



Original Article

Morpho-histochemical characterization of the salivary glands of semi-engorged *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae) female ticks



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ABSTRACT

This study presents the morphological and physiological characterization of the salivary glands of semi-engorged *Amblyomma triste* females. Unfed individuals were placed on New Zealand White rabbits for feeding and the females, after 4 days, were collected, dissected and the salivary glands were submitted to the application of histological (hematoxylin–eosin technique) and histochemical tests for the detection of protein (bromophenol blue technique), polysaccharides (periodic acid–Schiff technique), lipid (Nile blue technique) and calcium (von Kossa technique). The histological results show that the glandular tissue is composed by a system of ducts and three types of acini (I, II and III). The acini I are formed by a large central cell surrounded by several smaller agranular peripheral cells. Acini II are formed by cells **a**, **c1**, **c2**, **c3** and **c5**, which are full of secretion granules. Acini III are constituted by cells **d**, **e** and **f**; the former two contain secretion granules, the latter is agranular. The glandular histochemical composition was also verified. Data obtained here will certainly help in the understanding of the cellular morphology and of the general physiology of these organs in this specie, providing important information for the creation of scientific bases which will contribute for the development of more specific and efficient methods of control.

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1. Introduction

The ticks constitute a group of great medical–veterinary importance, once they cause significant blood losses to the animals infested and can also act as vectors of protozoa and bacteria, among other pathogens [1]. In this case, the species *Amblyomma triste* is responsible for the transmission of *Hepatozoon americanum*, *Rickettsia conorii* [2] and *Rickettsia parkeri* [3].

The tick species *A. triste* is spread throughout the Neotropics and has been reported in Ecuador [4], Argentina [5], Uruguay [6] and Brazil [7] infesting tapirs [8], dogs [9], capybaras [7], marsh deers [10], opossums [7] and human beings [6].

According to Moorhouse and Tatchell [11], the saliva is the primary route through which the micro-organisms are inoculated in the host's bloodstream. It is produced by the salivary glands; paired glands anterolaterally located in the idiosome. The females' salivary glands are composed of three types of acini and in males' of four types of acini [12–14,15].

Considering the fact that few morphological studies have been carried out on the species *A. triste* and the critical

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participation of the salivary glands in the biological success of the ticks, this study presents the morphophysiological description of the salivary glands of *A. triste* semi-engorged females, aiming to contribute to the obtention of information that help researchers get to better understand the physiology of this organ, as well as the nature of the compounds which compose the saliva and are synthesized by it; a fundamental step for the improvement of the existing control methods and/or the development of more efficient methods to control this ectoparasite.

2. Materials and methods

Ticks of *A. triste* ticks were collected from tick colonies maintained in controlled conditions (28°C, 80% humidity and 12 h photoperiod) at the Department of Animal Pathology, Veterinary College, UNESP – Jaboticabal, SP, Brazil. The couple of ticks were then placed in feeding chambers according to the methodology described by Bechara et al. [16]. Females weighing 22 mg in average were collected (about 4 days of feeding) and taken to the proposed procedures. Equipment from the Histology Laboratory of the Biology Department at the Institute, UNESP – Rio Claro, SP, Brazil, was used throughout the study.

Twenty-five individuals were maintained in refrigerator for thermal shock anesthesia, dissected in saline solution and the salivary glands were removed with the aid of camera lucida coupled to a Zeiss stereomicroscope.

The salivary glands were fixed in 4% paraformaldehyde. The material was then dehydrated in ethanol, embedded in Leica resin for 24 h and then transferred to plastic molds previously filled with polymerized Leica resin. After resin polymerization, the material was sectioned at 3 µm thickness slices and subjected to hematoxylin and eosin staining. Then, histochemical tests were applied to detect the presence of the following compounds: protein (bromophenol blue), as proposed by Pearse [17]; polysaccharides (PAS – periodic acid Schiff), as proposed by McManus [18] and counterstained with methyl green and calcium (von Kossa) as proposed by Junqueira and Junqueira [19] and lipid by Lison [20].

The glass slides with the salivary glands sections were examined using a Motic BA300 photomicroscope.

