Comparative morphology of the reproductive system and germ cells of *Amblyomma* ticks (Acari: Ixodidae): A contribution to Ixodidae systematics

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B.R. Sampieri [a], J.C.S. Moreira [a], F.A.R. Páez [a,b], M.I. Camargo-Mathias [a,*]

[a] *UNESP, Department of Biology, Av. 24A, 1515, Bela Vista. Rio Claro-SP, Brazil*
[b] *Universidad de Caldas, Facultad de Ciencias Exactas y Naturales, Calle 65 Nro 26-10, AA 275, Manizales, Caldas, Colombia*

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ABSTRACT

Among arthropods, ticks of the genus *Amblyomma* are of great medical and veterinary importance and present phylogenetic and taxonomic divergences given polymorphisms and phenotypic plasticity between subpopulations. Generally, the male reproductive system and spermatozoon exhibit diversified morphology and ultrastructure species-specific, bringing new possibilities for phylogenetic and taxonomic issues. Therefore, the present study aimed to describe and compare the morphology of the male reproductive system and its germ cells of *Amblyomma aureolatum*, *A. sculptum*, and *A. triste*, intending to identify possible diagnostic features. Couples of the three tick's species were kept in colony, infested on rabbits and collected over 12 days of feeding. The males had their reproductive systems dissected, fixed and processed for histology and scanning electron microscopy. The results obtained here allowed the description of spermiogenesis stages and the comparison of spermatids morphology in the last stage of development. Furthermore, the testis of *A. triste* present an isthmus connecting the distal region of both, while in the other two species this structure could not be observed. Some anatomical features were identified which can be used for taxonomic and phylogenetic studies, like the presence or absence of the isthmus connecting testis, spV cell shape, the shape of the operculum and the presence or absence of the rim on its base.

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1. Introduction

Ticks from the *Amblyomma* genus are of great medical and veterinary importance. Its ubiquitous geographic distribution, through the tropical and subtropical regions, and the occurrence of polymorphisms and phenotypic plasticity cause classification divergence between taxonomists. In the Americas, there are at least three "species complexes" within *Amblyomma*, namely: the "*cajennense* complex", the "*maculatum* complex" and the "*ovale* complex" and in Brazil, important species belonging to these complexes can be found, such as the *Amblyomma sculptum* (Berlese, 1888), the *A. triste* (Koch, 1844) and the *A. aureolatum* (Pallas, 1772), grouped respectively. Therefore, it is clear how difficult it is to identify and classify individuals from this genus as well as reinforce the importance of new studies that seek to find the solution for these issues [1,3–6].

Species complexes are groups of subpopulations whose individuals, in principle, are identified as the same species due to the similarity in diagnostic characters. However, wide geographic distribution, distinct ecological niches and important genetic differences show that, in most cases,

* Corresponding author. Tel.: +55 19 35264151; fax: +55 19 35340009.
E-mail address: micm@rc.unesp.br (M.I. Camargo-Mathias).

there is more than one species grouped in these complexes. The species classified in these *Amblyomma* complexes have subtle variations related to its external morphology that complicate their identification and relocation within the clade, in addition to the fact the material-type of these group fails to submit adequate quality of preservation and/or a shallow description [1–4,6].

The spermatozoon–male germ cells–have a diversified morphology and ultrastructure that differs depending on the animal species, therefore, assuming that specificity, thorough investigation of its form could be defined the phylum and even the male species that produced them [7,8]. In various groups of invertebrates, such as insects and annelids, the development of the germ cells, especially in spermiogenesis, as well as the morphology and the ultrastructure of the spermatozoon, shed scientific light on the resolutions for phylogenetic and taxonomic issues that could not be solved by the traditional taxonomy [8–13].

Based on the aforementioned, this study aimed to describe and compare the morphology of the male reproductive system of three species of tick from the *Amblyomma* genus that exist in Brazil, namely the *A. aureolatum*, the *A. sculptum*, and the *A. triste*, through histological and ultramorphological techniques in order to identify possible diagnostic characters at the species level.

2. Material and methods

2.1. Ticks

Conducting this study involved 10 couples (10 males and 10 females) of each tick species, the *A. aureolatum*, the *A. sculptum* and the *A. triste*. The *A. aureolatum* couples were obtained from colonies kept in Dr. Marcelo Bahia Labruna's laboratory, at the Faculdade de Medicina Veterinária e Zootecnia (FMVZ) at the University of São Paulo (USP), Sao Paulo, Brazil. The *A. sculptum* couples were collected in the campus of the UFSCar of Araras, São Paulo, Brazil, 22°18′37.0′íS 47°23′06.2′íW, and identified according to taxonomic keys established in the literature (Nava et al., 2014). The *A. triste* couples were obtained from colonies kept by Dr. Marcos Andre's team in the Departamento de Patologia Veterinária at the FCAV-UNESP, Jaboticabal, São Paulo, Brazil.

Three Rabbits (New Zealand White–Botucatu Variety) that had never been previously infested (age = 6 months; sex = females; weight average = 2.339 grams) were infested with the couples of each species of tick. The males of each species were collected from the host within more than 12 days of feeding (period counted from the tick first attachment to the host skin) a period in which developed germ cells can already be observed. After collection, the ticks were immediately dissected in PBS buffer and had their reproductive systems fixed so that microscopy techniques could be performed.

2.2. Histology

The male reproductive systems of five individuals of each species were fixed in 2.5% Glutaraldehyde in PBS (this fixative allows the germ cells to be better preserved) for

48 hours and dehydrated in escalating concentrations of ethanol (70, 80, 90 and 95%) for 15 minutes in each concentration. The samples were subsequently embedded in Leica historesin (embedding) for 72 hours and polymerized in the same historesin for later section of the blocks (3 μm) in a microtome. The latter were collected on glass slides and stained with hematoxylin and eosin stain (HE stain) for later photo-documentation on a Leica DM750 Photomicroscope.

2.3. Scanning Electron Microscopy (SEM)

The male reproductive systems of five individuals of each species were fixed in 2.5% Glutaraldehyde in PBS for 48 hours. The material was subsequently dehydrated in escalating concentrations of acetone (80, 85, 90, 95 and 100%) in five-minute baths in each concentration; during the 100% acetone procedure, specifically, two baths with the same time duration were run. After desiccation in a Critical Point Dryer, the specimens were pasted onto aluminum brackets with double-sided bonding tape, sputter-coated with gold, examined and photographed on a Hitachi TM3000 scanning electron microscope.

This study was approved by the Ethical Committee in Animal Use (Comitê de Ética de Uso Animal-CEUA) at the Instituto de Biociências, UNESP, Rio Claro-SP, Brazil, under process number 017/2012 and protocol 1422.

3. Results

The results obtained here show that the ticks male reproductive system studied herein, as well as those of other species of Ixodidae, are morphologically similar. However, significant differences can be observed occuring in particular gonads and cellular structures.

