

SPERMIOGENESIS AND STRUCTURE OF SPERMATOOA IN THE MITE *ERYTHRAEUS PHALANGOIDES* (ACARI, ERYTHRAEIDAE)

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ACROSOME
FORMATION
PERIPHERAL
CISTERNAE
OUTER SPERM
MEMBRANE

SUMMARY : The structure of early, intermediate and late spermatids as well as of the spermatozoon of the mite *Erythraeus phalangoides* is described. The acrosome and the attached acrosomal filament are formed during early spermiogenesis. The nuclear material, undergoing gradual condensation, also adheres to the acrosome. At the same time, the nucleus strongly elongates and the nuclear envelope almost completely disappears. Peripherally distributed, flattened and tubular cisternae appear in the late spermatid. These cisternae develop as invaginations of the plasmalemma and their membranes communicate with the plasma membrane. Immediately before entering the lumen of the testis, the spermatozoa develop an additional outer membrane formed on the outside of the plasmalemma.

During spermiogenesis mitochondria undergo a structural reorganization, with electron-dense granules appearing in their matrix.

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DU
SPERMATOZOÏDE

RÉSUMÉ : Les structures des spermatides jeunes, intermédiaires et âgés, et des spermatozoïdes sont décrites chez *Erythraeus phalangoides*. L'acrosome et son filament se forment précocement au cours de la spermiogénèse. Le matériel nucléaire soumis à une condensation graduelle, adhère aussi à l'acrosome et s'allonge beaucoup ; l'enveloppe nucléaire disparaît presque complètement. Des cisternes tubulaires et d'autres aplaties apparaissent tardivement à la périphérie des spermatides. Ces cisternes se forment par invagination du plasmalemme. Immédiatement avant d'entrer dans la lumière du testicule, les spermatozoïdes développent une membrane supplémentaire formée à l'extérieur du plasmalemme. Pendant la spermiogénèse, les mitochondries sont soumises à une réorganisation structurale au cours de laquelle des granules denses aux électrons apparaissent dans leur matrice.

INTRODUCTION

Recent publications, particularly those of ALBERTI (1980a, b) point to a considerable heterogeneity in the structure of acarine spermatozoa. This heterogeneity is especially pronounced in the order Acariformes (Actinotrichida), since representatives of the suborders Actinedida, Acaridida

and Oribatida differ from each other in both the general structure of the spermatozoa and the process of their formation. In the suborder Actinedida, which includes *Erythraeus phalangoides*, the spermatozoa usually contain clearly separated nuclear material, an acrosome with an acrosomal filament and peripherally distributed cisternae of various shapes which communicate with the cell membrane. Although the main stages of sper-

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miogenesis and the structure of spermatozoa in *Erythraeus phalangoides* were described by ALBERTI (1980b), such processes as the formation of the acrosome and the peripheral cisternae or the appearance of an additional outer membrane external to the plasmalemma require a more detailed presentation.

MATERIAL AND METHODS

Individuals of *Erythraeus phalangoides* were collected in July near Gdańsk (Northern Poland). The animals were fixed at room temperature for 90 min. in 3 % glutaraldehyde buffered with 0.09 M cacodylate, pH 7.4, postfixed in 1.4 % OsO_4 for 1^h, dehydrated in ethanol and propylene oxide and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Tesla BS 500 electron microscope.

RESULTS

Sections of the germinal part of the testis show groups of germ cells at the same stage of developments (Fig. 1). Spermatids are closely apposed to one another. Somatic cells only occasionally occur in these regions and are difficult to identify. Late spermatids are located near the lumen of the testis in deep invaginations of the plasmalemma of the somatic cells. During their transformation into spermatozoa, the spermatids lose their contact with the somatic cells and enter the lumen of the testis, where their surface becomes coated with a secretory material produced by the glandular cells of the testis.

