceto Antártica) e algumas ilhas (SCHUH; SLATER, 1995).

Pelo fato da maioria dos Heteroptera serem fitófagos, eles podem afetar diretamente os humanos, pois podem causar grandes danos às produções, por exemplo, de grãos ou frutos que são utilizados para consumo ou produção de medicamento, podem afetar a cadeia alimentar, ou mesmo transmitir algumas doenças às plantas. A importância econômica de vários Heteroptera também envolve muitas espécies que são benéficas, pois consomem insetos destrutivos de várias plantas. Algumas espécies são ectoparasitas de humanos e animais domésticos e poucos transmitem sérias doenças aos humanos, como por exemplo, a doença de Chagas (SCHUH; SLATER, 1995).

Além dos Heteroptera terrestres há os aquáticos e semi-aquáticos que são amplamente distribuídos, e surpreendem por sua capacidade de habitar uma extraordinária variedade de ecossistemas, sendo encontrados em habitats de água doce e marinho, e variada faixa de altitude entre 0 e 4.700 m (MELO, 2009).

Esses insetos possuem ciclo de vida direto (ovos, ninfas e adultos). Devido às peculiaridades morfológicas e características comportamentais desses insetos predadores, independentemente do estágio de desenvolvimento, são capazes de subjugar e consumir pequenos peixes e insetos. Isto confere a esse grupo evidente relevância para a estrutura trófica e transferência de nutrientes em ambientes de água doce, bem como

um potencial eminente para o controle das populações de vetores de doenças (SCHUH; SLATER, 1995; VIANNA et al., 2003).

Dentre as 80 famílias de Heteroptera, 23 são as representantes aquáticas e semi-aquáticas: Aepophilidae, Aphelocheiridae, Belostomatidae, Corixidae, Gelastocoridae, Gerridae, Hebridae, Helotrephidae, Hermatobatidae, Hydrometridae, Leptopodidae, Macroveliidae, Mesoveliidae, Naucoridae, Nepidae, Notonectidae, Ochteridae, Omaniidae, Paraphrynoveliidae, Pleidae, Potamocoridae, Saldidae e Veliidae. Dessas, foram analisadas no presente trabalho as famílias Belostomatidae, Gelastocoridae, Gerridae, Mesoveliidae, Notonectidae e Veliidae.

Os Heteroptera pertencentes à família **Belostomatidae** (Figura 1), muitas vezes referida como insetos gigantes d´água, estão distribuídos por todo mundo, embora sua maior diversidade esteja nos trópicos, normalmente associados com a vegetação encontrada em águas estagnadas ou de baixo fluxo. São conhecidos pelo comportamento do macho, pois este cuida dos ovos sem a cooperação da fêmea (PEREIRA et al., 1993; SMITH, 1997; TALLAMY, 2000; PAPESCHI; BRESSA, 2006; INADA et al., 2011).

Figura 1. Belostomatidae: *Abedus indentatus* macho com ovos depositados em seu dorso. (https://en.wikipedia.org/wiki/Belostomatidae)

Os insetos pertencentes à família **Gelastocoridae** (Figura 2), possuem hábito de vida semi-aquático, e são encontrados nas margens dos corpos d'água doce onde o solo é constantemente encharcado (BORROR et al., 1979; PEREIRA et al., 2007). As espécies desse gênero possuem hábito alimentar predador generalista, capturando pequenas presas que se encontram nas margens dos corpos d'água. Como não são adaptados à vida aquática, os gelastocorídeos evitam a submersão (MERRITT; CUMMINS, 1984).

Figura 2. Gelastocoridae: *Gelastocoris oculatus* (http://bugguide.net/node/view/361953/bgpage)

Heteroptera aquáticos pertencentes à família **Gerridae** (Figura 3), são conhecidos como insetos de passadas largas, pois se movimentam sobre a água usando simultaneamente as pernas medianas e posteriores. Vivem na superfície das lagoas, de córregos lentos, de pântanos, e de outras águas paradas. Podem mover-se muito

rapidamente, até 1,5 m/s, na superfície da água. O comprimento é bastante variável, de 1,6 a 36 mm, possuem pernas compridas e a forma do corpo é muito próxima do arredondado. Eles apresentam dieta insetívora, alimentando-se de pequenos insetos que eventualmente caem sobre a água. Seus ovos são depositados sobre objetos flutuantes (BORROR; DELONG, 1988).

Figura 3. Gerridae: *Gerris lacustris*
(http://www.discoverlife.org/mp/20q?search=Gerridae&flags=glean)

Os insetos da família **Notonectidae** (Figura 4) são comumente conhecidos como nadadores de costas, por nadarem ou saírem em disparada sobre suas costas. Além disso, eles são caracterizados por possuírem pernas posteriores adaptadas para a natação (UESHIMA, 1979).

Figura 4. Notonectidae: *Notonecta glauca*
(http://www.discoverlife.org/mp/20q?search=Notonectidae&flags=glean)

As espécies das famílias **Veliidae** e **Mesoveliidae** (Figura 5) são Heteroptera pequenos possuindo de 2 a 3 milímetros (MCKINSTRY, 1937) e passam a maior parte de seu ciclo de vida sobre a superfície da água (ANDERSEN, 1982). São insetos predadores, alimentando-se, principalmente, de pequenos artrópodes que nadam ou que caem sobre a superfície da água (HUNGERFORD, 1919; UESHIMA, 1979). Certas espécies podem ser economicamente e clinicamente importantes, agindo como potenciais predadores de larvas de anofelinos (FRICK, 1949) e pragas (NAKASUJI; DYCK, 1984). Estima-se que muitas espécies permanecem ainda não descritas, especialmente na subfamília Microveliinae (Veliidae) (POLHEMUS; POLHEMUS, 2007). No Brasil foram descritas quatro novas espécies do gênero *Paravelia* e uma espécie de *Rhagovelia* (MOREIRA, 2012; RODRIGUES; MOREIRA, 2016), mostrando a grande importância de estudos de taxonomia nessa Familia.

Figura 5. Veliidae: R*hagovelia* sp. (http://bugguide.net/node/view/1161986/bgpage)

2. Morfologia Testicular

Com relação à morfologia testicular em Heteroptera poucos trabalhos são encontrados na literatura. Para espécies aquáticas a morfologia é variável podendo ser arredondadas para espécies das famílias Gerridae e Veliidae, arredondadas/espiraladas para as famílias Belostomatidae e Notonectidae (gênero *Buenoa)* ou alongadas/espiraladas para as famílias Gelastocoridae e Notonectidae (gênero *Martarega*). Na maioria das espécies de Heteroptera aquáticos a morfologia testicular é semelhante quando comparadas espécies da mesma família, sendo a única exceção até o momento para espécies da família Notonectidae, na qual pode apresentar testículos com morfologia arredondada/espiralada para o gênero *Buenoa* ou alongada/espiralada para o gênero *Martarega* (PEREIRA et al., 2015).

A investigação da pigmentação da bainha peritoneal que reveste os testículos e os lobos testiculares é uma característica ainda pouco explorada em Heteroptera. As espécies aquáticas das famílias Belostomatidae, Gelastocoridae, Gerridae, Notonectidae e Veliidae apresentam bainha peritoneal transparente (CASTANHOLE et al., 2008; PEREIRA et al., 2015). Já outras espécies terrestres descritas na literatura apresentam bainha avermelhada, como por exemplo, *Hyalymenus* sp. e *Neomegalotomus pallescens* (Alydidae) (SOUZA et al., 2009) ou amarelada como em *Zicca annulata* (Coreidae) (SOUZA et al., 2007a). Não há um padrão que possa ser correlacionado com a cor da bainha, como por exemplo, a família à qual a espécie pertence, pois, por exemplo, na família Coreidae, observou-se espécies com bainha avermelhada, amarelada ou sem coloração (PEREIRA et al., 2015).

Outra estrutura estudada em Heteroptera são os lobos testiculares. Segundo a literatura o número pode variar de um a sete. As espécies *Rhagovelia tenuipes* e *R. zela* (Veliidae, PEREIRA el al., 2015) apresentam apenas um lobo testicular, *Limnogonus aduncus, Rheumatobates crassifemur crassifemur* (Gerridae, CASTANHOLE et al., 2008; PEREIRA el al., 2015), *Gelastocoris angulatus* e *G. f. flavus* (Gelastocoridae, PEREIRA el al., 2015), *Buenoa amnigenus, B. unguis, Martarega brasiliensis, M. membranacea, M. uruguayensis* (Notonectidae, PEREIRA el al., 2015) dois lobos, *Mormidae v-luteum* (Pentatomidae, SOUZA et al., 2008) três,

Oebalus poecilus e *O. ypisilongriseus* (Pentatomidae, SOUZA et al., 2008), *Zicca annulata* e *Chariesterus armatus* (Coreidae, SOUZA et al., 2007a) quatro, *Belostoma anurum* e *B. micantulum* (Belostomatidae, PEREIRA el al., 2015) cinco, *Antitheuchus tripterus* (Pentatomidae, SOUZA et al., 2007b) seis, *Nysius californicus* (Lygaeidae, SOUZA et al., 2007c), *Anasa bellator*, *Athaumastus haematicus*, *Dallacoris obscura*, *D. pictus, Leptoglossus gonagra, L .zonatus e Sphictyrtus fasciatus* (Coreidae, SOUZA et al., 2007a) sete, sendo sete considerado o número ancestral (GROZEVA; KUZNETSOVA, 1992).

3. Aspectos Citogenéticos

O primeiro estudo citogenético em Heteroptera data de 1891 com Henking, que realizou a análise morfológica da espermatogênese na espécie *Pyrrhocoris apterus* Linnaeus (Pyrrhocoridae) (PAPESCHI; BRESSA, 2006). Os Heteroptera possuem como característica geral cromossomos holocêntricos, ou seja, sem centrômero localizado (BUCK, 1967; COMINGS; OKADA, 1972a; MOTZOKO; RUTHMAN, 1984; RUFAS; GIMÉNEZ-MARTÍN, 1986; WOLF, 1996); a atividade cinética restrita aos finais dos cromossomos, mais especificamente, nas regiões teloméricas, sendo então denominados cromossomos telocinéticos (MOTZOKO; RUTHMAN, 1984; SCHRADER, 1935, 1940; HUGHES-SCHRADER; SCHRADER, 1961; GONZÁLEZ-GARCIA et al., 1996), presume-se que ocorra a terminalização dos quiasmas (JOHN; KING, 1985), embora haja trabalhos que discutam esse assunto (JONES, 1987; SOLARI; AGOPIAN, 1987), e a primeira divisão meiótica é reducional para os autossomos e equacional para os cromossomos sexuais. No leptóteno-zigóteno o cromossomo X é positivamente heteropicnótico e está localizado na periferia do núcleo. Após o diplóteno, as células aumentam de tamanho e o núcleo torna-se semelhante ao estado interfásico, sendo este denominado de “difuso”. No final da diacinese o cromossomo X torna-se isopicnótico. Na metáfase I o cromossomo X localiza-se no centro do anel formado pelos autossomos bivalentes. Na anáfase I os autossomos bivalentes dividem-se reducionalmente enquanto o cromossomo X divide-se equacionalmente. A segunda divisão segue-se diretamente após a telófase I, sem os

estágios restantes. Na metáfase II os autossomos dispõem-se no plano equatorial formando uma configuração em anel, com o cromossomo X no centro. Após a telófase II, as células filhas formadas, terão como destino o processo de espermiação, pela diferenciação dos espermatócitos e elongação dessas células (BRESSA et al., 2002).

Os Heteroptera possuem, além dos autossomos e cromossomos sexuais, os m-cromossomos (microcromossomos) e os cromossomos B. O comportamento meiótico desses cromossomos é diferente (UESHIMA, 1979; MANNA, 1984; PAPESCHI; MOLA, 1990; GONZÁLEZ-GARCIA et al., 1996; SUJA et al., 2000). Como regra, autossomos bivalentes são quiasmáticos (exceto para poucas espécies das famílias Saldidae, NOKKALA; NOKKALA, 1983; Nabidae, NOKKALA; NOKKALA, 1984a; Miridae, NOKKALA; NOKKALA, 1986a; Anthocoridae, NOKKALA; NOKKALA, 1986b e Microphysidae, NOKKALA; GROZEVA, 2000), e os bivalentes em forma de bastão orientam-se axialmente e segregam-se pré-reducionalmente. Por outro lado, os cromossomos sexuais são aquiasmáticos e comportam-se como univalentes na meiose dos machos (UESHIMA, 1979; GROZEVA; NOKKALA, 2001).

Wilson (1905) introduziu o termo m-cromossomo para descrever o pequeno par de cromossomos em Hemiptera com comportamento diferente dos autossomos e dos cromossomos sexuais durante a meiose. Em Heteroptera, os m-cromossomos foram primeiramente descritos nos Coreidae por Paulmier (1899). Subsequentemente foram encontrados em outras espécies das infraordens Nepomorpha, Leptopodomorpha e Pentatomomorpha (UESHIMA, 1979; GROZEVA; KUZNETSOVA, 1989). Os cromossomos B são reconhecidos como tais, pois aparecem em alguns indivíduos, em algumas populações, de uma determinada espécie causando polimorfismo cromossômico. Eles são supranumerários ou membros adicionais de um grupo cromossômico, não essencial para a célula ou indivíduo, mas, possivelmente prejudicial, pelo menos se presente em grande número. Cromossomos B foram encontrados em inúmeras espécies de plantas e animais em uma diversidade de grupos. Eles formam um grupo altamente heterogêneo, podem ser de vários tamanhos e variar grandemente no conteúdo de heterocromatina e estabilidade mitótica, e seus efeitos podem ser benéficos, prejudiciais ou neutros. Vários autores consideram os cromossomos B como parasitas ou egoístas (NOKKALA; NOKKALA, 2004).

O número de autossomos em Heteroptera varia de quatro (Belostomatidae) a 80 (Miridae), mas esses números não são típicos para a subordem (UESHIMA, 1979). É difícil dizer qual é o número cromossômico modal para a subordem inteira, pois todas as infraordens (Cimicomorpha, Dipsocoromorpha, Enicocephalomorpha, Gerromorpha, Leptopodomorpha, Nepomorpha e Pentatomorpha) dentro dos Heteroptera não foram estudadas citogeneticamente na mesma extensão (GROZEVA; NOKKALA, 1996).

Com relação aos sistemas cromossômicos do sexo há os simples XY/XX (74,7% das espécies) e X0/XX (14,8%) e os múltiplos (originados por fragmentação do cromossomo X e, menos frequentemente do Y, X_n0/X_nX_n, X_nY/X_nX_n e XY_n/XX, 10,3%) (UESHIMA, 1979; MANNA, 1984). Há, ainda, um sistema particular neo-XY (0,2%) (CHICKERING; BACORN, 1933; SCHRADER, 1940; JANDE, 1959).

Espécies da família **Belostomatidae** não possuem o par de m-cromossomos, o sistema cromossômico do sexo pode ser XY, X_nY ou o sistema neo-XY e o número modal de cromossomos para esta família é de 2n= 26 (PAPESCHI et al., 2006). Com relação à família **Gelastocoridae** somente uma espécie foi analisada citogeneticamente, *Gelastocoris oculatus*, com 2n= 30A + $X_1X_2X_3X_4Y$, não possuindo, portanto, m-cromossomos (UESHIMA, 1979).

A família **Gerridae** é caracterizada por possuir sistema cromossômico do sexo X0, disposição cromossômica em anel na metáfase meiótica e ausência de m-cromossomos. Apresenta, ainda, cromossomos holocêntricos, divisão pré-reducional dos autossomos e pós-reducional dos cromossomos sexuais. O número cromossômico modal é de 21 (20A + X0) ou 23 (22A + X0) (UESHIMA, 1979).

Nas espécies pertencentes à família **Notonectidae**, o gênero *Anisops* (Anisopinae) é caracterizado por sistema cromossômico do sexo X_1X_20 e apresenta um par de m-cromossomos, sendo o número diplóide de cromossomos de 26 para o macho e 28 para a fêmea. Espécies do gênero *Notonecta* (Notonectidae) analisadas possuem sistemas cromossômicos do sexo X0 e XY e 2n= 24 (20A+2m+XY) ou 26 cromossomos (22A+2m+XY) (UESHIMA, 1979).

As espécies da família **Veliidae**, *Hebrovelia* sp. e *Microvelia reticulata*, apresentam 2n= 21 cromossomos (20A+X0) (COBBEN, 1968), e as espécies *Velia*

currens (POISSON, 1936) e *V.* sp. (UESHIMA, 1979) 2n= 25 (24A+X0). Apesar de todas as espécies terem sido descritas apresentando sistema cromossômico do sexo X0, Takenouchi e Muramoto (1971) reportaram o sistema cromossômico do sexo XY para *Microvelia douglasi*, mas há necessidade de confirmação (UESHIMA, 1979).

4. Aspectos Ultraestruturais da Espermatogênese

Os espermatozoides são células geralmente pequenas, compactadas e altamente especializadas na fertilização do óvulo. São otimizados para transferir o material genético paterno para o óvulo, processo essencial na transmissão da hereditariedade e posterior desenvolvimento do organismo. Caracterizam-se por serem células dotadas de um flagelo, cujo papel principal é impulsioná-los através do meio fluido. É uma célula altamente complexa e tem passado por diversas modificações morfológicas ao longo dos processos evolutivos (BACCETTI; AFZELIUS, 1976).

A espermatogênese é o processo de diferenciação celular que ocorre nos túbulos seminíferos, esse processo é sincrônico e regular onde uma espermatogônia tronco é gradativamente diferenciada numa célula haplóide altamente especializada, o espermatozóide (JOHNSON, 1991; FRANÇA; RUSSELL, 1998).

Essa diferenciação envolve três classes de células germinativas: as espermatogônias que são células indiferenciadas, os espermatócitos e as espermátides. Nos adultos a espermatogênese é um processo contínuo que pode ser dividido em duas fases: a meiótica e a espermiogênese (COSTA; PAULA, 2003).

A fase meiótica envolve síntese de DNA nos espermatócitos em pré-leptóteno, síntese de RNA em espermatócitos em paquíteno e no final da meiose, quando vai ocorrer a divisão reducional, esses últimos geram espermátides haplóides (PARVINEN et al., 1991). Na fase da espermiogênese, as espermátides se diferenciam através de uma série de modificações morfológicas progressivas em espermatozoides altamente especializados. Estas modificações incluem desenvolvimento do acrossoma, condensação e alongamento do núcleo e formação da cauda espermática (RUSSELL et al., 1990).

Com relação às características ultraestruturais da espermatogênese dos Heteroptera já foram descritas algumas ultraestruturas como o acrossomo, o axonema, as mitocôndrias, o complexo mitocondrial (Nebenkern), os derivados mitocondriais, e o nucléolo em algumas espécies como *Gerris najas* (Gerridae) (WERNER; WERNER, 1993), *Notonecta glauca* (Notonectidae) (WERNER et al., 1988), *Euchistus heros* (FERNANDES et al., 2001), em espécies da família Pentatomidae (TRANDABURU, 1973; FERNANDES; BÁO, 1998; ARAUJO et al., 2011), Reduviidae (DOLDER, 1988; BÁO; DESOUZA, 1994), em Heteroptera aquáticos (AFZELIUS et al., 1985; LEE, 1991; LEE; LEE, 1992), e em insetos pertencentes a infraordem Leptopodomorpha (AFZELIUS et al., 1976), Cimicomorpha, Gerromorpha e Pentatomomorpha (DALLAI; AFZELIUS, 1980; ARAUJO et al., 2011).

Na maioria das espécies o **acrossomo** (Ac) é uma organela fundamental para fecundação (PHILLIPS, 1970). Além da fecundação, o acrossomo também está relacionado com a capacitação do espermatozoide onde suas enzimas podem funcionar para remover o mecanismo de proteção na superfície do espermatozoide e/ou na digestão de barreiras para a migração do espermatozoide dentro do trato reprodutor da fêmea (FAWCETT; ITO, 1965), sendo sua origem a partir do complexo de Golgi (YASUZUMI, 1974; BÁO et al., 1989).

Durante a espermiogênese é observado o aparecimento da vesícula pró-acrossomal, que determina o polo anterior da célula, estando localizada anteriormente ao núcleo, no espermatozoide (STANLEY, 1971; AZEVEDO et al., 1985; BÁO et al., 1989; ARAUJO et al., 2011). As enzimas acrossomais são, geralmente, armazenadas na forma de pró-enzimas e quando ocorre a reação acrossomal são liberadas e ativadas, participando, assim, do evento da fertilização (YANAGIMACHI, 1988; BACCETTI et al., 1989; TESARIK et al., 1990; GALLO et al., 1991).

Na literatura há poucos trabalhos que discutem a origem e o conteúdo do acrossomo, em Heteroptera. Essa estrutura foi analisada em *Gerris najas* (Gerridae) (WERNER; WERNER, 1993), *Notonecta glauca* (Notonectidae) (WERNER et al., 1988) e *Euchistus heros* (FERNANDES et al., 2001), onde foram observadas vesículas acrossomais formadas a partir do complexo de Golgi que, posteriormente, formarão o acrossomo, que com o alongamento da espermátide, determina o pólo anterior dessa.

Foram observadas mudanças nucleares onde o desenvolvimento do núcleo envolve mudanças na forma e no grau de condensação da cromatina. Inicialmente, a cromatina encontra-se dispersa e com uma eletrodensidade baixa, e ao decorrer da espermatogênese, assume um arranjo fibrilar, compacta e de alta eletrodensidade.

O estudo da organização estrutural do **axonema** (Ax) recebeu valiosas contribuições provenientes das inúmeras observações realizadas no flagelo do espermatozoide de *Drosophila* (PEROTTI, 1969; KIEFER, 1970; DALLAI; AFZELIUS, 1991). Na maioria dos insetos o padrão organizacional do axonema segue o esquema 9+9+2, que é o arranjo usual de 9+2 microtúbulos circundados por nove microtúbulos acessórios adicionais (PHILLIPS, 1970; WARNER, 1971; BACCETTI, 1972; FERNANDES, 1999). Em alguns mosquitos é comum o esquema 9+9+1, enquanto em efeméridas predomina o esquema 9+9+0 (PHILLIPS, 1970). Existem, ainda, esquemas que são considerados aberrantes, como por exemplo, em dípteros cecidomídeos em que o esquema é 13+0 (BACCETTI; DALLAI, 1976). Araujo et al. (2011) descreveram um padrão de cauda para algumas espécies da família Pentatomidae, onde o flagelo é composto de um axonema e dois derivados mitocondriais, o axonema segue o padrão de arranjo de microtúbulos de 9+9+2 (9 microtúbulos acessórios, 9 subtúbulos e 2 microtúbulos centrais). O autor descreve, também, que o axonema é cercado por uma membrana cisterna que na maturação do espermatozoide se reduz a duas pequenas cisternas de lados opostos ao axonema aderindo-o aos derivados mitocondriais. Entre os Heteroptera, a ultraestrutura de espermatozoides foi descrita em espécies da família Pentatomidae (TRANDABURU, 1973; FERNANDES; BÁO, 1998), Reduviidae (DOLDER, 1988; BÁO; DESOUZA, 1994), em Heteroptera aquáticos (AFZELIUS et al., 1985; LEE, 1991; LEE; LEE, 1992), e em insetos pertencentes a infraordem Leptopodomorpha (AFZELIUS et al., 1976), Cimicomorpha, Gerromorpha e Pentatomomorpha (DALLAI; AFZELIUS, 1980; ARAUJO et al., 2011) onde foi observado um padrão flagelar de 9+9+2. O padrão de localização dos derivados mitocondriais estando dispostos bilateralmente em relação ao axonema foi semelhante para todas essas famílias.

As **mitocôndrias** durante a espermiogenese assumem diferentes morfologias. Em insetos, a regularidade nas formas da mitocôndria é particularmente

notável (PHILLIPS, 1970). No decorrer da espermiogênese as mitocôndrias sofrem metamorfose, onde a estrutura típica da mitocôndria é completamente modificada. Nos estádios iniciais de diferenciação das espermátides ocorre um complexo processo de fusão e rearranjo das mitocôndrias formando o complexo mitocondrial que é frequentemente denominado "Nebenkern", especialmente em trabalhos mais antigos (PRATT, 1970; PHILLIPS, 1970; TANDLER; HOPPEL, 1972; BACCETTI, 1972; BACCETTI; AFZELIUS, 1976). Com o prosseguimento da espermatogênese, ocorre a divisão do complexo mitocondrial em dois derivados mitocondriais (DM), que no processo de alongamento da espermátide posicionam-se bilateramente em relação ao axonema (TOKUYASU, 1974).

Durante o processo de diferenciação os derivados mitocondriais são preenchidos ao longo de sua extensão por uma estrutura de natureza proteica, organizada num padrão paracristalino, e muitas espécies de insetos acumulam essas estruturas nas mitocôndrias durante a espermiogênese (PHILLIPS, 1970; WARNER, 1971; ROSATI et al., 1976; BÁO et al., 1992). Essa estrutura paracristalina, também conhecida como cristalóide, é formado por uma proteína rica em prolina, que foi designada de cristalomitina (BACCETTI et al., 1977). Em Heteroptera (famílias Gerridae, Pentatomidae e Reduviidae) foi evidenciado a presença de dois ou três corpos cristalinos no interior dos derivados mitocondriais (ARAUJO, 2011).

Várias são as funções sugeridas aos derivados mitocondriais dos espermatozoides de insetos, podendo participar no controle e regulação da forma do movimento flagelar e estando relacionado com o processo de estocagem e liberação de energia necessária para a motilidade flagelar (PHILLIPS, 1974; YASUZUMI, 1974; TOKUYASU, 1975). No entanto, Perotti (1973) contraria esta última afirmação, supondo que o material armazenado nos DM podem estar envolvidos na ativação e nutrição do ovócito após a fertilização.

