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Mg^{2+}-dependent ATPase activity in triatomine salivary glands (Heteroptera, Triatominae)

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ABSTRACT. Mg^{2+}-ATPase activity was detected in the three salivary glands of adult triatomines, males and females, of *Triatoma infestans* (Klug, 1834) and *Panstrongylus megistus* (Burmeister, 1835) (Heteroptera, Triatominae). A predominance of binucleated cells in D1 and D2 and mononucleated in D3 was observed, with bulky and polyploidy nuclei. ATPase activity was detected in the nuclei, possibly in euchromatin and nucleolus, where this enzyme probably acts in the transcription process. ATPase reaction was also evidenced in the nuclear membrane, which is probably associated with nuclear-cytoplasmatic transport. These characteristics indicate a high metabolism and protein synthesis, which must be essential to saliva production as well as in maintaining the hematophagy of triatomines.

KEYWORDS. Euchromatin, gland, nuclear membrane, nucleolus, phosphatase.

RESUMO. Atividade da enzima ATPase dependente de MG^{2+} em glândulas salivares de triatomíneos (Heteroptera, Triatominae). A atividade da enzima ATPase dependente de Mg^{2+} foi detectada nas três glândulas salivares de triatomíneos adultos, machos e fêmeas, de *Triatoma infestans* (Klug, 1834) e *Panstrongylus megistus* (Burmeister, 1835) (Heteroptera, Triatominae). Observou-se um predomínio de células binucleadas em D1 e D2, e mononucleadas em D3, com núcleos volumosos e poliplóides. Atividade da ATPase foi detectada no núcleo, provavelmente na eucromatina e no nucléolo, onde esta enzima atuaria no processo de transcrição. A atividade também foi evidenciada na membrana nuclear e, possivelmente, associada ao transporte núcleo-citoplasmático. Essas características indicam alto metabolismo e elevada síntese proteica, essenciais para a produção de saliva e manutenção da hematofagia de triatomíneos.

PALAVRAS-CHAVE. Eucromatina, glândula, membrana nuclear, nucléolo, fosfatase.

Triatomines are vectors of *Trypanosoma cruzi* (Chagas, 1909) the etiological agent of Chagas Disease. In Latin America, it has been estimated that there are six to seven million people infected by this protozoon (WHO, 2017) and 90–100 million are exposed to infection (Coura & Borges-Pereira, 2010).

Triatomines are hematophagous insects and it consists exclusively in the ingestion of blood in all life stages (five nymphal stages and adults). Eating activity is divided into two phases (probing and engorgement) (Soares *et al.*, 2006). Salivation is mandatory in both eating phases. This saliva is produced by salivary glands located in the thorax. In *Triatoma* and *Panstrongylus*, the salivary gland is formed by three pairs of independent units: D1 (anterior or main), D2 (median or supplementary), and D3 (posterior or accessory) (Barth, 1954).

Phosphatases are enzymes responsible for removing phosphate groups from specific substrates in a versatile, fast and easily reversible mechanism for maintaining cellular homeostasis. They are found in the cells of all organisms. Among the phosphatases, a group of ATPases has been described in the nucleus, where it acts in transcription and DNA repair. In the cytoplasm, ATPases can be found in the mitochondria, plastids chloroplasts, and peroxisomes. They may also act in the reconstitution of the endoplasmic reticulum and Golgi apparatus in transport vesicles and their fusion with the membrane in proteolysis. The disaggregation and unfolding of proteins are also necessary in the processes of cell division and cell death (apoptosis) (Ogura & Wilkinson, 2001).

There is considerable biochemical evidence of the existence of various types of ATPases distributed in various organelles and associated with various functions. However, data regarding the distribution of these enzymes in the salivary glands of insects are not found in the literature. Considering this limited literature, coupled with the importance of these secretory units during hematophagy and medical sanitary interests regarding these insects, the aim of this study was to analyze the distribution of magnesium-dependent ATPase in the secretory organs of two species: *Triatoma infestans* (Klug, 1834) and *Panstrongylus megistus* (Burmeister, 1835).