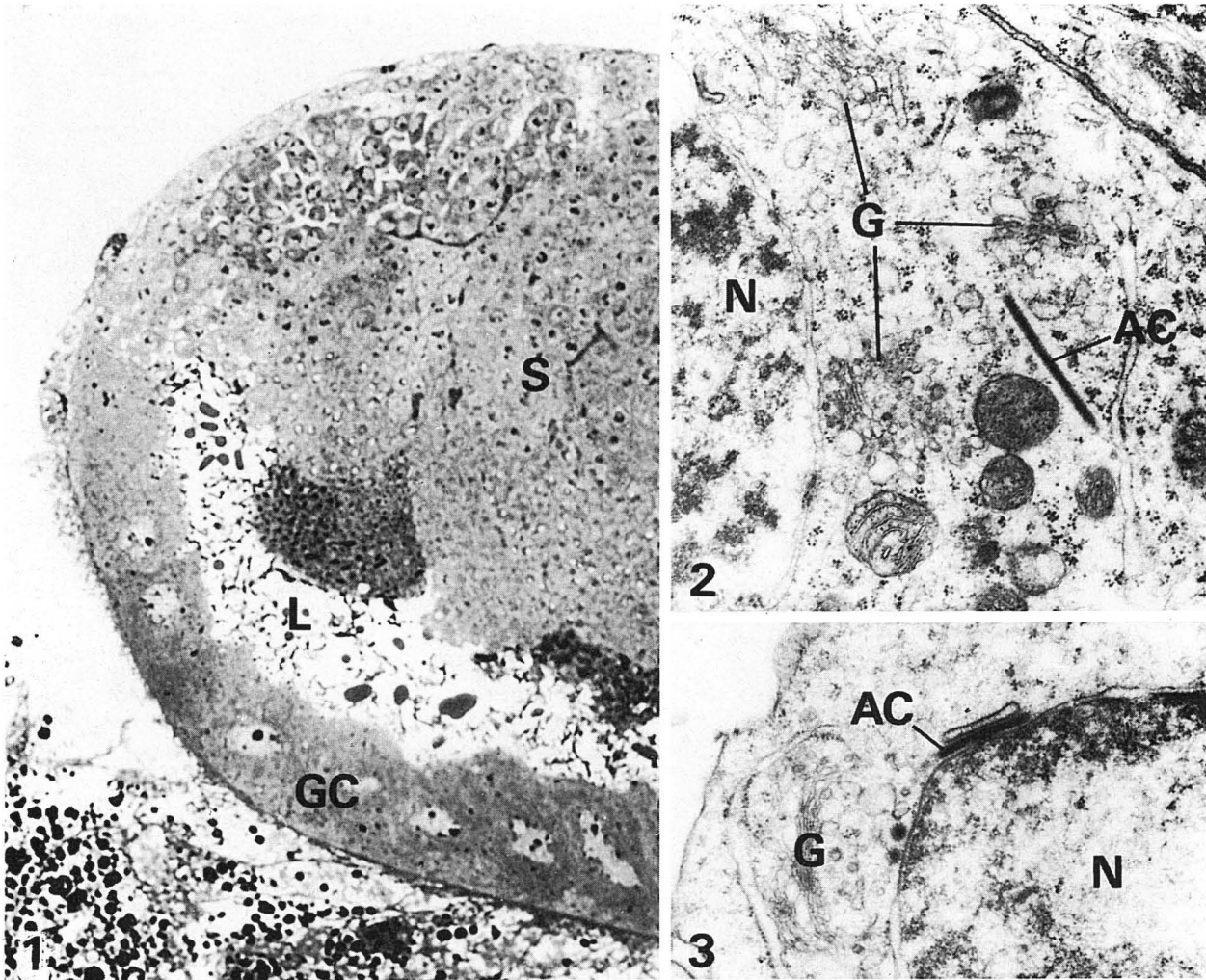
Spermiogenesis was divided into three stages : early, intermediate and late.

■ *The early spermatid.* The nucleus of the very early spermatid is spherical and centrally located. The electron-dense, compact chromatin initially forms clumps situated both near the nuclear envelope and in the central region of the nucleus. As soon as the primary spermatocytes are formed, an electron-dense acrosomal cisterna

appears in their cytoplasm (Fig. 2). When meiosis is completed the acrosomal cisterna adheres to the nucleus in the prospective anterior region. The acrosomal cisterna is separated from the cytoplasm by electron-lucent ER cisterna (Fig. 3). The two membranes of the nuclear envelope in that region are strictly parallel. Near the acrosomal cisterna a well-developed Golgi apparatus with electron-dense vacuoles can be observed.

Later, the nuclear material of the early spermatid starts to show a finely fibrillar texture and fills the entire nucleus except a space near its posterior pole. The nucleus takes an eccentric position by approaching the plasmalemma with its anterior pole (Fig. 4). The nucleus and the cell membrane are at this time separated only by the acrosomal cisterna. The acrosomal filament possessing a finely fibrillar structure can be observed running through the nucleus in a channel surrounded by the nuclear envelope (Figs. 4 and 5). Microtubules are occasionally found sparsely distributed along the cytoplasmic surface of the nuclear envelope at the anterior pole of the nucleus. At the posterior pole of the nucleus the nuclear envelope forms an invagination communicating with a cone of electron-dense material having a centriole located at its apex (Fig. 4).