3. Results

3.1. Histological analysis

3.1.1. Acinus I

The acini I are round-shaped and present a homogeneous cytoplasm slightly stained by eosin. The nuclei of the central and peripheral cells can also be observed, being round-shaped and strongly stained by hematoxylin (Fig. 1A).

3.1.2. Acinus II

a cells: these cells contain secretion granules strongly stained by eosin (Fig. 1A–D).

c1 cells: present secretion granules strongly stained by hematoxylin (Fig. 1D).

c2 cells: these cells are full of secretion granules which present little evident limit and are stained both by eosin and hematoxylin (Fig. 1B and C).

c3 cells: present larger secretion granules in relation to **c1** cells and are also strongly stained by hematoxylin (Fig. 1D).

c5 cells: in these cells the secretion granules are the largest observed for this type of acini, present various sizes and are strongly stained by hematoxylin (Fig. 1A–C).

3.1.3. Acinus III

d cells: the secretion granules of these cells present similar morphology, dimension and coloration to those of **a** cells of acini II (Fig. 1D and E).

e cells: these cells contain the largest secretion granules observed in the acini and are weakly stained by eosin (Fig. 1D and E).

f cells: the females in this feeding stage do not present secretion granules in these cells. The cytoplasm is homogeneous, weakly stained by eosin and the nuclei are round-shaped, presenting dispersed chromatin (Fig. 1E).

3.2. Histochemical analysis

The histochemical data are shown in Table 1.

4. Discussion

The interest in the study of the salivary glands of ixodid ticks has increased, once it is there that the synthesis of molecules with immunological and pharmacological properties responsible for the modulation of the hemostatic and immune-inflammatory systems of the host occurs, and these processes guarantee the success of spoliation [21]. Moreover, it is important to emphasize the importance of these organs in the storage and transmission of pathogens responsible for transmitting infections [22] to different groups of animals, including the human being [23]. Currently the molecules from the salivary glands have also been studied in order to be used for the treatment of diseases, such as cancer [24,25,26].

Considering the results obtained here, it was verified that the salivary glands of *A. triste* females follow the pattern already described in literature for other species of the Ixodidae family; i.e., a pair of non-compacted glandular masses, elongated and constituted by a secretory portion (acini) and an excretory one (system of ducts) without a reservatory for the storage of secretion [27,28,15].

Under the morphological and histochemical point of view, it was observed that the acini follow the description established in literature [13,22,15]; i.e., the presence of acini types I, II and III, the latter two granular.

The acini type I of *A. triste* females are also formed by two types of cells: the central – larger – and the peripheral, smaller and located around the central; corroborating data previously reported for other species of ticks [12,13,22,27,15].

In *A. triste* females, the acini I were stained only for protein, polysaccharides and neutral lipid, being weakly stained for protein and polysaccharides and moderately

