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SPERMIOGENESIS IN *Palembus dermestoides* (COLEOPTERA: TENEBRIONIDAE) WITH EMPHASIS ON THE FORMATION OF MITOCHONDRIAL DERIVATIVES

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ABSTRACT

The testes of *Palembus dermestoides* are formed by six follicles coated and interconnected by a peritoneal sheath. Long, cylindrical and slightly twisted spermatophores was observed. Spermiogenesis followed the usual sequence for insects. During spermiogenesis, the nucleus was transferred to the cell periphery and the mitochondria were grouped at the posterior pole of the cell. Two mitochondrial derivatives and two paracrystalline electrondense bodies were present close to the axoneme. The mitochondrial derivatives were surrounded by a single row of microtubules in immature spermatozoa. The axoneme had the typical 9+9+2 microtubular pattern of insects.

Key words: Coleoptera, differentiation, germ cells, nebenkern

INTRODUCTION

Spermiogenesis is a post-meiotic event during which spermatids undergo morphological alterations to produce spermatozoa. During spermatogenesis, the spermatogonia pass through successive, synchronic divisions which result in a clump of primary spermatocytes. The number of cells per clump or cyst is species-specific, but is usually 2^n, where n is the number of consecutive mitotic divisions that precedes meiosis [11,12,15]. The number of such divisions in a given group of insects often varies and tends to decrease in more advanced insect orders [11].

Insect spermatozoa are generally filamentous, and consist of an elongated head connected to a very long tail by a basal structure known as the control attachment [16]. The tail is formed by the axoneme and mitochondrial derivatives. In more evolved insect groups, the axoneme, which arises from a basal body, is usually of the 9+9+2 type [10,16] and is bordered by two mitochondrial derivatives formed by transformations of mitochondria [17].

Although spermiogenesis and spermatozoan morphology are similar among most insect species, many species also have certain distinctive characteristics. In this report, we describe the ultrastructural aspects of spermiogenesis in *Palembus dermestoides* (Coleoptera: Tenebrionidae).

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MATERIAL AND METHODS

Specimens of *P. dermestoides* collected in Limeira, SP, Brazil were maintained in the Department of Biology at UNESP.

For scanning electron microscopy (SEM), the testes of anesthetized specimens were removed and fixed in Karnovsky solution (2% paraformaldehyde and 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer). The tissues were then dehydrated in a graded ethanol series (70%-100%), and in 100% ethanol-acetone (1:1) solution, followed by four washes in 100% acetone. After drying, the samples were assembled on aluminium stubs, coated with gold, and examined and photographed with a Jeol JSM-P15 scanning electron microscope.

For transmission electron microscopy (TEM), the testes of three specimens were fixed in 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.4) for 2 h, and post-fixed with 1% osmium tetroxide in 0.2 M cacodylate buffer for 1 h at 4ºC and finally stained with 2% uranyl acetate for 12 h. Dehydration was done in a graded ethanol series (50%-100%), 100% acetone-100% ethanol (1:1), and 100% acetone. Samples were embedded in Epon-Araldite. Thin sections were stained with lead citrate (0.4%) and observed and photographed using a Zeiss EM9 S2 transmission electron microscope.

RESULTS

Scanning electron microscopy

The reproductive organs consisted of paired testes located in the middle-posterior region of the abdomen, paired seminal vesicles, and a common ejaculatory duct.

The testes were formed by six round follicles approximately 175 μm in diameter, connected to short efferent vessels (Fig. 1A). The follicles were coated and intercon-