The cytoplasm of early spermatid contains clusters of free ribosomes, canaliculi of rough endoplasmic reticulum (RER) and well-defined Golgi apparatus. The mitochondria are elongated and quite numerous, usually possessing two longitudinally oriented cristae. They commonly contain dense bodies and characteristic, electron-dense granules of considerable size (0.32 μm) located in the intracristal space near the end of mitochondrion. No such granules occur in the mitochondria of the somatic cells. The mitochondria are mostly situated peripherally, close to the plasmalemma of the spermatid. The early spermatids possess numerous cell processes which fill up the intercellular spaces (Fig. 5). The spermatids are interconnected by cytoplasmic bridges which contain a slightly denser cytoplasm with ER cisternae but without microtubules. These bridges are present until the stage of the late spermatid.



FIGS. 1-3 : *Erythraeus phalangoides* :

- 1) Section through the testis showing groups of spermatids at different stages of maturation (S), lumen of the testis (L), and glanular cells (GC) of the testicular wall. Light microscope. $\times 2,150$.
- 2) Perinuclear region of primary spermatocyte. Acrosomal cysterna (AC) can be seen in the vicinity of Golgi apparatus (G). N, nucleus. $\times 21,000$.
- 3) Part of early spermatid. The acrosomal cysterna (AC) adheres tightly to nucleus (N) and is separated from cytoplasm by electron-lucent cysterna. Note the close apposition of both membranes of nuclear envelope in the acrosomal region. G, Golgi elements. $\times 21,000$.

■ *The intermediate spermatid.* This stage (Fig. 6) is characterized by the elongation of the cell nucleus with concomitant disappearance of the nuclear envelope except for its fragment adjacent to the acrosomal cysterna. The nuclear material undergoes condensation and forms parallel threads which follow a roughly helical course.

Between these threads appear electron-dense granules of various diameters. At the anterior end the chromatin threads approach the acrosomal region and are attached to the electron-dense lamella adjacent to the arcuate acrosomal cysterna. In the cytoplasm, at some distance from the nuclear material, microtubules parallel to the long