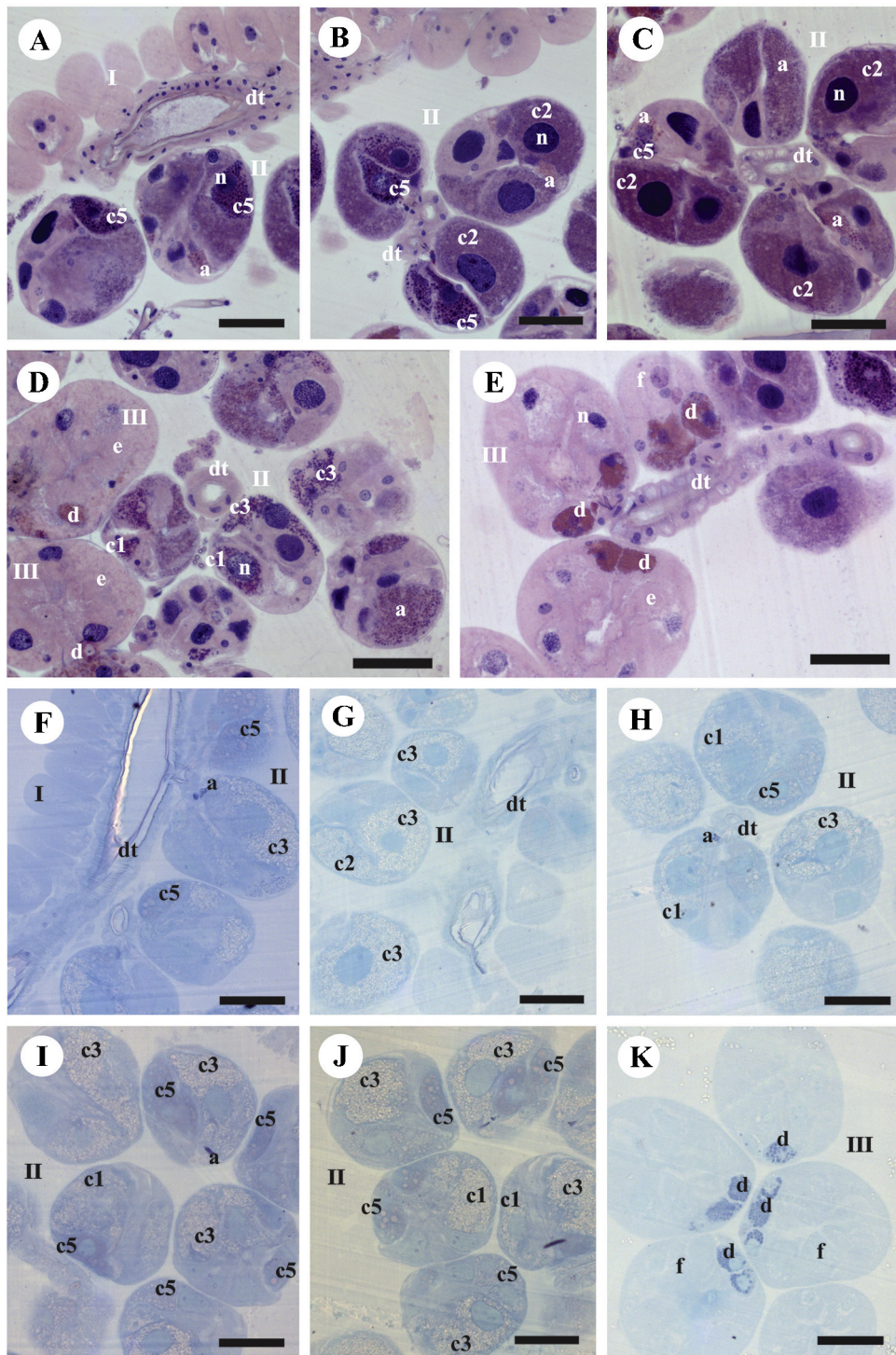


Fig. 1. Histological sections of the salivary glands of semi-engorged *Amblyomma triste* female ticks. (A–E) Hematoxylin and eosin (HE) staining. (A) Acini I and II. (B and C) Acini II. (D) Acini II and III. (E) Acini III. (F–K) Nile blue staining to detect lipids. (F) Acini I and II. (G–J) Acini II. (K) Acini III. n = nuclei; dt = duct; a, c1–c5, d, e, f = glandular cells. Scale bars: 20 μ m.

stained for lipid, elements found dispersed in the cytoplasm. This fact confirms the non-secretory characteristic of these acini, which according to literature would be a controversial function; and, according to some authors, able to

act on the osmoregulation, or on the hydric balance according to others [29]. Thus, the components here detected must be part of the physiology of the cells which compose these acini, participating in the metabolic routes which are

Table 1
Histochemistry analysis of the salivary glands of the semi-engorged *Amblyomma triste* females ticks.

