ULTRASTRUCTURE OF THE SPERMATHECA OF *Melipona bicolor bicolor* LEP. (HYMENOPTERA, APINAE, MELIPONINI)

ULTRA-ESTRUTURA DA ESPERMATECA DE Melipona bicolor bicolor Lep. (HYMENOPTERA, APINAE, MELIPONINI)

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ABSTRACT: The ultrastructural studies of the spermathecal wall of virgin and mated queens of *Melipona bicolor* showed that it is composed by a single layer of columnar pseudostratified epithelium lined internally by a thick cuticle and covered externally with an extensive tracheal network. Striking differences occur between the organ of the two life phases, although the general pattern of organization remain the same. Although vacuoles containing low electrondensity material are present in the mated queen epithelial cells, the ultrastructural features of the cells seem do not support a secretory function. Instead the ultrastructure of the cells point to a function in exchange of products directly with the hemolymph. An unexpected find was the presence of spermatozoa into the epithelial cells vesicles. Based upon the organ morphology a function of maintainance of an appropriated environment to the sperm cells is assigned to the spermatheca of *M. bicolor.*

UNITERMS: Stingless bee, Sperm storage, Reproduction, Spermatozoa.

INTRODUCTION

The spermatheca is one of the accessory reproductive organs of female insects, having the function of storage the sperm until the moment of eggs fertilization.

In *Apis mellifera* queens, the spermatheca, is a globular sac attached through a duct, to the beginning of the vagina. After the early works of Marchal (1894) and Bresslau (1905), few other contributions were substancial to the understanding of the morphology and physiology of this organ in the bees (SNODGRASS, 1956; FYG, 1960; KOENIGER, 1969; CAMARGO; MELLO, 1970; POOLE, 1970; DALLAI, 1975). Even in other hymenopteran, than bees, the available studies are restrict (CAMARGO-MATHIAS, 2000).

The studies accomplished describe the anatomy, histology and histochemistry of the organ, given a fairly complete picture of its structure, but they do not provide sufficient and definitive information about the organ physiology. Although is known that the spermatozoa, after copulation, are stored in the spermatheca, the different authors diverge about the relationships of them with the organ. Several hypothesis are present in literature, going from a single storage function to the responsibility by the sperm capacitation (KAULENAS, 1992).

The present report describes the spermatheca of virgin and mated queens of a stingless eusocial bee, and advance some interpretation of the morphological features in terms of the organ function.

MATERIAL AND METHODS

Spermathecae, in number of ten, were removed from virgin and mated queens of *Melipona bicolor bicolor* obtained from colonies maintained in the apiary of the Instituto de Biociências (UNESP), Rio Claro, SP, Brasil and Laboratório de Abelhas from the Instituto de Biociências (USP), São Paulo, SP, Brasil.

Three spermathecae were fixed in Karnovisky for Scanning Electron Microscopy (SEM), freeze-dried and after cleavage coated with gold for examination and photographing in a Philips SEM. Seven were fixed for Transmission Electron Microscopy (TEM) in 2,5% gluraldehyde in 0,1M Na Cacodylate buffer, pH 7.2, during

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at least 3 h. After washing in the buffer, the spermathecae were post-fixed in 1% osmium tetroxide in the same buffer. The dehydration was carried out in graded acetone concentrations (30% - 50%, 70%, 90%, 95%, 100%). A staining of the hole organ was done, during dehydration, with 2% uranyl acetate in 10% acetone, during 6 h. The embbeding was made in Epon Araldite. The thin sections were stained with lead citrate, and the examination and photographing in a Philips TEM.

To the fixatives of some spermathecae were add 0,5% ruthenium red.

Some thick sections were stained with 1% toluidine blue and examined and photographed with light microscopy (LM).

RESULTS

The spermatheca of *M. bicolor* is a small round, hollow organ, constituted by a continuous epithelial wall around the inner lumen (Fig. 1 A). The epithelium is covered, in the luminal surface, by a cuticular lining and outside by a thick basement membrane and an intricated net of tracheolar branches (Figs. 1 B, 2 A). Outside the basement membrane, lie a kind of serosa or peritoneal sheath constituted by layers of amorphous intercellular substances containing scattered elongated serosal cells (Fig. 2 B).