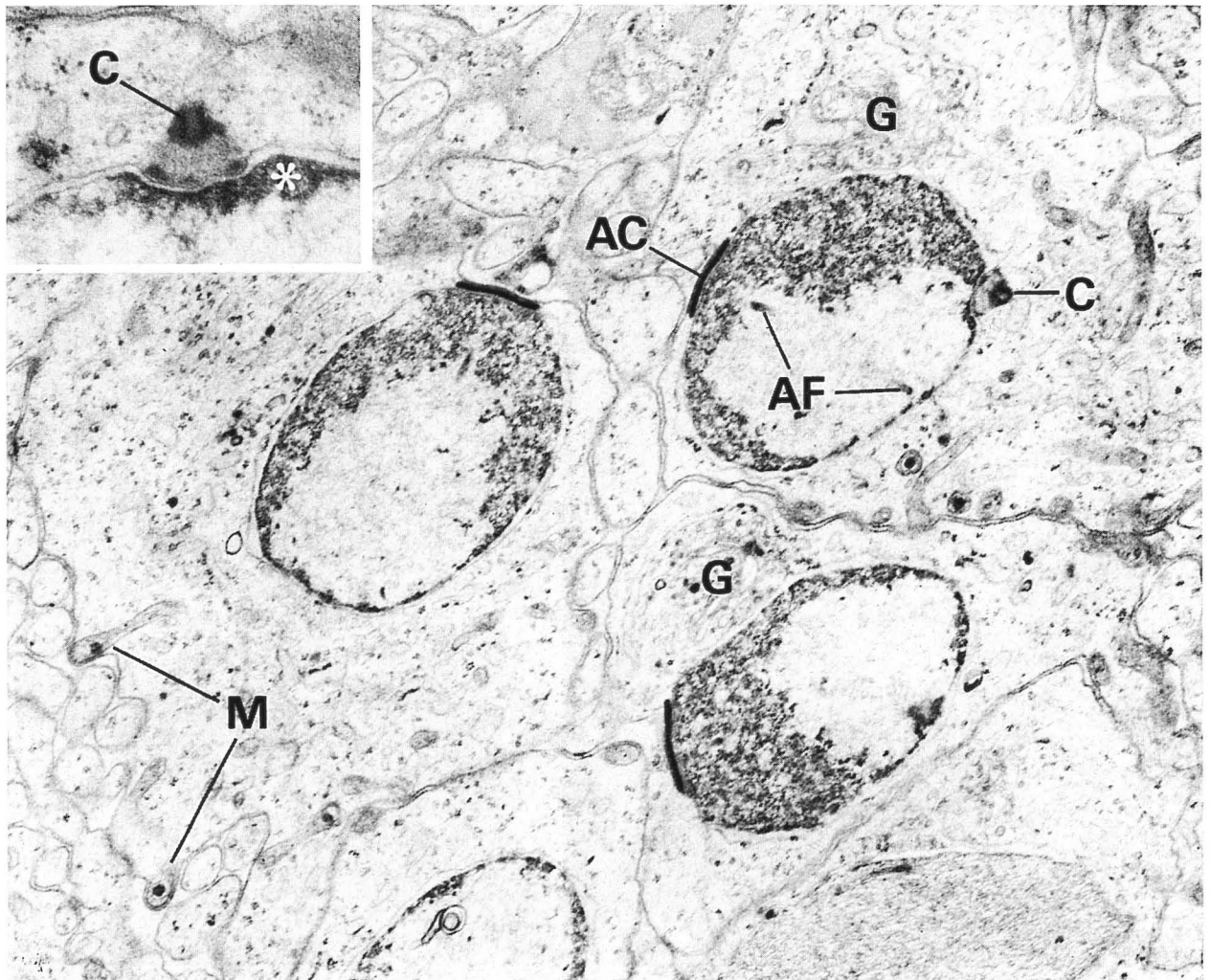


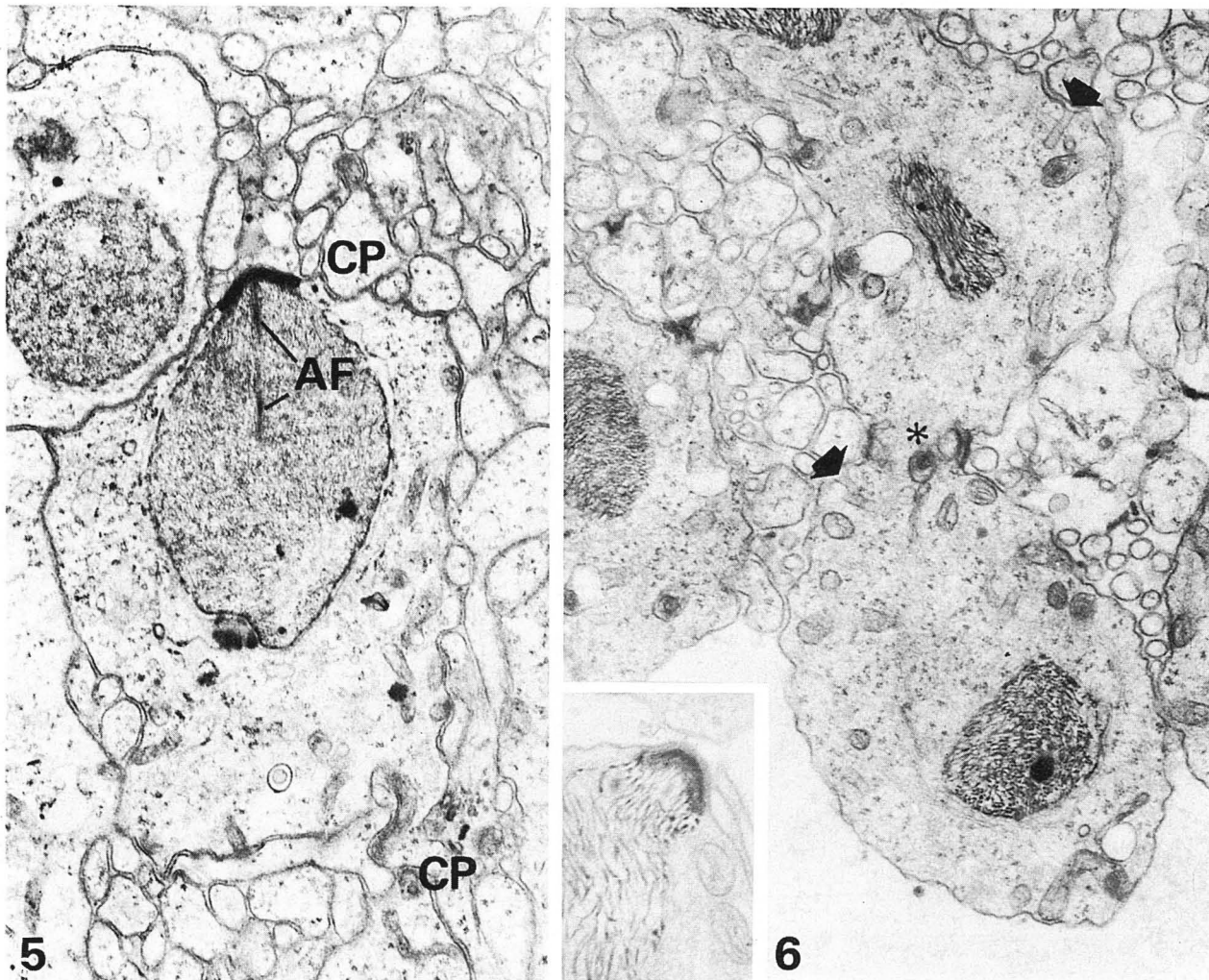
FIG. 4 : *Erythraeus phalangoides* :