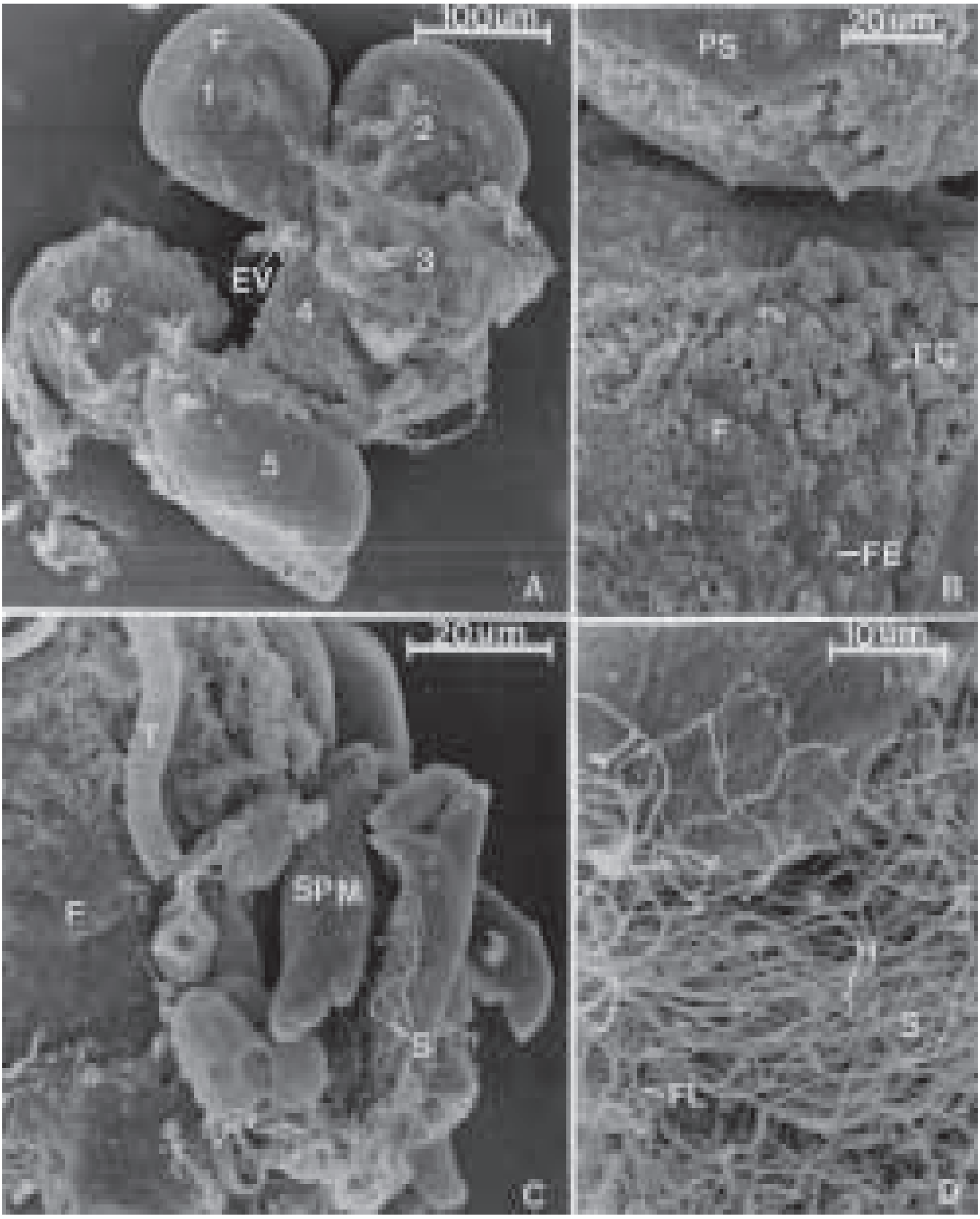

Figure 1. **A.** SEM micrograph of *P. dermestoides* testes, showing six follicles (F 1-6) and a short efferent vessel (EV). **B.** Detail of the terminal region of a follicle (F), showing the peritoneal sheath (PS), the outer surface of the follicular epithelium (FE), and fat cells (FC). **C.** Follicle (F) showing the spermatophore (SPM) and spermatozoa (S). T = tracheole. **D.** Spermatozoa (S) showing the head (H) and flagellum (FL),

nected by a peritoneal sheath of an adipose nature . Beneath this membrane, a follicular epithelium covered each follicle (Fig. 1B). Elongated, cylindrical spermatophores were observed (Fig. 1C) in the region where the follicles fuse to efferent vessels .

The spermatophore was a slightly twisted cylinder 57 to 60 μm long, which tapered at the extremities (Fig. 1C) and, in some cases, seemed to bifurcate. The spermatozoa were elongated structures with a head approximately 1.5 μm in diameter and a long flagellum (Fig. 1D).