3.1. Gonopore

The gonopore is found in the anteroventral region of the body distal to the anal opening. In the *A. aureolatum* species, the gonopore does not have roughness or setae on its surface, which is concave and rectangular, but with rounded corners and no lateral grooves (Fig. 1A, B). In the *A. sculptum* species, the gonopore can be observed as an elliptical/oval-shaped structure, with a smooth surface and no lateral grooves (Fig. 2A and B). The gonopore of the *A. triste* species on the other hand is rounded, with a concave surface and no setae; it is even laterally surrounded by evident marginal grooves of the ventral-anal scale (Fig. 3A and B).

### 3.2. Reproductive System and Germ Cells (ultramorphology)

The reproductive system of the three species studied here under scanning electronic microscopy, contains organs of similar morphology. The system exhibit a multilobed accessory gland complex located anteriorly to the body; a pair of seminal vesicles positioned antero-dorsally to the glandular complex each one connected to the vasa
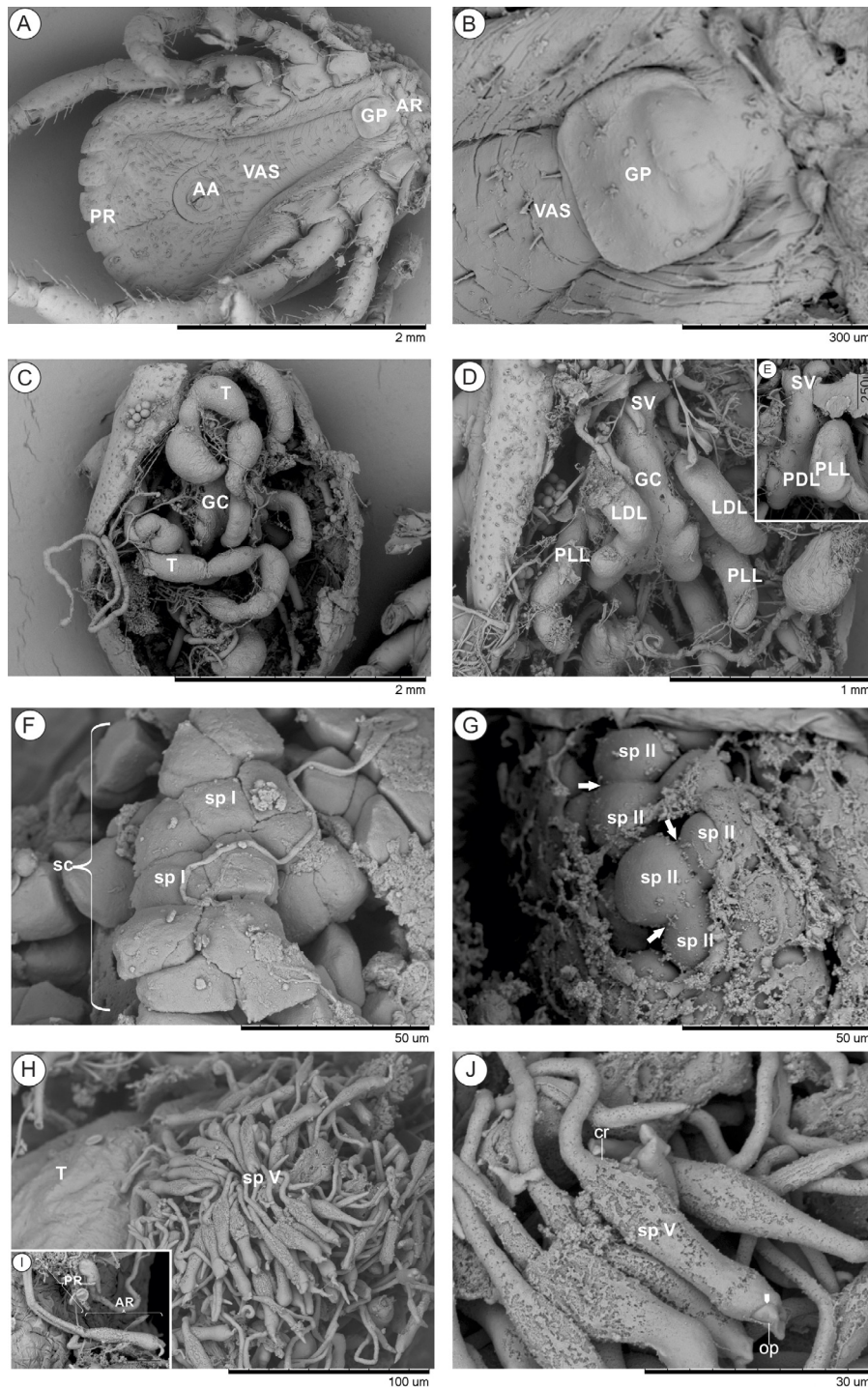
Fig. 1. External and internal ultramorphology of *Ambyomma aureolatum* male. (**A-B**) Overview and details of the ventral region, highlighting the gonopore (**GP**). (**C**) Testis (T) and gland complex (GC). (**D-E**) Detail of glandular complex and its lobes. (**F**) Spermatocysts (SC) housing spermatids in the first development stage (**sp I**). (**G**) spII spermatids showing cytoplasmic bridges (arrow). (**H-J**) Overview and detail of **spV**. **AA**= anal aperture; **AR** = anterior region; **arrow head** = operculum rim; **cr** = constriction region; **LDL** = Lateral Dorsal Lobe; **op** = operculum; **PDL** = Postero-Dorsal Lobe; **PLL**= Postero-Lateral Lobe; **PR**= posterior region; **SV** = seminal vesicle; **VAS** = ventral-anal shield.

defentia (Fig. 1D and E; 2C and D, 3C and D). The vasa differentia are short and positioned lateral to the gland complex connecting the seminal vesicles to the testes (Fig. 1D, 2D and E).