MATERIAL AND METHODS

Unfixed whole salivary glands of adult male and female *T. infestans and P. megistus* were studied (60 of each species; 30 of each sex). The insects were provided by the Insetário do Serviço Especial de Saúde de Araraquara, an organ of the Departamento de Epidemiologia da Faculdade de Saúde Pública da Universidade de São Paulo, SP, Brazil. The distribution of the dependent ATPase activity of Mg^{2+} was analyzed according to the technique proposed by Slater (1958) with modifications (Wegmann & Bankowsky, 1960).

The insects were dissected and the salivary glands removed. For the preservation of enzyme activity, the unfixed material was immediately incubated in a medium containing ATP as substrate. ATPase phosphate was released from the substrate and, after treatment with cobalt nitrate, a precipitate of cobalt phosphate formed in the enzyme-active site. This, treated with dilute ammonium sulfide, was converted to cobalt sulfide and visible by light microscopy as a dense granular deposition of dark color. The slides were mounted in glycerol, examined under a Jenaval Zeiss microscope, and photographed using a Sony Cyber Shot DSC-N1 8.1 Megapixel digital camera attached to the microscope.

A medium without substrate (ATP) was used as reaction control (Päutrat & Benköel, 1978).

RESULTS

Mg^{2+}-dependent ATPase was evidenced in the salivary glands of *T. infestans* and *P. megistus* (males and females) by light microscopy. In both sexes and species, the most intense reaction was observed in D1, and the least intense in D3 (Figs 1, 2). In these cells, bulky and highly polyploidy nuclei were observed, with a 97% of predominance of binucleated cells in D1 (Figs 2, 3, 11, 21, 29) and D2 (Figs 6, 14, 15, 23, 32, 33) and 96% of mononucleated cells in D3 (Figs 8, 9, 17, 18, 26, 27, 35, 36). 100 cells were counted in each gland.

In *T. infestans* salivary glands, the nucleus showed the most intense activity, observed by either dense and dark corpuscles inside the nucleus or by strong reactions in the nuclear membrane (Figs 1-18). Nuclear corpuscles were larger, unique and more intensely stained in males (Figs 1-9). In females, corpuscles were multiple and smaller (Figs 10-18). In both sexes, a thin granulation filled the nucleus. In the cytoplasm, no reaction was observed.

Similar results were also observed in *P. megistus* (Figs 19-36), where positive reaction was also observed in the nucleus. Dark corpuscles intensely stained were mainly observed in males, while in females a predominance of a thin granulation in the nucleus was visualized. In addition to the nuclear corpuscles, enzyme activity was also detected in the nuclear membrane in both sexes.

A total absence of enzyme activity was observed in salivary glands submitted to the control technique (incubation medium without ATP) in both sexes of the two species of triatomines studied [*T. infestans* (Figs 37-45) and *P. megistus* (Figs 46-54)].

DISCUSSION

Mg^{2+}-dependent ATPase activity was detected in the salivary glands of males and females of *T. infestans* and *P. megistus*. In both species and sexes, the enzyme reaction was more intense in D1 and less intense in D3. The difference in reactivity found the glands is associated with the cellular metabolism of these regions and, probably, with the biosynthesis of their different secretions. Similar results in acid phosphatase activity were observed (Anhê *et al.*, 2007).

Bulky and highly polyploidy nuclei were observed, with a predominance of binucleated cells in D1 and D2, and mononucleated cells in D3, as already observed (Barth, 1954; Anhê & Azeredo-Oliveira, 2008). According to Barth (1954), this increase in nuclear mass occurs because these organs are highly active and, in order to accelerate and regulate cellular regeneration after saliva secretion, they depend on bulky nuclei with extensive surfaces.