Transmission electron microscopy

The germ cells were interconnected by cytoplasmic bridges and formed cysts enclosed by somatic cells. The cystocytes or β-spermatogonia had a large round nucleus located in the center of the cell, and an obvious nucleolus and dispersed chromatin. The mitochondria were randomly distributed in the cell cytoplasm (Fig. 2A). The spermatocytes also had a spherical nucleus and a prominent nucleolus. In this phase, the mitochondria concentrated close to the nuclei (Fig. 2B).

The nuclei of early spermatids were displaced to the cell periphery, and the mitochondria concentrated at the opposite pole to form a cluster termed "nebenkern" (Fig. 3). The outer membrane of the mitochondria fused to form a single, large mitochondrial derivative in later spermatids (Fig. 4A).

During spermiogenesis, the chromatin condensed at the periphery of the nucleus, and the mitochondrial derivatives and centrioles moved to a position opposite to the acrosomal vesicle. Following this event, the mitochondrial derivatives divided into two portions of different electrondensity but similar size. The nuclear membrane developed a depression and thickening close to the basal body from which the axoneme would subsequently differentiate. The Golgi complex vesicles of the spermatids produced the acrosomic vesicle located at the apical pole of the nucleus. Acrosome formation was concomitant with the process of spermatid lengthening. The spermatid membranes in this phase had an irregular shape (Fig. 4A).