Three early spermatids. AC, acrosomal cisterna ; AF, acrosomal filament ; C, centriole ; G, Golgi complex ; M, mitochondria with electron-dense granules. $\times 17,100$.

Inset : The centriole (C) connected by electron-dense material with a concavity at the posterior pole of the nuclear envelope. Asterisk indicates condensed chromatin. $\times 39,500$.

axis of the nucleus can often be observed. The number of RER elements decreases. The mitochondria containing electron-dense granules are set parallel to the long axis of spermatid. The cell processes of the intermediate spermatid begin to disappear and the plasmalemma creases to form the cytoplasm-penetrating tubules (Figs. 6 and 7).

■ *The late spermatid.* The late spermatid (Figs. 8-11) is an elongated cell with a compact, homogeneous and electron-dense nucleus. The condensation of chromatin proceeds starting from the posterior pole of the nucleus. The nuclear material is adjacent to the acrosome in a somewhat eccentric position (Figs. 9 and 10), whereas the acrosomal filament is attached to the centre of the acrosomal cisterna and takes a spiral course



FIGS. 5-6 : *Erythraeus phalangoides* :

- 5) Early spermatid slightly older than that shown in fig. 4. The acrosomal filament (AF) attached to the curved acrosomal cisterna runs through the nucleus. The numerous cell processes (CP) of spermatids occupy the intercellular spaces. $\times 14,300$.
- 6) Two intermediate spermatids interconnected via an intercellular bridge (asterisk). The nuclei are devoid of envelopes and the nuclear material is fibrillar. In the left nucleus an electron-dense granule is visible. Arrows indicate the plasmalemma-derived tubules penetrating into the cytoplasm. $\times 18,700$.
- Inset : The acrosomal region of spermatid. $\times 24,200$.

around the nucleus to reach the postnuclear region of the spermatid. Sometimes the acrosomal filament penetrates through the cell nucleus (Fig. 9) ; in other cases the nuclear material forms processes that partially surround the filament (Fig. 11). Microtubules (approx. 40 per cell section) and mitochondria are located along the cell nucleus (Figs. 10 and 11). The spatial distribu-

tion of microtubules and mitochondria suggests some relation between these two cellular components. In cross-section mitochondria usually show a concentric structure with one crista (Figs. 9 and 11).

Two kinds of peripheral cisternae appear beneath the plasmalemma : flattened and tubular. The flattened cisternae are located in a single sub-