A general view of the spermathecal wall shows a single prismatic pseudostratified epithelium (Figs. 1 A, C and 2 A), in which the small, round to elliptical nuclei, form rows slightly displaced to the cell basal region (Figs. 1 A, C, 2 A). The cytoplasm is fulfilled by a labyrinth of the apical and basal plasmic membranes infolds that runs through all cell. The basement membrane and tracheoles penetrate to some extension, into the narrow extracellular compartiments formed by the basal infoldings (Figs. 2 A, B).

The morphological features of the spermatheca is basically the same in the virgin and fisogastric queens, but differences indicative of changed activity in the last ones, can be noted.

The labyrinth formed by the plasmic membrane infoldings, mainly in the basal region, turn around mitochondria, which show very plastic shapes, longitudinal cristae and dense matrix (Fig. 3 A, B). The lumen of the labyrinth is very narrow and it cytosolic surface is decorated with quantasomes (Fig. 3 B), mainly in virgin queens.

The epithelial cells of the spermathecae of virgin queens are lower and, have more round nuclei, with disperse chromatin and large nucleoli (Figs. 2 A, 3 A). In the epithelium of the mated queen are figures representative of intracellular digestion (Figs. 4 A, B, C) and amorphous material stained with the ruthenium red in the basal lamina and basal cytoplasm. Besides the spermathecae of mated queens presents huge vacuoles and multivesicular bodies in the apical region of the cells, fulfilled with low electrondensity material (Fig. 4C).

An unexpected finding in the epithelial cells of mated queens was the presence of spermatozoa into vesicles (Fig. 5 A, B, C). Besides unexpected, this finding is also difficult to explain since the spermathecal lumen is lined by a thick and apparently continuous cuticle, impossible of being crossed by the sperm cells. In spite of this, spermatozoa were found into the cytoplasm, always inside membrane bounded vesicles (Figs. 5 A, B, C).

The structures indicative of intracellular digestion seen in the epithelial cells, seem in some cases, be resulting of autophagy (Figs. 4 A, C) but in others seem result from sperm cell digestion, into the epithelial cells (Figs. 5C, D, E).

DISCUSSION

Within the insects the shape, arrangement and number of spermathecae is quite diverse (MATSUDA, 1976). However if considered their microscopic structure in various insect orders a great deal of similarities in the cellular organization can be find (AHMED; GILLOT, 1982).

The primary function of the spermatheca is storage the spermatozoa for the time between the mating and the eggs fertilization. In eusocial bees, this time could be very long, since the queen remains for long time laying eggs in the colonies after mating.

The ultrastructural features of the spermathecal epithelium are not typical of secretory function, although it could be expected from the function of storage the spermatozoa during long times (8 months to an year or more). By other hand, the possibility of an exchange of substances between epithelial cells and either the spermathecal lumen on one way, or the hemolymph on the other is supported by the epithelial cells structure. The pronounced apical invaginations of plasmic membrane and the extensive basal labyrinth, as well as its membrane decorations and the particularly high density of the mitochondrial matrix argue for active transport process (BERRIDGE; OSCHMAN, 1972). The basal labyrinth association with mitochondria is strongly reminiscent of the scalariform junctions described by Noirot-Timothée et al. (1979), involved in fluid transport in termite hindgut.

The labyrinth of plasmic membrane infoldings provided of quantasomes and associated with mitochondria are indicative of active exchange of substances with the hemolymph, that could in the same way be responsible

by the sperm maintenance. Substances must be actively absorbed from hemolymph being the decorations present in the cytosolic side of the infoldings membranes, constitued by enzymes actuating in the absorption process, as seen in the hindgut and rectal papillae (SMITH, 1968; MARTOJA; BALLAN-DUFRANÇAIS, 1984; BERRIDGE; OSCHMAN, 1972). The active transportation of substances through these membranes is also corroborated by the presence of numerous tracheolar branches and the intimate relationship of them with the epithelial cell, indicative of local high energy consumption. This feature seems to suggest that although the epithelial cell were not secretory they may absorbe nourishing substances from the hemolymph and pass them into the spermathecal lumen. The huge vacuoles present in the epithelial cells apical region, may contain the absorbed material before it be delivered in the lumen.

This conception, in certain way, goes against the idea of Poole (1970) that thinks that the spermatheca is an isolating structure in which the spermatozoa can be preserved during the necessary time without contact with the environment. Even if Poole is correct, the sperm isolation, do not remove the necessity of a milieu adequated to the sperm maintenance.