The testes, in turn, are connected to the vasa deferentia in its proximal region and extend (in length) laterally along the body. They are tubular organs that house, proximally, germ cells in their initial stages of development and
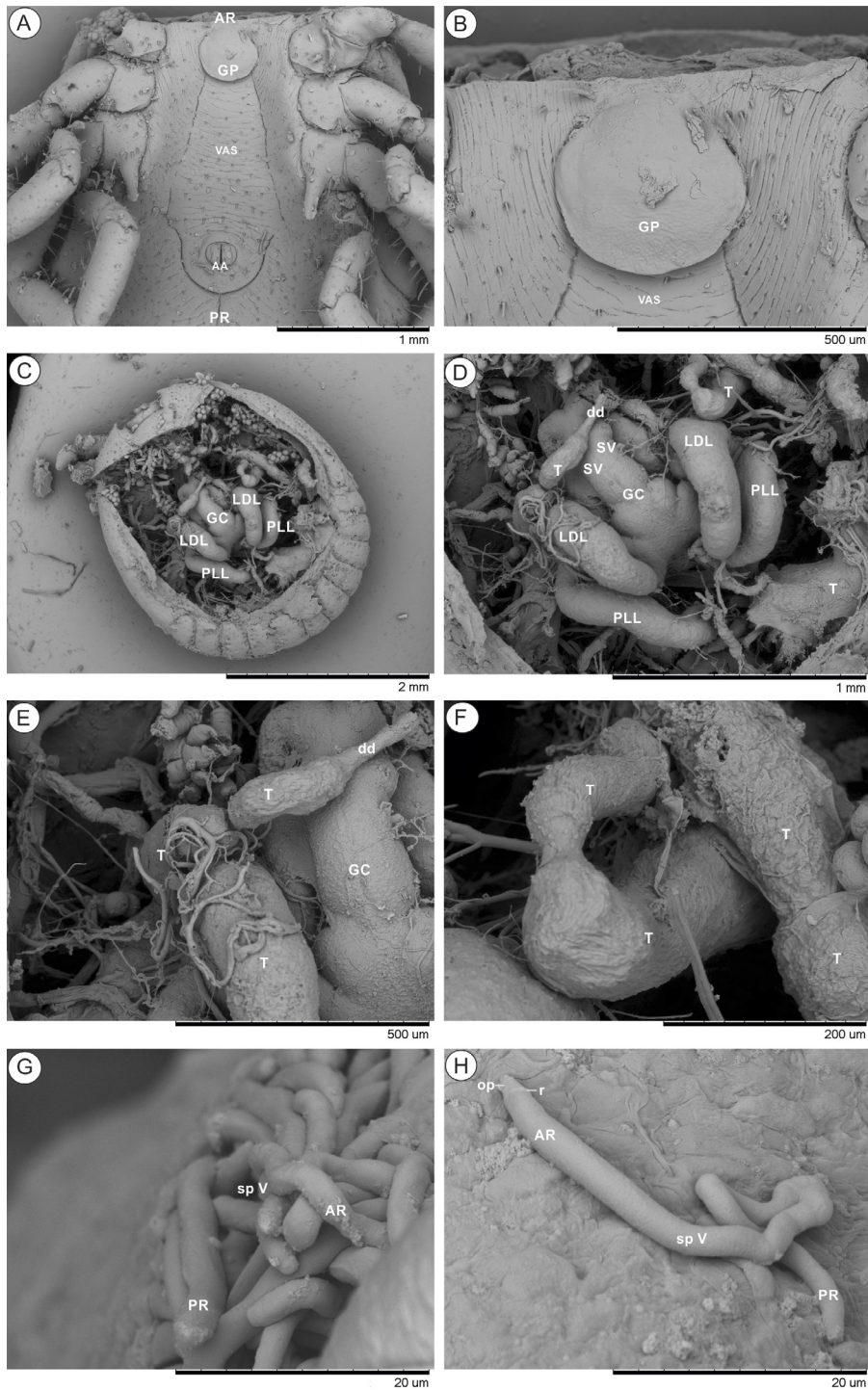
Fig. 2. External and internal ultramorphology of *Ambyomma sculptum* male. (**A-B**) Overview and details of the ventral region, highlighting the gonopore (**GP**). (**C-D**) Overview and Detail of gland complex (GC) and its lobes. (**E-F**) Testis (T) and vasa deferentia (dd). (**G-H**) Overview and detail of **spV**. **AA =** anal aperture; **AR =** anterior region; **cr =** constriction region; **LDL =** Lateral Dorsal Lobe; **op =** operculum; **PDL =** Postero-Dorsal Lobe; **PLL =** Postero-Lateral Lobe; **PR =** posterior region; **r =** operculum rim; **SV =** seminal vesicle; **VAS =** ventral-anal shield.

in the distal region, the cells are already in advanced stages of this process (Fig. 1F, G, H; 2E, F and G). In the *A. aureolatum* and *A. sculptum*, testes are separated and the only point of connection between them and the other organs