Acini	Cell types	Histochemistry tests				
		BB	PAS	NB		VK
				Neutral lipid	Acid lipid	
I		+ (Fig. 3A)	+ (Fig. 2A)	++ (Fig. 1F)	– (Fig. 1F)	– (Fig. 3G)
II	a	+++ (Figs. 3B and C)	– (Fig. 2B and F)	+++ (Fig. 1F, H and I)	– (Fig. 1F, H and I)	– (Fig. 3H)
	c1	+++ (Fig. 3B)	+++ (Fig. 2F)	– (Fig. 1H, I and J)	– (Fig. 1H, I and J)	+++ (Fig. 3I)
	c2	++ (Fig. 3C–E)	+ (Fig. 2B)	– (Fig. 1G)	– (Fig. 1G)	++ (Fig. 3H and J)
	c3	+++ (Fig. 3B–E)	+++ (Fig. 2C and G)	– (Fig. 1F–J)	– (Fig. 1F–J)	+++ (Fig. 3H and I)
	c5	+++ (Fig. 3B–E)	+++ or ++ or + or – (Fig. 2B–G)	– (Fig. 1F, H–J)	+++ or ++ or + (Fig. 1F and H–J)	+++ (Fig. 3J)
III	d	+++ (Fig. 3F)	– (Fig. 2H)	+++ (Fig. 1K)	– (Fig. 1K)	++ (Fig. 3K and L)
	e	+ (Fig. 3F)	– (Fig. 2H)	No observed	No observed	– (Fig. 3K and L)
	f	+ (Fig. 3F)	+ (Fig. 2H)	+ (Fig. 1K)	– (Fig. 1K)	– (Fig. 3K and L)

BB: bromophenol blue for detection of proteins; PAS: PAS for detection of polysaccharides; NB: Nile blue for detection of lipides; VK: von Kossa for detection of calcium; +++; strongly positive; ++: moderately positive; +: weakly positive; –: negative.

common to all the cells. Such hypothesis is confirmed by Fawcett et al. [22] who reported that the lipid present in the cells of acini I could be used as a source of energy, as the polysaccharides, which would also correspond to a source of energy, the glycogen.

In the acini II studied here, five cellular types were detected: **a**, **c1**, **c2**, **c3** and **c5**, from those established for other species: **a**, **c1**, **c2**, **c3**, **c4** and for *R. sanguineus* females and also types **c5** and **c6** [15]. Such variation is explained by the relation between the parasite – in this case *A. triste*, and the hosts; i.e., due to the type of immune-inflammatory response triggered by the hosts. It is known that the glandular morphophysiology, mainly of the secretory acini, which responsible for the synthesis and the release of molecules with the capacity to modulate the defense systems of the host, aiming to overcome the immune-inflammatory and hemostatic barriers [21].

In this study, it was verified that the **a** cells, under the histochemical point of view, follow the same pattern described in literature for other species of ticks [12,13,27,15]; i.e., the secretion granules of such cells are strongly stained for protein and lipid, with total absence of polysaccharides. According to literature, such cells secrete lipoprotein, which would participate in the formation of the cement cone [12,13], a structure that is not hydro-soluble, enabling the fixation of the parasite to the host which otherwise would be impaired by the water from sudoresis or from the environment in general.

The cells **c1** and **c3** contain secretion granules strongly stained for polysaccharides, protein and calcium, with total absence of lipid, following the same pattern of histochemical description found in literature for other species of ticks [12,13,27,15].

However, histochemical results for cells **c2** and **c5** differ in part from those found in literature [15]. The **c2** cells present granules moderately stained for protein and weakly stained for polysaccharides, as reported by other authors [15]; however, the presence of calcium (moderately stained granules) is a unheard of result for the constitution of the secretion synthesized by such cells, once this element had not been detected in cells **c2** belonging to other species of ticks [15].

Regarding the secretion granules of **c5** cells, strong staining was observed for protein and staining for polysaccharides varied from negative to strong. The granules were strongly stained for calcium, an unheard of result for these cells when compared to *R. sanguineus* females [14]. Moreover, staining for lipid was negative for the neutral and varied from weak to strong for the acid. It is important to emphasize that the detection of acid lipid had never been demonstrated before in literature concerning the histochemical composition of such cells.