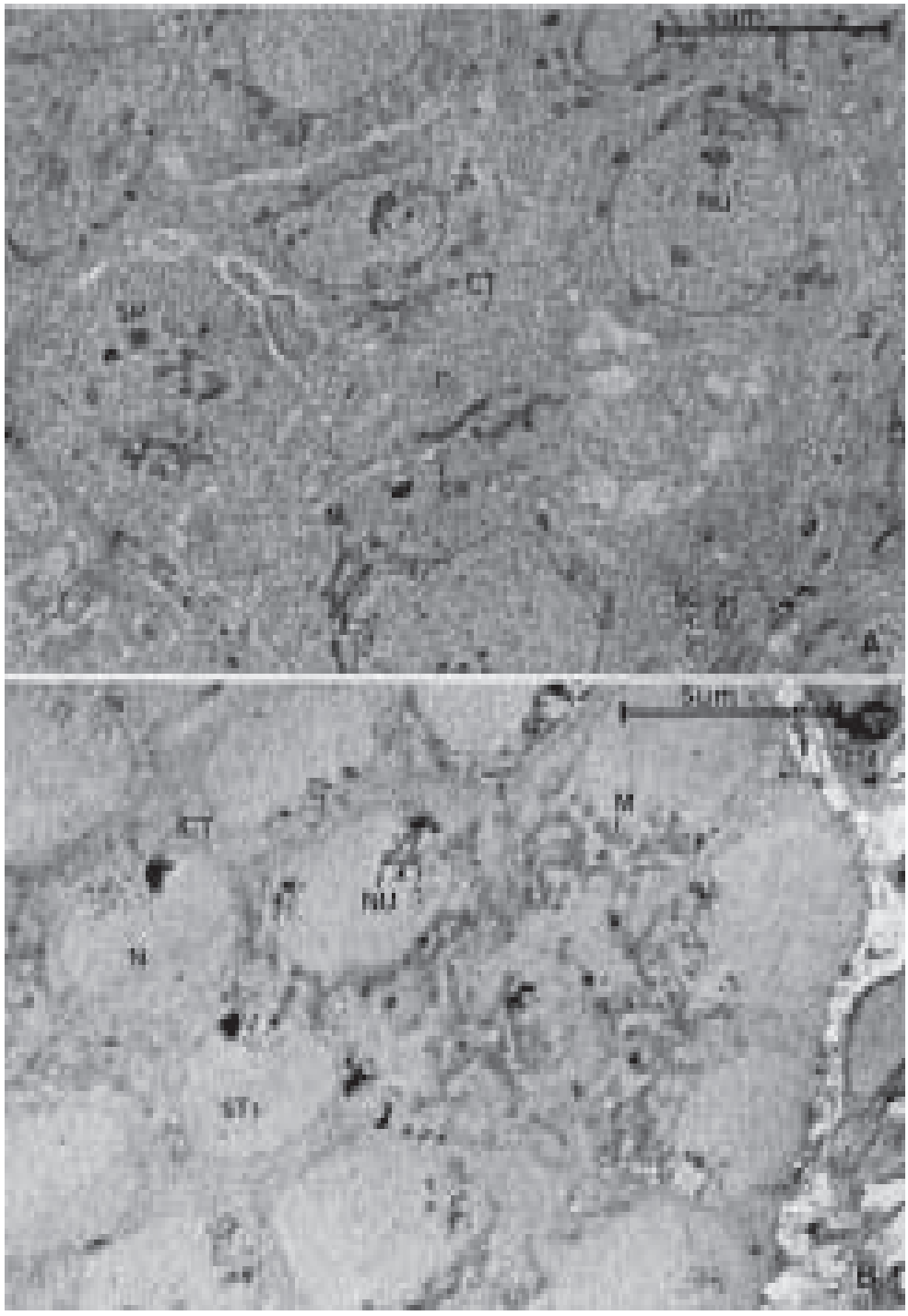

Figure 2. A. Spermatogonia (SP) of *P. dermestoides* interconnected by cytoplastic bridges (CB) to form cysts (C).The β-spermatogonium contains a large nucleus (N), a nucleolus (NU), disperse mitochondria (M), and somatic cells (SC) delimiting the cysts. **B.** Spermatocytes I (ST1), showing the mitochondria (M) concentrated close to the nucleus (N). NU=nucleolus, CT=cytoplasm.

Following elongation of the two mitochondrial derivatives parallel to the axoneme, the cristae and matrix underwent rearrangement. A deposit of paracrystalline electrondense material appeared in the matrix of each derivative at a position close to the axoneme (Fig. 4B).