Although secretory cells were not present in the spermathecal wall, the organ is provide with a small acessory spermathecal gland that seems functional and must contribute with it secretion, and the substances absorbed from hemolymph, to compose the stored spermatozoa environ.

Dallai (1975) and Camargo-Mathias (2000) report the presence of polysaccharides into the epithelial cells. The presence of polysaccharids is in accordance with the staining of the material coming from the hemolymph seen into the epithelial cells basal pole, by the ruthenium red.

The striking presence of spermatozoa into the epithelial cells remain a mistery, but it surely is not an artifact. Although it is incomprehensible how the sperm get into the epithelial cells, it seems sure that they are digested there.

In conclusion the spermatheca, as an organ that stored spermatozoa, seem to have also the function of maintain an appropriated environment to them through absorption of substances directy from the hemolymph, and maybe of eliminating some defective spermatozoa.

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RESUMO: O estudo da ultra-estrutura da parede da espermateca de rainhas virgens e fecundadas de *Melipona bicolor* mostrou que esta é composta de um epitélio colunar pseudo-estratificado, coberto internamente por uma cutícula espessa e externamente por uma extensa rede traqueal. O padrão-geral de organização é o mesmo nas duas fases da vida das rainhas, mas algumas diferenças estão presentes nas células. Apesar de estarem presentes vacúolos contendo material de baixa densidade eletrônica nas células das rainhas fecundadas, o aspecto geral destas não é característico de células secretoras. A ultra-estrutura das células, aponta mais para uma função de troca de substâncias diretamente com a hemolinfa. Um aspecto incomum é a presença de espermatozóides em vesículas no interior das células epiteliais. Com base na morfologia do órgão, pode se dizer que a sua função, básicamente, é a de manter um ambiente adequado para a sobrevivência dos espermatozóides no seu interior.

UNITERMOS: Abelha sem ferrão, Armazenamento de esperma, Reprodução, Espermatozóide.

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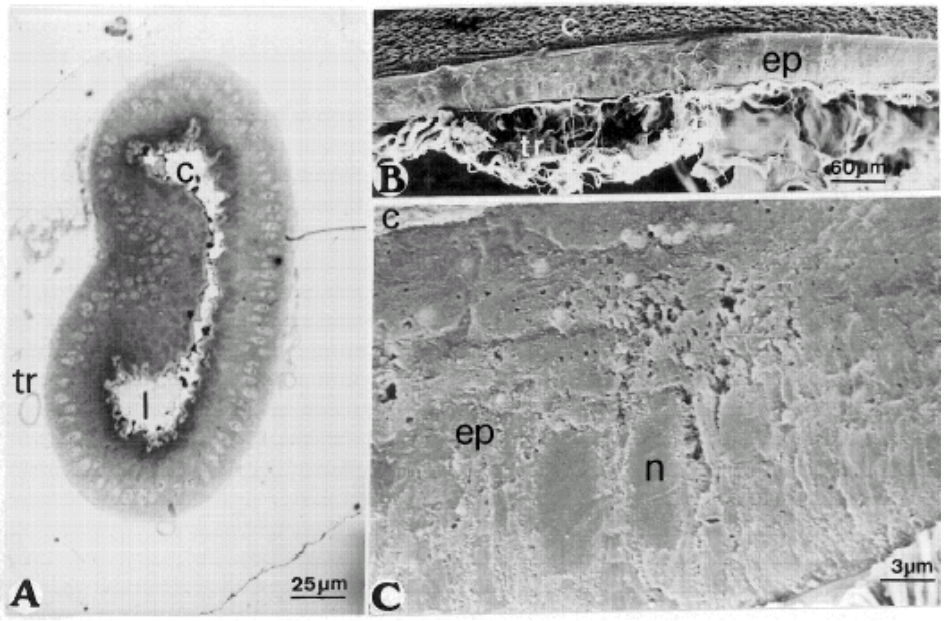

Figura 1. General features of the spermathecal wall. A. Light micrograph of a section of the spermatheca of a virgin queen. B. and C. Scanning electron micrographs of the spermatheca wall showing the inner cover of cuticle (c) of the epithelium (ep) and the outter cover of tracheoles (tr). L = lumen; n = epithelial cells nuclei.