of the reproductive system are the vasa deferentia (Fig. 4A and B). However, in the *A. triste* species, there is an elongation of the tissue that covers the organs connecting both. (Fig. 3E, F and 4C).
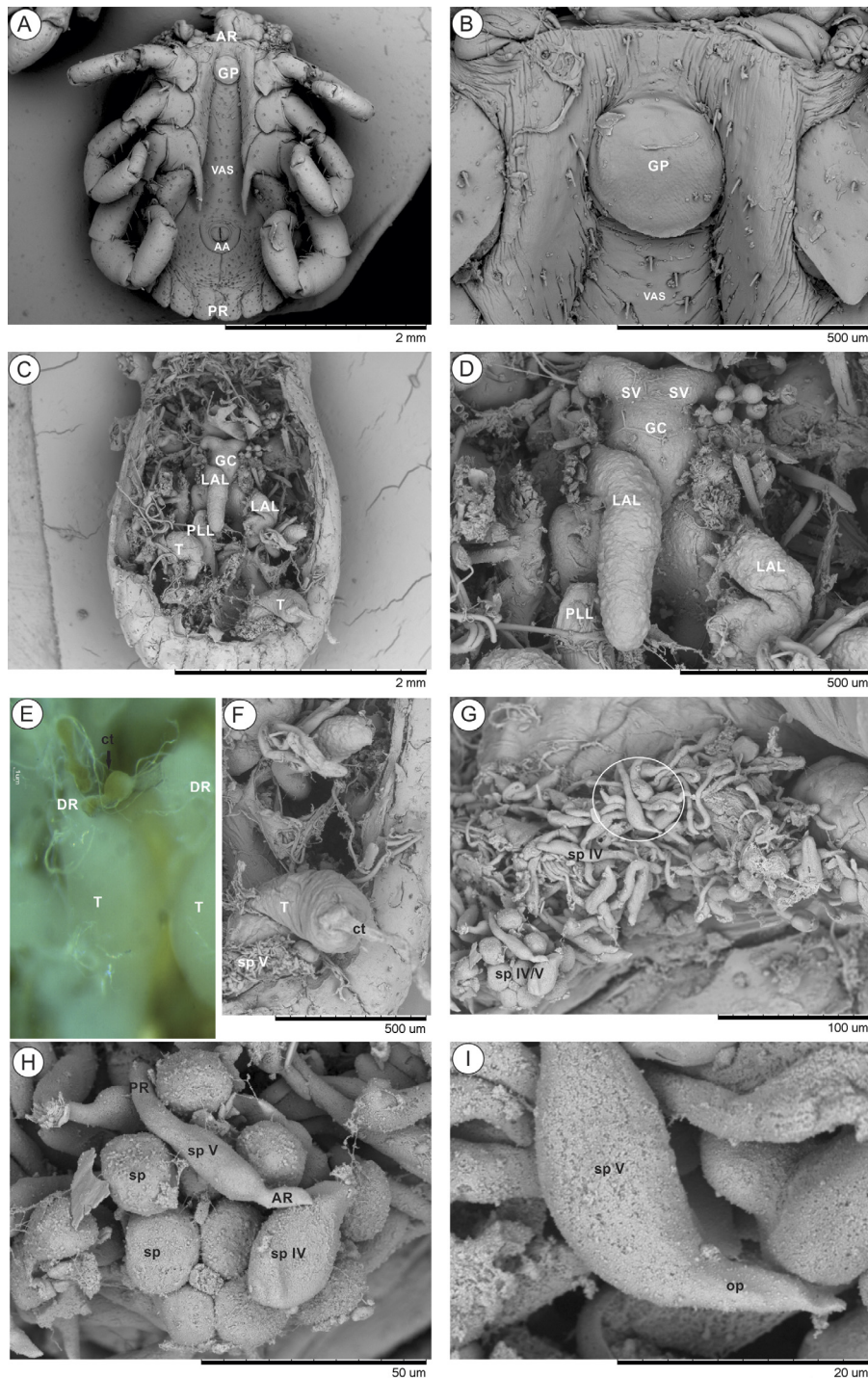
Fig. 3. External and internal ultramorphology of *Ambyomma triste* male. (**A-B**) Overview and details of the ventral region, highlighting the gonopore (**GP**). (**C-D**) Overview and Detail of gland complex (**GC**) and its lobes. (**E**) Photomicrograph in stereomicroscope of the paired testis distal region, where it can be observed the isthmus connecting both. (**F**) Ultramorphology of the same region shown in **E**, where the isthmus is broken. (**G-H**) Spermatids **sp IV** e **sp V**, overview and detail. (**I**) **Sp V** and its rim in detail. **AA** = anal aperture; **AR** = anterior region; **cr** = constriction region; **ct** = isthmus; **DR** = distal region; **LAL** = Antero-Lateral Lobe; **LDL** = Lateral Dorsal Lobe; **op** = operculum; **PLL** = Postero-Lateral Lobe; **PR** = posterior region; **r** = operculum rim; **SV** = seminal vesicle; **VAS** = ventral-anal shield.
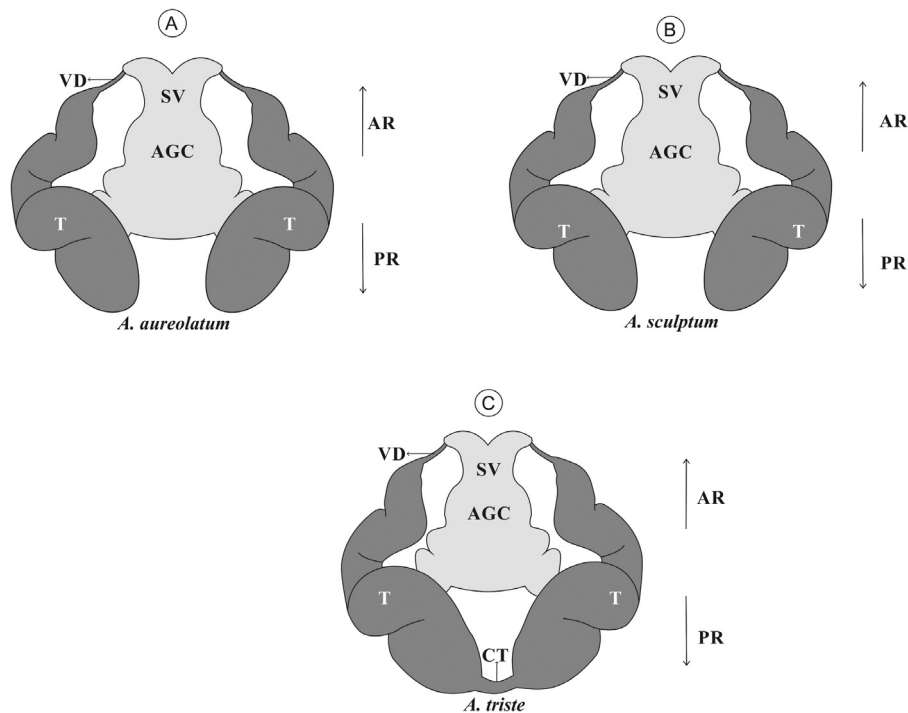
Fig. 4. Schematic Drawing of male reproductive system of *A. aureolatum*, *A. sculptum* e *A. triste* species. The main difference observed is the isthmus (CT) between testis in *A. triste*. **AGC** = accessory gland complex; **AR** = anterior region; **CT** = isthmus; **PR** = posterior region; **SV** = seminal vesicule; **T** = testis; **VD** = vasa deferentia. Not in scale.

The germ cells, which occurs along the entire length of the testes, are spermatids in differentiation process, which are in different stages of development depending on the region analyzed. In the proximal region, the compacted spermatids are deposited inside the spermatocysts (Fig. 1F). In the median region, the spermatids are bigger, less compacted and shows a rounded shape and cytoplasmic bridges (Fig. 1G). The late spermatids can be found distally in the testes, which are elongated cells that can be divided into the two anterior and posterior regions. In the anterior region an operculum can be observed, which varies in shape according to the species, and the posterior region has a tail-like shape (Fig. 1I and J; 2G and H; 3G and H).

In *A. aureolatum*, the two regions of the late spermatids are very distinct, marked by a constriction in their median portion; while the operculum has the shape of a parabola, on its base an edge that faces toward the inside of the cell can be seen (Fig. 1I, 1J and 5A). In *A. sculptum*, the anterior and posterior regions of these cells are distinct. In the anterior region, the conical operculum with a lighter rim on its base can be observed, whereas the extremity of the posterior one has a rounded shape tail-like (Fig. 2G and H, 5B). The regions of the spermatids in the *A. triste* species are very distinct. In the anterior region there is also a long and sharp operculum (which looks like the bird beak), but it has no evident rim on its base (Fig. 5C). The anterior region differs because of a slight median constriction in the cell, which gives the posterior region a tail-like shape (3G and H).

A summary of the comparative results obtained from the SEM of the reproductive system and its germ cells can be seen in Figs. 4 and 5.

3.3. Reproductive system and Spermiogenesis (histology)

The accessory gland complex of the species studied herein have well-developed anterior (ADL) and posterior (PDL) dorsal lobes, with narrow lumen and secretory cells whose cytoplasm is filled with granules. These granules are strongly stained by the hematoxylin (Fig. 6A and C; 7A, B and D; 8A). Two lateral dorsal lobes (LDL) can also be observed which most of their cells show cytoplasm filled with basophilic secretion. However, there are others whose secretion has the shape of granules similar to the other lobes (Fig. 6A; 7C; 8A, B and C). In addition to these, only in *A. aureolatum* can the anterior ventral lobe (AVL) also be observed, in which the secretory cells are present (Fig. 1A). In the accessory gland complex, pairs of seminal vesicles, which extend in length along the entire extension of the complex, can be observed, presenting a simple cuboidal epithelium overlay and showing a lumen filled with spermatids (Fig. 6C; 7A and B; 8A and C).

The testes of the three species studied herein have a similar morphology and also have germ cells in the same stages of development. By relating the feeding stage and the development of the germ cells, five different stages of the spermiogenesis process can be observed according to the following description. All five stages described here are summarized in Fig. 9.