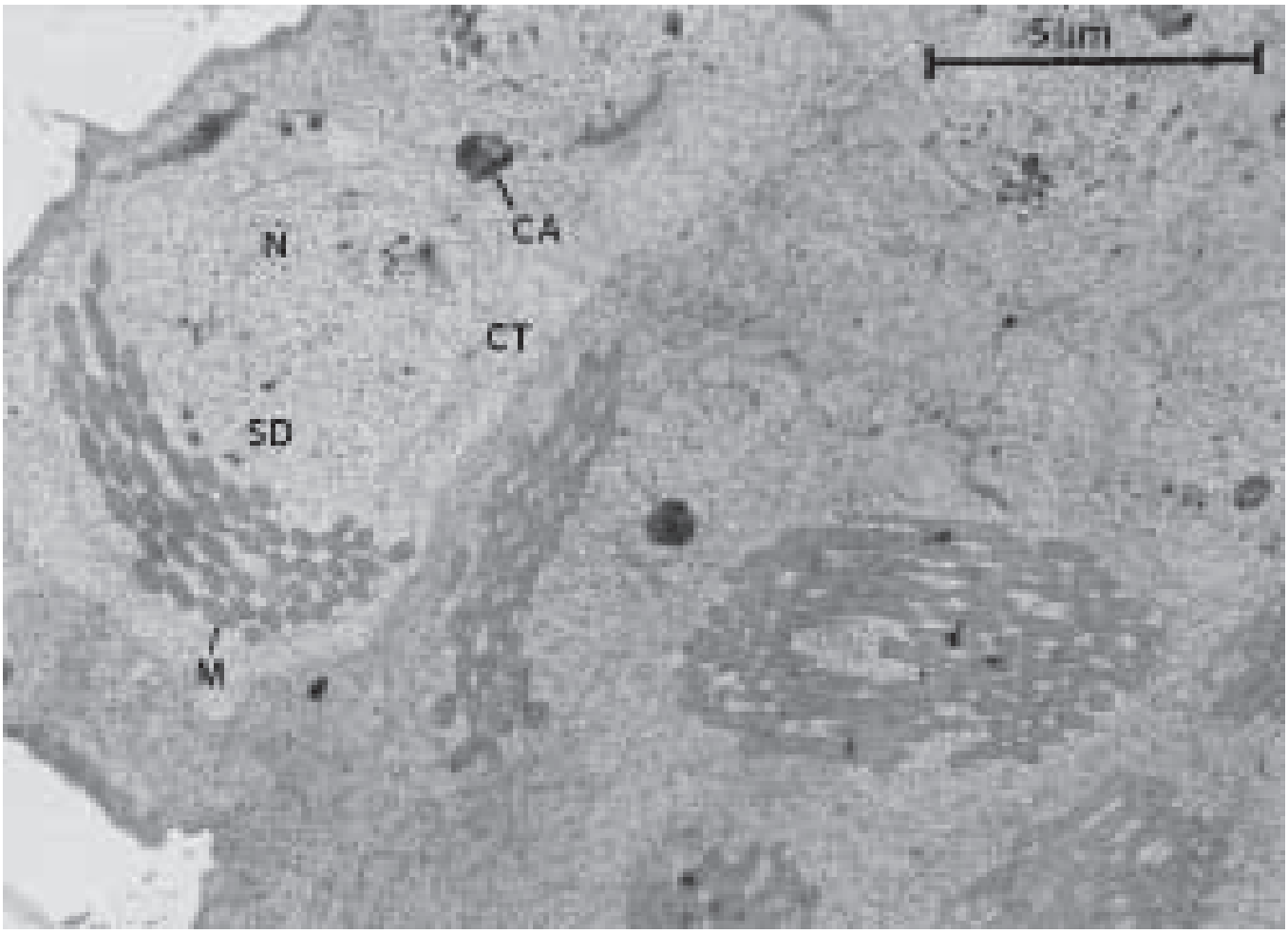

Figure 3. Early spermatids (SD) showing the concentrated and polarized mitochondria (M). CT=cytoplasm, N=nucleus, CA = centriole adjuntic.

During differentiation, the elongation of the mitochondrial derivatives usually began alongside the basal nucleus, laterally to the basal body, and then proceeded parallel to the axoneme. It was unclear whether the mitochondrial derivatives extended to the distal end of the axoneme or ended before reaching this region. The axoneme consisted of a 9 (outer row of single microtubules) + 9 (intermediate row of double microtubules) + 2 (central single microtubules) pattern of microtubules (Fig. 4B).

In immature spermatozoa, myelinic structures and two mitochondrial derivatives of different diameters but similar electrondensity were observed. The tail of these immature spermatozoa also contained two small, round accessory bodies located laterally to the axoneme (Fig. 4B).

DISCUSSION

Most male reproductive organs consist of a pair of testes with paired seminal vesicles and an ejaculatory duct. Each testis consists of a series of follicles which vary in number from one in Coleoptera to over 100 in Acrididae [5].

The spermatophores are a primitive method of insemination in insects. Although not common in Coleoptera [5], spermatophores have been observed in the coleopteran species *Alphitobius diaperinus*, *Thanatophilus sinuatus*, *Tribolium castaneum* and *Photinus* fireflies [3,9,14,21].

The arrangement of the mitochondrial cristae, and the quantity and distribution pattern of electrondense material within the mitochondrial derivatives are characteristic of each species [1,18]. The crystalline structure of part of the mitochondrial derivative matrix is suggestive of the local accumulation of proteins, probably enzymes. Their location close to the axoneme suggests that these enzymes may be involved in releasing energy for tail beating [19]. As in *D. melanogaster*, the entire sperm enters the egg at fertilization. Tokuyasu [19] suggested that the paracrystalline body contributes to fertilization, perhaps through an action on sperm motion.

The 9+9+2 microtubule pattern of *P. dermestoides* is characteristic of the more differentiated groups of insects [16], including the orders Orthoptera - C*onocephalus*