As células germinativas possuem em seu citoplasma o acúmulo de um material denominado **corpo cromatóide** (CB) ou "nuage" (PARVINEN, 2005). O CB é considerado um complexo macromolecular que, provavelmente, desempenha um papel de coordenador do controle pós-transcricional de produtos gênicos em células germinativas masculinas haploides e, também, funciona como um centro de

determinação dos destinos de RNAm (PARVINEN, 2005; KOTAJA et al., 2006; KOTAJA; SASSONE-CORSI, 2007).

Alguns autores acreditam que o CB origina-se a partir de um material existente entre aglomerados de mitocôndrias, presente no citoplasma das células germinativas (FAWCETT et al., 1970). No entanto, trabalhos mais recentes defendem a origem do CB a partir da fragmentação do material nucleolar durante o processo de espermatogênese (COMINGS; OKADA, 1972; ANDERSON, 1978; ANDONOV, 1990; PERUQUETTI et al., 2008; PERUQUETTI et al., 2010; PERUQUETTI et al., 2011). Portanto, ainda existem muitas dúvidas em relação à origem e função do corpo cromatóide.

Outra estrutura que vem sendo estudada em Heteroptera é o **nucléolo** e outras estruturas que são impregnadas pelos íons prata. O tamanho do nucléolo está relacionado com a atividade biossintética da célula, portanto, o tamanho e o número de nucléolos e corpos pré-nucleolares dependem das características funcionais das células e podem refletir, então, em diferenças metabólicas e funcionais (TAVARES; AZEREDO-OLIVEIRA, 1997; SOUZA et al., 2007a,b).

Na maioria das espécies de artrópodos, os nucléolos dissociam-se no diplóteno ou diacinese. Portanto, os corpúsculos impregnados pela técnica Ag-NOR, específica para proteínas associadas ao RNAr, não são visíveis da metáfase I à telófase I. Essas marcações vão reaparecer no início da formação das espermátides, indicando o reinício das funções transcricionais de RNA ribossomal e, finalmente, desaparecem no final da formação das espermátides (BRESSA et al., 2003). Entretanto, a literatura relata algumas exceções como, por exemplo, em *Asellus aquaticus* (isopoda), em que os corpúsculos Ag-NOR foram visualizados durante todo o processo de espermatogênese (DI CASTRO et al., 1983), em *Callicrania seoanei* (Orthoptera), da intercinese até a pró-metáfase II (SANTOS et al., 1987) e em *Triatoma infestans* e *T. sordida* (Heteroptera, Reduvidae), presentes até a metáfase I (TAVARES; AZEREDO-OLIVEIRA, 1997). Finalmente em *Carlisis wahlbergi* (Heteroptera, Coreidae) os corpúsculos Ag-NOR foram observados até a metáfase II, sendo denominado de semi-persistência nucleolar (FOSSEY; LIEBENBERG, 1995), enquanto em *Acanthocoris sordidus* (Heteroptera, Coreidae) e *Coptosoma punctissimum* (Heteroptera, Plataspidae),

os nucléolos foram detectados nas placas metafásicas de espermatócitos primários e secundários (YOSHIDA, 1947).

Em Heteroptera aquáticos temos a descrição do comportamento nucleolar em *Limnogonus aduncus* (Gerridae) sendo encontrada marcações em autossomos, e um corpúsculo nucleolar em células em fase de anáfase, na espermatogênese observa-se os corpúsculos nucleolares em, praticamente, todas as fases (CASTANHOLE et al., 2008) e em *Brachymetra albinerva*, *Halobatopsis platensis*, *Cylindrostethus palmaris* (Gerridae) foi observado células com um ou vários corpúsculos, que ao se desorganizarem foi observado ao redor da bainha pericromossômica no final da metáfase I. As espermátides arredondadas apresentaram dois corpúsculos próximos um ao outro, mas que com o alongamento um desses corpúsculos permanece na região da cabeça e o outro migra para a parte inicial da cauda. Ao final do processo de espermiogênese essas marcações não foram mais visualizadas (CASTANHOLE et al., 2010).

Diante dessas informações a nossa hipótese é que a partir da análise ultraestrutural dos testículos dos Heteroptera possamos caracterizar todas as etapas e estruturas durante a espermatogênese.

II. OBJETIVOS

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Objetivo Geral

O objetivo geral do presente projeto foi de analisar a espermatogênese de espécies das famílias Belostomatidae, Gerridae, Mesoveliidae, Notonectidae e Veliidae (Heteroptera) por meio de análises ultraestruturais.

Objetivos Específicos

a) Avaliar o comportamento nucleolar durante a espermatogênese;

b) Avaliar o comportamento mitocondrial durante a espermatogênese;

c) Analisar a ultraestrutura do acrossomo, axonema, corpo cromatóide, complexo mitocondrial e derivados mitocondriais;

d) Comparar a espermatogênese das espécies das diferentes famílias analisadas e associar os resultados com as famílias ou habitat.

III. CAPÍTULOS

ARTIGO I

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Study of nucleolar behavior during spermatogenesis in *Martarega brasiliensis* (Heteroptera, Notonectidae)

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ABSTRACT. Few cytogenetic studies have been undertaken using aquatic heteropterans and the nucleolar behavior of these insects has been described in only four species, *Limnogonus aduncus*, *Brachymetra albinerva*, *Halobatopsis platensis*, and *Cylindrostethus palmaris*. The nucleolus is a cellular structure related to biosynthetic

Study of nucleolar behavior during spermatogenesis of *Martarega brasiliensis* (Heteroptera, Notonectidae)

Running title: **Nucleolar behavior of *Martarega brasiliensis***

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ABSTRACT

Cytogenetic studies in aquatic heteropteran are fairly low. The nucleolar behavior of these insects was described in only four species, out more, *Limnogonus aduncus*, *Brachymetra albinerva*, *Halobatopsis platensis*, *Cylindrostethus palmaris*. The nucleolus is a cellular structure related to the biosynthetic activity and presents a peculiar behavior in the heteropteran of Triatominae subfamily, persisted during all stages of meiosis. Thus, this study aims to analyze the spermatogenesis of *Martarega brasiliensis*, with emphasis on nucleolar behavior. Were used 20 adult males of *M. brasiliensis*, collected in Municipal reservoir the city of São José do Rio Preto, Sao Paulo, Brazil. The species were fixed in methanol: acetic acid (3:1) and then dissected,

the testicles were extracted, torn apart and impregnated with silver ions. *M. brasiliensis* showed nuclei in prophase composed by the nucleolus and nucleolar corpuscles that varied from one to four, emphasizing that this insect has great synthetic activity during meiosis. The analysis of cells in metaphase I showed that *M. brasiliensis* presents nucleolar organizing region (NOR) in at least one autosome. Furthermore, was not observed the phenomenon of nucleolar persistence. All spermatids presented nucleolar markings that varied in number and position according to the stage of elongation. Moreover, was also possible highlight the presence of a vesicle in spermatids. Thus, this paper describes the nucleolar behavior of *M. brasiliensis* and highlights important characteristics during spermatogenesis, increasing thus the knowledge about the biology of these aquatic heteropteran

Keywords: Aquatic Heteroptera; Spermiogenesis; Meiosis

INTRODUCTION

The Order Hemiptera is composed of three suborders: Auchenorrhyncha, Heteroptera and Sternorrhyncha (Cryan and Urban, 2012). The suborder Heteroptera has seven infra-orders with about 80 families, most of which occurs in all continents (except Antarctica) and some islands.

The heteropteran exhibit great variation in occupancy of habitats can be found in terrestrial environments, aquatic or semi-aquatic and in form of feeds, may be phytophagous, hematophagous or predators (Gullan and Cranston, 2008). Thus, these insects have great economic importance (when agricultural pests) and epidemiological (as vectors of Chagas disease). Moreover, these insects are impotantes models for cellular studies because they present some peculiarities as holocentric chromosomes and inverted meiosis for sex chromosomes (Ueshima, 1979).

Aquatic and semi-aquatic heteropteran are widely distributed and surprised by his extraordinary ability to inhabit a variety of ecosystems, being found in habitats of freshwater and marine, and varied altitude range between 0 and 4,700 m (Melo, 2009).

Cytogenetic studies in aquatic heteropteran are greatly reduced (Castanhole et al., 2008, 2010). The nucleolar behavior of these insects is known only for the species *Limnogonus aduncus*, *Brachymetra albinerva*, *Halobatopsis platensis*, *Cylindrostethus palmaris* (Castanhole et al., 2008, 2010). The nucleolus is a cellular structure related to the biosynthetic activity, therefore, the size and number of nucleoli and nucleolar corpuscles present in the nucleus depends on the functional characteristics of the cells and may then reflect on metabolic and functional differences (Tavares and Azevedo-Oliveira, 1997; Souza et al., 2007a, b; Alevi et al., 2013).

In eukaryotes, during spermatogenesis the nucleolus dissociates at the end of prophase and reorganizes in telophase (González-Garcia and Rufas 1995). However, in the heteropteran of Triatominae subfamily reports exist of the persistence of the nucleolus or nucleolar corpuscles during the whole meiosis, a phenomenon termed as nucleolar persistence (Tartarotti and Azeredo-Oliveira, 1999; Alevi et al., 2013, 2014a).

Thus, this study aims to analyze the spermatogenesis of *Martarega brasiliensis*, with emphasis on nucleolar behavior.

MATERIAL AND METHODS

Were used 20 adult males of *M. brasiliensis*, collected in Municipal reservoir the city of São José do Rio Preto, Sao Paulo, Brazil, with the aid of net and transported in pots, to the Laboratory of Cytogenetics and Molecular Genetics of Insects (LACIMI) of UNESP / IBILCE. The species were fixed in methanol: acetic acid (3:1) and then dissected being the testicles extracted, torn apart and impregnated with silver ions seconds Howell and Black (1980). The best images were captured in the microscope ZEISS Axio Scope A1 using the program for the analysis of images AXIO VISION LE version 4.8.

RESULTS

Through analysis cytochemical by impregnation by silver ions was observed nucleolar behavior during meiosis (Figure 1a-f and 2a-h). During prophase I, beyond the nucleolus with rounded morphology (Figure 1a-e, arrowhead), have been observed nucleolar corpuscles arranged in the cell nuclei that ranged from one to four (Figure 1b-e, arrows). Cells in metaphase I presented markings of nucleolar organizer regions (NOR) in the telomeric region of one of the autosomes (Figure 1 f, arrow). In cells in anaphase I have been observed various corpuscles with different sizes and shapes (Figure 2a, arrows), and late migration of the sex chromosome (Figure 2a, arrowhead). Cells in telophases I presented up to two marks (Figure 2b, arrows). Regarding spermiogenesis, spermatids at early stages were rounded and presented nucleolar marking only in the region beginning of the formation of the tail (Fig. 2c, arrow). During the elongation of spermatids was observed the presence of nucleolus (Figure 2d, arrow) and nucleolar corpuscles (Figure 2d, asterisks). Furthermore, was observed the presence of a large vesicle and rounded at the anterior region of the head of early spermatids (Figure 2c-e, arrowheads). The spermatids rod shaped presented two corpuscles (Figure 2f, asterisks), discrete impregnations in the head (Figure 2f, arrow) and a tag dividing the spermatid longitudinally (Figure 2f, arrowhead). In the final phase observed spermatids elongated rod shaped with persistence of nucleolar corpuscles (Figure 2g, arrow) and elongated spermatids with marking in the anterior region of the head (Figure 2h, arrow).

DISCUSSION

The *Martarega* genus belonging to the family Notonectidae, are popularly known as swimmers from back, because they have rear legs adapted for swimming (Ueshima, 1979).

During meiosis of *M. brasiliensis*, we observed the nucleologenesis this insect. This Heteroptera showed nuclei in prophase I composed by the nucleolus and nucleolar corpuscles that varied from one to four. This variation in the number of nucleolar corpuscles is related to the synthetic activity of the cells (Tavares and Azeredo-Oliveira, 1997, Bressa et al., 2003). Alevi et al. (2013) analyzed the nucleolar markings of two species of triatomine bugs (*Triatoma lenti* and *T. melanocephala*) and found that the

species with the highest number of markings showed great synthetic activity. Thus, it is possible to observe that *M. brasiliensis* has great synthetic activity during meiosis. However, we emphasize that this variation in the number of nucleolar corpuscles may be related to transcriptional need of the cell during cell division.

Although the technical impregnation by silver ions is applied extensively to studies of NORs (Howell and Black, 1980), the nature holocentric of the chromosomes of hemipterans makes it difficult the marking of NORs. The technique of silver ions mark the NORs that were active in the previous interphase, thus by analyzing the cells in metaphase I we observed that *M. brasiliensis* presents RON at least one autosome. This feature was also observed in the chromosomes of *L. aduncus* (Castanhole et al., 2008).

The phenomenon of nucleolar persistence is characterized by the presence of the nucleolus or nucleolar corpuscles in metaphase (Alevi et al., 2014a). *M. brasiliensis* showed no nucleolar persistence because during metaphase I was observed only the presence of RON. This same feature was also observed for *L. aduncus* (Castanhole et al., 2008). The presence of nucleolar corpuscles during the phases of meiosis have been characterized in other insects as of the Order Hemiptera: *Carlisis wahlbergi* (Fossey and Liebenberg, 1995) *Acanthocoris sordidus, Coptosoma punctissimum* (Yoshida, 1947) and 21 species of the Triatominae subfamily (Alevi et al. 2014a). Alevi et al. (2014a) propose that the nucleolar persistence may be a synapomorphy of the subfamily Triatominae.

During spermiogenesis, all spermatids presented nucleolar marks that varied in number and location in accordance with the phase of elongation. The presence of nucleolar corpuscles during all stages of spermatogenesis had already been observed in aquatic Heteroptera (Castanhole et al., 2008). In species *B. albinerva*, *H. platensis* and *C. Palmaris* were observed cells with one or more corpuscles that during the disorganization turning around of the pericromosomic sheath. The round spermatids presented two close corpuscles during stretching corpuscles remains in the head and the other migrates to the initial part of the tail. At the end of spermiogenesis process these markings were not visualized (Castanhole et al., 2010).

Although nucleolar marks were observed in spermatids of *M. brasiliensis*, recently Alevi et al. (2014b) suggested that during spermiogenesis the cells hemipterans not have transcriptional activity. The authors highlight the relationship of nucleolar markings with regions of constitutive heterochromatin and suggest that the nucleolus is inactivated by epigenetic factors and that the organelle chromatoid body supplies all transcriptional activity during cell differentiation. Thus, we emphasize the importance of further studies with specific epigenetic markers to discuss the nucleolar material observed in *M. brasiliensis* has transcriptional activity.

A peculiarity observed during spermiogenesis of *M. brasiliensis* is the presence of a vesicle in spermatids, not observed in terrestrial species (Souza et al., 2007a, b, 2008, 2009) and aquatic (Castanhole et al., 2008, 2010) studied. Thus, we emphasize that ultrastructural studies are needed to describe this structure in more detail in order to clarify its relationship with the formation of sperm.

Thus, this paper describes the nucleolar behavior of *M. brasiliensis* and highlights important characteristics during spermatogenesis, increasing thus the knowledge about the biology of these aquatic heteropteran

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REFERENCES

Alevi KCC, Castro NFC, Lima ANC, Ravazi A, et al. (2014a). Nucleolar persistence during spermatogenesis of the *Rhodnius* (Hemiptera, Triatominae). *Cell. Biol. Int.* 38: 977-980.

Alevi KCC, Mendonça PP, Pereira NP, Rosa JA, et al. (2013). Análise das possíveis Regiões Organizadoras Nucleolares e da atividade nucleolar em *Triatoma melanocephala* e *T. lenti*, importantes vetores da doença de Chagas. *Rev. Ciênc. Farm. Básica Apl.* 34: 417-421.

Alevi KCC, Mendonça PP, Pereira NP, Rosa JA, et al. (2014b). Is there post-meiotic transcriptional activity during hemiperan spermiogenesis? *Inv. Rep. Dev.* DOI: 10.1080/07924259.2014.889767.

Bressa MJ, Fumagalli E, Ituarte S, Frassa MV, et al. (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 MMU, Pereira LLV, Bicudo HEMC, Costa LAA, et al. (2008). Heteropicnotic chromatin and nucleolar activity in meiosis and spermigenesis of *Limnogonus aduncus* (Heteroptera, Gerridae): a stained nucleolar organizing region that can serve as a model for studying chromosome behavior. *Gen. Mol. Res.* 7: 1398-1407.

Castanhole MMU, Pereira LLV, Souza HV, Itoyama MM (2010). Spermatogenesis and karyotypes of three species of water striders (Gerridae, Heteroptera). *Gen. Mol. Res.* 9: 1343-1356.

Cryan JR, Urban JM (2012). Higher-level phylogeny of the insect order Hemiptera: is Auchenorrhyncha really paraphyletic? *Syst. Ent.* 37: 7-21.

Fossey A, Liebenberg H (1995). Meiosis and nucleolar structures in the stink bug *Carlisis wahlbergi* Stal (Coreidae: Heteroptera). *Cytobios* 81: 7-15.

González-Garcia JM, Rufas JS (1995). Nucleolar cycle and localization of NORs in early embryos of *Parascaris univalens*. *Chromosoma* 104: 287–297.

Gullan PJ, Cranston PS (2008). Os insetos: um resumo de entomologia. 3rd ed. Roca, São Paulo.

Howell WM, Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* 36: 1014-15.

Melo MC (2009). Biodiversidad de Heteroptera (Hemiptera) acuáticas y semiacuáticas de la Patagonia argentina. *Rev. Soc. Entomol. Argent.* 68: 177–185.

Souza HV, Arakaki RLM, Dias LN, Lima ASM, et al. (2007a). Cytogenetical Aspects of Testicular Cells in Economically Important Species of Coreidae Family. *Cytologia* 72: 49-56.

Souza HV, Bicudo HEMC, Itoyama MM (2007b). Study of chromosomal and nucleolar aspects in testes of Nysius californicus (Heteroptera:Lygaeidae). *Gen. Mol. Res.* 6: 33-40.

Souza HV, Caatanhole MMU, Bicudo HEMC, Costa LAA, et al. (2008). Morphological patterns of the heteropycnotic chromatin and nucleolar material in meiosis and spermiogenesis of some Pentatomidae (Heteroptera). *Gen. Mol. Biol.* 31: 686-691.

Souza HV, Souza FB, Maruyama SRC, Castanhole MMU, et al. (2009). Meiosis, spermatogenesis and nucleolar behavior in the seminiferous tubules of Alydidae, Coreidae and Rhopalidae (Heteroptera) species. *Gen. Mol. Res.* 8: 1383-1396.

Tartarotti E, Azeredo-Oliveira MTV (1999). Patterns of nucleolar activity during spermatogenesis of two triatomines, *Panstrongylus megistus* and *P. herreri*. *Caryologia* 52: 177-184.

Tavares MG, Azeredo-Oliveira MTV (1997). Pattern of Nucleolar Activity During Spermiogenesis In Triatomines (Heteroptera-Reduviidae) As Analysed By Silver-Staining. *Cytobios* 89: 93-103.

Ueshima N (1979). Hemiptera II: Heteroptera. In: Animal Cytogenetics. Vol. 3. Insecta, 6. Gebruder Borntraeger, Berlin, 1-117.

Yoshida TH (1950). Remarkable behavior of the nucleolus in meiosis of *Acanthocoris sordidus* Thumb. (Heteroptera) (in Japanese*). Kromosomo* 7: 273-282.

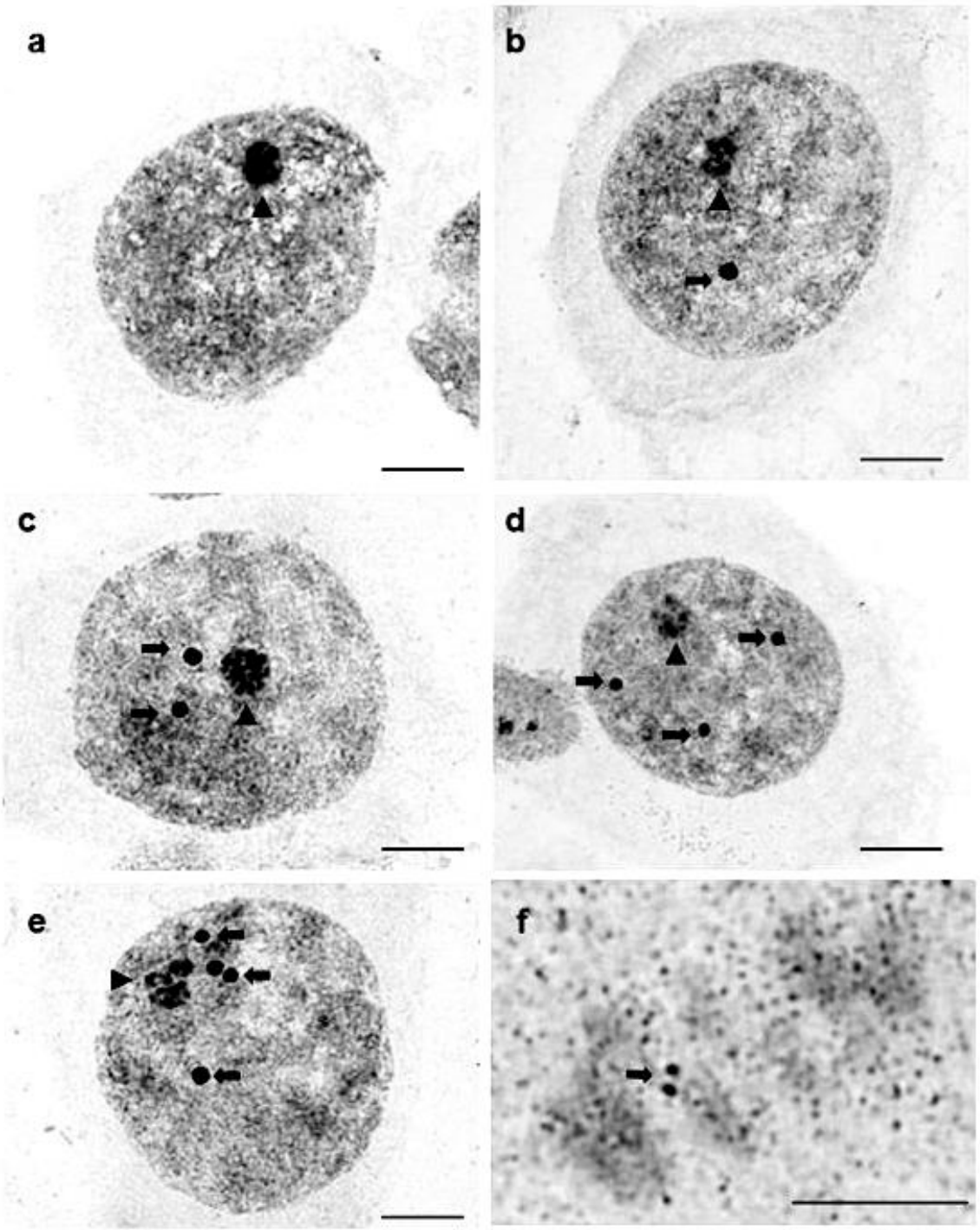

Figure 1. Cells of the seminiferous tubules of *M. brasiliensis*, impregnated by silver ions. a-e) Prophase I. Note the presence of rounded nucleolus (arrowhead) and nucleolar corpuscles (arrow) that varied in number: a) absence of nucleolar corpuscles; b) one corpuscle; c) two corpuscles; d) three corpuscles and e) four corpuscles. f) Metaphase I. Note the presence of nucleolar organizer regions (arrow) in the telomeric region of one autosome. Bar: 10 μm.