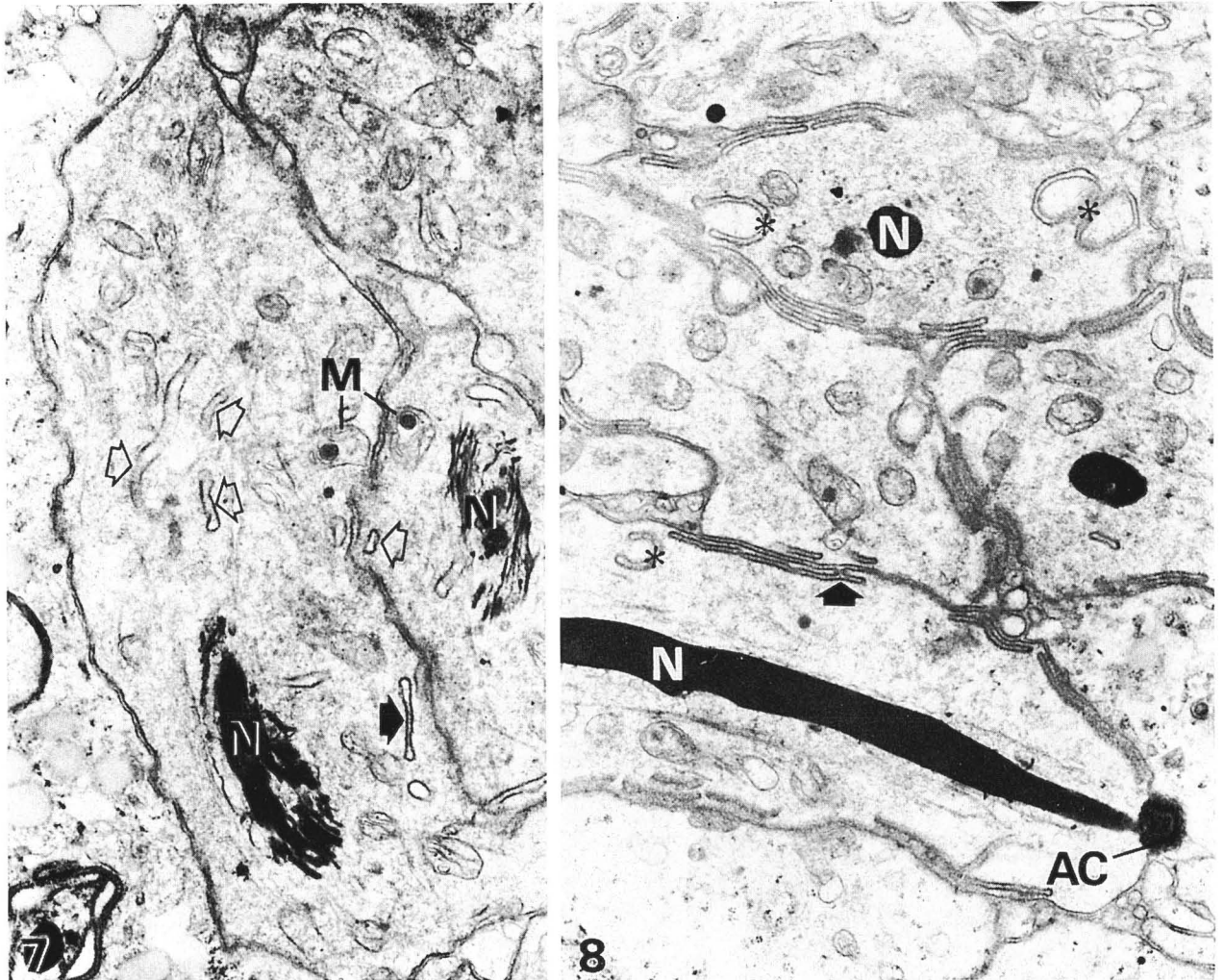


FIG. 7-8 : *Erythraeus phalangoides* :

- 7) Oblique section through the intermediate spermatid. In the cytoplasm the plasmalemma-derived tubules (light arrows) are running. Some of them (dark arrow) are wider and located near the plasmalemma. M, mitochondria containing intracristal electron-dense granules ; N, nucleus. $\times 18,000$.
- 8) Cross sections (top) and longitudinal section (bottom) through the late spermatids. The flattened cisternae connected with plasmalemma (arrow) and U-shaped or circular membranous structures (asterisks) are seen. AC, acrosome ; N, nucleus. $\times 15,400$.

plasmalemmal layer (Figs. 9-11). The electron-lucent tubular cisternae are less numerous than the flattened and lie more centrally, frequently under the margins of the flattened cisternae (Figs. 9 and 11). The membranes of both types of peripheral cisternae are joined with plasmalemma (Fig. 9) and covered with particles, 50 nm in diameter, arranged in rows running along the spermatid. Apart from the peripheral cisternae,

U-shaped or circular membranous structures were occasionally observed (Fig. 8).