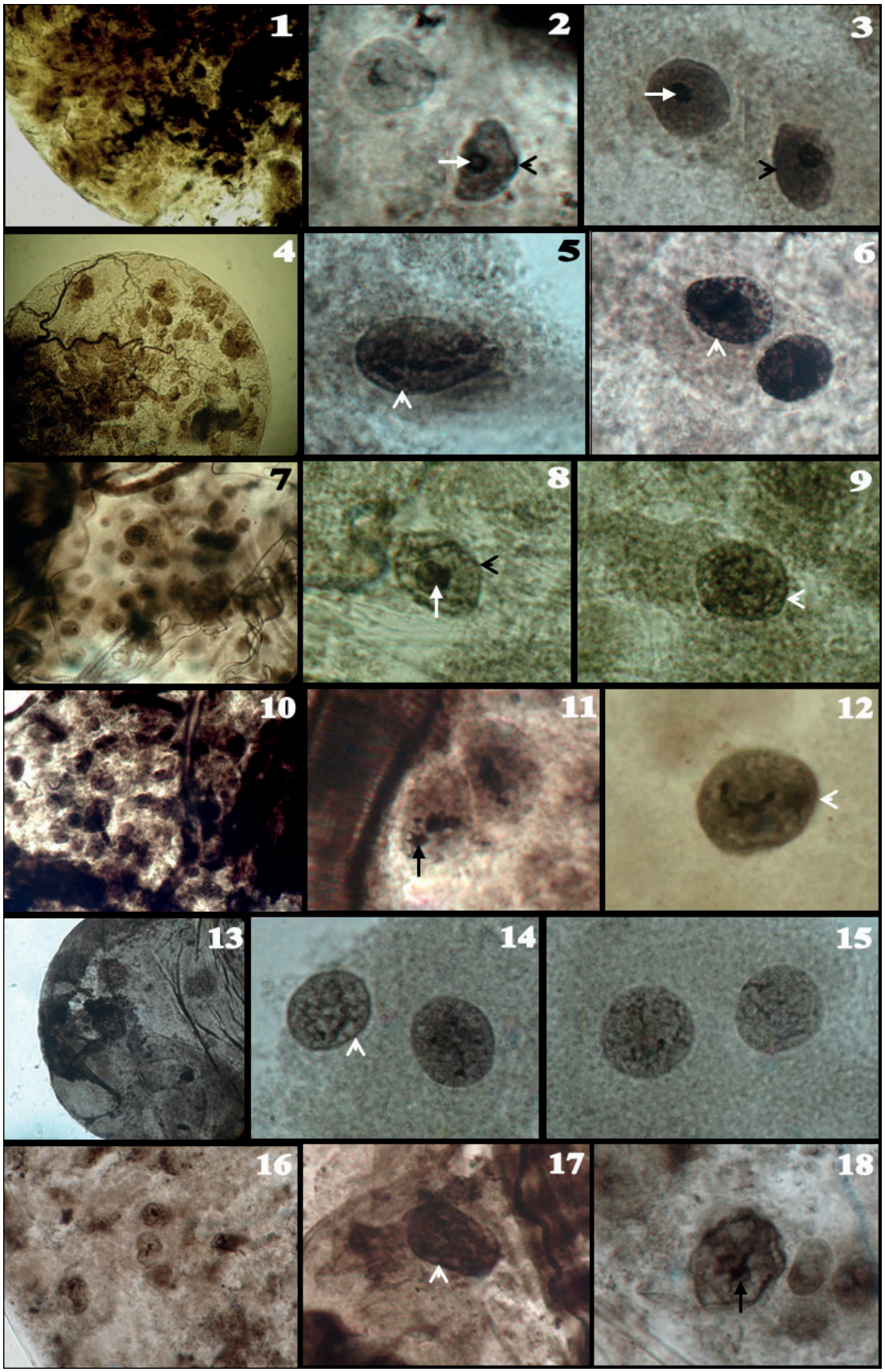
In the nucleus, the most intense response was observed as dark corpuscles, probably characteristic of nucleolar corpuscles, and as a fine granulation distributed throughout the nucleus, suggesting activity in the euchromatin (Anhê & Azeredo-Oliveira, 2008). Moreover, enzymatic activity was positive in the nuclear membrane and negative in the cytoplasm. Similar results were observed in the Malpighian tubules (Azeredo-Oliveira & Mello, 1986) and salivary glands (unpublished data) of triatomines.