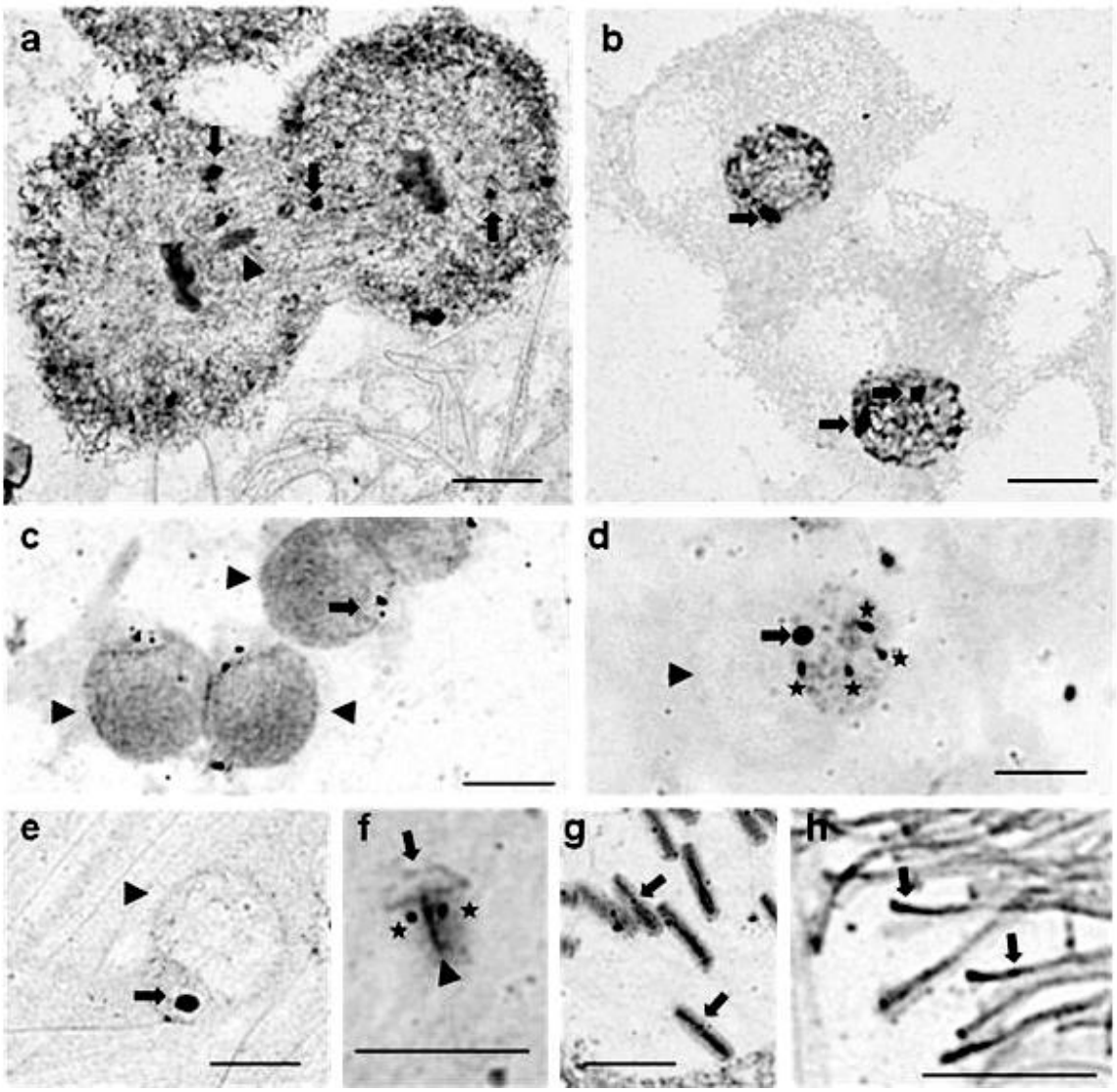

Figure 2. Cells of the seminiferous tubules of *M. brasiliensis*, impregnated by silver ions. a) Anaphase I with various corpuscles of different sizes and shapes (arrows) and late migration of the sex chromosome (arrowhead). b) Telophases with up to two marks (arrows). c) Early spermatids with marking in the posterior region (arrows) and vesicle in the anterior region of the head (arrowhead). d) Initial round spermatids with a nucleolus (arrow) and four nucleolar corpuscles (asterisk). Note the presence of large and rounded vesicles (arrowhead). e) Elongating spermatids with marking in the posterior region (arrow) and vesicles in the anterior head (arrowhead); f) spermatids rod shaped presented two corpuscles (asterisks), discrete impregnations in the head (arrow) and a tag dividing the spermatid longitudinally (arrowhead). g) spermatids elongated rod shaped with persistence of positive silver corpuscles (arrow); h) elongated spermatids with marking in the head region (arrow). Bar = 10 micrometres

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Research Article Open Access

Histological and Ultrastructural Analysis of Spermatogenesis in *Gelastocoris flavus flavus* (Heteroptera: Nepomorpha)

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Abstract

Studies on the ultrastructural aspects of spermatogenesis and, specifically, the structure of sperm in aquatic Heteroptera are scarce. Therefore, the objective of this study was to analyse of the histology and ultrastructure of spermatogenesis. Semi-fine sections of the testicles of adult male *Gelastocoris flavus flavus* were stained with toluidine blue or impregnated with silver ions, and ultra-fine sections were analysed by transmission electron microscopy. The ultrastructural features observed during spermatogenesis of the species showed the presence of several small mitochondria uniformly distributed in the cytoplasm of cells in prophase I. These mitochondria then came together to form fewer, larger structures, which converged and formed the mitochondrial complex. Later, this mitochondrial complex was divided into two structures, termed mitochondrial derivatives, which were arranged bilaterally to the axoneme. The axoneme showed a flagellar pattern of 9+9+2. A vesicle was observed that originated in the early stages of spermiogenesis and was composed of many argyrophilic granules that united to form a single structure. This vesicle contained some highly stained structures in its interior. Thus, this paper describes histological and ultrastructural characteristics during spermatogenesis, contributing to the reproductive knowledge of these aquatic Heteroptera.

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Keywords: argyrophilic granules, mitochondrial derivative, axoneme, nucleolus

Introduction

In both vertebrates and invertebrates, studies addressing the ultrastructural aspects of spermatogenesis and, specifically, the structure of the sperm comprise a vast and growing body of literature. In insects, the study of the ultrastructural aspects of spermiogenesis and sperm began in the 1970s, when the methods for electron microscopy of biological material were optimised [1, 2, 3], but in Heteroptera, studies are still scarce. Some ultrastructures involved in spermatogenesis, such as the acrosome, axoneme, mitochondria and chromatoid body, are described in the literature.

In most species, the acrosome (Ac) is an organelle essential for fecundation [1]. In addition to fecundation, the acrosome is also related to the sperm's ability to remove the protective mechanism on the surface of ovule and/or to digest barriers to the migration of sperm into the female reproductive tract [4]. Acrosomes originate from the Golgi complex [5, 6].

Research on the structural organisation of the axoneme (Ax) has received valuable contributions from numerous observations made in the sperm flagellum of *Drosophila* [7, 8, 9]. In most insects, the organisational pattern of the axoneme follows the pattern 9+9+2, which is the usual arrangement of 9+2 microtubules surrounded by nine additional accessory microtubules [1, 2, 10]. In some mosquitoes, the pattern 9+9+1 is common, whereas in mayflies, the pattern 9+9+0 predominates [1]. There are also patterns that are considered aberrant; for example, in dipterous of the Cecidomyiidae family, the schema is 13+0 [11].

Another ultrastructure often analysed during spermatogenesis is the mitochondria, which assume different morphologies. In insects, the regularity in the mitochondrial shape is particularly notable [1]. During spermatogenesis, the mitochondria undergo metamorphosis, where the typical structure of mitochondria is completely modified. In the initial stages of differentiation, a complex fusion process occurs, and mitochondria rearrange, forming the mitochondrial complex. This complex is often termed "Nebenkern," especially in older studies [1, 2, 12, 13, 14]. As spermatogenesis continues, the mitochondrial complex divides into two mitochondrial derivatives (MD), which are positioned bilaterally to the axoneme during the spermatozoid stretching process [15].

During differentiation, the mitochondrial derivatives are filled along their length by a protein structure that is organised in a paracrystalline pattern, and many species of insects accumulate these structures in mitochondria during spermiogenesis [1, 10, 16, 17]. This paracrystalline structure is also known as a crystalloid and is formed by a protein rich in proline [18]. In Heteroptera (Pentatomidae, Reduviidae and Gerridae family), two or three crystalline bodies within the mitochondrial derivatives have been observed [19].

Various functions have been suggested for the mitochondrial derivatives of sperm in insects; for example, they may participate in the control and regulation of flagellar movement or shape, or they may be involved in the storage and release of the energy required for flagellar motility [5, 10, 21]. However, Perotti [22] contradicted this last statement, relating the mitochondrial derivatives to paternal cytoplasmic inheritance and presuming that the material stored in the MDs may be involved in the activation and nutrition of the oocyte after fertilisation.

In addition to the structures mentioned previously, the cytoplasm of germ cells contains a build-up of a material termed a chromatoid body (CB) or “nuage” [23]. The CB is a macromolecular complex that is thought to play a coordinating role in the post-transcriptional control of gene products in haploid male germ cells and also to function as a centre for the determination of mRNA [23, 24, 25].

Some authors believe that the CB originates from a material between clusters of mitochondria that is present in the cytoplasm of germinal cells [26]. However, more recent studies describe the origin as coming from the fragmentation of nucleolar material during the process of spermatogenesis [27, 28, 29, 30, 31, 32, 33, 34]. Other structures that have been studied are the nucleolus and other structures that are impregnated with silver ions. The size of the nucleolus is related to the biosynthetic activity of the cell; therefore, the size and number of nucleoli and pre-nucleolar bodies depend on the metabolic and functional characteristics of the cells [35, 36, 37, 38, 39, 40, 41, 42].

In most species of arthropods, the nucleoli dissociate at the diplotene stage or diakinesis. Therefore, the corpuscles impregnated by the Ag-NOR technique, which are specific proteins associated with rRNA, are not visible from metaphase to telophase I.

These markings reappear at the beginning of spermatid formation, indicating that the transcriptional functions of ribosomal RNA have resumed, and they finally disappear at the end of spermatid formation [43]. However, the literature reports some exceptions; for example, in *Asellus aquaticus* (Isopoda) and species of the genus *Rhodnius* (Hemiptera), Ag-NOR corpuscles were visualised during the entire process of spermatogenesis [44, 42]; in *Callicrania seoanei* (Orthoptera), Ag-NORs were observed in interkinesis until pro-metaphase II [45]; and in *Triatoma infestans* and *T. sordida* (Hemiptera), the markings were present until metaphase I [35]. Furthermore, in *Carlisis wahlbergi* (Heteroptera, Coreidae), nucleolar semi-persistence (the presence of nucleolar corpuscles during metaphase) was observed until metaphase II [46], whereas for *Acanthocoris sordidus* (Heteroptera, Coreidae) and *Coptosoma punctissimum* (Heteroptera, Plataspidae), the nucleoli were detected in metaphase plates of primary and secondary spermatocytes [47].

With the objective of broadening the information known histological and ultrastructural about aquatic Heteroptera spermatogenesis, we analysed the species *Gelastocoris flavus flavus*.

Material and Methods

Testicles of *G. f. flavus* males were extracted and immediately fixed in Karnovsky fixative solution for a period of 3 to 48 hours. Samples were then washed twice in Millonig buffer, and post-fixation was performed with Osmium Tetroxide 1%:Millonig (1:3) for 2 hours in a refrigerator. The material was washed thoroughly with bidistilled water and then dehydrated in a series of solutions with increasing concentrations of acetone until a concentration of 100% was reached. A pre-infiltration in araldite:acetone (1:1) was performed overnight at room temperature. Afterwards, infiltration in araldite was performed for 2 hours at 37 °C and was then continued for 48 hours at 60 °C. Semi-fine and ultra-fine sections were obtained using a Leica Ultracut UCT ultramicrotome. The semi-fine sections were stained with toluidine blue pH4.0 [48] or silver ions (Ag-NOR) (Howell & Black [49], with modifications). The ultra-fine sections were collected on *grids* and then contrasted with 2% uranyl acetate for 20 minutes [50], followed by 2% lead citrate solution with 1 N sodium hydroxide for 6

minutes [51]. The results of transmission electron microscopy were documented by electron micrographs obtained from a CM100 Philips transmission electron microscope at the Center of Electron Microscopy, Institute of Biosciences, Botucatu, UNESP – IBB Campus Botucatu. São Paulo, Brazil.

Results

The images obtained from semi-fine sections and ultramicrographs showed several rounded chromatoid bodies (CBs) with different sizes in prophase I (Figures 1a and 2a, b). These were located near pore complexes of the nuclear envelope (Figure 2d, e). Small mitochondria were also observed that were uniformly dispersed within the cytoplasm (Figures 1a and 2a-c). The mitochondria were starting to come together to form larger structures (Figure 1c) and were moving to one side of the cell (Figures 1c-e and 2c), forming the mitochondrial complex (MC). The MC is a unique structure in that it is rounded and contains a tangle of mitochondria (Figures 1h, i). During the elongation of spermatids, the MC was divided in two sections, forming the mitochondrial derivatives (MDs), which were initially very close to each other and bilaterally flanked the axoneme (Ax) (Figures 1l and 2f, g). In a later stage, the MDs separated, with the Ax between them (Figures 1n-p and 2 f-i). At the end of the process, the MDs completely surrounded the Ax (Figure 2j). A crystalloid structure was also seen in the interior of the MDs (Figure 2f,g). In Figure 2k, supernumerary elements were observed (the presence of four Ax and two mitochondrial derivatives in the same tail). Another structure observed in the cell nucleus at prophase I was the nucleolus (Figure 2a, b), which was rounded and more electrodense than the other structures, with less electrodense interior regions. As shown in Figure 3a-b, a cell in prophase I was also observed with various markings that were silver-positive, small, rounded and dispersed throughout the cytoplasm. In the early stages of spermiogenesis, many argyrophilic granules could be seen that were strongly coloured and of different sizes (Figure 1f, g). They began to unite into a single vesicle, which was rounded and heavily stained (Figure 1h, k). In cells impregnated with silver ions, rounded structures of different sizes were observed; the structures united and formed a single structure that was also rounded and located adjacent to the MC (Figure 3c-h). Observation of the silver-

impregnated sections revealed a more highly impregnated region inside the vesicle (Figure 3h-m). As shown in Figures 1q, r and 3n, o longitudinal sections of the spermatid head were observed and showed the vesicle with markings. Toluidine blue staining indicated that the interior of the vesicle contained intensely stained filaments with an irregular morphology (Figure 1m, r). The organisational pattern of the axoneme was verified by transmission electron microscopy to have the 9+9+2 pattern (Figure 2j). As shown in Figure 1s-v, cysts containing sperms at different stages of development were observed.

Discussion

The literature regarding the ultrastructural aspects of Heteroptera spermatogenesis is extremely limited, yet understanding these aspects is of fundamental importance, because some structures cannot be analysed with light microscopy. In the species analysed in this study, argyrophilic granules were observed both by toluidine blue staining and by silver ion markings. These granules were present in the cytoplasm of prophase I and spermatids, and the granules came together to form a vesicle. Because these structures have not been described in other aquatic Heteroptera, more studies are necessary to determine the contents of these granules and their function. The vesicles observed in spermatids are most likely the nucleus and acrosome; however, it was not possible to determine the position of these structures in spermatids with the techniques used here. Further studies should be performed to determine accurate positions of the mentioned structures, both in spermatids and in sperm of *G. f. flavus*.

The acrosome, for example, was analysed in *Gerris najas* (Gerridae) [52], *Notonecta glauca* (Notonectidae) [53] and *Euchistus heros* [54], and it was observed that acrosome vesicles were formed from the Golgi complex. Changes in the form and degree of chromatin condensation were also observed. Initially, chromatin was dispersed and had a low electrodensity, but during the course of spermatogenesis, the chromatin assumed a compact fibril arrangement and developed a high electrodensity.

Acrosome enzymes are usually stored as proenzymes and are released and activated when the acrosome reaction occurs, thus participating in the event of fertilisation [55, 56, 57, 58]. Souza and Itoyama et al. [59] analyzed the

acromosomogensis in six species of Heteroptera, by means of Periodic Acid Schiff (PAS) and have found that, in general, the behavior of periodic acid Schiff-positive granules for all of the species analyzed is similar. In the beginning of spermiogenesis, there is a central granule that migrates to one of the extremities of the spermatid, and later, it becomes elongated and cannot be distinguished in the spermatozoa.

CBs of *G. f. flavus* were observed near the pore complexes of the nuclear envelope. This observation agrees with recent work in various species showing that CBs originate from the fragmentation of nucleolar material, such as, for example, in observations by Comings and Okada [27] and Andonov [29] in rats, by Anderson (1978) in the blue fox, by Peruquetti et al. [30, 31, 32] in *Rattus novergicus, Mus musculus*, gerbils and amphibians, and in triatomines by Silistino-Souza et al. [34].

Initial analysis of CB in Hemiptera hematophagous suggest that the formation of this organelle starts in spermatocytogenesis [60]. Furthermore, the authors suggest that the nucleolar persistence phenomenon observed in these insects is essential for CB formation, which presents extreme importance during the spermiogenesis these vectors, since during this phase of spermatogenesis the nucleolus shows no transcriptional activity [61].

Regarding the axoneme, the flagellar pattern observed for *G. f. flavus* was 9+9+2 (9 accessories, 9 doublets and 2 central microtubules). Araujo et al. [19, 62] observed that for some species of the Pentatomidae and Largidae family, the flagellum consists of an axoneme and two mitochondrial derivatives, and the axoneme follows a microtubule arrangement pattern of 9+9+2. This pattern was also described for other species of Heteroptera: Pentatomidae [63, 64], Reduviidae [65, 66], aquatic Heteroptera [67, 68, 69], Leptopodomorpha [70], Cimicomorpha, Gerromorpha, and Pentatomomorpha [19, 71] and is thus the default for this suborder.

It is known that insect mutants may exhibit disorders in spermiogenesis [72, 73] and that ambient conditions such as temperature, humidity and food availability may influence the normal process of spermiogenesis [74, 75]. The presence of aberrant spermatids in insect spermiogenesis does not appear to be a rare event. Caetano [76], for example, described *Atta capigura* and *A. sexdens rubropilosa* (Formicidae) sperm with supernumerary elements. The supernumerary elements found in the *G. f. flavus* species

may be caused by possible stress during collection, by the condition of the environment where they were collected or due to natural occurrence. However, more study is necessary to conclusively determine why occurs supernumerary elements during spermatogenesis.

Thus, this paper describes histological and ultrastructural characteristics during spermatogenesis, contributing to the reproductive knowledge of these aquatic Heteroptera.

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References

1. Phillips DM (1970) Insect sperm: their structure and morphogenesis. J Cell Biol 44: 243-277.
2. Baccetti B (1972) Insect sperm cell. Adv in Insect Phys 9: 315-397.
3. Messias JR (1990) Aspectos Ultra-Estruturais da espermiogênese de *Crysomya megacephala* Fab (Diptera: Calliphoridae). Dissertação. Universidade Estadual de Campinas. Campinas, São Paulo, Brasil.
4. Fawcett DW, Ito S (1965) The fine structure of bat spermatozoa. Amer J Anat 116: 567-610.
5. Yasuzumi G (1974) Electron microscope studies on spermiogenesis in various animal species. Intern Rev Cytol 37: 53-119.
6. Báo SN, Quagio-Grassiotto I, Dolder H (1989) Acrosome formation in *Ceratitis capitata* (Diptera, Tephritidae). Cytobios 58: 93-100.

7. Perotti ME (1969) Ultrastructure of the mature sperm of *Drosophila melanogaster* Meig. J Submicrosc Cytol 1: 171-196.
8. Kiefer RG (1970) Development, organization and degeneration of the *Drosophila* sperm flagellum. J Cell Sci 6: 177-194.
9. Dallai R, Afzelius BA (1991) Sperm flagellum of *Dacus oleae* (Gmelin) (Tephritidae) and *Drosophila melanogaster* (Drosophilidae) (Diptera). Int J Insect Morphol Embryol 20: 215-222.
10. Warner FD (1971) Spermatid differentiation in the blowfly *Sarcophaga bullata* with particular reference to flagelar morphogenesis. J Ultrast Res 35: 210-232.
11. Baccetti B, Dallai R (1976) The spermatozoan of arthropoda. XXVII. Uncommon axoneme patterns in different species of the cecidomyid flies. J Ultrast Res 55: 50-69.
12. Pratt S (1970) Formation and differentiation of the Nebenkern in spermatios of na hemipteran insect, *Murgantia iristionia*. In: Baccetti, B (ed) Comparative Spermatology, Academic Press, London, pp 301-310.
13. Tandler B, Hoppel C (1972) Mitochondria in spermatogenesis. In: Tandler, B. and Hoppel, C (ed) Mitochondria, 1rd edn., Academic Press, New York and London, p. 36-39.
14. Baccetti B, Afzelius BA (1976) The biology of the sperm cell. Basel press, New York.
15. Tokuyasu KT (1974) Dynamics of spermiogenesis in *Drosophila melanogaster*. III. Relation between axoneme and mitochondrial derivatives. Exp Cell Res 84: 239-250.

16. Rosati F, Selmi G, Mazzini M (1976) Comparative observation on the mitochondrial derivatives of insect sperm. J Submicrosc Cytol 8: 56-67.
17. Báo SN, Lins U, Farina B, de Souza W (1992) Mitochondrial derivatives of *Culex quinquefasciatus* (Culicidae) spermatozoon: Some new aspects evidenced by cytochemistry and image processing. J Struct Biol 109:4 6-51.
18. Baccetti B, Dallai R, Pallini V, Rosati F (1977) Afzelius, B.A. Protein of insect sperm mitochondrial crystals: crystallomitin. J Cell Biol 73: 594-600.
19. Araújo VA, Lino-Neto J, Ramalho FS, Zanuncio JC, Serrão JE (2011) Ultrastructure and heteromorphism of spermatozoa in five species of bugs (Pentatomidae: Heteroptera). Micron 42: 560-567.
20. Phillips DM (1974) Structural variants in invertebrate sperm flagella and their relationship to motility. In: Sleigh, MA (ed) Cilia and Flagella, Academic Press, London, pp 379-402.
21. Tokuyasu KT (1975) Dynamics of spermiogenesis in *Drosophila melanogaster*. V. Head-tail alignment. J Ultrast Res 50: 117-129.
22. Perotti ME (1973) The mitochondrial derivative of the spermatozoon of *Drosophila* before and after fertilization. J Ultrast Res 44:181-198.
23. Parvinen M (2005) The chromatoid body in spermatogenesis. International J Androl 28: 189-201.
24. Kotaja N, Bhattacharyya SN, Jaskiewics L, Kimmins S, Parvinen M, Filipowicz W, Sassone-Corsi P (2006) The chromatoid body of male germ cells: Similarity with processing bodies and presence of Dicer and microRNA pathway components. Proc Natl Acad Sci 103: 2647-2652.

25. Kotaja N, Sassone-Corsi P (2007) The chromatoid body: a germ-cell specific RNAprocessing centre. Mol Cell Biol 8: 85-90.
26. Fawcett DW, Eddy EM, Phillips DM (1970) Observations on the fine structure and relationships of the chromatoid body in mammalian spermatogenesis. Biol Reprod 2: 129-153.
27. Comings DE, Okada TA (1972) The chromatoid body in mouse spermatogenesis: Evidence that it may be formed by the extrusion of nucleolar components. J Ultrast Res 39: 15-23.
28. Anderson K (1978) Fine structure of spermatogonia and spermatocytes in the blue fox (*Alopex lagopus*). Acta Vet Scand 19: 229-242.
29. Andonov M (1990) Further study of the chromatoid body in rat spermatocytes and spermatids. Zeit Zellfor Mikrosk Anat 104: 46-54.
30. Peruquetti RL, Assis IM, Taboga SR, Azeredo-Oliveira MTV (2008) Meiotic nucleolar cycle and chromatoid body formation during the rat (*Rattus novergicus*) and mouse (*Mus musculus*) spermiogenesis. Micron 39: 419-425.
31. Peruquetti RL, Taboga SR, Azeredo-Oliveira MTV (2008) Characterization of Mongolian gerbil chromatoid bodies and their correlation with nucleolar cycle during spermatogenesis. Reprod Dom Anim 45: 399-406.
32. Peruquetti RL, Taboga SR, Santos LRS, Oliveira C, Azeredo-Oliveira MTV (2011) Nucleolar cycle and chromatoid body formation: Is there a relationship between these two processes during spermatogenesis of *Dendropsophus minutus* (Amphibia, Anura)? Micron 42: 87-96.

33. Morielle-Souza A, Taboga SR, Azeredo-Oliveira MTV (2010) Ultrastructural analysis of the nucleolar aspects at spermiogenesis in triatomines (Heteroptera, Triatominae). Micron 41: 791-786.
34. Silistino-Souza R, Peruquetti RL, Taboga SR, Azeredo-Oliveira MTV (2012) Chromatoid body: Remnants of nucleolar proteins during spermatogenesis in triatomine (Heteroptera, Triatominae). Micron 43: 954-960.
35. Tavares MG, Azeredo-Oliveira MJV (1997) Pattern of nucleolar activity during spermatogenesis in triatomines (Heteroptera: Reduviidae) as analysed by silver staining. Cytobios 89: 93-103.
36. Souza HV, Bicudo HEMC, Costa LAA, Itoyama MM (2007) A study of meiosis and spermatogenesis in different testicular lobes of *Antiteuchus tripterus* (Heteroptera, Pentatomidae). Eur J Ent 104: 353-362.
37. Souza HV, Bicudo HEMC, Itoyama MM (2007) Study of chromosomal and nucleolar aspects in testes of *Nysius californicus* (Heteroptera: Lygaeidae). Gen Mol Res 6: 33-40.
38. Castanhole MMU, Pereira LLV, Souza HV, Bicudo HEMC, Costa LAA, Itoyama MM (2008) Heteropicnotic chromatin and nucleolar activity in meiosis and spermiogenesis of *Limnogonus aduncus* (Heteroptera, Gerridae): a marked RON revealing aspect of the chromosomal behavior. Gen Mol Res 7: 1398-1407.
39. Castanhole MMU, Pereira LLV, Souza HV, Itoyama MM (2010) Spermatogenesis and karyotypes of three species of water striders (Gerridae, Heteroptera). Gen Mol Res 9:1343-1356.

40. Castanhole MMU, Pereira LLV, Souza HV, Itoyama MM (2012) Spermatogenesis of riffle bugs, *Rhagovelia whitei* and *Rhagovelia* sp. (Veliidae), and backswimmers *Martarega* sp. (Notonectidae). Gen Mol Res 11: 2003-2020.
41. Alevi KCC, Mendonça PP, Pereira NP, Rosa JA, Azeredo-Oliveira MTV (2013b) Análise das Regiões Organizadoras Nucleolares e da atividade nucleolar em *Triatoma melanocephala* e *T. lenti*, importantes vetores da doença de Chagas. Rev Cienc Farm Básica e Aplicada 34: 417-21.
42. Alevi KCC, Castro NFC, Lima AC, Ravazi A, Morielle-Souza A, Oliveira J, Rosa JA, Azeredo-Oliveira MTV (2014) Nucleolar persistence during spermatogenesis of the genus *Rhodnius* (Hemiptera, Triatominae). Cell Biol Intern 38: 977-980.
43. Bressa MJ, Papeschi AG, Fumagalli E, Van Doesburg PH, Larramendy ML (2003) Cytogenetic and nucleolar meiotic cycle analyses in *Dysdercus imitator* Blõte, 1931 (Pyrrhocoridae, Heteroptera) from Argentina. Folia Biol 5: 135-141.
44. Di Castro M, Prantera G, Cipriani L, Rocchi A (1983) Silver staining analysis of nucleolar-organizer activity during spermatogenesis of *Asellus aquaticus* (Crustacea, Isopoda). Genetica 60: 163-166.
45. Santos J, Sentis C., Fernandez-Piqueras J (1987) Pattern of nucleolar organizer region activity during male meiosis in *Callicrania seoanei* (Orthoptera) as analyzed by silver staining: evidences for a possible reactivation in the period between the two meiotic divisions. Genome 29: 516-518.
46. Fossey A, Liebenberg H (1995) Meiosis and nucleolar structures in the stink bug *Cartisis wahlbergi Stal* (Coreidae: Heteroptera). Cytobios 81: 7-15.