■ *The spermatozoon.* The maturation of the late spermatid involves condensation of its cytoplasm and formation of an additional outer membrane which surrounds the entire cell (Figs. 12-14). The initial fragments of the additional membrane appear at the same time at numerous sites bet-

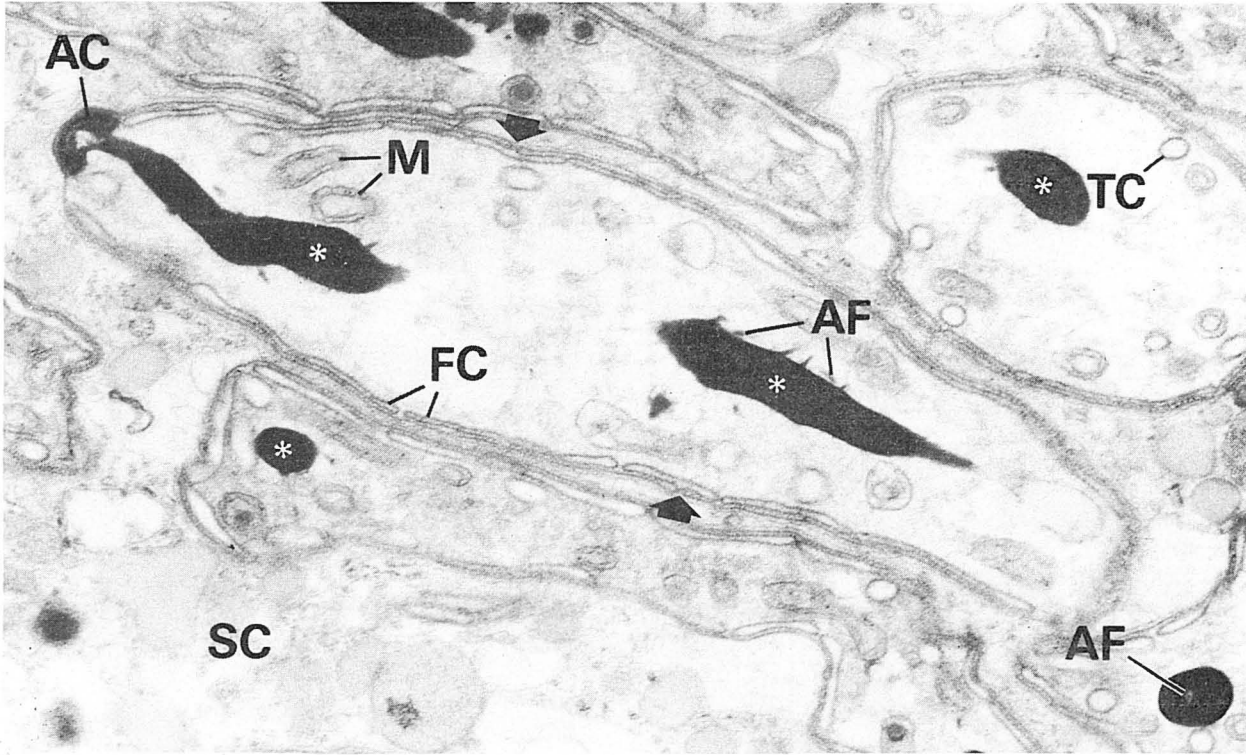


FIG. 9 : *Erythraeus phalangoides* :

Nearly longitudinal section through the late spermatid. Both the nucleus (asterisks) and the acrosomal filament (AF) are connected with the acrosomal cisterna (AC). Tubular (TC) and flattened (FC) peripheral cisternae communicating with the plasmalemma (arrows) are visible. AF, acrosomal filament located inside nucleus ; M, mitochondria ; SC, somatic cell. $\times 18,100$.

ween the cell membrane of the spermatozoon and that of the somatic cells (Fig. 12). Shortly afterwards these fragments fuse to produce a uniform layer, becoming slightly thicker than the plasmalemma or the membrane of the peripheral cisternae. When the formation of the outer membrane is completed (Fig. 13), the spermatozoa are released into the lumen of the testis.