Concerning the function of the cells from **c** group, the secretion produced by them would be related to the modulation of the host's defense system [12,13]. In addition, the presence of calcium justifies this function, once the literature discusses the presence and the function of this element in the salivary glands of several species of ticks [30–33,34]. According to Jaworski et al. [30], Brossard and Wikel [31], Steen et al. [33] and Jittapalpong et al. [34], the calcium binding protein is among the components secreted by the saliva of the ticks, functioning as facilitators of the feeding process through their immunosuppressing and antihemostatic actions [30,31,34]. In addition, the calreticulins would modulate the immunity of the host, mimetizing or inducing the self-immunity [33]. The calreticulins could also inhibit the host's hemostatic response through the binding with calcium ions, co-factors of the enzymes involved in the process of coagulation; however, in this case the calreticulins would bind to calcium only in the host's organism; opposing the results here obtained, demonstrating the presence of calcium in the secretion of some cells of the salivary glands [33].

Concerning the acini type III, it was verified that they are constituted by three cellular types: the **d**, **e** and **f** cells, corroborating data reported in literature for other species of ticks [12,13,22,27,15].

In this study, **d** cells presented secretion granules strongly stained for protein and neutral lipid, moderately stained for calcium and negative for polysaccharides. The **e** cells were weakly stained for protein and negative for polysaccharides and calcium.

Data obtained here showing the absence of polysaccharides both for the granules of **d** and **e** cells corroborate