Studies in the literature show the presence of Mg^{2+}-dependent ATPase in the nucleoli of rat hepatocytes (Siebert, 1966), mouse hepatocytes (Buchwalow & Unger, 1977), the Malpighian tubules of insects (Azeredo-Oliveira & Mello, 1986; Azeredo-Oliveira *et al.*, 2012), and cultures of human fibroblasts (Fox *et al.*, 1981), where ultrastructural analysis indicated that positive activity occurred in the fibrillar centers.

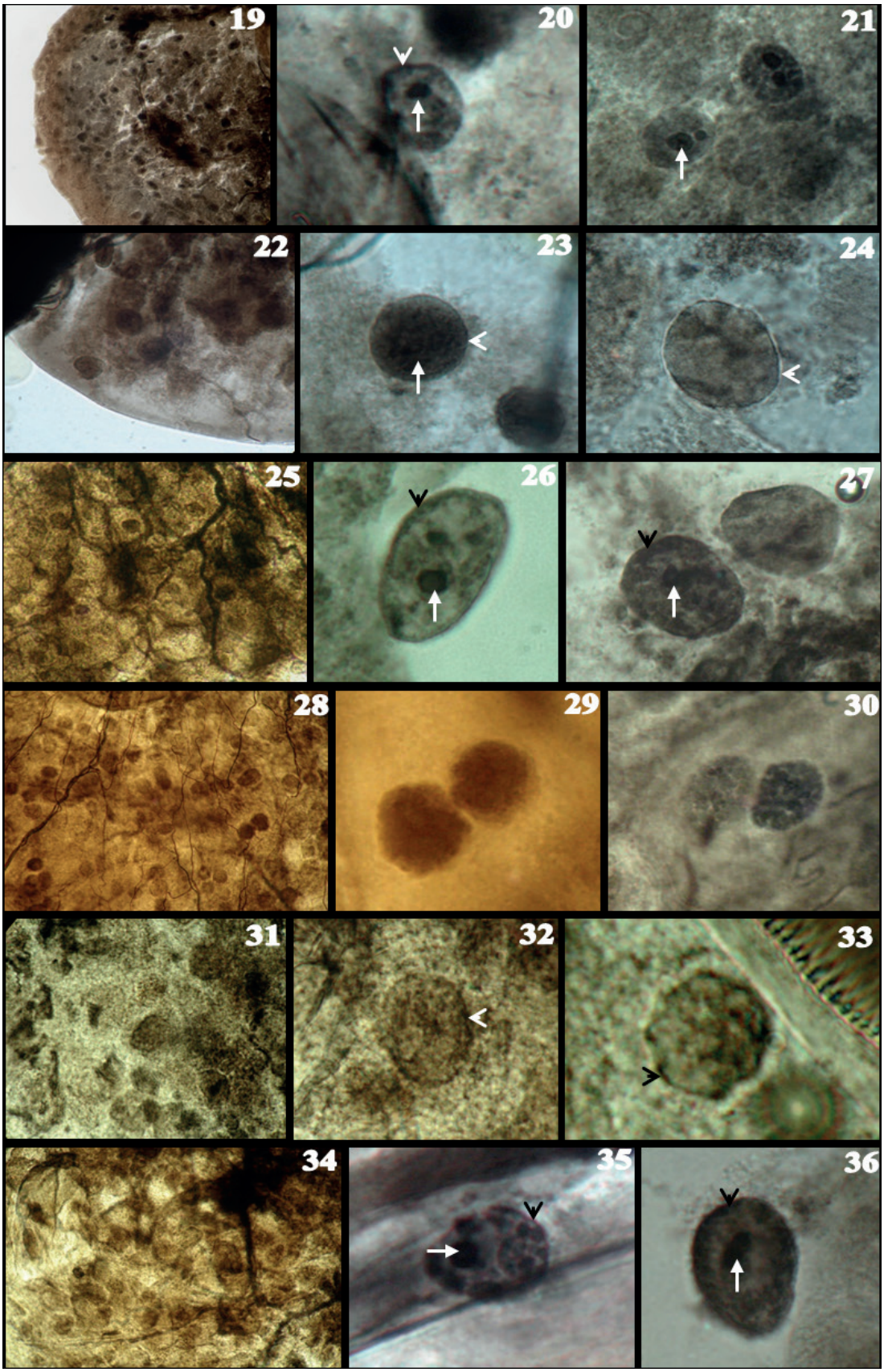
Karsenti & Gounon (1979) demonstrated Mg^{2+}-dependent ATPase activity in lampbrush chromosomes in the oocytes of *Pleurodeles waltl* (Michahelles, 1830) suggesting an activity in chromosome contraction and distension during the transcription process. In addition, Alberts & Sternglanz (1977) suggested enzyme activity in the distension of DNA during replication. Other studies have described the presence of ATPase in chromatin, which would participate in the regulation of transcription, DNA repair, recombination of homologs, and their condensation (Shen *et al.*, 2000; Lusser & Kadonaga, 2003).