47. Yoshida, T (1947) Unusual type of the nucleolus observed in a bug, *Acanthocoris sordidus*. J Fac Sci 9: 243-249.

48. Mello MLS, Vidal BC (1980) Práticas de Biologia Celular. FUNCAMP Editora Edgard Blücher LTDA, Campinas, São Paulo, Brasil.

49. Howell WM, Black DA (1980) Controlled silver staining of nucleolus organizer regions with protective colloidal developer: I-step method. Experientia 36: 104-105.

50. Watson ML (1958) Staining tissue section of electron microscopy with heavy metals. J Biophys Biochem Cytol 4: 475-478.

51. Venable JH, Coggeshall R.A (1965) A simplified lead citrate stain for use in electron microscopy. J Cell Biol 25: 407- 408.

52. Werner G, Werner K (1993) Changes in the nucleus, endoplasmic reticulum, Golgi apparatus, and acrosome during spermiogenesis in the waterstrider, *Gerris najas* deg. (Heteroptera : Gerridae). Int J Insect Morphol Embryol 22: 521-534.

53. Werner G, Afzelius BA, Mosler B (1988) Acrosome formation during spermiogenesis in *Notonecta glauca* L. (Heteroptera). J Submic Cytol Pathol 20: 123-35.

54. Fernandes AP, Curi G, Báo SN (2001) Nuclear changes and acrosome formation during spermiogenesis in *Euchistus heros* (Hemiptera: Pentatomidae). Tiss Cell 33: 286-293.

55. Yanagimachi R (1988) Mammalian fertilization. In: Knobil, E and Neill, J (ed) The Physiology of Reproduction. 1rd edn. Raven Press, New York, p. 135-185.

56. Baccetti B, Burrini AG, Collodel G, Piomboni P, Renieri T, Sensini C (1989) Lacalization of acrosomal enzymes in Arthopoda, Echinodermata and Vertebrata. J Submic Cytol Pathol 21: 385-389.

57. Tesarik J, Drahorad J, Testart J, Mendonza C (1990) Acrosin activation follows its surface exposure and precedes membrane fusion in human sperm acrosome reaction. Development 110: 391-400.

58. Gallo J, Escalier D, Grellier P, Precigout E, Albert M, David G, Schrevel J (1991) Characterization of a monoclonal antibody to human proacrosin and its use in acrosomal staus evaluation. J Histochem Cytochem 39: 273-282.

59. Souza HV, Itoyama MM (2010). Study of Acrosome Formation, Interspecific and Intrspecific, in the Testicular Lobes of Some Pentatomid Species. J Insect Sci 10: 132.

60. Borgueti AO, Alevi KCC, Silistino-Souza R, Rosa JA, Azeredo-Oliveira MTV. Immunofluorescence and ultrastructural analysis of the chromatoid body during spermatogenesis of *Triatoma platensis* and *T. rubrovaria* (Hemiptera, Triatominae). Micron 74: 44-46.

61. Alevi KCC, Mendonça PP, Pereira NP, Rosa JA, Azeredo-Oliveira. Is there post-meiotic transcriptional activity during hemipteran spermiogenesis? Inv Rep Dev 58: 193-198.

62. Araújo VA, Báo SN, Lino-Neto J (2012) Polymorphism of spermatozoa in *Largus rufipennis* Laporte 1832 (Heteroptera: Pyrrhocoroidea: Largidae). Acta Zoologica 93: 239-244.

63. Trandaburu V (1973) The acrosome and the centriole adjunct in *Eurydema ventralis* Kol. (Heteroptra, Pentatomidae). Trav Mus Nat Hist Nat Grig Antipa 13: 137-145.

64. Fernandes AP, Báo SN (1998) Spermiogenesis in phytophagous bug (Hemiptera: Pentatomidae): an ultrastructural study. J Submic Cytol Pathol 30: 485-493.

65. Dolder H (1988) Cytoskeletal bridges between organelles in sperm flagellum of *Triatoma infestans* (Hemiptera, Reduviidae). J Ultrast Mol Struct Res 101: 159–164.

66. Báo SN, de Souza W (1994) Structural specialization in the flagellum of the spermatozoon of the bloodsucking bug (*Rhodnius prolixus*, Hemiptera, Reduviidae). Tiss Cell 26: 299-308.

67. Afzelius BA, Dallai R, Lindskog P (1985) Spermatozoa of saldid bugs (Insecta, Hemiptera, Leptopodomorpha). J Ultrast Res 90: 304-312.

68. Lee CE (1991) Morphological and phylogenetic studies on the true water bugs (Hemiptera: Heteroptera). J Nat Hist Soc Korea 21: 1-183.

69. Lee YH, Lee CE (1992) Ultrastructure of spermatozoa and spermatogenesis in Nepomorpha (Insecta: Heteroptera) with special reference to phylogeny. Zool Sci 9: 971–981.

70. Afzelius BA, Baccetti B, Dallai R (1976) The giant spermatozoon of Notonecta. J Submic Cytol Pathol 8: 149-161.

71. Dallai R, Afzelius BA (1980) Characteristics of the sperm structure in Heteroptera (Hemiptera, Insecta). J Morphol 164: 301-309.

72. Báo SN (1987) Estudo ultra-estrutural da espermiogênese no mutante "olho-róseo" comparativo a linhagem selvagem de *Ceratitis capitata* Weidmann (Diptera, Tephritidae). Dissertação. Universidade Estadual de Campinas, Campinas, São Paulo, Brasil.

73. Báo SN, Dolder J (1991) Abnormal characteristics observed in spermatic cells during spermiogenesis in the Ra mutant of *Ceratitis capitata* (Diptera, Tephritidae). Rev Bras Gen 14: 287-297.

74. Szollosi A (1976) Influence of infra-optimal breeding temperature on spermiogenesis of locust *Locusta migratoria* I. Abnormalities in differentiation of cytoplasmic organelles. J Ultrast Res 54: 202-214.

75. Szollosi A (1976) Influence of infra-optimal breeding temperature on spermiogenesis of locust Locusta migratoria II. Abnormalities in differentiation of the nucleus. J Ultrast Res 54: 215-223.

76. Caetano FH (1980) Ultraestrutura dos espermatozóides de *Atta capiguara* e *Atta sexdens rubropilosa* (Formicidae). Naturalia 5: 105-111.

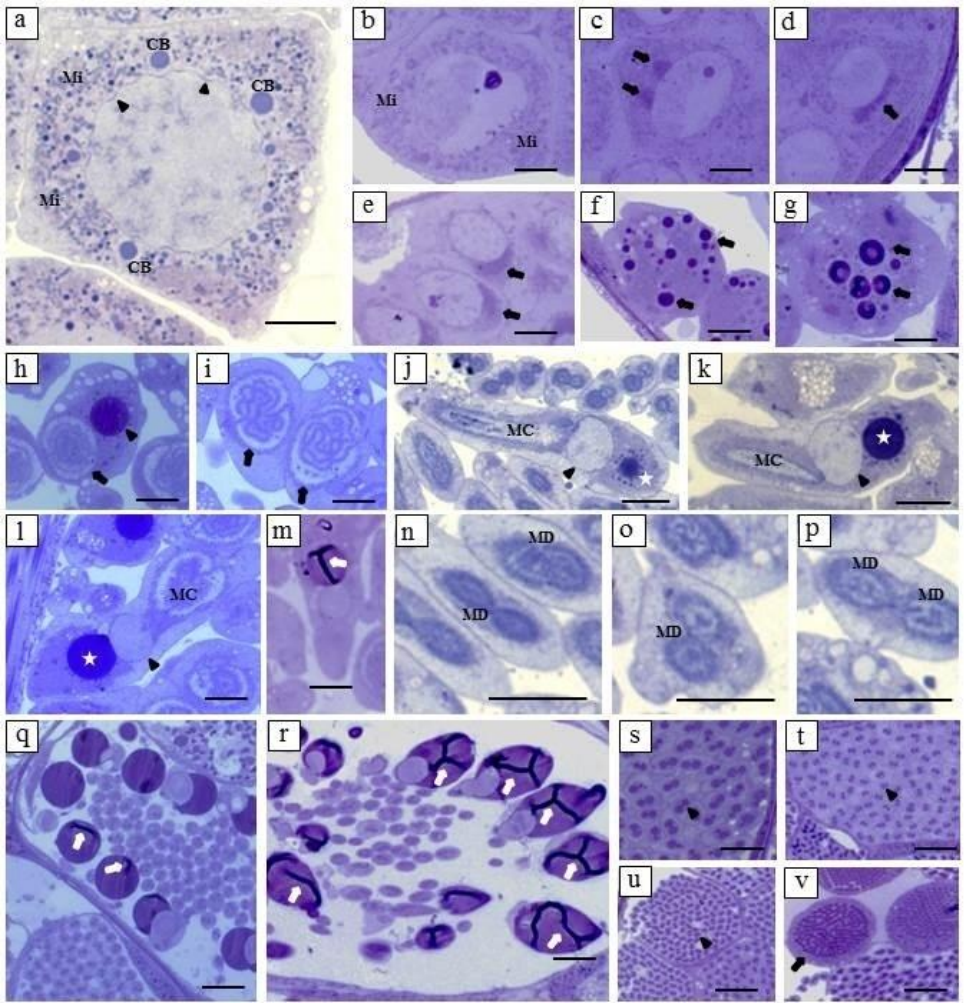

Figure 1. Semi-fine sections of *Gelastocoris flavus flavus* testicle stained with toluidine blue. **a)** Prophase I, showing the chromatoid bodies (CB) rounded, of varying sizes and with sharp metachromasia. Near the CB are pore complexes (arrowheads) and mitochondria (Mi); **b-c)** mitochondrial complex formation; in "b", mitochondria (Mi) are small, isolated and dispersed throughout the cytoplasm. In "c", mitochondria are beginning to unite on one side of the cell (arrows); **d,e)** mitochondrial complex formation (arrows); **f-g)** early spermatids with several argyrophilic granules of different sizes (arrows); **h)** union of argyrophilic granules forming a vesicle (arrowhead) and the mitochondrial complex (arrow); **i)** spermatids with the mitochondrial complex (arrows); **j-l)** spermatids in elongation showing the mitochondrial complex (MC), a highly stained vesicle (asterisk) and a vesicle without staining (arrowhead); **m)** vesicle containing a more intensely coloured region (arrow); **n-p)** cross-section of a tail showing the mitochondrial derivative (MD); **q-r)** longitudinal section of the head, containing a vesicle with more highly stained interior regions (arrows); **s-u)** cross-section of different regions of the tail showing the mitochondrial derivative (arrowheads); **v)** cyst with spermatozoids (arrow). Bars: 10 µm.

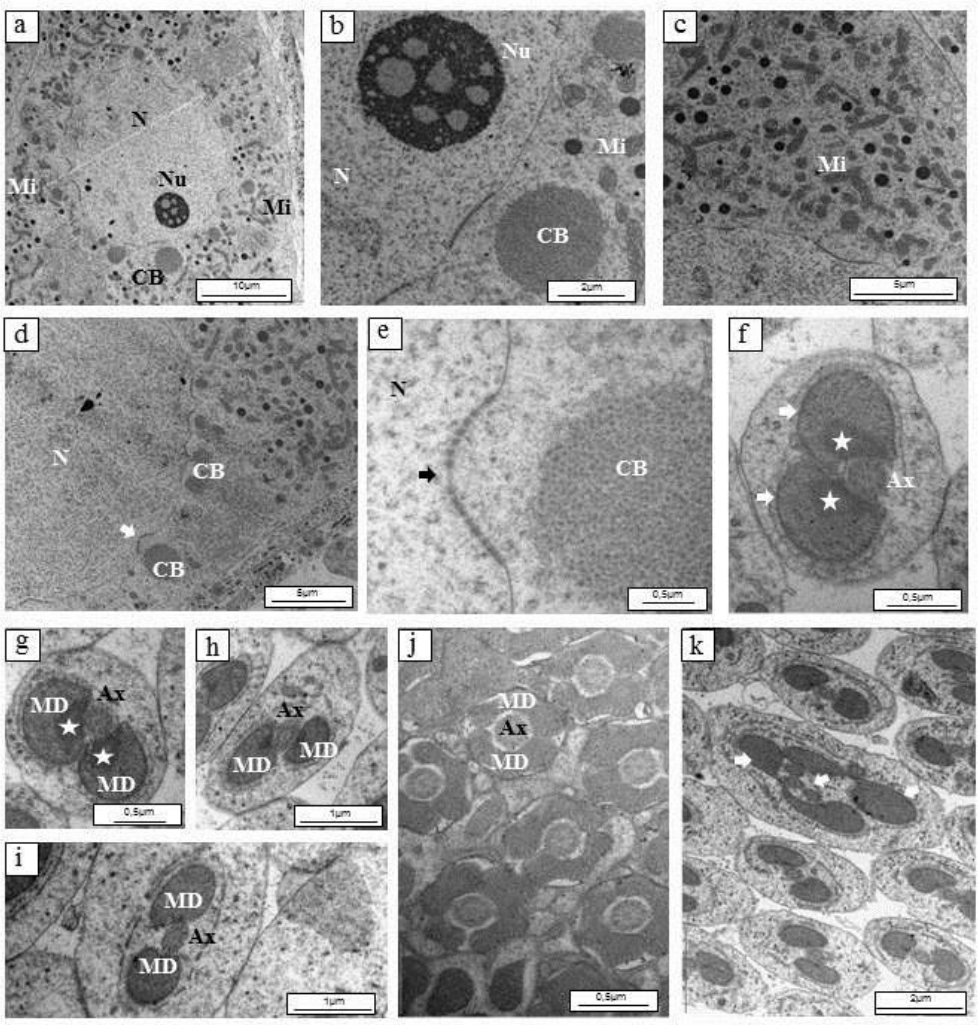

Figure 2. Electron micrographs of *Gelastocoris flavus flavus* testicular cells. **a)** Cell in prophase I showing the nucleolus (Nu), which is more electrodense than the other structures and has less electrodense interior regions (this region, in more detail, can be viewed in b); **a-c)** mitochondria (Mi) of different sizes and enlargements. Note that in "c", mitochondria are primarily located on one side of the cell, CB: chromatoid body, N: nucleus, Nu: nucleolus; **d,e)** the chromatoid body (CB) and the pore complex (arrows). In "e", these structures are shown at a higher magnification; **f)** cross section of tail, showing the mitochondrial derivatives (arrows) and the presence of interior crystalloids (asterisks) and the axoneme (Ax); **g-j)** tail formation process in relation to the mitochondrial derivative (MD) and the axoneme (Ax). In "g", the mitochondrial derivatives are close, and the axoneme is still moving to the central region of the tail; note the presence of crystalloids (asterisks); **j)** axoneme surrounded by the mitochondrial derivative; **k)** supernumerary elements (arrows). Bars: 10 µm.

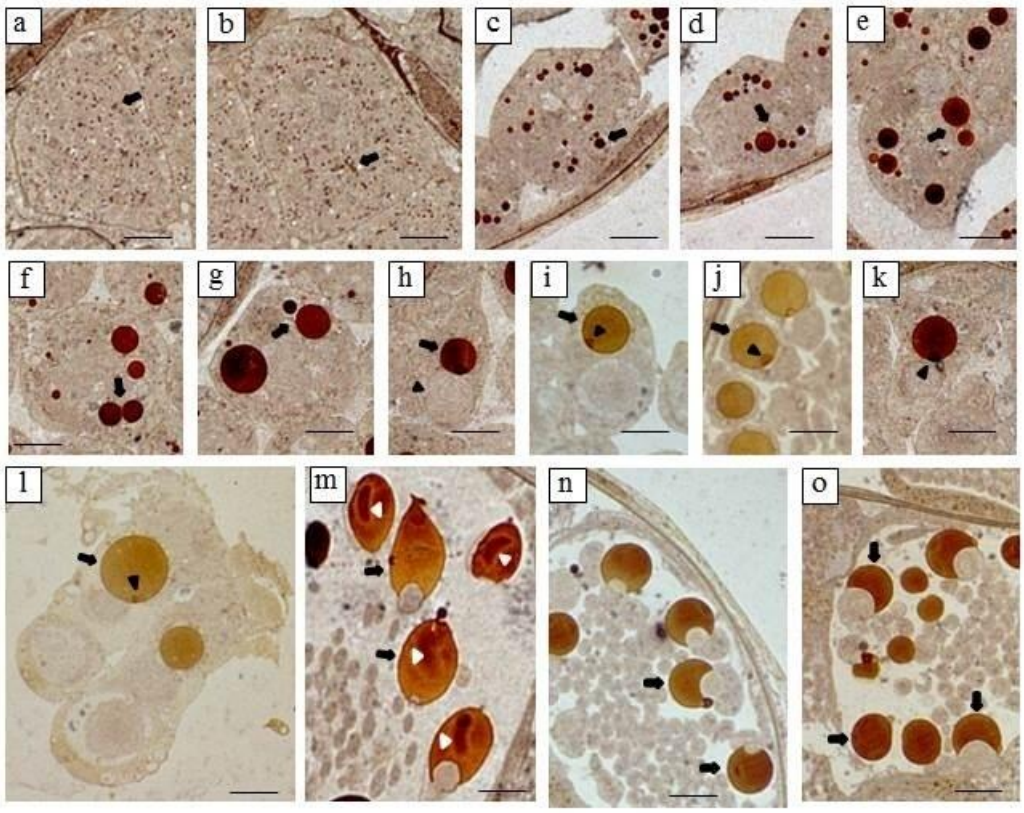

Figure 3. Semi-fine sections of *Gelastocoris flavus flavus* testicle impregnated with silver ions. **a,b)** Prophase I with several positive silver markings (arrows); **c-e)** spermatids with several silver-positive argyrophilic granules (arrows); **f,g)** spermatids showing silver-positive argyrophilic granules uniting and forming larger and rounded vesicles (arrows); **h)** spermatid with a silver-positive vesicle (arrow) and the mitochondrial complex (arrowhead); **i-m)** silver-positive vesicle (arrows) with stronger interior staining, which internalises with spermatid elongation (arrowheads); **n,o)** longitudinal section of the head of the spermatids, with a silver-positive vesicle (arrows). Bars: 10 µm

ARTIGO III

Ultrastructure of spermatogenesis of *Martarega uruguayensis* (Heteroptera)

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Abstract

The insects of the Notonectidae family, commonly known as backswimmers, are important for tropical countries because they are predators of mosquito larvae and pupae of *Aedes aegypti*. They therefore provide natural biological control of the mosquitoes that transmit tropical diseases such as yellow fever, dengue fever, the Zika virus, and the Chikungunya virus. Studies on ultrastructural aspects of spermatogenesis and, more specifically, on the structure of the sperm in aquatic Heteroptera are still scarce; for this reason, the objective of this study was to analyze the ultrastructures of spermatogenesis using transmission electron microscopy on the testes of adult male *Martarega uruguayensis* specimens. The microscopic analysis revealed the formation of mitochondrial derivatives, the microtubule organization pattern within the axoneme

(9+9+2), and the presence of very argyrophil granules which combine to form a single structure. The study revealed the ultra-structural characteristics to be similar to those of other Heteroptera described in the literature.

Keywords: Axoneme; Argyrophil granules; Mitochondrial derivative; Spermatogenesis; Spermiogenesis

Introduction

The suborder Heteroptera has seven infraorders with approximately 80 families. Most occur on all continents except Antarctica and some islands [1]. In addition to terrestrial Heteroptera, there are also widely distributed aquatic and semi-aquatic species that have adapted to live in an extraordinary variety of ecosystems: species have been found in freshwater and marine habitats alike and at altitudes ranging from 0 m to 4,700 m [2].

The insects of the Notonectidae family are commonly known as backswimmers, as they have hind legs adapted for swimming. They are important for tropical countries because the species are predators of mosquito larvae and pupae of *Aedes aegypti*; therefore, they serve as a natural biological control of the mosquitoes that transmit tropical diseases such as yellow fever, dengue fever, the Zika virus, and the Chikungunya virus [3-5].

Research addressing the ultrastructural aspects of spermatogenesis makes up a broad and growing field of study; however, in the case of Heteroptera, research is still scarce. Only a few structures, such as the acrosome, axoneme, and mitochondria, have been described [6,7].

The acrosome is an essential organelle for fertilization: it removes the protective mechanism on the surface of sperm as well as the digestion barriers in order for sperm to migrate into the female reproductive tract [6-9]. The axoneme is an ultrastructure that has been studied in several species of insects, and in most organizational patterns, the axoneme follows the scheme of 9+2 microtubules

surrounded by 9 additional accessories [6,8,10]. This is the flagellar pattern reported for all Heteroptera species described in the literature [11].

Another ultrastructure that is analyzed during spermatogenesis is the mitochondria, which, during spermatogenesis, can assume different morphologies. During spermatogenesis, the mitochondria undergo metamorphosis, which is a complex process of fusion and rearrangement of mitochondria that forms the mitochondrial complex. As spermatogenesis continues, the mitochondrial complex divides into two mitochondrial derivatives which, in the sperm elongation process, are positioned bilaterally relative to the axoneme [6, 8, 12 - 15].

In light of the substantial biological importance of the Notonectidae family in tropical countries, this paper presents information on the spermatogenesis-related ultrastructures of *Martarega uruguayensis* as a tool for better understanding the reproductive biology this species.

Material and Methods

Testicles of *Martarega uruguayensis* males were extracted and immediately fixed in a Karnovsky fixative solution for 3 hours. Samples were then washed twice in a Millonig buffer, and post-fixation was performed using Osmium Tetroxide 1% Millonig (1:3) for 2 hours in a refrigerator. The material was washed thoroughly with double-distilled water and then dehydrated in a series of solutions with increasing concentrations of acetone until a concentration of 100% was reached. As part of the pre-infiltration in araldite:acetone (1:1) was applied overnight at room temperature. Next, the product was infiltrated in araldite for 2 hours at 37°C, followed by another 48 hours at 60°C. Semi-fine and ultra-fine sections were obtained using a Leica Ultracut UCT ultramicrotome. The semi-fine sections were stained with toluidine blue at a pH of 4.0 [16] or using silver ions (Ag-NOR) as the method offered by Howell and Black [17], with modifications. The ultra-fine sections were collected on grids and then contrasted using 2% uranyl acetate for 20 minutes [18], followed by a 2% lead citrate solution with 1 N sodium hydroxide for 6 minutes [19]. The results of transmission electron microscopy were documented as electron micrographs obtained using a CM100 Philips

Transmission Electron Microscope at the Center for Electron Microscopy within the Institute of Biosciences, at the São Paulo State University (UNESP) Botucatu Campus in Botucatu, São Paulo, Brazil.

Results and Discussion

Testicular cells stained with toluidine blue revealed early spermatids with the presence of several small mitochondria homogeneously distributed throughout the cytoplasm and a large nucleolus that was rounded and intensely stained (Figure 1a). These same structures were observed in the electron micrographs, which revealed the nucleolus to be more electrodense than the other structures (Figure 2a-b). In Figure 2c, the mitochondria can be observed in greater detail. As spermatogenesis progresses, the mitochondria begin to group together (Figures 1b,c e 2a,b) and concentrate on only one side of the cell (Figure 1c). The mitochondria then combine to form a single mitochondrial complex (Figure 1d-h), which subsequently divides into two mitochondrial derivatives (Figure 2d). They are initially positioned on opposite sides of the axoneme; they then increase in size and wrap around the axoneme (Figure 2e,f) and present irregular morphology (Figure 2f).

The process in which the mitochondria undergo metamorphosis to form the mitochondrial complex and the subsequent mitochondrial derivative has been described for several species [6,8,12-15]. They are involved in the control and regulation of flagellar movement and provide the energy required for the motility of the tail [20-22].

Figure 1k still shows cysts containing sperm. Spermatogenesis in cysts has been described for Heteroptera since the first studies with light microscopy [3]. Electron micrographs of *M. uruguayensis* showed the microtubule pattern of the axoneme to be 9+9+2 (9 accessories, 9 doublets, and 2 central microtubules; Figure 2d), results which are consistent with other Heteroptera described in the literature [11, 23-25].

The use of silver impregnation ions identified several silver-positive argyrophil granules. They were rounded, intensely impregnated, and evenly distributed throughout the cytoplasm (Figure 3a,b). At the beginning of spermiogenesis, there are fewer argyrophil granules, but they are larger in size (Figures 1d,e and 3c) relative to prophase

I (Figures 1a,b and 3a,b). In spermatids, they are disposed around the mitochondrial complex (Figure 1d,e), and as spermatogenesis progresses, these granules unite, this union can be seen in Figure 3d-h. At the end of the process, the argyrophil granules come together to form a single vesicle that is rounded and strongly stained (Figures 1f,g and 3i) and which is located beside the mitochondrial complex (Figure 1f,g), and posteriorly in the anterior region of the spermatid (Figure 1h-j and 3l,m).

The material stained with toluidine blue and via impregnation by silver ions showed the the vesicle to include more heavily stained areas (Figure 1h,i and 3j-n), and less intensely impregnated regions (Figure 3l,m).

The argyrophil granules observed after the toluidine blue stain and with markings by silver ions were first described in aquatic Heteroptera by Alevi et al [11]; however, their content remains unknown, and more studies are necessary to determine the function and content of these granules.

Conclusion

These findings suggest that the ultrastructural characteristics of *Martarega uruguayensis* are similar to those of other Heteroptera described in the literature. The 9+9+2 flagellar pattern is consistent with other species described in the literature, and therefore seems to be the standard for Heteroptera species. The mitochondria are grouped in a way that forms a mitochondrial complex, which then divides into two mitochondrial derivatives around the axoneme.