In the anterior region of the testes the spermatocysts can be observed, which are cysts covered by a simple squamous epithelium, housing spermatids at the same stage. The spermatids from this region will be referred to as spermatids I (**sp I**). Sp I are small cells in which it is not
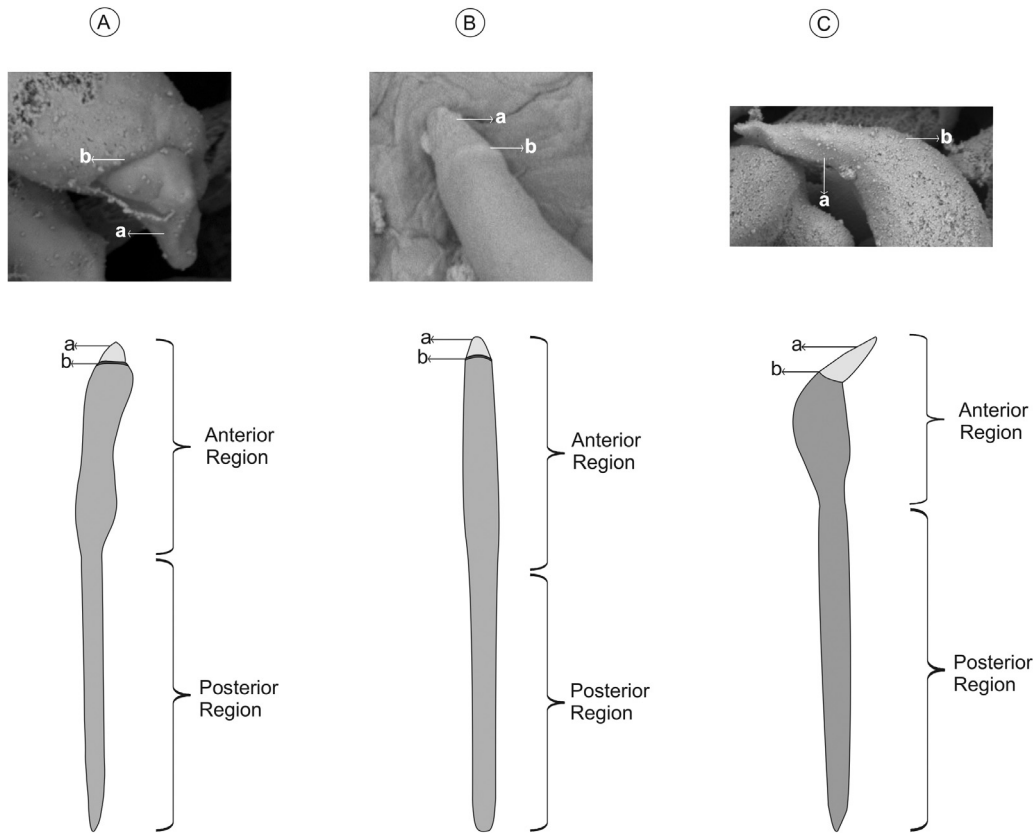
Fig. 5. Schematic drawing of **sp V** of the three species studied, (**A**) *A. aureolatum*, (**B**) *A. sculptum* and (**C**) *A. triste*. Main difference: operculum morphology (**a**) and form, presence or absence of the rim (**b**).

possible to observe its limits, exhibiting a cytoplasm strongly stained by eosin and a round interphase nucleus stained by the hematoxylin (Fig. 6D).

In the testes median region, the spermatids are still inside the spermatocysts. These cells are in stage II (**sp II**) and already have an evident cellular limit and a round or polygonal shape due to the packaging process inside the spermatocyst. They exhibit a large, round nucleus containing one or two nucleoli. It is possible to observe cytoplasmic bridges between some of the spermatids (Fig. 7E and F).

Between the testes median and distal region, the organization and arrangement of the germ cells into the organ undergo changes. The spermatocysts can no longer be observed and the spermatids are in three distincts stages, namely: spermatid III (**sp III**), IV (**sp IV**) and V (**sp V**). Each of those stages have characteristics of their own. The sp III are rounded, showing an oval-shaped nucleus with the heterochromatin with some condensation points in one of the cell poles. Close to the cell's internal limit, a thick, darker, eosin-colored band can be observed, which has a striated form. In addition, it is possible to notice a structure that looks like a thin, dark thread at the cell pole anterior to the nucleus, which belongs to the acrosome vesicle (Fig. 6E and F; 7G; 8H).

The sp IV undergo a cell-elongation process that changes their previous morphology, including the the nucleus. The cytoplasm is medially stained by the eosin, but it is still possible to observe the cell's external limit. In the sp IV proximal region, the nucleus is strongly stained by the hematoxylin, which evidences a high condensation level of chromatin and the occurrence of an elongation process known as the nuclear process. In the cytoplasm a membranous cisternae can be observerd, which also follows the lengthening of the cell (Fig. 6E and G; 7H; 8G).

Sp V is the last stage in the spermiogenesis. The cell's external limit is visible and there is a slender structure in the center of the cell, which is probably the result of the cisternae fusion observed in the sp IV. The nucleus is located in the cell periphery and is strongly stained by the hematoxylin, following the morphology of the cell (Fig. 6H; 7I; 8F). In the extremity of the anterior region of the sp V a structure that is possibly the operculum can be observed (in greater detail on the SEM) (Fig. 6H, 7I and 8F).