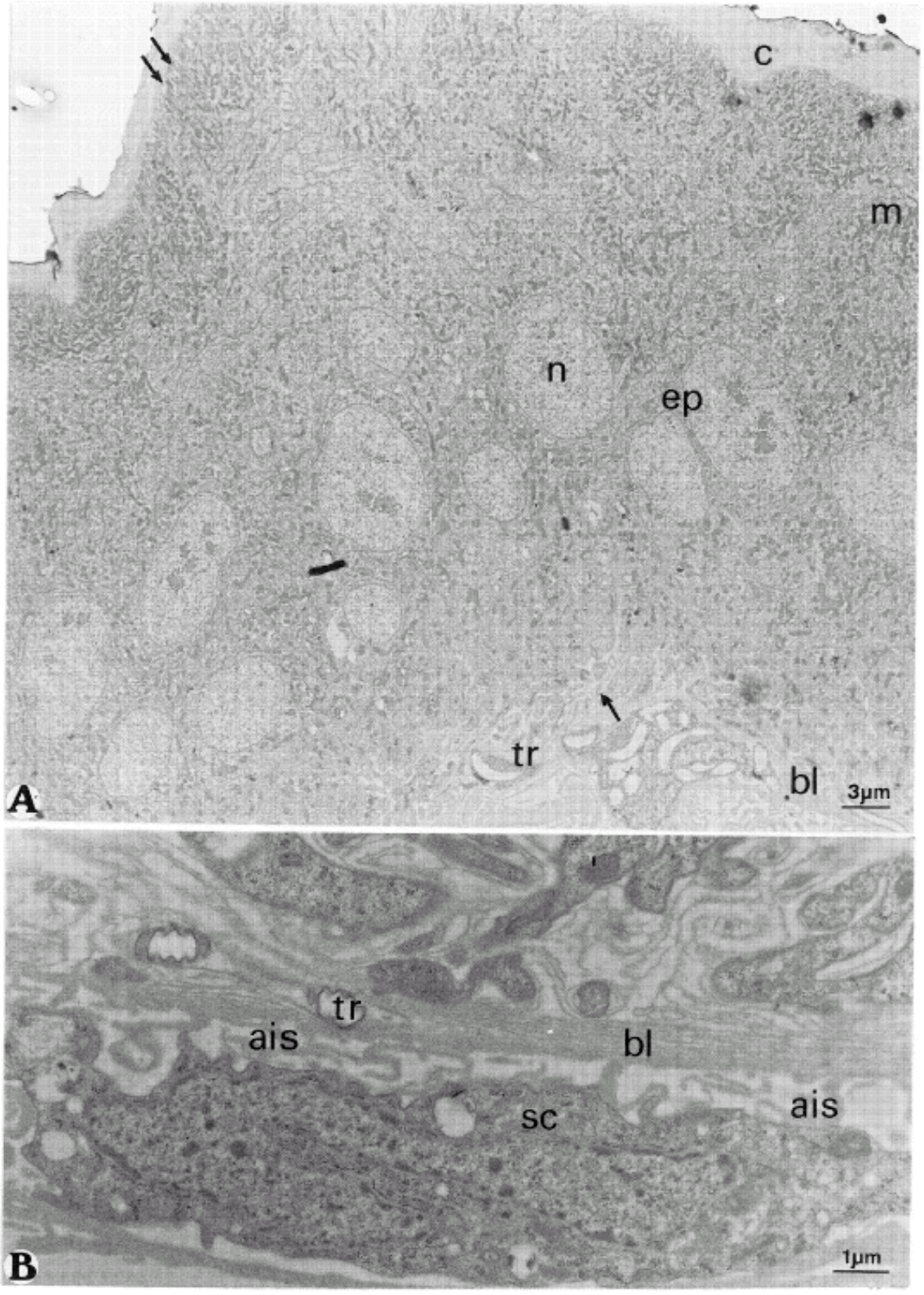

Figura 2. Transmission electron microscopy (TEM) micrographs of the spermathecal epithelium (ep). A. Low magnification, general view of the epithelium (ep) showing the richness in internal membranes resulting from basal (single arrows) and apical (doble arrows) invaginations of the cell plasmic membrane. B. Basal region of the epithelium showing the serosal cell (sc) and amorphous intercellular substance (ais). bl = basal lamina; tr = tracheoles; n = nucleus; c = cuticle.