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References

1. Schuh TT, Slater JA (1995) True bugs of the world (Hemiptera: Heteroptera) Classification and natural history, Cornell UNIVERSITY press, Ithaca, New York, xii, 338 pp.

2. Melo MC (2009) Biodiversidad de Heteroptera (Hemiptera) acuáticas y semiacuáticas de la Patagonia argentina. Revista de la Sociedad Entomológica Argentina, v. 68 (1-2), p. 177-185.

3. Ueshima N (1979) Animal cytogenetics, Insecta 6, Hemiptera: Heteroptera. Gebruder Borntraeger: Berlin, Stuttgart, 117 pp.

4. Panicker KN, Rajagopalan PK (1977). Biologial control potential of Anisops bouvieri Kir (Hemiptera, Notonectidae) Indian J. Med. Res. 66: 772-776.

5. Ellis RA, Borden JH (1970) Predation by *Notonecta undulata* Say (Hemiptera: Notonectidae) on larvae of yellow fever mosquito. Ann Entomol Soc Amer. 63(4):963-973.

6. Phillips DM (1970) Insect sperm: their structure and morphogenesis. J Cell Biol 44: 243-277.

7. Fawcett DW, Ito S (1965) The fine structure of bat spermatozoa. The American journal of anatomy, v. 116 p. 567-610.

8. Baccetti B (1972) Insect sperm cell. Adv in Insect Phys 9: 315-397.

9. Messias JR (1990) Aspectos Ultra-Estruturais da espermiogênese de *Crysomya megacephala* Fab (Diptera: Calliphoridae). Dissertação. Universidade Estadual de Campinas. Campinas, São Paulo, Brasil.

10. Warner FD (1971) Spermatid differentiation in the blowfly Sarcophaga bullata with particular reference to flagellar morphogenesis. J Ultrastruct Res 35: 210-232.

11. Alevi KCC, Pereira LLV, Moreira FFF, Barbosa JF, Taboga SR and Itoyama MM (2015) Histological and ultrastructural analysis of spermatogenesis in *Gelastocoris flavus flavus* (Heteroptera: Nepomorpha). Entomol. Ornithol. Herpetol. 4:3.

12. Baccetti B (1970) Comparative Spermatology. Academic, London, p. 301-310.

13. Tandler B, Hoppel C (1972) Mitochondria in spermatogenesis. In: Tandler B, Hoppel C (eds.) Mitochondria, (1stedn.), Academic Press, New York and London, pp. 2: 36-39.

14. Baccetti B, Afzelius BA (1976) The biology of the sperm cell. Monogr Dev Biol 1-254.

15. Tokuyasu KT (1974) Dynamics of spermiogenesis in *Drosophila melanogaster*. Relation between axoneme and mitochondrial derivatives. Exp Cell Res 84:239-250.

16. Mello MLS, Vidal BC (1980) Práticas de Biologia Celular. FUNCAMP Editora Edgard Blücher LTDA, Campinas, São Paulo, Brasil.

17. Howell WM, Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36:1014-1015.

18. Watson ML (1958) Staining of tissue sections for electron microscopy with heavy metals. J Biophys Biochem Cytol 4: 475-478.

19. Venable JH, Coggeshall R (1965) A simplified lead citrate stain for use in electron microscopy. J Cell Biol 25: 407-408.

20. Phillips DM (1974) Structural variants in invertebrate sperm flagella and their relationship to motility. In: Sleigh MA (eds) Cilia and Flagella, Academic Press,London, pp. 2: 379-402.

21. Yasuzumi G (1974) Electron microscope studies on spermiogenesis in various animal species. Int Rev Cytol 37: 53-119.

22. Tokuyasu KT (1975) Dynamics of spermiogenesis in *Drosophila melanogaster*. V. Head-tail alignment. J Ultrastruct Res 50: 117-129.

23. Araújo VA, Báo SN, Lino-Neto J (2012) Polymorphism of spermatozoa in Largus rufipennis Laporte 1832 (Heteroptera: Pyrrhocoroidea: Largidae). Acta Zoologica 93: 239-244.

24. Dallai R, Afzelius BA (1980) Characteristics of the sperm structure in Heteroptera (Hemiptera, Insecta). J Morphol 164: 301-309.

25. Fernandes AP, Báo SN (1998) Spermiogenesis in phytophagous bug (Hemiptera, Pentatomidae): an ultrastructural study. J Submicrosc Cytol Pathol 30: 485-493.

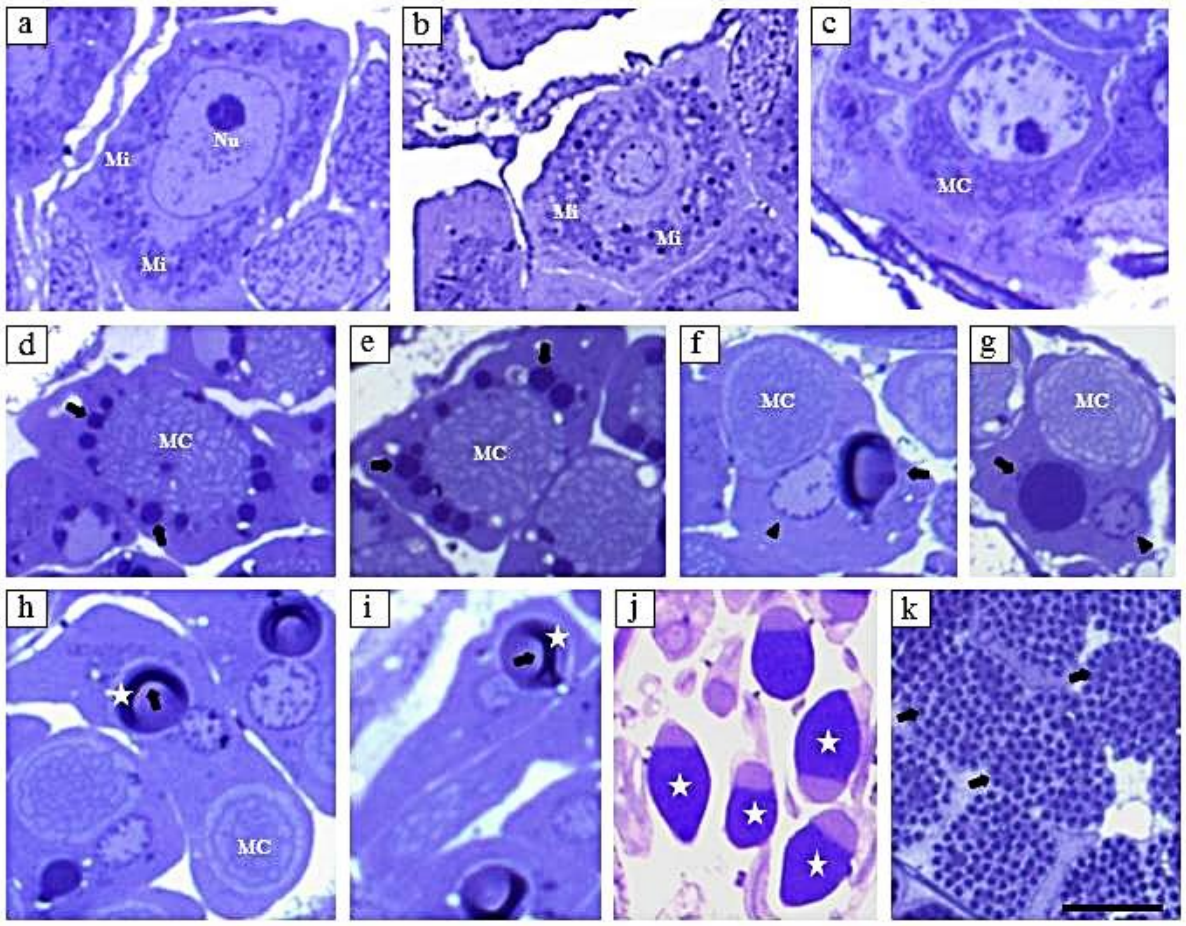

Figure 1. Semi-fine sections of *Martarega uruguayensis* testicle stained with toluidine blue. **a,b)** Prophase I showing mitochondria scattered throughout the cytoplasm (Mi) and a rounded and heavily stained nucleolus (a, Nu); **c)** mitochondrial complex formation (MC); **d,e)** early spermatids with several argyrophil granules of different sizes (arrows) around the mitochondrial complex (MC); **f,g)** spermatids with mitochondrial complex (MC), a more intensely stained vesicle (arrows), and other non-stained vesicles (arrowhead); **h,i)** stretched spermatid showing vesicle (asterisk) in the anterior region with a more intensely stained region inside (arrows) and a mitochondrial complex (MC), **j)** longitudinal head section showing a vesicle with sharp metachromasia (asterisk); **k)** cysts with spermatozoids (arrow). Bar: 10 μm.

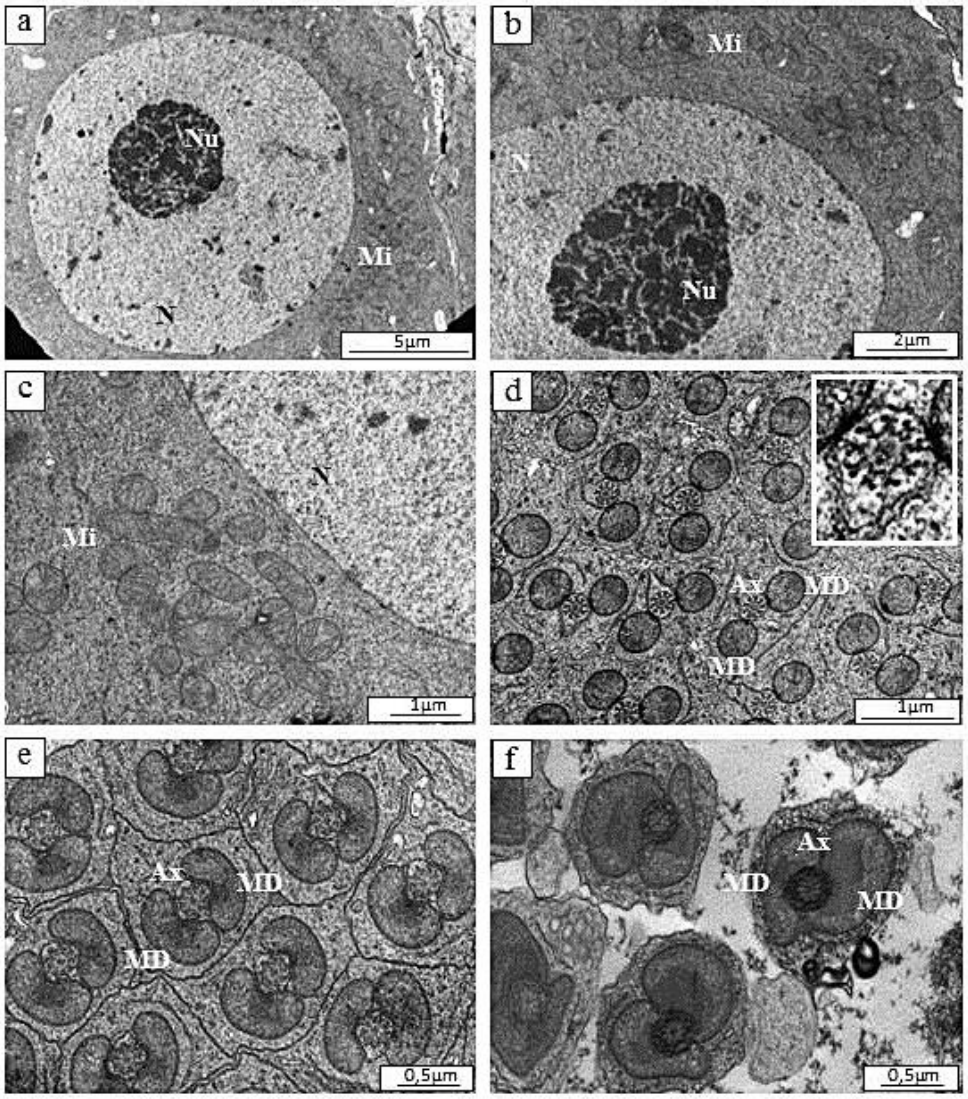

Figure 2. Electron micrographs of testicular cells from *Martarega uruguayensis*. **a,b)** Cell in prophase I showing the nucleolus (Nu) to be more eletrodense than the other structures, and mitochondria (Mi) migrating to one side of the cell; **c)** arrangement of the mitochondria (Mi) for the initial formation of the mitochondrial complex; **d)** mitochondrial derivatives (MD) arranged bilaterally relative to the axoneme (Ax), and the axoneme with its 9+9+2 pattern; **e,f)** mitochondrial derivatives (MD) wrapped around the axoneme (Ax);

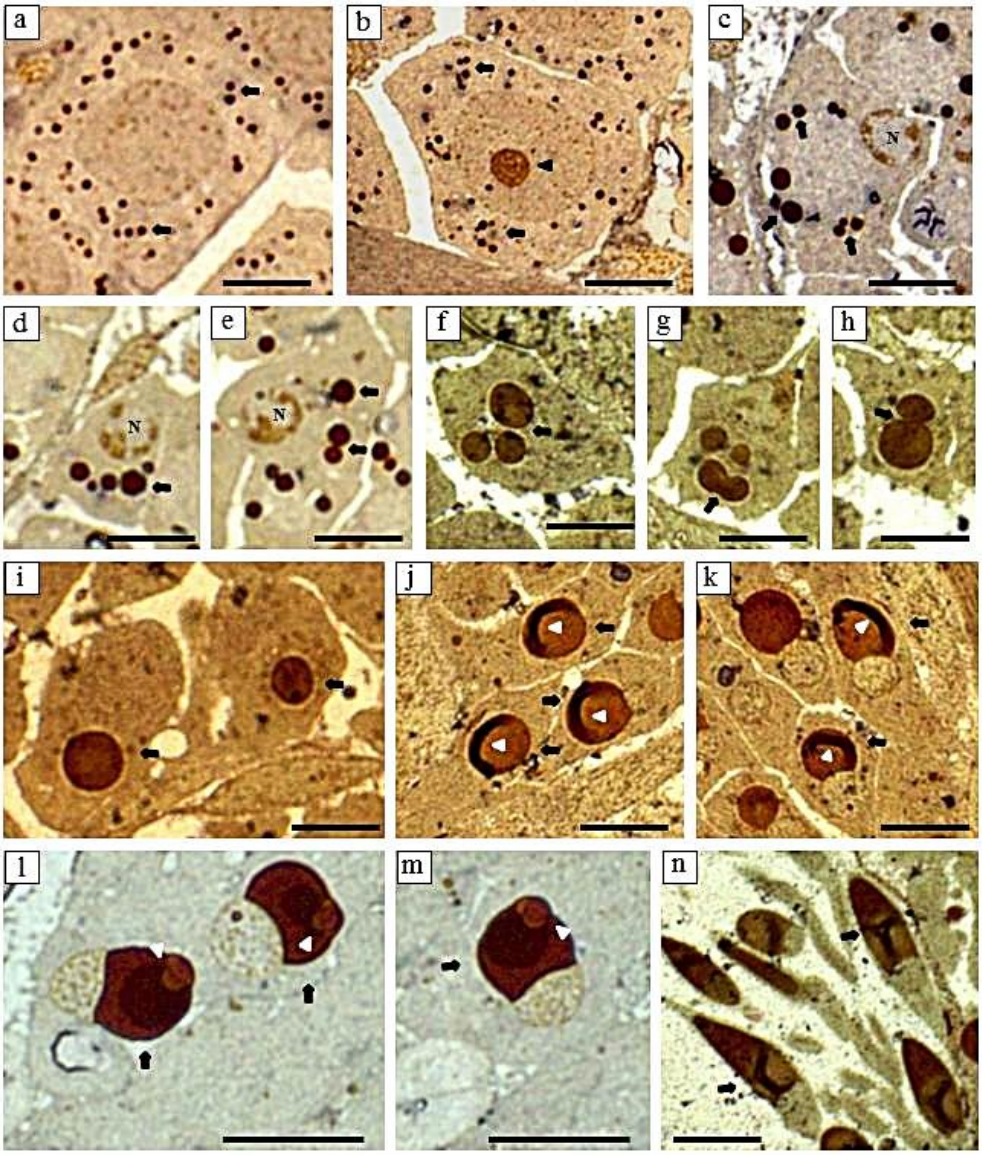

Figure 3. Semi-fine sections of *Martarega uruguayensis* testicles impregnated with silver ions. **a,b)** Prophase I with silver-positive markings (arrows); In figure **b**, note the nucleolus with rounded morphology (arrowhead); **c-e)** spermatids with several silver-positive argyrophil granules (arrows) and a nucleolus (N); **f,h)** fusion of silver-positive argyrophil granules (arrows); **i-k)** spermatid with silver-positive vesicle (arrows) with more intense staining evident inside (arrowhead); **l-n)** spermatid with silver-positive vesicle (arrows) with two regions inside, one of which is less intensely impregnated (arrowhead); Bars: 10 μm

ARTIGO IV

Spermatogenesis in aquatic Heteroptera: An ultrastructural spermiogenesis approach to family Belostomatidae, Notonectidae, Gerridae, Mesoveliidae e Veliidae

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Abstract

Were analyzed the ultrastructures present in the spermatids of nine species of aquatic Heteroptera through transmission electron microscopy. We can observe in the early spermatids a presence of chromatoid body located near the nucleus, the formation of mitochondrial complex and mitochondrial derivatives, the flagellar pattern of the axoneme of 9+9+2, and the process of abscission of the cytoplasm. The synapomorphic features of the group were observed for all species, being them a) two opposite bridges in the axoneme connect the flattened cisterns adherent to the internal side of each mitochondrial derivative to the intertubular material; b) flagellar pattern of the axoneme of 9+9+2; c) accessory bodies are absent all along the flagellum. The most interesting feature, was the difference in size between the mitochondrial derivatives presented in species of the genus *Buenoa* and *Martarega*, which belong to Notonectidae family.

Keywords: Axoneme, mitochondrial derivative, chromatoid body.

1. Introduction

Hemiptera is the fifth largest group of insects, with approximately 82.000 described species (CRYAN and URBAN, 2012). This order is composed of four suborders, being them Auchenorryncha, Coleorryncha, Sternorryncha e Heteroptera (FORERO, 2008), constituting one of the most numerous groups among the hemimetabolous, with its monophyly being recognized based on specific buccal structures of the mandible and the maxila (FORERO, 2008).

The suborder Heteroptera has seven infraorders with approximately 80 families, no other group of insects has such habitat diversity, like the Heteroptera, because it occurs in all continents, except Antarctica and some islands (SCHUH and SLATER, 1995).

Among the 80 families of Heteroptera, 23 are aquatic and semi-aquatic representatives: Aepophilidae, Aphelocheiridae, Belostomatidae, Corixidae, Gelastocoridae, Gerridae, Hebridae, Helotrephidae, Hermatobatidae, Hydrometridae, Leptopodidae, Macroveliidae, Mesoveliidae, Naucoridae, Nepidae, Notonectidae,

Ochteridae, Omaniidae, Paraphrynoveliidae, Pleidae, Potamocoridae, Saldidae e Veliidae. These insects are surprising for their ability to inhabit an extraordinary variety of ecosystems, been found in freshwater and marine habitats (MELO, 2009).

The spermiogenesis's ultrastructural characteristics of this suborder are considered as a tool, which can support in the elucidation of the evolutionary processes of these insects. However, the relations of sinapomorphy among the suborders of Hemiptera are still controversial, possibly the limited number of taxa to date. In Auchenorryncha suborder a typical sperm pattern is found in many species being, possibly, a synapomorphy of the suborder (DALLAI et al., 2016a), but we can find modifications in some species which do not have accessory bodies, and this can be an autapomorphy of the species (DALLAI, 1979; DALLAI et al., 2016a).

Some interspecific differences are found in the acrosome and axoneme of the Sternorryncha suborder (DALLAI et al., 2016a). For the Heteroptera suborder some synapomorphies are described in the literature, as the presence of two opposite bridges in the axoneme connecting the flattened cisterns adherent to the internal side of each mitochondrial derivative to the intertubular material of the doublets 1–2 and 4–5, flagellar pattern of the axoneme of 9+9+2 and absence of accessory bodies in the flagellum (DALLAI et al., 2016a), but a larger number of species should be analyzed to confirm this information.

Therefore, the aim of this paper was to analyze the ultrastructural characteristics during spermatogenesis of aquatic Heteroptera species of Belostomatidae, Notonectidae, Gerridae, Mesoveliidae e Veliidae families, in order to characterize the synapomorphies of the gametes of Heteroptera.

2. Material and Methods

Testicles of *Belostoma* sp. (Belostomatidae), *Martarega brasiliensis* (Notonectidae), *Martarega membranacea* (Notonectidae), *Buenoa amnigenus* (Notonectidae), *Rheumatobates crassifemur crassifemur* (Gerridae), *Limnogonus profugus* (Gerridae), *Limnogonus aduncus aduncus* (Gerridae), *Mesovelia mulsanti* (Mesoveliidae), *Rhagovelia tenuipes* (Veliidae) were extracted and fixed in Karnovsky

fixative solution for 3 hours. Samples were then washed twice in a Millonig buffer, and post-fixation was performed using Osmium Tetroxide 1% and Millonig buffer (1:3) for 2 hours in a refrigerator.

The material was washed thoroughly with double-distilled water and then dehydrated in a series of solutions with increasing concentrations of acetone until a concentration of 100% was reached. As part of the pre-infiltration in araldite:acetone (1:1) was applied overnight at room temperature. Next, the product was infiltrated in araldite for 2 hours at 37°C, followed by another 48 hours at 60°C. Ultra-fine sections were obtained using a Leica Ultracut UCT ultramicrotome. The ultra-fine sections were collected on grids and then contrasted using 2% uranyl acetate for 20 minutes (WATSON, 1958), followed by a 2% lead citrate solution with 1 N sodium hydroxide for 6 minutes (VENABLE and COGGESHALL, 1965).

The results of transmission electron microscopy were documented as electron micrographs obtained using a Jeol JEM – 100 CXII Transmission Electron Microscope at the Electron Microscopy Laboratory, Universidade de São Paulo – Faculdade de Medicina de Ribeirão Preto, USP-FMRP. Ribeirão Preto, São Paulo, Brazil.

3. Results

The analysis of early spermatids of all species revealed a nucleolus (Nu) rounded and more electrodensing than other structures (Figure 1a, b), the chromatoid body (CB) located near the nucleus (Figure 1a) and mitochondria (Mi) located in only one side of the cell (Figure 1b), initiating the formation of the mitochondrial complex (MC) (Figure 1c), which presented inside of it, small points more electrondense (Figure 1d).

During spermiogenesis the mitochondrial complex (MC) is divided into two mitochondrial derivatives (MD), which are initially rounded and positioned bilaterally to the axoneme (Figure 2a). However, with the development of spermatids the mitochondrial derivatives have different morphologies, depending on the species/family: *Belostoma* sp.(Belostomatidae), *R. tenuipes* (Veliidae), *L. profugus* (Gerridae) and *L. a. aduncus* (Gerridae) have mitochondrial derivatives of similar size,

positioned bilaterally to the axoneme, involving it partially (Figura 2b-d); *M. brasiliensis* (Notonectidae) and *M. membranacea* (Notonectidae) have the axoneme positioned at the center of the mitochondrial derivatives (Figure 2e); *B. amnigenus* (Notonectidae) and *R. c. crassifemur* (Gerridae) have mitochondrial derivatives of different sizes (Figure 2 f, g) and *M. mulsanti* (Mesoveliidae) has the mitochondrial derivatives completely enveloping the axoneme (Figure 2h, i). Inside the mitochondrial derivatives of all species analyzed, can be observed the presence of two or three regions with paracrystalline structures (Figure 2d and 3c).

Regarding the beginning of the organization of the axoneme, it was possible to observe early spermatids with triple microtubules (Figure 3a) and two central microtubules (Figure 3a). With the development of spermatid, the axoneme begins to acquire the flagellar pattern of 9+9+2, being 9 accessory microtubules, 9 doublet of subtubules and 2 central microtubules, with presence of intertubular material (Figure 3b,c). In addition, it was possible to identify two opposite bridges in the axoneme, which are connecting the flattened cisterns adherent to the internal side of each mitochondrial derivative to the intertubular material of the doublets 1–2 and 4–5 of the axoneme (Figure 3c). Cytoplasmic projections were also observed (Figure 3d), which are a mechanism by which spermatocytes lose cytoplasmic material, it was also possible to identify the nucleus (N), acrosome (Ac) and the centriole adjuvant in a longitudinal section of the spermatid (Figure 3e,f).

4.Discussion

Knowing the ultrastructural aspects of aquatic Heteroptera is extremely importante for environmental monitoring. Recently, Kheirallah (2015) used the specie *Anisops sardeus* (Notonectidae) as a biomarker for aquatic pollutants. In this paper, the author shows that the bioaccumulation of heavy metals, in particular Cu, Zn and Hg, in the testicles modifies aspects of ultrastructures such as mitochondria and nucleolus, resulting, therefore, in a useful tool to evaluate the degree of water pollution.

Dias et al. (2016) also emphasizes the importance of ultrastructural studies to evolution area, because the structure of the spermatozoon is relatively more stable and less influenced by environmental pressures than the external morphology.

All species analyzed showed early spermatids with a nucleolus (Nu) rounded and more electrodensing than the other structures, as was also evidenced by Alevi et al. (2015) for the *Gelastocoris flavus flavus* (Gelastocoridae) specie. Analysis of the nucleolar pattern can produce important data for environmental monitoring, due to changes in the morphology of the nucleolus what are related to the presence of pollutants in the water (KHEIRALLAH, 2015).