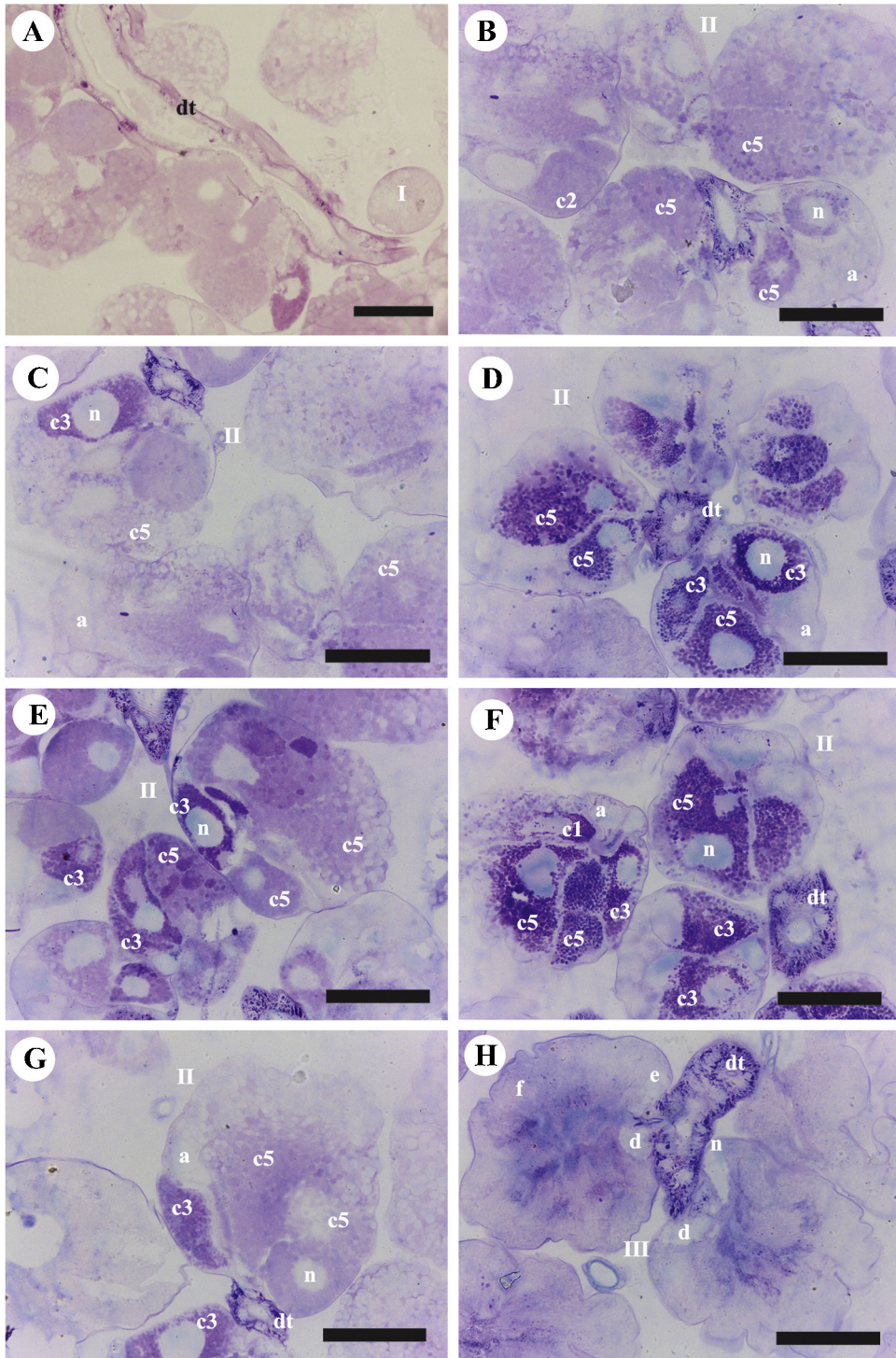


Fig. 2. Histological sections of the salivary glands of semi-engorged *Amblyomma triste* female ticks. (A–H) PAS staining to detect polysaccharides. (A) Acini I. (B–G) Acini II. (H) Acini III. dt = duct; a, c1–c5, d, e, f = glandular cells. Scale bars: 20 μ m.

those found by Camargo-Mathias and Furquim [15] for *R. sanguineus* females and Nunes et al. [27] for *A. cajennense* females. According to literature, d and e cells of acini III of the salivary glands of ixodid ticks are involved

with the secretion of lipoprotein-derived substances. These lipoproteins, as those secreted by the a cells of acini II, participate in the formation of the cement cone, structures which, along with the buccal structures of the ectoparasite,