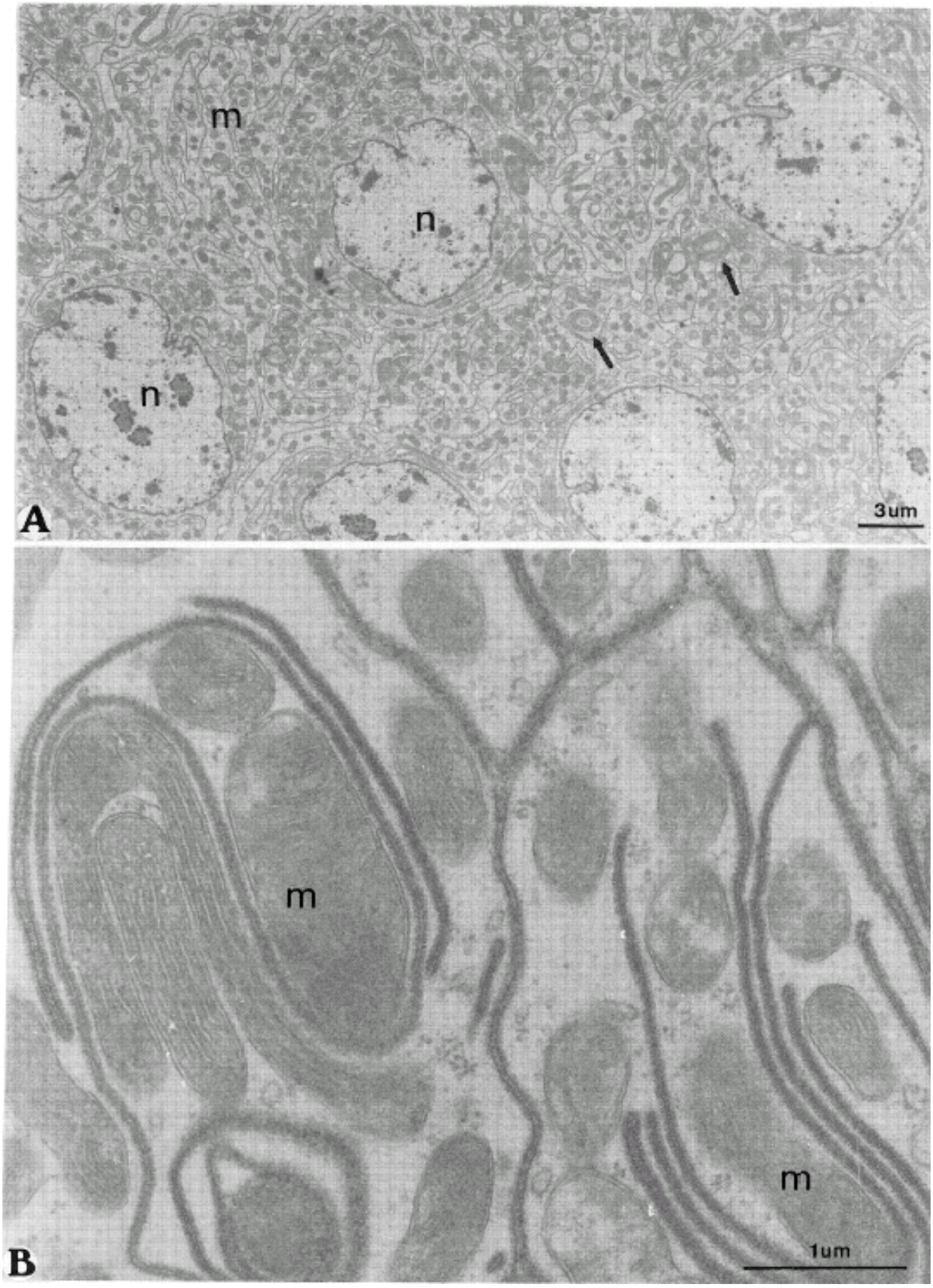

Figura 3. TEM micrographs of the internal membranous labyrinth from virgin queen spermatheca. A. General view of the medium height region of the epithelial cell, showing the richness in smooth membranes (arrows) and mitochondria (m). B. In the cell basal pole, notice the cytosolic decoration of the membranes with quantasomes and the association with mitochondria (m). n=nuclei.