Another ultrastructure observed in early spermatids was chromate body (CB).This cytoplasmic organelle is located near the nucleus and is considered as a macromolecular complex, which probably coordinates the post-transcriptional control of gene products in spermatids and functions as a center for determining mRNA destinations, according to Parvinen (2005), Kotaja et al. (2006) and Kotaja; Sassone-Corsi (2007) since during spermiogenesis the nucleolus, although present, does not present transcriptional activity (ALEVI et al., 2015; BORGUETTI et al., 2015).The knowledge about this cytoplasmic organelle in Hemiptera is very limited (ALEVI et al., 2015). However, important discoveries were made, as characterization of the fibrillarin protein in CB (BORGUETTI et al., 2015), which corroborates the theory of the appearance of CB from nucleolar material (PERUQUETTI et al., 2008; PERUQUETTI et al., 2010; PERUQUETTI, 2011; ALEVI et al., 2015).

Regarding the Heteroptera's axoneme (AX), it is known that in early spermatids the microtubules begin to organize, being possible to evidence triple microtubules and two central microtubules (MENCARELLI et al., 2014). With the development of spermatid, the axoneme begins to assume an organizational pattern of 9+9+2, being 9 accessory microtubules, 9 doublet of subtubules and 2 central microtubules, with presence of intertubular material (ARAUJO et al., 2011; 2012). All species analyzed in this study also presented this pattern, which is considered a synapomorphy for the order Hemiptera (ARAUJO et al., 2011; DALLAI et al., 2016a)

All species analyzed showed the same pattern of migration of the mitochondria to one of the poles of the cell, which cluster to form the mitochondrial complex, which

subsequently divides into two mitochondrial derivatives, already described in the literature for Heteroptera (PRATT, 1970; PHILLIPS, 1970; TANDLER and HOPPEL, 1972; BACCETTI and AFZELIUS, 1976; DALLAI et al., 2016a). The only difference observed was the size of the mitochondrial derivatives of the species *B. amnigenus* (Notonectidae) and *R. c. crassifemur* (Gerridae).

For the family Notonectidae an interesting fact was the difference between the genus *Buenoa* and *Martarega*, the species of *Martarega* have mitochondrial derivatives of similar sizes and the species of *Buenoa* present mitochondrial derivatives of different sizes.

In species of the genus *Drosophila* and *Zaprionus*, the difference in mitochondrial derivative sizes has been correlated with phylogenetic data to understand the radiation of Drosophilidae (MOJICA et al., 2000; REGO et. al., 2016). The difference found in the species of the Notonectidae family could be used in the future in conjunction with evolutionary data, with the purpose of better understanding the evolutionary process of this family.

During the process of differentiation of spermatids, the inside of the mitochondrial derivatives present a structure of protein nature, organized in a paracrystalline pattern and many species of insects accumulate these structures in the mitochondrial derivatives during spermiogenesis (PHILLIPS, 1970; WARNER, 1971; ROSATI et al., 1976; BÁO et al., 1992). This paracrystalline structure is also known as crystalloid and is formed by a protein rich in proline denominated of crystalomitina (BACCETTI et al., 1977). In Heteroptera of the families Pentatomidae, Reduviidae and Gerridae it was evidenced the presence of two or three crystalline bodies within the mitochondrial derivatives (ARAUJO et al., 2011). In the species analyzed in the present study, the paracrystalline structure was also present, partially filling the interior of the mitochondrial derivatives.

In the analyzed species it was possible to observe cytoplasmic projections forming large spirals, being an interesting mechanism of abscision of the excess of cytoplasm. This is an important process related to sperm formation, where large membrane spirals involving pieces of cytoplasm are left behind during the maturation of spermatids (PHILLIPS, 1970).

Another observed ultrastructure was the adjunct of the centriole, whose structure gives rise to a flagellar axoneme, being the proteins of the pericentriolar material responsible for producing microtubules that are involved in the elongation and formation of the spermatid tail (DALLAI et al., 2016b).

All the ultrastructures described in the analyzed species were similar to those described in the literature for Heteroptera, corroborating the synapomorphic characteristics presented by Dallai et al (2016a), being them: a) two opposite bridges in the axoneme connecting the flattened cisterns adherent to the internal side of each mitochondrial derivative to the intertubular material; b) flagellar pattern of the axoneme of 9+9+2; c) accessory bodies absent all along the flagellum.

The ultrastructural characteristics presented in this work are important from the environmental and evolutionary point of view, because these data can be used as a comparative tool for environmental assessment of water receiving domestic and agricultural waste, as well as helping to understand the evolutionary relationship of aquatic Heteroptera, emphasizing the importance of new studies, with emphasis, mainly, in the characterization of the size of the mitochondrial derivatives.

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References

Alevi K.C.C., Mendonça P.P., Pereira N.P., Rosa J.A., Azeredo-Oliveira M.T.V.(2014). Is there post-meiotic transcriptional activity during hemipteranspermiogenesis? Invertebrate Reproduction & Development. http://dx.doi.org/10.1080/07924259.2014.889767

Alevi K.C.C., Pereira L.L.V., Moreira F.F.F., Barbosa J.F., Taboga S.R., Itoyama M.M. (2015). Histological and Ultrastructural Analysis of Spermatogenesis in *Gelastocoris flavus flavus* (Heteroptera: Nepomorpha). Entomology, Ornithology & Herpetology .http://dx.doi.org/10.4172/2161-0983.1000157

Araújo V.A., Báo S.N., Lino-Neto J. (2012). Polymorphism of spermatozoa in *Largus rufipennis* Laporte 1832 (Heteroptera: Pyrrhocoroidea: Largidae). Acta Zoologica. http://dx.doi.org/10.1111/j.1463-6395.2011.00559.x

Araújo V.A., Lino-Neto J., Ramalho F.S., Zanuncio J.C., Serrão J.E. (2011). Ultrastructure and heteromorphism of spermatozoa in five species of bugs (Pentatomidae: Heteroptera). Micron. http://dx.doi.org/10.1016/j.micron.2011.02.001

Afzelius B.A., Baccetti B., Dallai R. (1976). The giant spermatozoon of Notonecta. Journal of Submicroscopic Cytology and Pathology.8 149–161.

Afzelius, B.A. Dallai, R., Lindskog, P. (1985). Spermatozoa of saldid bugs (Insecta, Hemiptera, Leptopodomorpha). Journal of Ultrastructure Research. http://dx.doi.org/10.1016/S0022-5320(85)80008-3

Baccetti, B. (1972). Insect sperm cell. Advances in Insect Physiology. http://dx.doi.org/10.1016/S0065-2806(08)60279-9

Baccetti, B.; Afzelius, B.A. (1976). The biology of the sperm cell. Basel, New York, Karger, 254p.

Baccetti B., Dallai R., Pallini V., Rosati F., Afzelius B.A. (1977). Protein of insect sperm mitochondrial crystals: crystallomitin. The Journal of Cell Biology.73 594-600.

Báo S.N., Lins U., Farina B., De Souza W. (1992). Mitochondrial derivatives of *Culex quinquefasciatus* (Culicidae) spermatozoon: Some new aspects evidenced by cytochemistry and image processing. Journal of Structural Biology. http://dx.doi.org/10.1016/1047-8477(92)90066-J

Báo S.N., De Souza W. (1994). Structural specialization in the flagellum of the spermatozoon of the bloodsucking bug (Rhodnius prolixus; Hemiptera, Reduviidae). Tissue Cell. http://dx.doi.org/10.1016/0040-8166(94)90016-7

Borgueti A.O., Alevi K.C.C., Silistino-Souza R., Rosa J.A., Azeredo-Oliveira M.T.V. (2015). Immunofluorescence and ultrastructural analysis of the chromatoid body during spermatogenesis of *Triatoma platensis* and *T. rubrovaria* (Hemiptera, Triatominae). Micron. http://dx.doi.org/10.1016/j.micron.2015.04.002

Cryan J.R., Urban J.M. (2012). Higher-level phylogeny of the insect order Hemiptera: is Auchenorrhyncha really paraphyletic? Sistematic Entomology. http://dx.doi.org/10.1111/j.1365-3113.2011.00611.x

Dallai R., Afzelius B.A. (1980). Characteristics of the sperm structure in Heteroptera (Hemiptera, Insecta). Journal of Morphology.
http://dx.doi.org/10.1002/jmor.1051640306

Dallai R. (1979). An overview of atypical spermatozoa in insects. In *The Spermatozoon*, ed. DW Fawcett, JM Bedford, pp. 253–65. Baltimore, MD: Urban Schawarzenberg

Dallai R., Gottardo M., Beutel R.G. (2016). Structure and Evolution of Insect Sperm: New Interpretations in the Age of Phylogenomics. Annu. Rev. Entomol. http://dx.doi.org/10.1146/annurev-ento-010715-023555

Dallai R., Paoli F., Mercati D., Lupetti P. (2016). The centriole adjunct of insects: Need to update the definition. Tissue and Cell.http://dx.doi.org/10.1016/j.tice.2016.02.001

Dias G., Lino-Neto J., Mercati D., Dallai R. (2016). The sperm structure and spermiogenesis of the heteropteran *Coptosoma scutellatum* (Geoffroy) with emphasis on the development of the centriole adjunct. Micron. http://dx.doi.org/10.1016/j.micron.2015.12.005

Dolder H. (1988). Cytoskeletal bridges between organelles in sperm flagellum of Triatoma infestans (Hemiptera, Reduviidae). Journal of Ultrastructure and Molecular Structure Research. http://dx.doi.org/10.1016/0889-1605(88)90005-5

Fernandes A.P., Báo S.N.(1998). Spermiogenesis in phytophagous bug (Hemiptera: Pentatomidae): an ultrastructural study. Journal of Submicroscopic Cytology and Pathology. 30 (4) 485-493.

Forero D. (2008). The systematics of the Hemiptera. Revista Colombiana de Entomologia. 34 (1), 1-21.

Howell W.M., Black D.A. (1980). Controlled silver staining of nucleolus organizer regions with protective colloidal developer: I-step method. Experientia. http://dx.doi.org/10.1007/BF01953855

Kheirallah D.A. (2015). Ultrastructure biomarker in *Anisops sardeus* (Heteroptera: Notonectidae) for the assessment and monitoring of Water Quality of Al-Mahmoudia Canal, Western Part of Nile Delta, Egypt. Journal of Bioscience and Applied Research. 1 (6) 326-334.

Kotaja N., Bhattacharyya S.N., Jaskiewics L., Kimmins S., Parvinen M., Filipowicz W., Sassone-Corsi P. (2006). The chromatoid body of male germ cells: Similarity with processing bodies and presence of Dicer and microRNA pathway components. Proceedings of the National Academy of Sciences of the United States of America.http://dx.doi.org/10.1073/pnas.0509333103

Kotaja N., Sassone-Corsi P. (2007). The chromatoid body: a germ-cell specific RNA-processing centre. Nature reviews Molecular cell biology. http://dx.doi.org/10.1038/nrm2081

Lee C.E. (1991). Morphological and phylogenetic studies on the true water bugs (Hemiptera: Heteroptera). Journal of Natural History Society of Korea.21 1–183.

Lee Y.H., Lee C.E. (1992). Ultrastructure of spermatozoa and spermatogenesis in Nepomorpha (Insecta: Heteroptera) with special reference to phylogeny. Zoological Science. 9 971-981.

Melo M.C. (2009). Biodiversidad de Heteroptera (Hemiptera) acuáticas y semiacuáticas de la Patagonia argentina. Revista de la Sociedad Entomológica Argentina. 68 (1-2), 177-185.

Mello M.L.S., Vidal B.C. (1980). Práticas de Biologia Celular. FUNCAMP Editora Edgard Blücher LTDA, Campinas, São Paulo, Brasil.

Mojica J.M., File-Emperador S., Bruck D.L. (2000). Sperm bundle and spermatozoon ultrastructure in two species of the cardini group of Drosophila. Invertebrate Reproduction & Development. http://dx.doi.org/10.1080/07924259.2000.9652413

Parvinen M. (2005). The chromatoid body in spermatogenesis. International Journal of Andrology. http://dx.doi.org/10.1111/j.1365-2605.2005.00542.x

Peruquetti R.L., Assis I.M., Taboga S.R., Azeredo-Oliveira M.T.V. (2008). Meiotic nucleolar cycle and chromatoid body formation during the rat (*Rattus novergicus*) and mouse (*Mus musculus*) spermiogenesis. Micron. http://dx.doi.org/10.1016/j.micron.2007.02.009

Peruquetti R.L., Taboga S.R., Azeredo-Oliveira M.T.V. (2010). Characterization of Mongolian gerbil chromatoid bodies and their correlation with nucleolar cycle during spermatogenesis. Reproduction in Domestic Animals.http://dx.doi.org/10.1111/j.1439-0531.2008.01204.x

Peruquetti R.L., Taboga S.R., Santos L.R.S.,Oliveira C., Azeredo-Oliveira M.T.V. (2011). Nucleolar cycle and chromatoid body formation: Is there a relationship between these two processes during spermatogenesis of Dendropsophusminutus (Amphibia, Anura)?.Micron.http://dx.doi.org/10.1016/j.micron.2010.07.009

Phillips D.M.,1970. Insect sperm: their structure and morphogenesis. The Journal of Cell Biology.44 243-277.

Pratt S. (1970). Formation and differentiation of the Nebenkern in spermatios of nahemipteran insect, *Murgantia iristionia*. In: BACCETTI, B. Comparative Spermatology. Academic, London, p. 301-310,

Rego L.N.A.A., Alevi K.C.C., Azeredo-Oliveira M.T., Madi-Ravazzi L. (2016). Ultrastructural features of spermatozoa and their phylogenetic application in Zaprionus (Diptera, Drosophilidae). FLY. http://dx.doi.org/10.1080/19336934.2016.1142636

Rosati F., Selmi G., Mazzini M. (1976). Comparative observation on the mitochondrial derivatives of insect sperm. Journal of submicroscopic cytology.8 56-67.

Schuh T.T., Slater J.A. (1995). True bugs of the world (Hemiptera: Heteroptera) Classification and natural history, Cornell UNIVERSITY press, Ithaca, New York, xii, 338 pp.

Tandler B., Hoppel C. (1972). Mitochondria in spermatogenesis. In: Tandler, B.; Hoppel, C. Mitochondria, 1ª Ed., New York and London, Academic Press, p. 36-39.

Tokuyasu K.T. (1974). Dynamics of spermiogenesis in *Drosophila melanogaster*. III. Relation between axoneme and mitochondrial derivatives.Experimental Cell Research.http://dx.doi.org/10.1016/0014-4827(74)90402-9

Trandaburu V. (1973). The acrosome and the centriole adjunct in Eurydema ventrali sKol.(Heteroptra, Pentatomidae). Travaux Du Muséum National D´Histoire Naturalle Grigore Antipa.13 137–145.

Venable J.H., Coggeshall R. (1965). A simplified lead citrate stain for use in electron microscopy. J Cell Biol. 25 407-408.

Warner F.D. (1971). Spermatid differentiation in the blowfly *Sarcophaga bullata* with particular reference to flagelar morphogenesis. Journal of Ultrastructure Research. http://dx.doi.org/10.1016/S0022-5320(71)80153-3

Watson, M.L., 1958. Staining of tissue sections for electron microscopy with heavy metals. J Biophys Biochem Cytol. http://dx.doi.org/10.1083/jcb.4.6.727

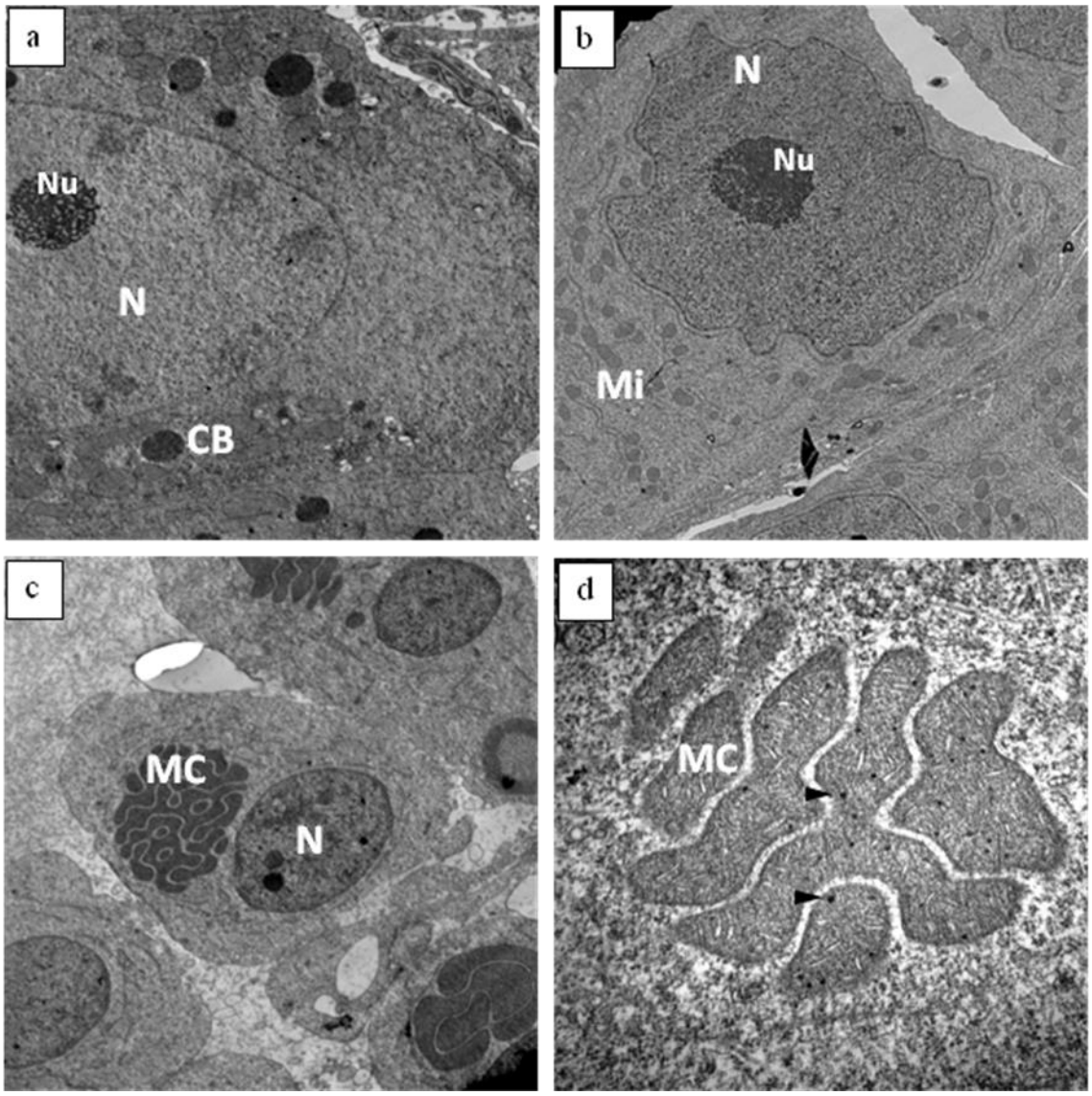

Figure 1. Electron micrographs of testicular cells from *Martarega brasiliensis* (a), *Mesovelia mulsanti* (b) and *Belostoma sp.* (c,d). **a,b)** Early spermatids showing the nucleus (Nu), nucleolus (Nu) more eletrodense than the other structures, chromoid body (CB) in the cytoplasm region and mitochondria (Mi) migrating to one side of the cell; **c)** spermatid with mitochondrial complex (MC) and nucleus (N); **d)** mitochondrial complex (MC) with a more eletrodense dots inside (arrowhead). Magnification: a) 7750; b)6000; c) 7750; d)35970.

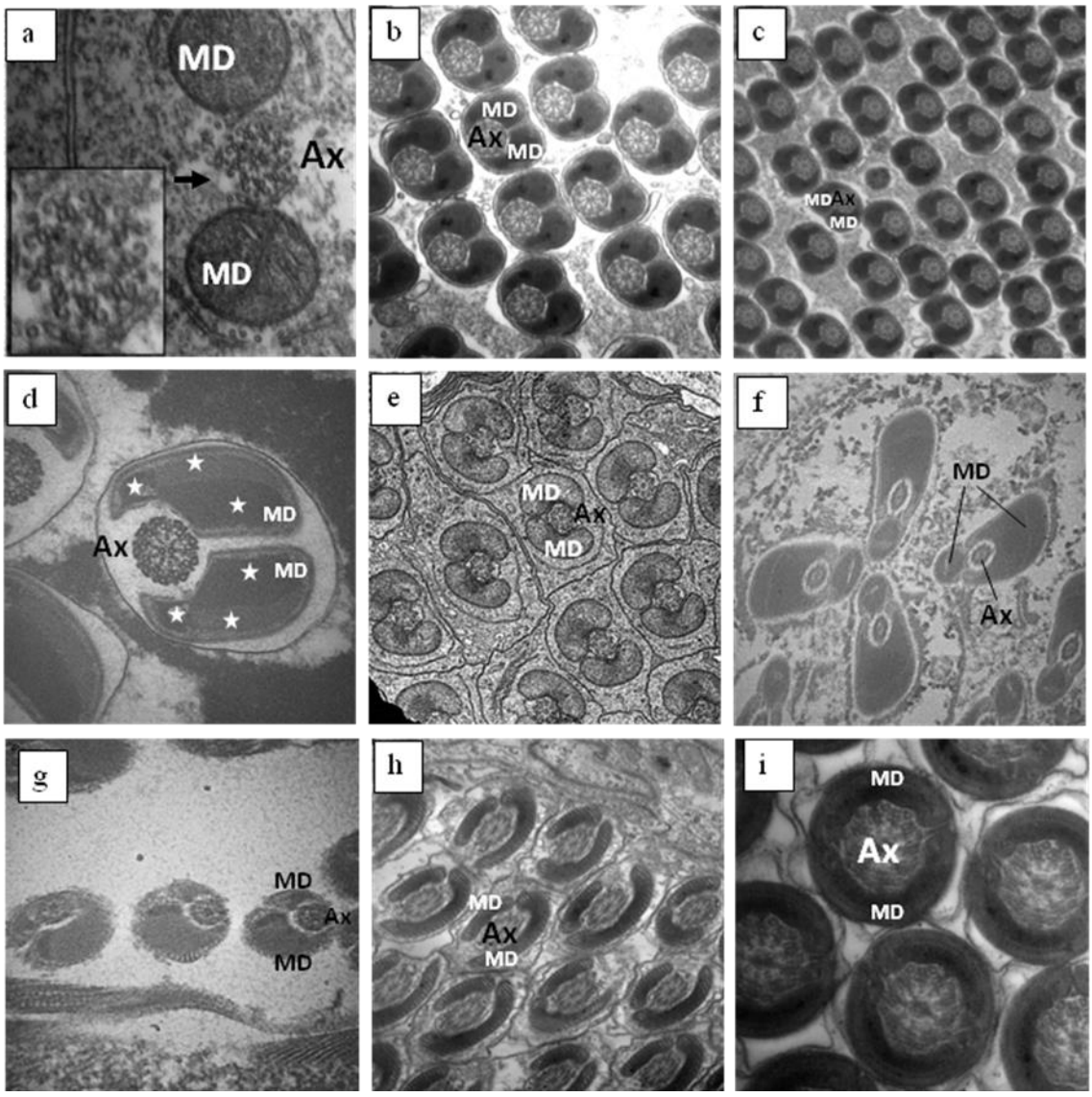

Figure 2. Electron micrographs of testicular cells from *Belostoma* sp. (a,b), *Rhagovelia tenuipes* (c), *Limnogonus profugus* (d), *Martarega brasiliensis* (e), *Buenoa amnigenus* (f), *Rheumatobates crassifemur crassifemur* (g) and *Mesovelia mulsanti* (h,i). **a)** Early spermatids with mitochondrial derivatives (MD) arranged bilaterally relative to the axoneme (Ax) which presents triple microtubules (arrow); **b-d)** mitochondrial derivatives (MD) of similar sizes arranged bilaterally relative to the axoneme (Ax), observe in Figure d the presence of three areas with paracrystalline structures (asterisk); **e)** mitochondrial derivatives (MD) of similar sizes with the axonema (Ax) positioned in the center; **f,g)** mitochondrial derivatives (MD) of different sizes; **h-i)** mitochondrial derivatives (MD) that will prolong until completely surround the axoneme. Magnification: a) 77500; b) 46460; c) 12930; d)100000; e) 46460; f)27000; g)50000; h)27800; i)100000

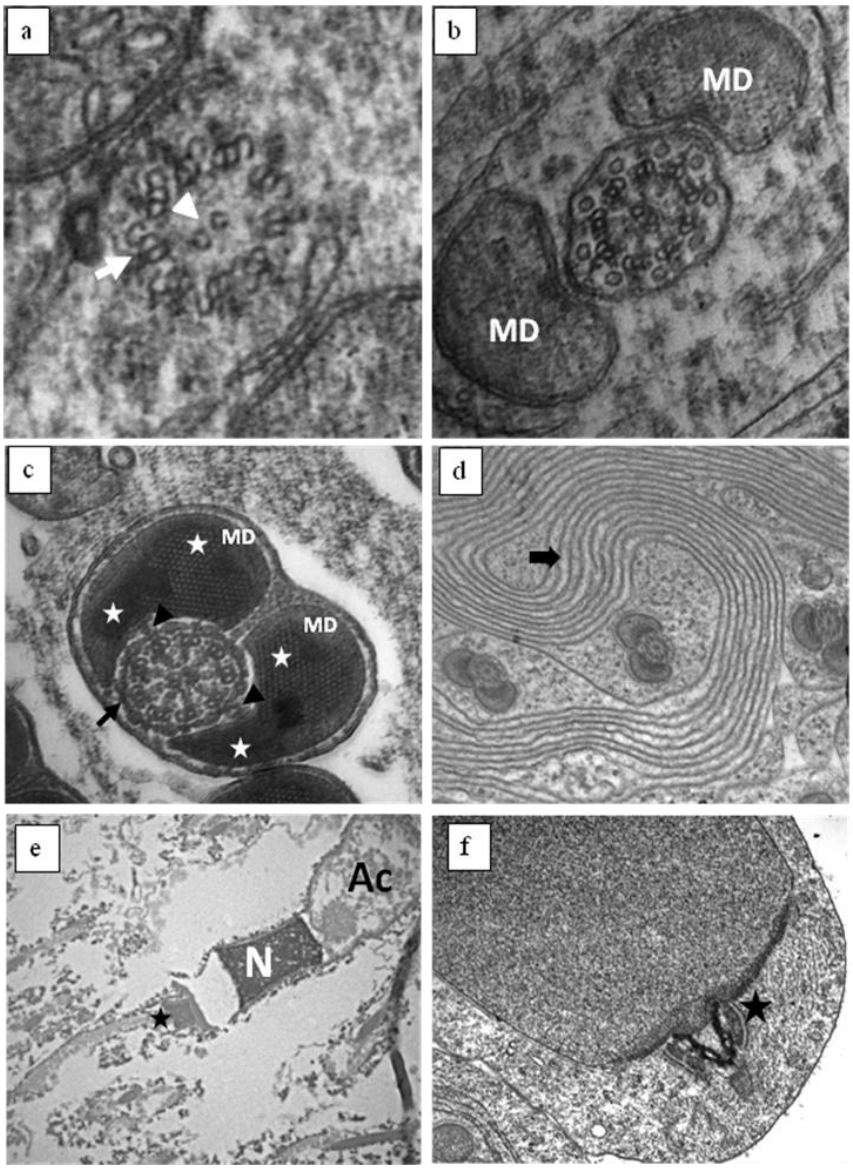

Figure 3. Electron micrographs of testicular cells from *Martarega brasiliensis* (a and f), *Mesovelia mulsanti* (b and d), *Belostoma* sp. (c) and *Martarega membranacea* (e). **a)** spermatid with axonema composed of triple microtubules (arrow) and two central microtubules (arrowhead); **b)** mitochondrial derivatives (MD) arranged bilaterally relative to the axoneme (Ax), and the axoneme with its 9+9+2 pattern; **c)** 9+9+2 flagellar axoneme, intertubular material (arrow), mitochondrial derivatives (MD) with the presence of two regions with paracrystalline structures(asterisk) and two opposite bridges connecting the flattened cisterns adherent to the internal side of each mitochondrial derivative to the intertubular material of the doublets 1–2 and 4–5 (arrowhead); **d)** Spermatid with cytoplasmic extensions (arrow); **e,f)** longitudinal section of spermatid showing acrosome (Ac), nucleus (N), and the centriole adjunct (asterisk), observe in Figure f the centriole adjunct in greater detail. Magnification: a)60000; b)77500; c) 129300; d) 21560; e) 10000; f) 21560.