4. Discussion

The knowledge regarding the morphology and development of the male reproductive system of ticks have been increased during the last decades, however its evident the lack of comparative analyses addressing phylogenetic and taxonomic issues. The data available in the literature are old and aimed different purposes, like the morphology and the spermatogenesis description or even the physiology of
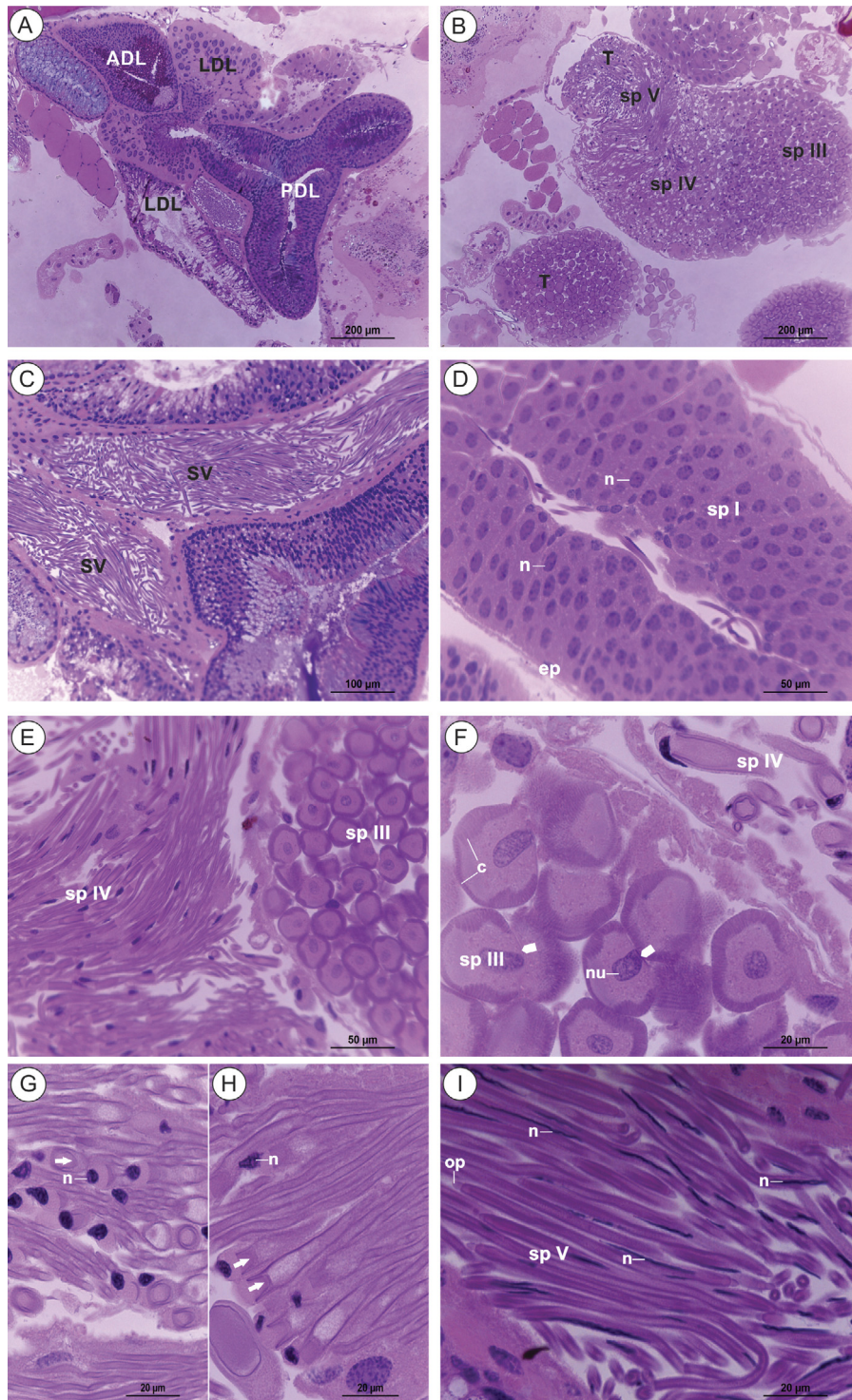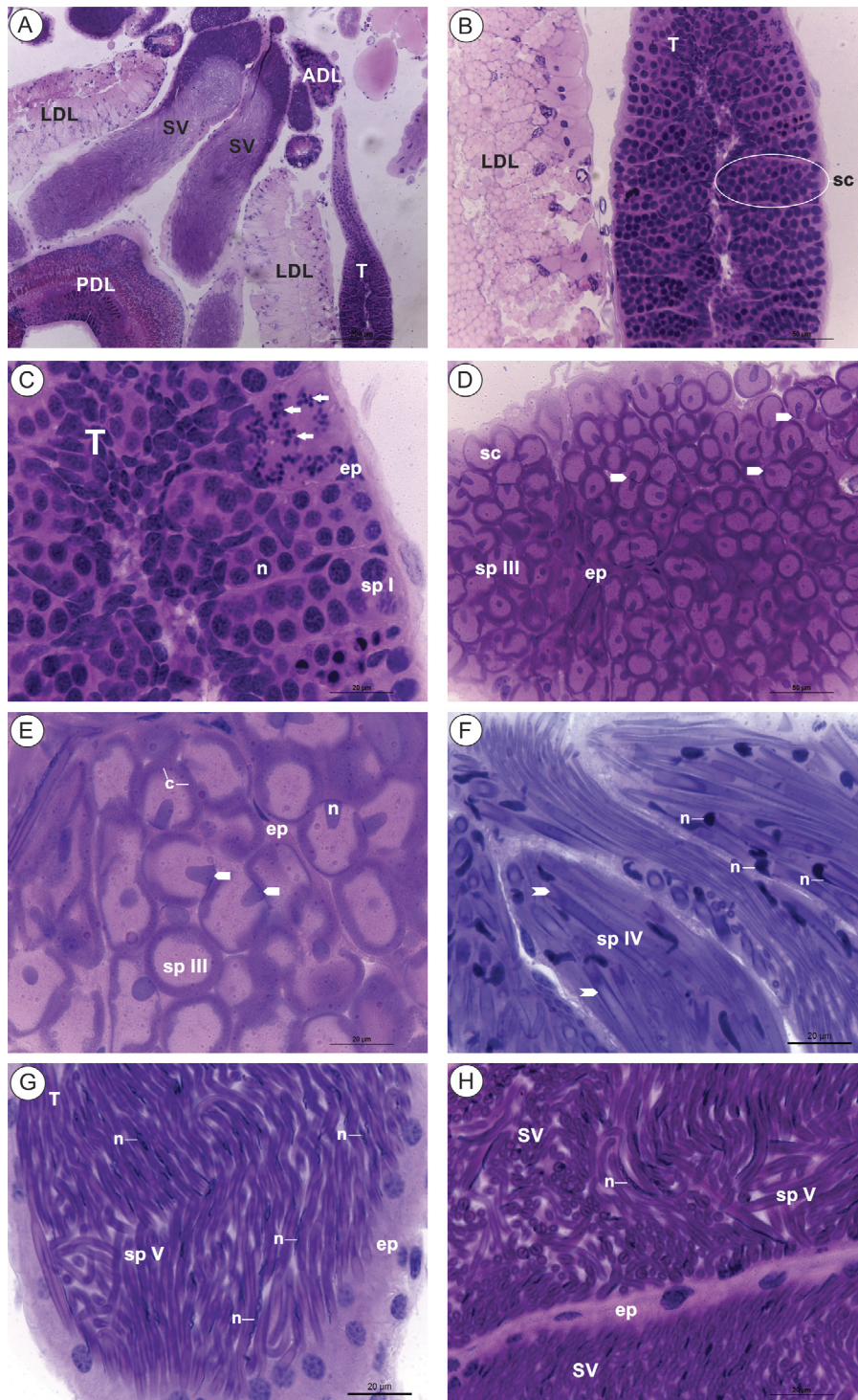
Fig. 6. Histological photomicrograph of the gland complex and testis of *A. aureolatum*. (**A**) Overview of the gland complex and its lobes. (**B**) Section of the testes distal region (T) showing spermatids in different stages of development. (**C**) Detail of seminal vesicles. (**D**) Testes proximal region with immature spermatids. (**E-F**) Detail of spermatids III e IV. (**G-H**) Detail of spermatids IV during cellular elongation process. (**I**) Spermatids V inside the seminal vesicle, showing the helical nucleus (n) and the operculum (op). **ADL** = Antero-Dorsal Lobe; **c** = cisternae; **arrow head** = acrossomal vesicle; **ep** = epthelium; **LDL** = Latero-Dorsal Lobe; **PDL** = Postero-Dorsal Lobe; **sp III** = spermatid III; **sp IV** = spermatid IV; **sp V** = spermatid V; **SV** = seminal vesicle; **VAL** = Ventro-Anterior Lobe.
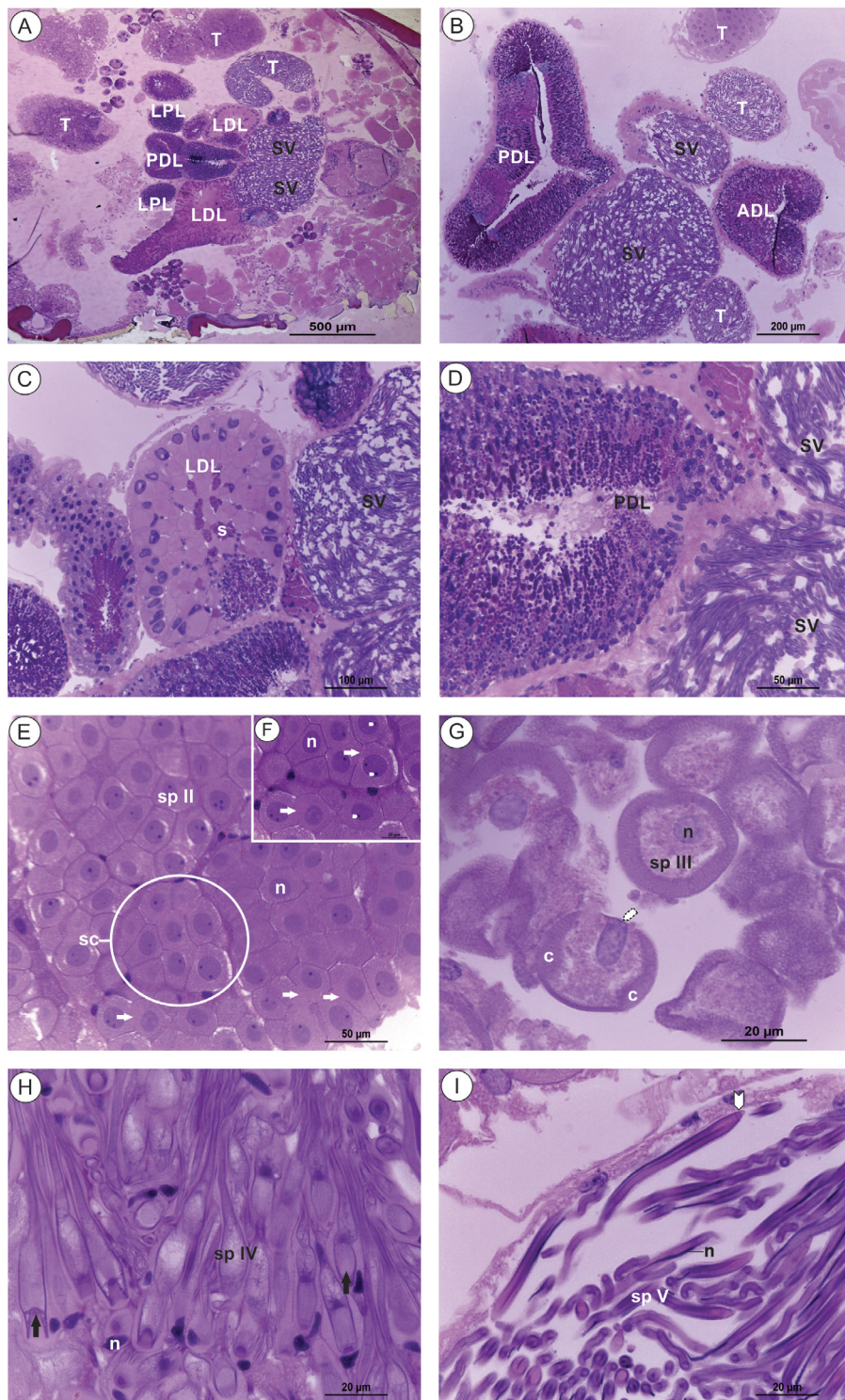
Fig. 7. Histological photomicrograph of the gland complex and testis of *A. sculptum*. (**A-B**) Overview of the gland complex showing the seminal vesicles (SV) and the lobes. (**C-D**) Detail of the LDL during transition from granular type to agranular type. (**E-F**) Spermatids II showing cytoplasmic bridges (arrows) between cells. (**G**) Spermatids III full of cisternae and showing the acrossomal vesicle (arrow head). (**H**) Spermatids IV during elongation process, which notes the membrane complex (arrow). (**I**) Sp V showing helical nucleus (n) and the operculum (arrow head). **ADL** = Antero-Dorsal Lobe; **c** = cisternae; **arrow head** = acrossomal vesicle; **ep** = epthelium; **LDL** = Latero-Dorsal Lobe; **PDL** = Postero-Dorsal Lobe; **sc** = spermatocyst; **sp III** = spermatid III; **sp IV** = spermatid IV; **sp V** = spermatid V; **SV** = seminal vesicle; **VAL** = Ventro-Anterior Lobe.