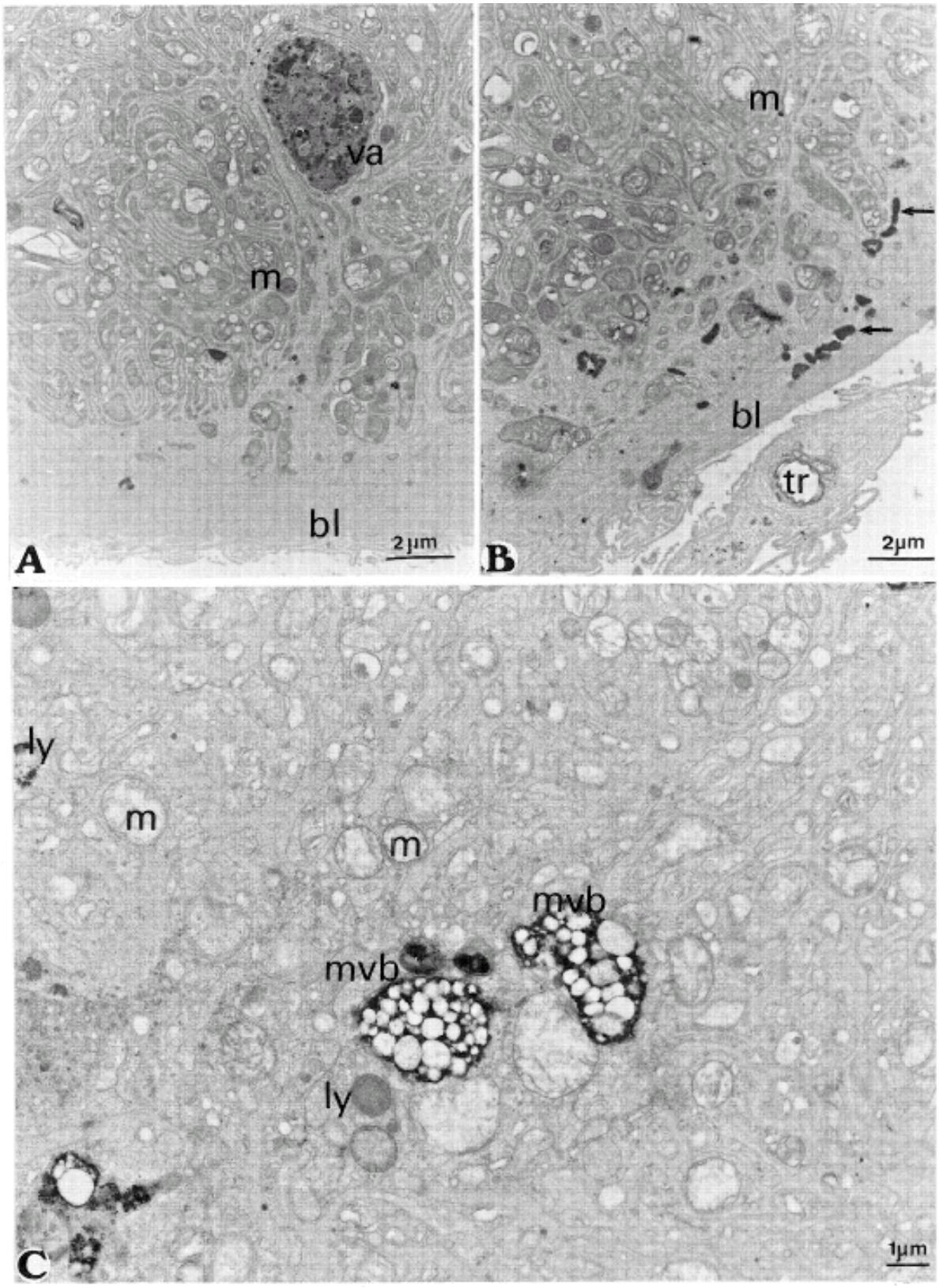

Figura 4. TEM cellular features of the spermatheca of mated queen epithelial cells. A. Basal pole of the cell showing the thick and lamellar basal lamina (bl) and autophagic vacuoles (va). B. Presence of material stained by ruthenium red (arrows) into the basal lamina (bl). C. Several structures representative of intracellular digestion, as multivesicular bodies (mvb) and lysosomes (ly). m = mitochondria.