IV. DISCUSSÃO GERAL

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Conhecer os aspectos da espermatogênese de Heteroptera aquáticos é de extrema importância. Dias et al. (2016) ressalta essa importância para a área de evolução, uma vez que a estrutura do espermatozoide é relativamente mais estável e menos influenciada pelas pressões ambientais do que a morfologia externa, dessa forma dados citogenéticos e ultraestruturais poderão futuramente serem utilizados, em conjunto com dados evolutivos, com a finalidade de compreender melhor o processo evolutivo das famílias estudadas.

Com relação ao comportamento nucleolar da espécie *Martarega brasiliensis* os corpúsculos nucleolares observados em células de Prófase I (de um a quatro) comprovam uma grande atividade sintética das células nessa fase da divisão celular, uma vez que os corpúsculos nucleolares estão relacionados com a atividade celular (TAVARES; AZEREDO-OLIVEIRA, 1997, BRESSA et al., 2003). Em células na fase de Metáfase I foi possível identificar as regiões organizadoras nucleolares (NOR) na região telomérica de um dos autossomos. Descrições de marcações de NOR, na metáfase I, de Heteroptera aquáticos são raras, sendo observadas em poucas espécies como, por exemplo, em *Limnogonus aduncus* na qual houve marcação em um autossomo (CASTANHOLE et al., 2008). Nessa espécie foi possível ainda observar em Anáfase I vários corpúsculos nucleolares persistindo até a fase de Telófases I, em *Asellus aquaticus* (Isopoda), foram visualizados durante todo o processo de espermatogênese (DICASTRO et al., 1983), em *Callicrania seoanei* (Orthoptera), da intercinese até a pró-metáfase II (SANTOS et al., 1987) e em *Triatoma infestans* e *T. sordida* (Heteroptera, Reduviidae), as marcações estavam presentes até a metáfase I (TAVARES; AZEREDO-OLIVEIRA, 1997) e, finalmente, em *Carlisis wahlbergi* (Heteroptera, Coreidae) a semi-persistência nucleolar (presença de corpúsculos nucleolares durante as metáfases) foi observada até a metáfase II (FOSSEY; LIEBENBERG, 1995), enquanto em *Acanthocoris sordidus* (Heteroptera, Coreidae) e *Coptosoma punctissimum* (Heteroptera, Plataspidae), os nucléolos foram detectados nas placas metafásicas de espermatócitos primários e secundários (YOSHIDA, 1947).

Uma das diferenças observadas durante a espermiogênese de *Martarega brasiliensis* foi a presença de uma vesícula nas espermátides iniciais e em alongamento, não observada nas espécies terrestres (Pentatomidae, SOUZA et al., 2007b, 2008; Coreidae, SOUZA et al., 2007c), sendo portanto necessária análises ultraestruturais para melhor compreensão dessa estruturas.

Estudos relacionados aos aspectos ultraestruturais da espermatogênese de Heteroptera aquáticos são escassos, porém de fundamental importância para o entendimento de aspectos observados, por exemplo, em material preparado por esmagamento, pois algumas estruturas não são possíveis de serem analisadas em microscopia de luz. Além disso, conhecer os aspectos ultraestruturais de Heteroptera aquáticos é de extrema importância para monitoramento ambiental. Recentemente, Kheirallah (2015) utilizou a espécie *Anisops sardeus* (Notonectidae) como biomarcador para poluentes aquáticos. Nesse trabalho, o autor mostra que a bioacumulação de metais pesados, em particular Cu, Zn e Hg, nos testículos modifica os aspectos de ultraestruturas como, por exemplo, mitocôndrias e nucléolo, resultando, portanto, em uma ferramenta útil para avaliar o grau de poluição da água.

Algumas ultraestruturas da espermatogênese dos Heteroptera já foram descritas como o acrossomo, axonema, mitocôndrias, complexo mitocondrial (Nebenkern), derivados mitocondriais, e nucléolo em algumas espécies de Heteroptera como *Gerris najas* (Gerridae) (WERNER; WERNER, 1993), *Notonecta glauca* (Notonectidae) (WERNER et al., 1988), *Euchistus heros* (FERNANDES et al., 2001), em espécies da família Pentatomidae (ARAUJO et al., 2011; TRANDABURU, 1973; FERNANDES; BÁO, 1998), Reduviidae (DOLDER, 1988; BÁO; DESOUZA, 1994), em Heteroptera aquáticos (AFZELIUS et al., 1985; LEE, 1991; LEE; LEE, 1992), e em insetos pertencentes a infraordem Leptopodomorpha (AFZELIUS et al., 1976), Cimicomorpha, Gerromorpha e Pentatomomorpha (DALLAI; AFZELIUS, 1980; ARAUJO et al., 2011).

Todas as espécies analisadas apresentaram espermátides iniciais com um nucléolo (Nu) arredondado e mais eletrodenso que as outras estruturas. A análise do padrão nucleolar pode gerar dados importantes para o monitoramento ambiental, uma

vez que alterações no padrão da morfologia do nucléolo estão relacionadas com a presença de poluentes na água (KHEIRALLAH, 2015).

Com relação ao axonema (AX) dos Heteroptera, sabe-se que em espermátides inicias os microtúbulos começam a se organizar sendo possível evidenciar triplos microtúbulos e os dois microtúbulos centrais (MENCARELLI et al., 2014). Com o desenvolvimento da espermátide, os triplos microtúbulos passam a assumir um padrão organizacional de 9+9+2, sendo 9 microtúbulos acessórios, 9 dupletos de subtúbulos e 2 microtúbulos centrais, com presença de material intertubular (ARAUJO et al., 2011, 2012). Esse padrão também foi descrito para outras espécies de Heteroptera (Pentatomidae, TRANDABURU, 1973; FERNANDES; BÁO, 1998; Reduviidae, DOLDER, 1988; BÁO; DESOUZA, 1994; Heteroptera aquáticos, AFZELIUS et al., 1985; LEE, 1991; LEE; LEE, 1992; Leptopodomorpha, AFZELIUS et al., 1976; Cimicomorpha, Gerromorpha, e Pentatomomorpha, DALLAI; AFZELIUS, 1980; ARAUJO et al., 2011). Todas as espécies analisadas nesse trabalho também apresentaram esse padrão que é considerado uma sinapomorfia para a ordem Hemiptera (ARAUJO et al., 2011; DALLAI et al., 2016a)

As mitocôndrias durante a espermatogênese assumem diferentes morfologias Em insetos a regularidade nas formas da mitocôndria é particularmente notável (PHILLIPS, 1970). No decorrer da espermatogênese as mitocôndrias passam por mudanças onde sua estrutura típica é completamente modificada. Nos estágios iniciais de diferenciação ocorre um complexo processo de fusão e rearranjo das mitocôndrias formando o complexo mitocondrial também denominado de “Nebenkern”, em trabalhos mais antigos (PRATT, 1970; PHILLIPS, 1970; TANDLER; HOPPEL, 1972; BACCETTI, 1972; BACCETTI; AFZELIUS, 1976; DALLAI et al., 2016a). Com o prosseguimento da espermatogênese, ocorre a divisão do complexo mitocondrial em dois derivados mitocondriais (DM), que no processo de alongamento do espermatozóide posicionam-se bilateralmente em relação ao axonema (TOKUYASU, 1974). Todas as espécies analisadas seguiram o mesmo padrão de migração das mitocôndrias para um dos polos da célula, fusão para a formação dos complexos mitocondriais e divisão deste em dois derivados mitocondriais. A única diferença observada foi no tamanho dos derivados mitocondriais das espécies *B. amnigenus* da família Notonectidae e *R. c.*

crassifemur da família Gerridae. Para a família Notonectidae um dado interessante foi a diferença entre os gêneros *Buenoa* e *Martarega*, uma vez que as espécies do gênero *Martarega* apresentaram derivados mitocondriais de tamanhos semelhantes e a espécie do gênero *Buenoa* apresenta derivados mitocondriais de tamanhos diferentes.

Em espécies do gênero *Drosophila e Zaprionus* a diferença nos tamanhos dos derivados mitocondriais tem sido correlacionado com dados filogenéticos para entender a radiação desses gêneros (MOJICA et al., 2000; REGO et. al., 2016), sendo assim a diferença encontrada nas espécies da família Notonectidae poderá futuramente ser utilizada em conjunto com dados evolutivos com a finalidade de compreender melhor o processo evolutivo dessa família.

Durante o processo de diferenciação das espermátides os derivados mitocondriais são preenchidos ao longo de sua extensão por uma estrutura de natureza proteica, organizada num padrão paracristalino, e muitas espécies de insetos acumulam essas estruturas nas mitocôndrias durante a espermiogênese (PHILLIPS, 1970; WARNER, 1971; ROSATI et al., 1976; BÁO et al., 1992). Essa estrutura paracristalina também é conhecida como cristalóide e é formado por uma proteína rica em prolina, que foi designada de cristalomitina (BACCETTI et al., 1977). Em Heteroptera das famílias Pentatomidae, Reduviidae e Gerridae foi evidenciado a presença de dois ou três corpos cristalinos no interior dos derivados mitocondriais (ARAUJO, 2011). Nas espécies analisadas no presente trabalho, a estrutura paracristalina também estava presente, preenchendo parcialmente o interior dos derivados mitocondriais.

Várias são as funções sugeridas aos derivados mitocondriais dos espermatozoides de insetos, podendo participar no controle e regulação da forma do movimento flagelar e estando relacionado com o processo de estocagem e liberação de energia necessária para a mobilidade flagelar (PHILLIPS, 1974; YASUZUMI, 1974; TOKUYASU, 1975). No entanto, Perotti (1973) contraria esta última afirmação, supondo que o material armazenado nos DM pode estar envolvido na ativação e nutrição do ovócito após a fertilização.

Outra ultraestrutura evidenciada em espermátides iniciais foi o corpo cromatóide (CB). Essa ultraestrutura citoplasmática encontra-se localizada próxima ao núcleo e é considerada como um complexo macromolecular que, provavelmente, desempenha um

papel de coordenador do controle pós-trancricional de produtos gênicos em espermátides e funciona como um centro de determinação dos destinos de RNAm (PARVINEN 2005; KOTAJA et al., 2006; KOTAJA, SASSONE-CORSI 2007) uma vez que durante a espermiogênese o nucléolo, embora presente, não apresenta atividade transcricional (ALEVI et al., 2015; BORGUETTI et al., 2015). Os conhecimentos sobre essa organela citoplasmática em Hemiptera são bastante limitados (ALEVI et. al., 2015). No entanto, importantes descobertas foram realizadas, como a caracterização da presença da proteína fibrilarina no CB (BORGUETTI et al., 2015), o que corrobora a teoria do surgimento do CB a partir de material nucleolar (PERUQUETTI et al., 2008a,b; PERUQUETTI, 2011, ALEVI et al., 2015).

Nas espécies *Belostoma* sp. (Belostomatidae), *Martarega brasiliensis* (Notonectidae), *M. membranacea* (Notonectidae), *Buenoa amnigenus* (Notonectidae), *Rheumatobates crassifemur crassifemur* (Gerridae), *Limnogonus profugus* (Gerridae), *L. aduncus aduncus* (Gerridae), *Mesovelia mulsanti* (Mesoveliidae), *Rhagovelia tenuipes* (Veliidae) foi possível observar projeções citoplasmáticas formando grandes espirais, sendo este um interessante mecanismo de abscisão do excesso de citoplasma. Esse é um importante processo relacionado à formação do espermatozoide sendo observado também em Gafanhotos e Mamíferos, onde grandes espirais de membrana envolvendo pedaços de citoplasma são deixados para trás durante a maturação das espermátides (PHILLIPS, 1970). Outra ultraestrutura observada nessas espécies foi o adjunto do centríolo, cuja estrutura dá origem a um axonema flagelar, sendo as proteínas do material pericentriolar responsáveis pela produção de microtúbulos que estão envolvidos no alongamento e formação da cauda da espermatide (DALLAI et al. 2016b).

Todas as ultraestruturas descritas nas espécies analisadas foram semelhantes às descritas na literatura para Heteroptera, corroborando as características sinapomórficas apresentadas por Dallai et al (2016a) sendo elas: a) a presença de duas pontes que ligam o material intertubular do axonema flagelar às cisternas achatadas que aderem aos lados internos dos derivados mitocondriais; b) padrão flagelar do axonema de 9+9+2 e c) ausência de corpos acessórios.

As características ultraestruturais apresentadas nesse trabalho são importantes do ponto de vista ambiental e evolutivo, pois esses dados poderão ser utilizados como ferramenta comparativa para avaliação ambiental de águas que recebem resíduos domésticos e agrícolas, assim como auxiliar no entendimento da relação evolutiva dos Heteroptera aquáticos, ressaltando a importância de novos estudos, com ênfase, principalmente, na caracterização do tamanho dos derivados mitocondriais.

V. CONCLUSÕES

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As análises do comportamento nucleolar de *Martarega brasiliensis* e o trabalho de ultraestrutura realizado com as espécies de Heteroptera aquáticos, pertencentes às famílias Belostomatidae (*Belostoma sp.)*, Gelastocoridae (*Gelastocoris flavus flavus*), Gerridae (*Rheumatobates crassifemur crassifemur*, *Limnogonus profugus*, *L. a. aduncus)*, Notonectidae (*Buenoa amnigenus, Martarega brasiliensis, M. membranacea* e *M. uruguayensis)*, Veliidae (*Rhagovelia tenuipes)* e Mesoveliidae (*Mesovelia mulsanti*) permitiu-nos concluir que:

Comportamento nucleolar de *Martarega brasiliensis:*

- Apresenta de nenhuma à quatro marcações em Prófase I;
- Número de corpúsculos está relacionado com a atividade sintética das células;
- Células em Metáfase I possuem regiões organizadoras nucleolares na região telomérica de um dos autossomos;
- As marcações prata positivas ocorrem em todas as fases da espermiogenese.

Ultraestrutura da espermatogênese:

- Todas as espécies analisadas apresentaram um padrão fagelar para o axonema de 9+9+2 sendo, portanto o padrão para as espécies de Heteroptera aquáticos;
- As mitocôndrias se agrupam formando um complexo mitocondrial, que posteriormente se divide em dois derivados mitocondriais;
- Os derivados mitocondriais são preenchidos por uma estrutura proteica paracristalina;
- Os derivados mitocondriais assumem diferentes morfologias, dependendo da espécie/família: *Belostoma* sp. (Belostomatidae), *R. tenuipes* (Veliidae), *L. profugus* e *L. a. aduncus* (Gerridae) e *G. f. flavus* (Gelastocoridae) possuem derivados de tamanhos semelhantes, posicionados bilateralmente ao axonema, envolvendo-o parcialmente pelas extremidades; *M. uruguayensis M. brasiliensis* e *M. membranacea* (Notonectidae) possuem o axonema posicionado no centro dos derivados; *B. amnigenus* (Notonectidae) e *R. c. crassifemur* (Gerridae) apresentaram um derivado maior que o outro e em *M. mulsanti* (Mesoveliidae)

os derivados mitocondriais vão se prolongando e envolvendo completamente o axonema;

- A formação do acrossomo ocorre no início da espermiogênese com a presença de vesículas acrossomais que se unem formando uma única estrutura, localizada na região anterior da cabeça da espermátide que se prolonga pela lateral do espermatozóide acompanhando o núcleo até o final deste;
- As vesículas observadas em cortes ultrafinos corados com Azul de Toluidina ou impregnados por íons prata podem estar relacionadas com a formação do acrossomo;
- O flagelo pode apresentar elementos supranumerários;
- O início da formação do corpo cromatóide ocorre nos primeiros estágios de diferenciação.

Podemos concluir, portanto que as características se mostram semelhantes às de outros Heteroptera descritos na literatura, com exceção da morfologia e do tamanho dos derivados mitocondriais que se mostraram diferentes dependendo da família/gênero analisado.

VI. REFERENCIAS

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AFZELIUS, B.A.; BACCETTI, B.; DALLAI, R. The giant spermatozoon of Notonecta. **Journal of Submicroscopic Cytology and Pathology**, v. 8 p. 149–161, 1976.

AFZELIUS, B.A.; DALLAI, R.; LINDSKOG, P. Spermatozoa of saldid bugs (Insecta, Hemiptera, Leptopodomorpha).**Journal of Ultrastructure Research**, v. 90 p. 304–312, 1985.

ALEVI, K.C.C.; MENDONÇA, P.P.; PEREIRA, N.P.; ROSA, J.A.; AZEREDO-OLIVEIRA, M.T.V. Is there post-meiotic transcriptional activity during hemipteran spermiogenesis? **Invertebrate Reproduction & Development**, v. 58, n. 3, p. 193-198, 2014.

ALEVI, K.C.C., PEREIRA, L.L.V., MOREIRA, F.F.F., BARBOSA, J.F., TABOGA, S.R., ITOYAMA, M.M., Histological and Ultrastructural Analysis of Spermatogenesis in *Gelastocoris flavus flavus* (Heteroptera: Nepomorpha). **Entomology, Ornithology & Herpetology** .4 (3)1-6, 2015.

ANDERSEN, N. M. The semiaquatic bugs (Hemiptera, Gerromorpha) phylogeny,adaptations, biogeography and classification. **Entomonograph**, 3 1-455, 1982.

ANDERSON, K. Fine structure of spermatogonia and spermatocytes in the blue fox (Alopexlagopus). **Acta Veterinaria Scandinavica** (Denmark), v. 19(2), p. 229-242, 1978.

ANDONOV, M. Further study of the chromatoid body in rat spermatocytes and spermatids. **Zeitschriftfür Zellforschung und mikroskopische Anatomie**, v. 104, p. 46-54, 1990.

ARAÚJO, V.A.; LINO-NETO, J.; RAMALHO, F.S.; ZANUNCIO, J.C.; SERRÃO, J.E. Ultrastructure and heteromorphism of spermatozoa in five species of bugs (Pentatomidae: Heteroptera). **Micron**, v. 42 p. 560–567, 2011.

AZEVEDO, C.; LOBO-DA-CUNHA, A.; OLIVEIRA, E. Ultrastructure of the spermatozoon in *Gibbula umbilicalis* (Gastropoda, Prosobranchia), with special reference to acrosomal formation. **Journalof submicroscopic cytology**, *v. 17 p. 609-614, 1985.*

BACCETTI, B. Insect sperm cell, **Advances in Insect Physiology**, *v. 9 p. 315-397, 1972.*

BACCETTI, B.; AFZELIUS, B.A. The biology of the sperm cell. Basel, New York, Karger, 254p., 1976.

BACCETTI, B.; BURRINI, A.G.; COLLODEL, G.; PIOMBONI, P.; RENIERI, T.; SENSINI, C. Lacalization of acrosomal enzymes in Arthopoda, Echinodermata and Vertebrata. **Journal of Submicroscopic Cytology and Pathology**, v. 21 p. 385-389, 1989.

BACCETTI, B.; DALLAI, R. The spermatozoan of arthropoda. XXVII. Uncommon axoneme patterns in different species of the cecidomyid flies. **Journal of Ultrastructure Research**, v. 55 p. 50-69, 1976.

BACCETTI, B.; DALLAI, R.; PALLINI, V.; ROSATI, F. AFZELIUS, B.A. Protein of insect sperm mitochondrial crystals: crystallomitin. **The Journal of Cell Biology**, v. 73 p. 594-600, 1977.

BÁO, S.N.; DE SOUZA, W. Structural specialization in the flagellum of the spermatozoon of the bloodsucking bug (*Rhodnius prolixus*; Hemiptera, Reduviidae). **Tissue Cell**, v. 26 n. 3 p. 299–308, 1994.

BÁO, S.N.; LINS, U.; FARINA, B.; DE SOUZA, W. Mitochondrial derivatives of *Culex quinque fasciatus* (Culicidae) spermatozoon: Some new aspects evidenced by cytochemistry and image processing. **Journal of Structural Biology**, v. 109 p. 46-51, 1992.

BÁO, S.N.; QUAGIO-GRASSIOTTO, I.; DOLDER, H. Acrosome formation in *Ceratitis capitata* (Diptera, Tephritidae). **Cytobios**, v. 58 p. 93-100, 1989.

BORGUETI, A. O.; ALEVI, K. C. C.; SILISTINO-SOUZA, R.; ROSA, J. A.; AZEREDO-OLIVEIRA, M. T. V. Immunofluorescence and ultrastructural analysis of the chromatoid body during spermatogenesis of *Triatoma platensis* and *T. rubrovaria* (Hemiptera, Triatominae). **Micron**, v. 74, p. 44-46, 2015.

BORROR, D.J.; DE LONG, D.M.; TRIPLEHORN, A.C. An Introduction to the Study of Insects. Sauders College Publishing, Philadelphia. 1979.

BORROR, D.J.; DELONG, D.M. Introduçãoaoestudo dos insetos. São Paulo, Brasil: Ed. Edgard Blucher LTDA., 653 pp., 1988.

BRESSA, M.J.; FUMAGALLI, E.; ITUARTE, S.; FRASSA, M.V.; LARRAMENDY, M.L. Meiotic studies in *Dysdercus* Guérin Méneville 1831 (Heteroptera: Pyrrhocoridae). II. Evidence on variations of the diffuse stage between wild and laboratory-inbred populations of *Dysdercuschaquency* Freiberg, 1948. **Hereditas**, v. 137, p. 125-131, 2002.

BRESSA, M.J.; PAPESCHI, A.G.; FUMAGALLI, E.; VAN DOESBURG, P.H.; LARRAMENDY, M.L. Cytogenetic and nucleolar meiotic cycle analyses in *Dysdercus*

imitator Blõte, 1931 (Pyrrhocoridae, Heteroptera) from Argentina. **Folia Biologica** (Krakow), v. 51, p. 135-141, 2003.

BUCK, R.C. Mitosis and meiosis in *Rhodnius prolixus*: the fine structure of the spindle and difuse kinetochore. **Journal of Ultrastructure Research**, v. 18, p. 489-501, 1967.

CASTANHOLE, M. M. U.; PEREIRA, L. L. V.; SOUZA, H. V.; BICUDO, H. E. M. C.; COSTA, L. A. A.; ITOYAMA, M. M. Heteropicnotic chromatin and nucleolar activity in meiosis and spermiogenesis of *Limnogonus aduncus* (Heteroptera, Gerridae): a marked RON revealing aspect of the chromosomal behavior. **Genetics and Molecular Research**, v. 7, n. 4, p.1398-1407, 2008.

CASTANHOLE, M.M.U.; PEREIRA, L.L.V.; SOUZA, H.V.; ITOYAMA, M.M. Spermatogenesis and karyotypes of three species of water striders (Gerridae, Heteroptera).**Genetics and Molecular Research**, v. 9, p. 1343-1356, 2010.

CHICKERING, A.M.; BACORN, B. Spermatogenesis in the Belatomatidae. IV. Multiple chromosomes in *Lethocerus.* **Papers of the Michigan Academy of Science, Arts and Letters**, v. 17, p. 529-534, 1933.