Fig. 8. Histological photomicrograph of the gland complex and testis of *A. triste*. (**A**) Overview of the gland complex showing the seminal vesicles (**SV**) and the lobes. (**B**) Detail of the proximal region of the testes (T). (**C**) Detail of spermatids I. (**D-E**) Detail of spermatids III full of cisternae and showing the acrossomal vesicule (arrow head). (**F**) Spermatids IV during elongation process, including the nuclear process (n). (**G-H**) Sp V showing helical nucleus (n). **ADL** = Antero-Dorsal Lobe; **c** = cisternae; **arrow head** = acrossomal vesicle; **ep** = epthelium; **LDL** = Latero-Dorsal Lobe; **PDL** = Postero-Dorsal Lobe; **sc** = spermatocyst; **sp III** = spermatid III; **sp IV** = spermatid IV; **sp V** = spermatid V; **SV** = seminal vesicle; **VAL** = Ventro-Anterior Lobe.
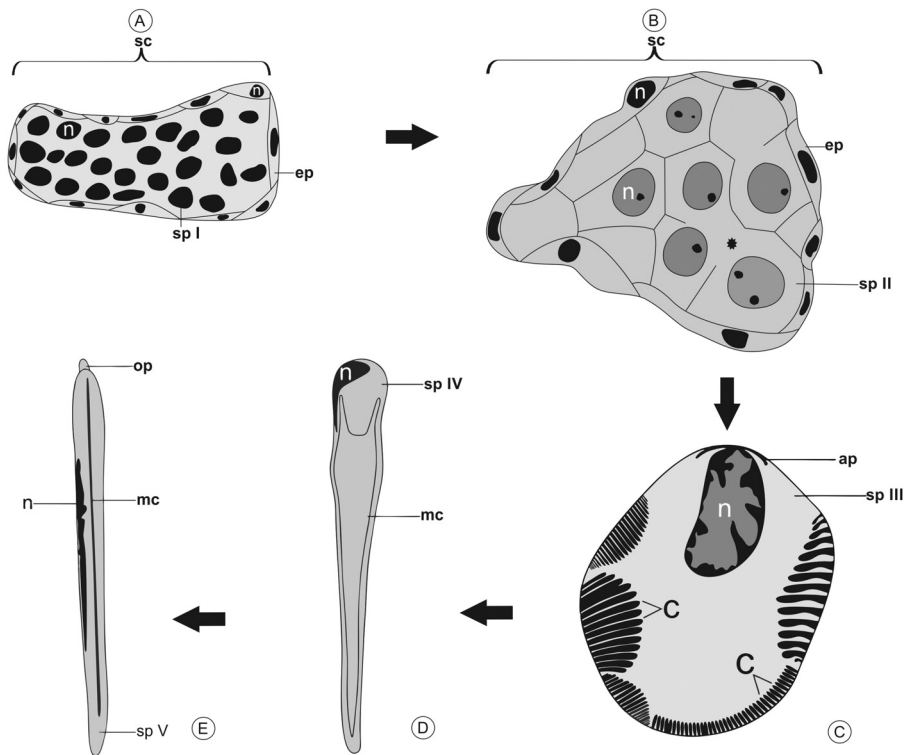
Fig. 9. Schematic drawing of spermiogenesis process in the genus *Amblyomma*. (**A**) First development stage, **sp I**, into spermatocysts. (**B**) Second stage, **sp II**, characterized by the cellular limit and bridges (*). (**C**) Third stage, **sp III**, which cisternae and acrossomal vesicle are observed. (**D**) Fourth stage, **sp IV**, characterized by the elongation and nuclear process. (**E**) Final stage, **sp V**. **ap** = acrosssomal vesicle; **c** = cisternae; **ep** = epithelium; **n** = nucleus; **mc** = membrane complex; **op** = operculum; **sc** = spermatocyst.