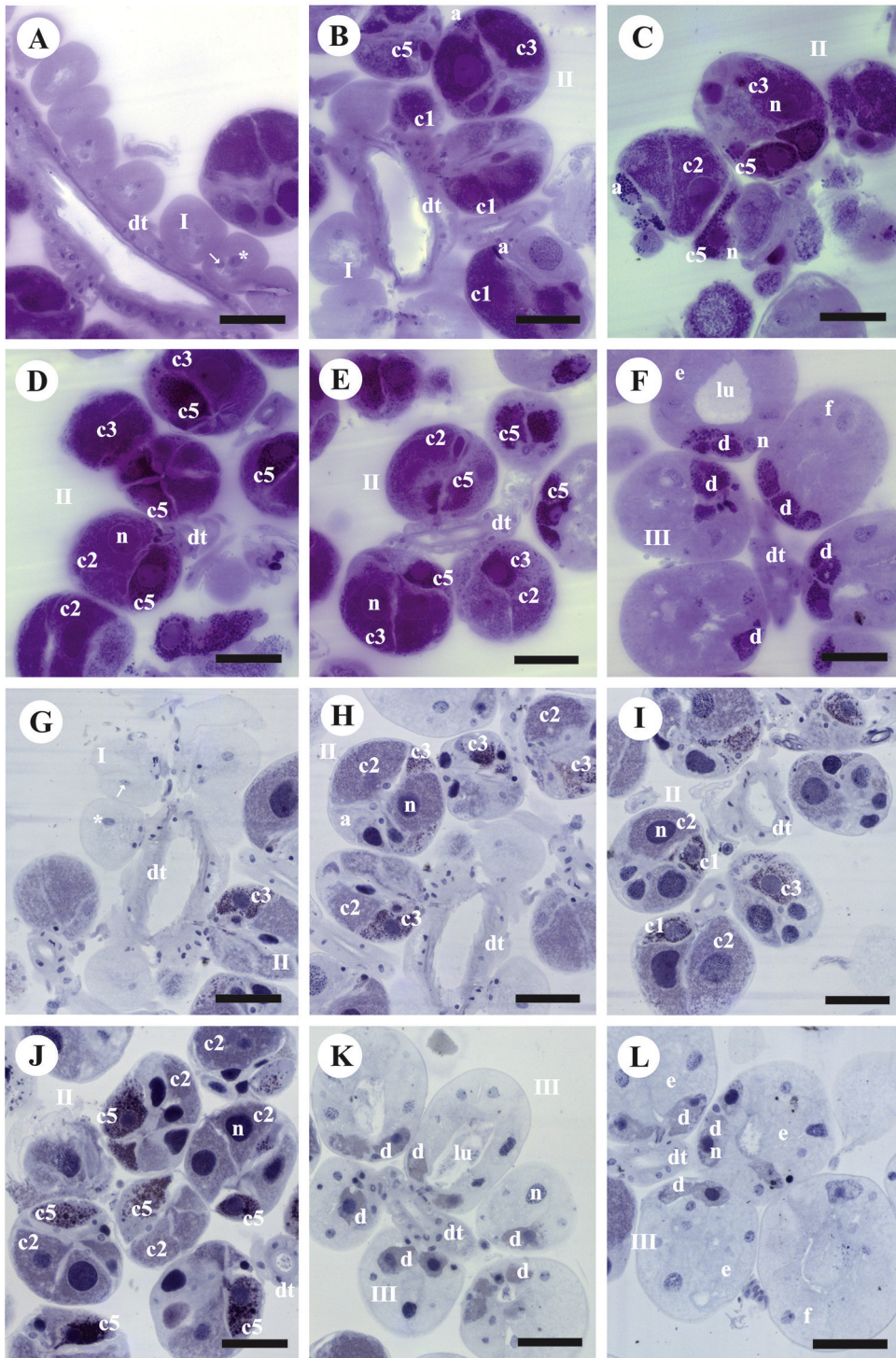


Fig. 3. Histological sections of the salivary glands of semi-engorged *Amblyomma triste* female ticks. (A–F) Bromophenol blue staining to detect proteins. (A) Acini I. (B) Acini I and II. (C, D and E.) Acini II. (F) Acini III. (G–L) Von Kossa staining to detect calcium. (G) Acini I. (H, I, J) Acini II. (K) L. Acini III. *n* = nuclei; dt = duct; *a*, *c1*–*c5*, *d*, *e*, *f* = glandular cells. Scale bars: 20 μ m.

promote its fixation on the host [12,13,22,35,27,15]. Therefore, according to the histochemical data it can be inferred that the secretion synthesized by such cells in *A. triste* females would perform this function.

The histological and histochemical data obtained for *f* cells show that they have already completed the secretory phase and are in the beginning of the osmoregulatory phase, since they do not present dispersed secretion

granules in the cytoplasm and their lumen is slightly dilated. The morphophysiological alterations suffered by acini III, affecting exclusively **f** cells during the feeding process of female ixodid ticks are widely reported in literature [12,13,27,15]. Such alterations occur in order to allow the excess of water and mineral salts to be eliminated via salivation through their transportation from the hemolymph to **f** cells and from there to the acinar lumen and to the duct system [12,13,36].

This shows that *A. triste* females, here analyzed after 4 days of feeding, are beginning their phase of great blood consumption. In this sense, it is established here that both the feeding behavior and the morphophysiology of the salivary glands of *A. triste* females, especially **f** cells, are similar to those of *R. sanguineus* females. According to Camargo-Mathias and Furquim [15], with 2 days of feeding **f** cells are in full secretory activity and with 4 days they are starting the phase of osmoregulation.

Still concerning **f** cells, the histochemical tests applied showed that their cytoplasm were weakly stained for protein, polysaccharides and for neutral lipid, and presented no reaction to acid lipid and calcium, elements which are part of their metabolism.

5. Conclusion

Considering the histochemical and histological data obtained in this study, it is clear that the salivary glands of *A. triste* females follow a morphophysiological description similar to those of other species of ixodid ticks, not previously described for this species of tick. Also showed that this salivary glands physiology differ from those reported in the literature since the histochemical tests detected the presence of calcium in the **c2** cells granules (moderately positive) and **c5** cells granules (strongly positive), as well as the presence of acid lipids (staining varied from weakly to strongly positive) in **c5** cells granules. Data obtained here will certainly help in the understanding of the cellular morphology and of the general physiology of these organs in this species, bringing critical information for the creation of scientific bases which will contribute for the creation of more specific and efficient methods of control.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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