COBBEN, R.H. Evolutionary trends in Heteroptera. Part I. Eggs, architecture of the shell, gross embryology and eclosion. **Centre for Agricultural Publishing and documentation**, Wageningen, 475 p, 1968.

COMINGS, D.E.; OKADA, T.A. Holocentric chromosomes in Oncopeltus: kinetochore plates are present in mitosis but absent in meiosis. **Chromosoma** (Berl.), v. 37, p. 177-192, 1972a.

COMINGS, D.E.; OKADA, T.A. The chromatoid body in mouse spermatogenesis: Evidence that it may be formed by the extrusion of nucleolar components. **Journal Ultrastructure Research**, v. 39 n.1 p. 15-23, 1972b.

COSTA, D.S.; PAULA, T.A.R. Espermatogênese em mamíferos. **Scientia.** 4: 53-72, 2003.

CRYAN, J. R.; URBAN, J. M. Higher-level phylogeny of the insect order Hemiptera: is Auchenorrhyncha really paraphyletic? **Sistematic Entomology**, v. 37, p. 7-21, 2012.

DALLAI, R.; AFZELIUS, B.A. Characteristics of the sperm structure in Heteroptera (Hemiptera, Insecta). **Journal of Morphology**, v. 164 p. 301–309, 1980.

DALLAI, R.; AFZELIUS, B.A. Sperm flagellum of *Dacus oleae* (Gmelin) (Tephritidae) and *Drosophila melanogaster* (Drosophilidae) (Diptera)**. International Journal of Insect Morphology and Embryology**, v.20 p. 215-222, 1991.

DALLAI, R., GOTTARDO, M., BEUTEL, R.G. Structure and Evolution of Insect Sperm: New Interpretations in the Age of Phylogenomics. **Annual Review of Entomology v** 61 p.1–23, 2016a.

DALLAI, R.; PAOLI, F.; MERCATI, D.; LUPETTI, P. The centriole adjunct of insects: Need to update the definition. **Tissue and Cell**, v.48 (2) p. 104-113, 2016b.

DIAS, G.; LINO-NETO, J.; MERCATI, D.; DALLAI, R. The sperm structure and spermiogenesis of the heteropteran *Coptosoma scutellatum* (Geoffroy) with emphasis on the developmentof the centriole adjunct. **Micron,** v 82 33-40, 2016.

DI CASTRO, M.; PRANTERA, G.; CIPRIANI, L.; ROCCHI, A. Silver staining analysis of nucleolar-organizer activity during spermatogenesis of *Asellus aquaticus* (Crustacea, Isopoda). **Genetica**, v. 60, p. 163-166, 1983.

DOLDER, H. Cytoskeletal bridges between organelles in sperm flagellum of *Triatoma infestans* (Hemiptera, Reduviidae). **Journal of Ultrastructure and Molecular Structure Research**, v. 101 p. 159–164, 1988.

FAWCETT, D. W.; EDDY, E. M.; PHILLIPS, D. M. Observations on the fine structure and relationships of the chromatoid body in mammalian spermatogenesis. **Biology of Reproduction**, v.2(1), p. 129-153, 1970.

FAWCETT, D.W.; ITO, S. The fine structure of bat spermatozoa. **The American journal of anatomy**, v. 116 p. 567-610, 1965.

FERNANDES, A.P.; BÁO, S.N. Spermiogenesis in phytophagous bug (Hemiptera: Pentatomidae): an ultrastructural study. **Journal of Submicroscopic Cytology and Pathology**, v. 30 n. 4p. 485-493, 1998.

FERNANDES, A.P.; CURI, G.; BÁO, S.N. Nuclear changes and acrosome formation during spermiogenesis in Euchistusheros (Hemiptera: Pentatomidae).**Tissue & Cell**, v. 33 n. 3 p. 286-293, 2001.

FERNANDES, A.P. Estudo ultraestrutural e citoquímico da espermiogênese de percevejos da família pentatomidae (Hemiptera). Campinas, Tese de Mestrado. Universidade Estadual de Campinas. 109p., 1999.

FORERO, D. The systematics of the Hemiptera. **Revista Colombiana de Entomologia**, v. 34, n. 1, p. 1-21, 2008.

FOSSEY, A.; LIEBENBERG, H. Meiosis and nucleolar structures in the stink bug *Cartisis wahlbergi* Stal (Coreidae: Heteroptera) **Cytobios**, v.81, p. 7-15, 1995.

FRANÇA, L.R.; RUSSELL, L.D. The testis of domestic animals. In: MARTINEZ-GARCIA F.; REGADERA, J. (Eds), **Male Reproduction: a multidisciplinary overview**. Churchill Livingstone, Madrid.p.197-219, 1998.

FRICK, K. E.. The biology of *Microvelia capitata* Guérin, 1857, in the Panama Canal Zone and its role as a predator on anopheline larvae (Veliidae: Hemiptera). **Annals of the Entomological Society of America,** 42: 77-100. 1949

GALLO, J.; ESCALIER, D.; GRELLIER, P.; PRECIGOUT, E.; ALBERT, M.; DAVID, G.; SCHREVEL, J. Characterization of a monoclonal antibody to human proacrosin and its use in acrosomalstaus evaluation. **Journal of Histochemistry and Cytochemistry**, v. 39 p. 273-282, 1991.

GONZÁLEZ-GARCIA, J.M.; ANTONIO, C.; SUJA, J.A.; RUFAS, J.S. Meiosis in holocentric chromosomes: kinetic activity is randomly restricted to the chromatid ends of sex univalents in *Graphosoma italicum* (Heteroptera). **Chromosome Research**, v. 4, p. 124-132, 1996.

GROZEVA, S.M.; KUZNETSOVA, V.G. Karyotypes and some structural properties of the reproductive system of bugs of the subfamily Artheneinae (Heteroptera, Pentatomorpha, Lygaeidae). **Entomologicheskoye Obozreniye**, n. 4, p. 700-709, 1989.

GROZEVA, S.M.; KUZNETSOVA, V.G. The reproductive system of the some primitive families of pentatomomorphan bugs (Heteroptera). In: **Advances in regulation of insect reproduction (Bennettova B, Gelbic I and Soldan T, eds.)** Institute of Entomology, Czechoslovak Acadademy of Sciences, Prague, 97-102, 1992.

GROZEVA, S.; NOKKALA, S. Chromosomes and their behaviour in two families of the primitive infraorder Dipsocoromorpha (Heteroptera). **Hereditas**, v. 125, p. 31-36, 1996.

GROZEVA, S.; NOKKALA, S. Chromosome numbers, sex determining systems, and patterns of the C-heterochromatin distribution in 13 species de Lace Bugs (Heteroptera, Tingidae). **Folia Biologica** (Krakow), v. 49, n. 1-2, p. 29-41, 2001.

HUGHES-SCHRADER, S.; SCHRADER, F. The Kinetochore of the Hemiptera. **Chromosoma**, v. 12, p. 327-350, 1961.

HUNGERFORD, H. B. The biology and ecology of aquatic and semiaquatic Hemiptera. **University of Kansas Science Bulletin** 11: 1-328, 1919.

INADA, K., KITADE, O., MORINO, H. Paternity analysis in an egg-carrying aquatic insect *Appasus major* (Hemiptera: Belostomatidae) using microsatellite DNA markerse.**Entomological Science.** v. 14, p. 43–48. 2011.

JANDE, S.S. An analysis of the chromosomes in four species of the family Belostomatidae (Heteroptera, Cryptocerata). **Research Bulletin (NS) Panjab University**, v. 10, p. 25-34, 1959.

JOHN, B.; KING, M. Pseudoterminalization, terminalization and non-chiasmate modes of terminal association. **Chromosoma,** v. 92, p. 89-99, 1985.

JOHNSON, L. Spermatogenesis. In; **Reproduction in domestic animals.**4 ed. New York: Academic Press. p. 173-219. 1991.

JONES, G.H. Chiasmata. In: Monees PB, ed., **Meiosis**. Orlando: Academic Press, p. 213-244, 1987.

KHEIRALLAH, D.A. Ultrastructure biomarker in *Anisops sardeus* (Heteroptera: Notonectidae) for the assessment and monitoring of Water Quality of Al-Mahmoudia Canal, Western Part of Nile Delta, Egypt. **Journal of Bioscience and Applied Research**. v.1(6) 326-334, 2015.

KIEFER, R.G. Development, organization and degeneration of the *Drosophila* sperm flagellum. **Journal of Cell Science**, v. 6 p. 177-194, 1970.

KOTAJA N.; BHATTACHARYYA S.N.; JASKIEWICS L.; KIMMINS S.; PARVINEN M.; FILIPOWICZ W.; SASSONE-CORSI P. The chromatoid body of male germ cells: Similarity with processing bodies and presence of Dicer and microRNA pathway components. **Proceedings of the National Academy of Sciences** v. 103 n. 8 p. 2647-2652, 2006.

KOTAJA, N.; SASSONE-CORSI, P. The chromatoid body: a germ-cell specific RNA-processing centre. **Nature reviews – Molecular Cell Biology**, v.8, p. 85-90, 2007.

LEE, C.E. Morphological and phylogenetic studies on the true water bugs (Hemiptera: Heteroptera). **Journal of Natural History Society of Korea**, v. 21 p. 1–183, 1991.

LEE, Y.H.; LEE, C.E. Ultrastructure of spermatozoa and spermatogenesis in Nepomorpha (Insecta: Heteroptera) with special reference to phylogeny. **Zoological Science**, v. 9 p. 971–981, 1992.

MANNA, G.K. **Chromosomes in evolution in Heteroptera.** In: SHARMA, A.K., ed. Chromosomes in evolution of Eukaryotic groups. Boca Ratton Florida USA: CRCPress, p. 189-225, 1984.

MCKINSTRY, A. P. Some New Species Microvelia (Veliidae, Hemiptera). **Journal of the Kansas Entomological Society**, v. 10 (1) p. 30-36, 1937.

MELO, M.C. Biodiversidad de Heteroptera (Hemiptera) acuáticas y semiacuáticas de la Patagonia argentina. **Revista de la Sociedad Entomológica Argentina**, v. 68 (1-2), p. 177-185, 2009.

MENCARELLI, C.; MERCATI, D.; DALLAI, R.; LUPETTI, P. Ultrastructure of the sperm axoneme and molecularanalysis of axonemal dynein in Ephemeroptera (Insecta). **Cytoskeleton**, 71:328–39, 2014.

MERRITT, R.W., CUMMINS, K.W. An Introduction to the Aquatic Insects of North America. Kendall/Hunt, Iowa 1984.

MOJICA, J.M.; FILE-EMPERADOR, S.; BRUCK, D.L. Sperm bundle and spermatozoon ultrastructure in two species of the cardini group of Drosophila. **Invertebr. Repr. Dev.,** v.37 (2) p. 147-55, 2000.

MOREIRA, F.F.F.; BARBOSA, J.F.; RIBEIRO, J.R.I.Veliidae (Insecta, Heteroptera, Gerromorpha) from southeastern Brazil: three new species from Rio de Janeiro State, a new species group for Neotropical *Rhagovelia* Mayr, and notes on distribution and synonymy **Revista Brasileira de Entomologia, v**.56 (2) 2012.

MOTZKO, D.; RUTHMAN, A. 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. **European Journal of Cell Biology**, v. 33, p. 205-216, 1984.

NAKASUJI, F.; DYCK, V. A. Evaluation of the role of *Microvelia douglasi atrolineata* (Bergroth) (Heteroptera: Veliidae) as predator of the brown planthopper *Nilaparvata lugens* (StÅl) (Homoptera: Delphacidae). **Researches on Population Ecology,**26: 134-149.1984.

NOKKALA, S.; GROZEVA, S. Aquiasmatic male meiosis in *Myrmedobia coleoptrata* (Fn.) (Heteroptera, Microphysidae). **Caryologia**, v. 53, n. 1, p. 5-8, 2000.

NOKKALA, S.; NOKKALA, C. Achiasmatic male meiosis in two species of *Saldula* (Saldidae, Hemiptera). **Hereditas**, v. 99, n. 1, p. 131-134, 1983.

NOKKALA, S.; NOKKALA, C. Achiasmatic male meiosis in the Heteropteran genus *Nabis* (Nabidae, Heteroptera), **Hereditas**, v. 101, p. 31-35, 1984a.

NOKKALA, S.; NOKKALA, C. Achiasmatic male meiosis of collochore type in the heteropteran family Miridae. **Hereditas**, v. 105, p. 193-297, 1986a.

NOKKALA, S.; NOKKALA, C. Achiasmatic male meiosis in *Anthocoris nemorum* (Hemiptera, Anthocoridae). **Hereditas**, v. 105, p. 287-289, 1986b.

NOKKALA, S.; NOKKALA, C. Interaction of B chromosomes with A or B chromosomes in segregation in insects. **Cytogenetic and Genome Research**, v. 106, p. 394-397, 2004.

PAPESCHI A.G., BRESSA, M.J., Evolutionary cytogenetics in Heteroptera. **Journal of Biological Research.** v. 5 p. 3 – 21, 2006.

PAPESCHI, A.G.; MOLA, L.M. Meiotic studies in *Acanonicus hahni* (Coreidae, Heteroptera). I. Behaviour of univalents in desynaptic individuals. **Genetica**, v. 80, p. 31-38, 1990.

PARVINEN, M. The chromatoid body in spermatogenesis. **International Journal of Andrology**, v. 28, p. 189-201, 2005

PARVINEN, M.; SÖDER, O.; MALI, P.; FRÖYSA, B.; RITZÉN, E. M. In Vitro Stimulation of Stage-Specific Deoxyribonucleic Acid Synthesis in Rat Seminiferous Tubule Segments by Inter leukin-l α. **Endocrinology**, 129(3) p. 1614-1620, 1991.

PAULMIER, F.C. The spermatogenesis of *Anasa tristis***. Journal of Morphology,** v. 15 suppl., p. 224-272, 1899.

PEREIRA, D.L.V., MELO, A.L., HAMADA, N. Keys to families and genera of gerromorpha and nepomorpha (Insecta: Heteroptera) in the central Amazonia, Brazil. **Neotropical Entomology**, v. 36(2) p. 210-228, 2007.

PEREIRA, L.L.V.; ALEVI, K.C.C.; CASTANHOLE, M.M.U.; MOREIRA, F.F.F.; BARBOSA, J.F.; ITOYAMA, M.M. Cytogenetics Analysis and Testis Morphology of Aquatic Species of the Families Belostomatidae, Gelastocoridae, Gerridae, Notonectidae, and Veliidae (Heteroptera). **Journal of Insect Science**, 15 (1):21, 2015.

PEREIRA, M.H., SILVA, R.E., AZEVEDO, A.M.S., MELO, M.L., PEREIRA, L.H. Predation of *Biomphalaria glabrata* during the development of *Belostoma anurum* (Hemiptera, Belostomatidae). **Revista do Instituto de Medicina Tropical de São Paulo**, v. 35 n 5 p. 405-409, 1993.

PEROTTI, M.E. The mitochondrial derivative of the spermatozoon of *Drosophila* before and after fertilization. **Journal of Ultrastructure Research**, v.44 p. 181-198, 1973.

PEROTTI, M.E. Ultrastructure of the mature sperm of *Drosophila melanogaster* Meig. **Journal of submicroscopic cytology**, v.1 p. 171-196, 1969.

PERUQUETTI, R.L.; ASSIS, I.M.; TABOGA, S.R.; AZEREDO-OLIVEIRA, M.T.V. Meiotic nucleolar cycle and chromatoid body formation during the rat (*Rattus novergicus*) and mouse (*Mus musculus*) spermiogenesis. **Micron**, v. 39, p. 419-425, 2008.

PERUQUETTI, R.L.; TABOGA, S.R.; AZEREDO-OLIVEIRA, M.T.V. Characterization of Mongolian gerbil chromatoid bodies and their correlation with nucleolar cycle during spermatogenesis. **Reproduction in Domestic Animals**, 45 (3) p. 339-406, 2010.

PERUQUETTI, R.L.; TABOGA, S.R.; SANTOS, L.R.S.; OLIVEIRA, C.; AZEREDO-OLIVEIRA, M.T.V. Nucleolar cycle and chromatoid body formation: Is there a relationship between these two processes during spermatogenesis of *Dendropsophus minutus* (Amphibia, Anura)?.**Micron**, v. 42 p. 87-96, 2011.

PHILLIPS, D.M. Insect sperm: their structure and morphogenesis. **The Journal of Cell Biology**, v. 44 p.243-277, 1970.

PHILLIPS, D.M. Structural variants in invertebrate sperm flagella and their relationship to motility. In: SLEIGH, M.A. Cilia and Flagella London: Academic Press, p. 379-402, 1974.

POISSON, R. Nouvelles observations sur le processus espermatogénétique dans les éléments sexuels d' Hemípteres aquatiques. **Archives the Zoologie Experimentale et Generale**, v.78, p. 133-194, 1936.

POLHEMUS, J. T.; POLHEMUS, D. A. Global trends in the description of aquatic and semiaquatic Heteroptera species, 1758-2004.**Tijdschriftvoor Entomologie,** 150: 271-288.2007.

PRATT, S. Formation and differentiation of the Nebenkern in spermatios of nahemipteran insect, *Murgantia iristionia*. In: BACCETTI, B. Comparative Spermatology. Academic, London, p. 301-310, 1970.

REGO, L.N.A.A., ALEVI, K.C.C., AZEREDO-OLIVEIRA, M.T., MADI-RAVAZZI, L. Ultrastructural features of spermatozoa and their phylogenetic application in Zaprionus (Diptera, Drosophilidae). **FLY**,v.10 (1) p.47–52, 2016.

RODRIGUES, H. D. D.; MOREIRA, F.F.F.Four new species of *Paravelia* (Hemiptera: Heteroptera: Veliidae) from Brazil, with notes on previously described species. **The Canadian Entomologist**, v. 148 (6) pp. 642-667, 2016

ROSATI, F.; SELMI, G.; MAZZINI, M. Comparative observation on the mitochondrial derivatives of insect sperm. **Journal of submicroscopic cytology**, v.8 p. 56-67, 1976.

RUFAS, J.S.; GIMÉNEZ-MARTÍN, G. Ultrastructure of the kinetochore in *Graphosoma italicum* (Hemiptera: Heteroptera). **Protoplasma**, v. 32, p. 142-148, 1986.

RUSSELL, L.D.; ETTLIN, R.A.; SINHA-HIKIM, A.P.; CLEGG, E.D. Histological and histopathological evaluation of the testis. **Cache River Press**, 1990.

SANTOS, J.; SENTIS, C.; FERNANDEZ-PIQUERAS, J. Pattern of nucleolar organizer region activity during male meiosis in *Callicrania seoanei* (Orthoptera) as analyzed by silver staining: evidences for a possible reactivation in the period between the two meiotic divisions. **Genome**, v. 29, p. 516-518, 1987.

SCHRADER, F. Notes on the mitotic behaviour of long chromosomes. **Cytologia** (Tokio), v. 6, p. 422-430, 1935.

SCHRADER, F. The formation of tetrads and the meiotic mitoses in the male of *Rhytidolomia senilis* Say (Hemiptera, Heteroptera). **Journal of Morphology**, v. 67, p. 123-141, 1940.

SCHUH, T. T.; SLATER, J.A. True bugs of the world (Hemiptera: Heteroptera) Classification and natural history, Cornell UNIVERSITY press, Ithaca, New York, xii, 338 pp., 1995.

SMITH, R.L. Evolution of paternal care in the giant waterbugs (Hemiptera: Belostomatidae). In: Choe JC, Crespi BJ (eds) The Evolution of Social Behavior in Insects and Arachnids Sociality, Cambridge University Press, Cambridge pp 116–149, 1997.

SOLARI, A.; AGOPIAN, S. Recombination nodules, synaptonemal complexes and heterochromatin in the hemipteran *Triatoma infestans*. **Microscopia Eletronica y Biologia Celular**, v. 11, p. 179-195, 1987.

SOUZA, H. V.; BICUDO, H. E. M. C.; COSTA L. A. A.; ITOYAMA, M. M.A study of meiosis and spermatogenesis in different testicular lobes of *Antiteuchus tripterus* (Heteroptera, Pentatomidae). **European Journal of Entomology**, v. 104, p. 353-362, 2007a.

SOUZA, H.V.; BICUDO, H. E. M. C.; ITOYAMA, M.M. Study of chromosomal and nucleolar aspects in testes of *Nysius californicus* (Heteroptera: Lygaeidae). **Genetics and Molecular Research**, v. 6, n. 1, p. 33-40, 2007b.

SOUZA, H.V.; ARAKAKI, R.L.M.; DIAS, L.; MURAKAMI, A.S.; COSTA, L.A.A.; BICUDO, H.E.M.C.; ITOYAMA, M.M. Cytogenetical Aspects of Testicular Cells in Economically Important Species of Coreidae Family (Heteroptera). **Cytologia**, v. 72, p. 49-56, 2007c.

SOUZA, H. V.; CASTANHOLE, M. M. U.; BICUDO, H. E. M. C.; COSTA, L. A. A.; ITOYAMA, M. M. Morphological patterns of the heteropycnotic chromatin and nucleolar material in meiosis and spermiogenesis of some Pentatomidae (Heteroptera). **Genetics and Molecular Biology**, v.31, n. 3, p. 686-691, 2008.

SOUZA, H.V.;SOUZA, F.B.;MARUYAMA, S.R.C.;CASTANHOLE, M.M.U.; ITOYAMA, M.M. Meiosis, spermatogenesis and nucleolar behavior in the seminiferous tubules of Alydidae, Coreidae and Rhopalidae (Heteroptera) species. **Genetics and Molecular Research**, 1383-1396, 2009.

STANLEY, H.P. Fine structure of spermiogenesis in the elasmobranch fish *Squalus suckleyi I.* Acrosome formation, nuclear elongation and differentiation of the midpiece axis. **Journal of Ultrastructure Research**, v. 36 p. 86-102, 1971.

SUJA, J.A.; DEL CERRO, A.L.; PAGE, J.; RUFAS, J.S.; SANTOS, J.L. Meiotic sister chromatid cohesion in holocentric sex chromosomes of three heteropteran species is maintained in absence of axial elements. **Chromosoma**, v. 109, p. 35-43, 2000.

TAKENOUCHI, Y.; MURAMOTO, N. A study of the chromosomes in three species of heteropteran insects (Anthocoridae and Veliidae: Heteroptera). (In Japanese). **Journal of Hokkaido University of Education**, IIB, v. 22, p. 23-25, 1971.

TALLAMY, D. W. Sexual selection and the evolution of exclusive paternal care in arthropod. **Animal Behaviour.**v. 60 p. 559-567, 2000.

TANDLER, B.; HOPPEL, C. Mitochondria in spermatogenesis. In: TANDLER, B.; HOPPEL, C. Mitochondria, 1ª Ed., New York and London, Academic Press, p. 36-39, 1972.

TAVARES, M. G.; AZEREDO-OLIVEIRA, M.J.V. Pattern of nucleolar activity during spermatogenesis in triatomines (Heteroptera: Reduviidae) as analysed by silver staining. **Cytobios**, v. 89, p. 93-103, 1997.

TESARIK, J.; DRAHORAD, J.; TESTART, J.; MENDONZA, C. Acrosin activation follows its surface exposure and precedes membrane fusion in human sperm acrosome reaction. **Development**, v. 110 p. 391-400, 1990.

TOKUYASU, K.T. Dynamics of spermiogenesis in *Drosophila melanogaster*. III. Relation between axoneme and mitochondrial derivatives. **Experimental Cell Research**, v. 84 p. 239-250, 1974.

TOKUYASU, K.T. Dynamics of spermiogenesis in *Drosophila melanogaster*. V. Head-tail alignment. **Journal of Ultrastructure Research**, v. 50 p. 117-129, 1975.

TRANDABURU, V. The acrosome and the centriole adjunct in EurydemaventralisKol.(Heteroptra, Pentatomidae). **Travaux Du Muséum National D´Histoire Naturalle Grigore Antipa,** v. 13 p. 137–145, 1973.

UESHIMA, N. Animal cytogenetics, Insecta 6, Hemiptera: Heteroptera. GebruderBorntraeger: Berlin, Stuttgart, 117 pp., 1979.

VIANNA, G. J. C. and MELO A.L. Distribution patterns of aquatic and semi aquatic Heteroptera in Retiro das Pedras, Brumadinho, Minas Gerais, Brazil. **Lundiana**, v.4, n.2, p.125-128, 2003

WARNER, F.D. Spermatid differentiation in the blowfly *Sarcophaga bullata* with particular reference to flagelar morphogenesis. **Journal of Ultrastructure Research**, v. 35 p. 210-232, 1971.

WERNER G.; AFZELIUS, B.A.; MOSLER B. Acrosome formation during spermiogenesis in *Notonecta glauca* L. (Heteroptera). **Journal of Submicroscopic Cytology and Pathology**, v. 20 n. 1 p. 123-35, 1988.

WERNER, G.; WERNER, K. Changes in the nucleus, endoplasmic reticulum, Golgi apparatus, and acrosome during spermiogenesis in the waterstrider, *Gerris najas* deg. (Heteroptera : Gerridae). **International Journal of Insect Morphology and Embryology**. v. 22 p. 521-534, 1993.

WILSON, E.B. Studies of chromosomes II. The paired microchromosomes, idiochromosomes and heterotrophic chromosomes in Hemiptera. **The Journal of Experimental Zoology.**, v. 2, p. 507-545, 1905.

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

YANAGIMACHI, R. Mammalian fertilization. In: KNOBIL, E; NEILL, J. The Physiology of Reproduction. New York: Raven Press, p. 135-185, 1988.

YASUZUMI, G. Electron microscope studies on spermiogenesis in various animal species. **International Review of Cytology**, v. 37 p. 53-119, 1974.

YOSHIDA, T. Unusual type of the nucleolus observed in a bug, *Acanthocoris sordidus*. **Journal of the Faculty of Science- Hokkaido University**.(Zool.), v. 9, p. 243-249, 1947.