the system as a hole, but in none of the published work, an evolutionary approach was given. Additionally, these studies contain data on a few species, which makes difficult any comparative analyses [21–25].

Ticks male reproductive system presents some peculiarities as its morphology, but generally, it seems to be very similar among the species studied to date. According to Soneshine [22,26] the system is composed by a multilobed accessory gland complex which houses dorsally a pair of seminal vesicles, connected laterally to a pair of vasa deferentia, extending then in a pair of tubular testes or in a single horseshoe-shaped testis depending on the species.

The same anatomical pattern was observed in ticks from the Ixodidae family as well as in Argasidae family and in the *Ixodes* (Prostriata) genus. However, the studies conducted so far have pointed out an important difference regarding testes morphology. In the Argasidae ticks *Ornithodoros savignyi*, *O. kelleyi* and *Argas persicus* the authors described the testis as a single horseshoe-shaped organ, whereas in *O. moubata* species it was observed a pair of testes conected by a thin isthmus in its distal region and yet, in the *Ar. reflexus* species, a pair of testes was observed totally individualized, without any kind of interconnection [22,26].

Studies on specimens of the *Ixodes* genus have shown that they have a distally interconnection between these organs, while most of representatives of the Ixodidae family, for all the species studied to date, have a pair of individualized testes [22,26,28].

During this study only *A. triste* specimens presented the testes connected in its distal region by a thin tubular-shaped isthmus. For the first time this kind of organization was observed in a tick species from the *Amblyomma* genus, which indicates an apomorphic character among this group of ticks, since most of the Argasidae species, considered basal, present a "single testis" as a product of the plesiomorphic condition. Thus the "separated testes" character would be derived or apomorphic when compared to the "paired testes".

As regards spermatogenesis, this occurs in the same way in ticks from the *Amblyomma* genus, as is demonstrated in this work and in studies conducted by Reger [21] and Wüest et al. [24] on *A. dissimilli* and *A. hebraeum*, respectively. It is important to emphasize that the development process of the germ cells described in the present study is the spermiogenesis, which is the final stage from a series of morphological changes that spermatids go through until it become a mature spermatozoon. Thus, the data obtained to date makes it possible to conclude that: a) the cells in the last stage of spermiogenesis in the male genital tract are late spermatids, classified herein as sp V; and b) the spermatogenesis ends at the nymph instar or when adult is in fasting feeding stage, depending on the species under consideration.

In fed *A. triste* individuals, a portion of the testis was observed to contain some spermatocytes II, a fact that was unreported for ticks from the *Amblyomma* genus in

these stages of life and feeding, indicating that spermatogenesis in this species must depend on the adult tick's nutritional state. Anholeto et al. [29] and Sampieri et al. [28] observed spermatocytes II in *A. aureolatum* but not in *A. sculptum.*

With the exception of this subtle difference, spermiogenesis in three species occurred in the same way, being with the sp I (originated from the meiosis of spermatocytes II) which, due to hypertrophy, started to occupy larger areas in the cysts. Following the accentuated hypertrophy event from which originates sp II, the cells become round and show striations, which remain internally attached to the cell limit, also known as cisternae. These structures were also observed by Reger [21] in *A. dissimili*, by Wüest [24] in *A. hebraeum* and by Brinton et al. [23] in *Dermacentor andersoni* which have the same arrangement inside the cell; a characteristic that is apparently shared by the Ixodidae family.

The sp III observed in the three species studied herein had a thin thread anterior to the nucleus, strongly stained by hematoxylin. This thread represents part of the acrosomal complex and probably represents the acrosomal vesicle itself. Reger [21] assumed that this structure would be the acrosomal vesicle in the sp III of *A. dissimili*; In this case, if it is confirmed that this thin thread is in fact the acrosomal vesicle, this would be a feature (a synapomorphy) shared among Arachnida, including free-living mites [31,32].

The sp IV undergo accentuated morphological changes from the sp III, such as cell elongation, high condensation of the nuclear material, fusion of the cisternae in a membranous complex, development of the acrosomal vesicle and formation of the operculum, which are changes that were also observed by other authors when studying spermiogenesis in ticks from the Ixodidae and Argasidae families [21,26].

In addition, in the sp IV, changes that give the nucleus the appearance showed in the sp V (phylliform and helical) also seem to occur in the spermatids of water and phytophagous mites, where this organelle loses its envelope and start to be called as chromatin body. Similarly, the membranous complex formed from the cisternae fusion in the sp IV matches with the descriptions made by the same authors when studying these same water and phytophagous mites [23,26,30–32].

Therefore, regardless of the fact that spermiogenesis in ticks only ends in the female genital tract after mating, the late spermatids or sp V observed in the male genital tract have ultramorphological characteristics which allows us to make a distinction between the three species studied herein, making spermiotaxonomy a useful tool for ticks.

Thus, based on the external cell surface morphology, it was possible to schematically propose a comparison between the sp V of each species, showing how they are different from one another, and find evidence that the sp V of *A. sculptum* is similar to the *A. hebraeum* studied by Wüest et al. [24]. Whereas *A. aureolatum* and *A. triste* sp V exhibits characteristics that until now were never observed in any other studied species from the *Amblyomma* genus.

5. Conclusions

The results obtained herein confirm that there is a species-specific connection between the morphology of ticks male germ cells, setting precedents for the necessity of a thorough investigation regarding the ultrastructure of these cells with spermiotaxonomic and spermiocladistic purposes.

Finally, comparing the morphology of male ticks' reproductive system–as well as its germ cells–can contribute toward understanding problems related to their classification and evolution, especially in groups that present probable polyphyletism, as is the case in the species from the *Amblyomma* genus.

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