Ultrastructural cytochemical studies have demonstrated ATPase activity in the nuclear membrane, specifically in the pore complex (Yasuzumi & Tsubo, 1966; Klein & Afzelius, 1966; Yasuzumi *et al*., 1967; Chardonnet & Dales, 1972; Silakova *et al*., 1977; Berrios *et al*., 1983). Furthermore, Agutter *et al.* (1977) found that ATPase activity depends on RNA, suggesting the presence of the enzyme in the nucleocytoplasmic transport of RNPs.

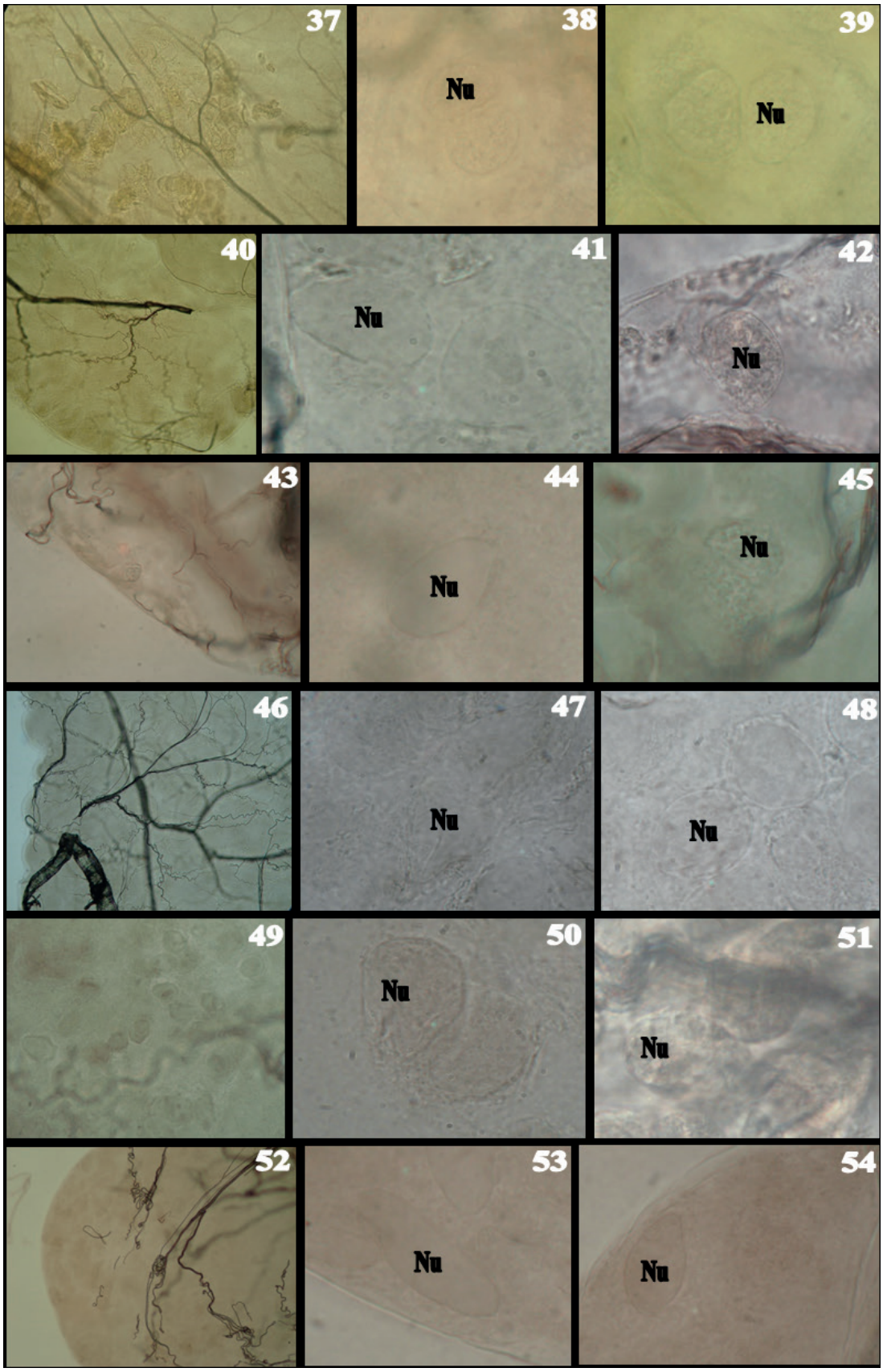
Finally, in the salivary glands of triatomines, the results suggested the activity in nucleolus, euchromatin, and nuclear membrane. Although its physiological function