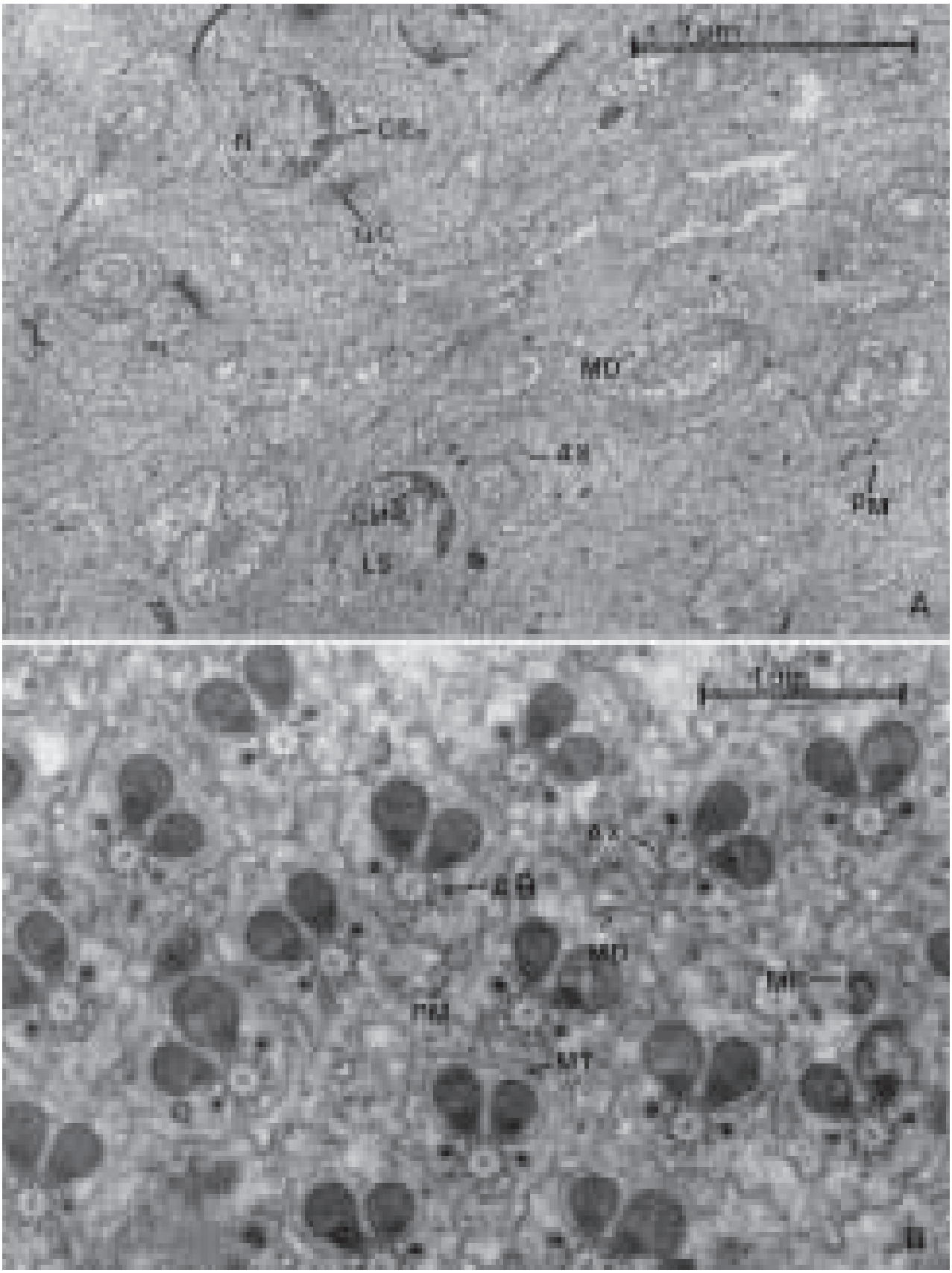

Figure 4. **A.** Spermatids in an intermediate stage of differentiation showing condensed chromatin (CHR) at the periphery of the nucleus (N), mitochondrial derivatives (MD) divided into two portions, and a centriole (CE) near the nucleus. Note the irregular plasma membrane (PM) around the spermatids, LS = later spermatids. **B.** Section of immature spermatozoan tails showing the axoneme (AX) formed by 9 single outer microtubules, 9 intermediate double microtubules and 2 central single microtubules, as well as the presence of an accessory body (AB) and mitochondrial derivatives (MD) surrounded by a single row of microtubules (MT). AX = axoneme, GC=Golgi complex, MF=myelinic figures.

saltator [7], *Myogrillus* sp. [6], *Eyprepoenemis plorans* [13], Diptera - *Drosophila melanogaster* [20], Odonata - *Enallagma cheliferum* [8], Hemiptera - *Rhodnius prolixus* [2] and Hymenoptera - *Atta capiguatta* and *A. sexdens rubropilosa* [4].

The myelinic figures are involved in the reabsorption of portions of cytoplasm that must be eliminated during the decrease in the size of the sperm cell that occurs during the transformation of spermatids into spermatozoa [6].

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