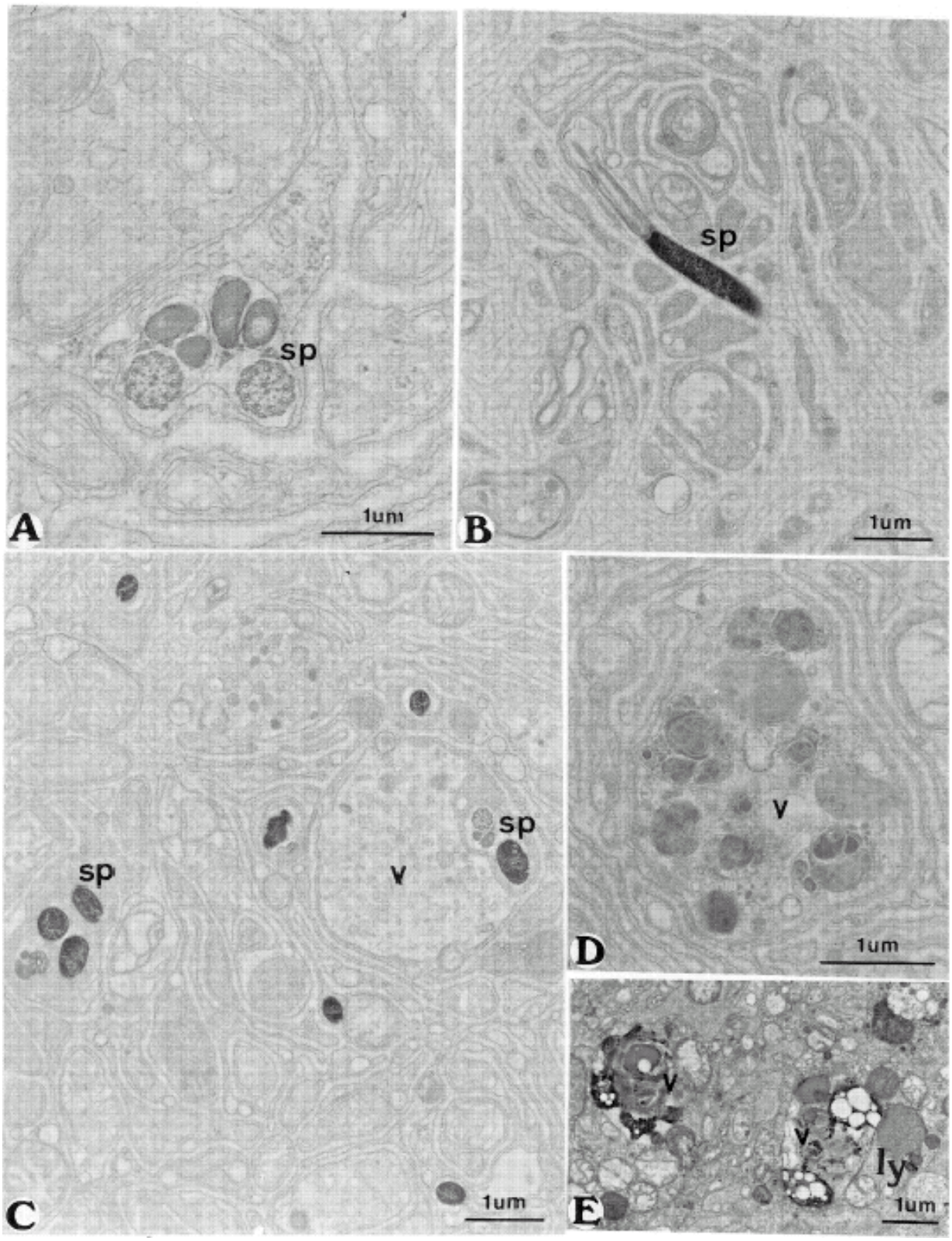

Figura 5. TEM micrographs showing spermatozoa (sp) into vesicles in the epithelial cells. A. Apparently intact spermatozoon tails (sp) into the epithelial vacuoles. B. Spermatozoon (sp) showing the head apparently in process of decondensation. C. Features indicative of spermatozoa (sp) digestion (v). D. and E. Digestive vacuoles (v) with wastes possibly resulting from spermatozoa digestion. ly = lysosome.