Figs 1-18. *Triatoma infestans* (Klug, 1834), salivary glands of males (Figs 1-9) and females (Figs 10-18) submitted to cytochemical reaction for Mg^{2+}-dependent ATPase. General view and detail of anterior (Figs 1-3, 10-12), median (Figs 4-6, 13-15), and posterior (Figs 7-9, 16-18) salivary gland. Arrows indicate dark nuclear corpuscles with a strong cobalt sulfide precipitation. The arrowheads indicate a positive response in the nuclear envelope. Magnifications: Figs 1, 4, 7, 10, 13, 16 = 270x; Figs 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18 = 1344x.



Figs 19-36. *Panstrongylus megistus* (Burmeister, 1835), salivary glands of males (Figs 19-27) and females (Figs 28-36) submitted to cytochemical reaction for Mg^{2+}-dependent ATPase. General view and detail of anterior (Figs 19-21, 28-30), median (Figs 22-24, 31-33), and posterior (Figs 25-27, 34-36) salivary gland. Arrows indicate dark nuclear corpuscles with a strong cobalt sulfide precipitation. The arrowheads indicate a positive response in the nuclear envelope. Magnifications: Figs 19, 22, 25, 28, 31, 34 = 270x; Figs 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35, 36 = 1344x.



Figs 37-54, Salivary glands of male *T. infestans* (Figs 37-45) and *P. megistus* (Figs 46-54) submitted to the control method (absence of substrate) of cytochemical for Mg^{2+}-dependent ATPase. General view and detail of anterior (Figs 37-39; 46-48), median (Figs 40-42; 49-51), and posterior (Figs 43-45; 52-54) salivary gland. Note the absence of enzyme activity in the nucleus (Nu) and cytoplasm. Magnifications: Figs 37, 40, 43, 46, 49, 52 = 270x; Figs 38, 39, 41, 42, 44, 45, 47, 48, 50, 51, 53, 54 = 1344x.

is not yet fully understood, the literature suggests that the enzyme is necessary for the transcriptional processes involving euchromatin and the nucleolus. Activity in the nuclear membrane may be related to the processes of nuclear-cytoplasmic transport of substances, because gland cells undergo intense protein synthesis and high active metabolism (Barth, 1954). The difference in response to the ATPase activity may be related to differential cellular metabolism of these three salivary glands, and probably the biosynthesis of different secretions. Females showed a less intense activity than males, which is relation to the predominance of euchromatin in the nuclei, as already observed in salivary gland (Anhê & Azeredo-Oliveira, 2008) and Malpighin tubules (Azeredo-Oliveira, 1990). Control reaction conducted in the absence of substrate (ATP) showed no activity in the cells and thus confirms the reliability of the technique.

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