The mature spermatozoon is elongated (approx. $11.4 \mu\text{m}$ in length and $2.8 \mu\text{m}$ in width) and slightly distended in its posterior part. Its anterior apex (Fig. 14) is occupied by a more or less eccentrically located acrosome adjacent to the elongated cell nucleus, around which the acrosomal filament is helically coiled. The plasmalemma, communicating with the peripheral cisternae, is covered by the outer membrane. The mitochondria with intracrystal electron-dense gra-

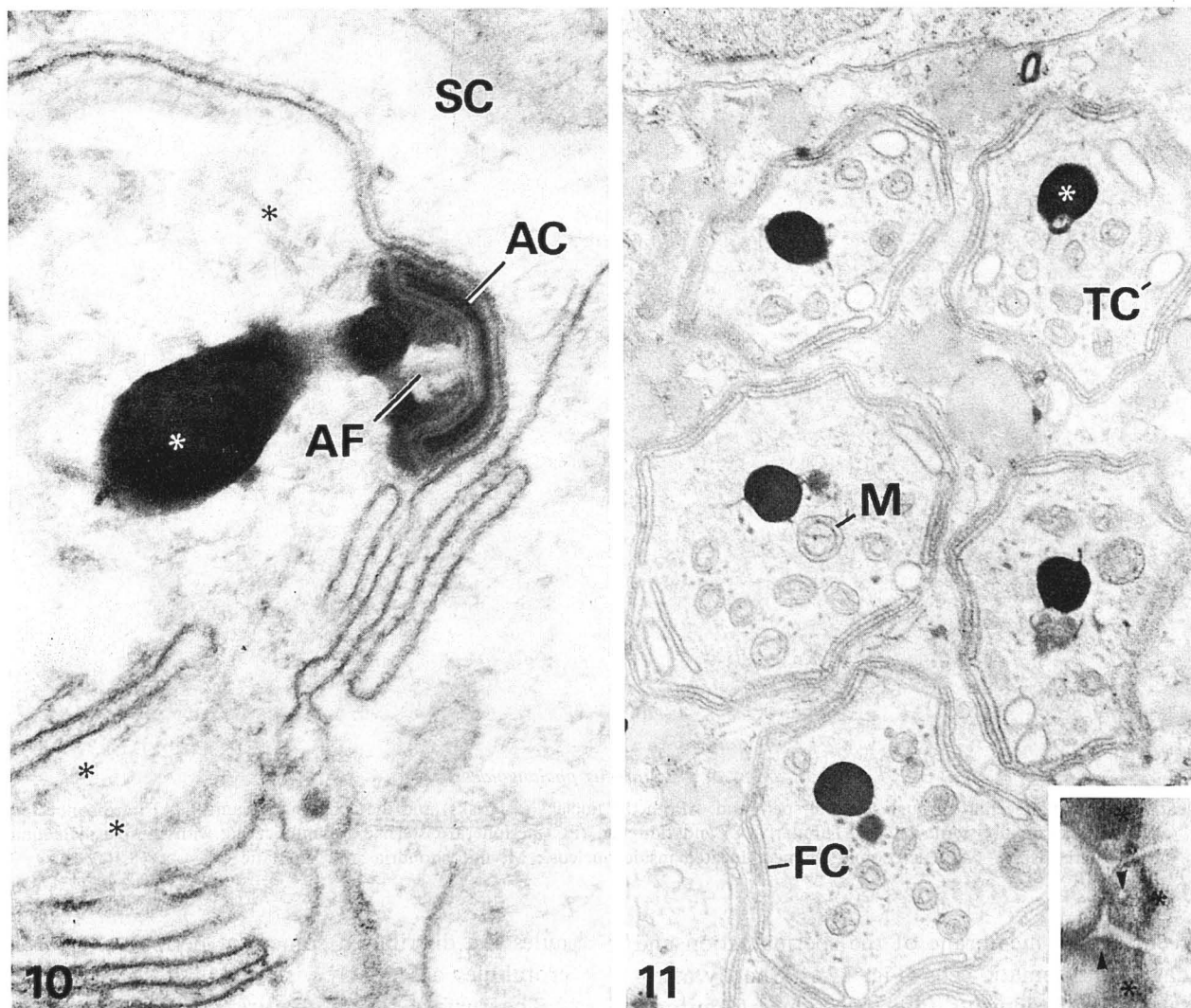
nules are distributed somewhat peripherally. Microtubules are poorly visible.

The surface of the mature spermatozoon lying in the testicular lumen is often coated with one of the components of the secretory material produced by the glandular part of the testis (secretory material type 1, WITTE and STORCH, 1973).

DISCUSSION

The results obtained in the present study are fully consistent with those reported by ALBERTI (1980b). Nevertheless, the presence of some specific structures seems to require a more detailed discussion.

Although the spermatozoa of *Erythraeus phalangoides* lack flagella, as do those of all other



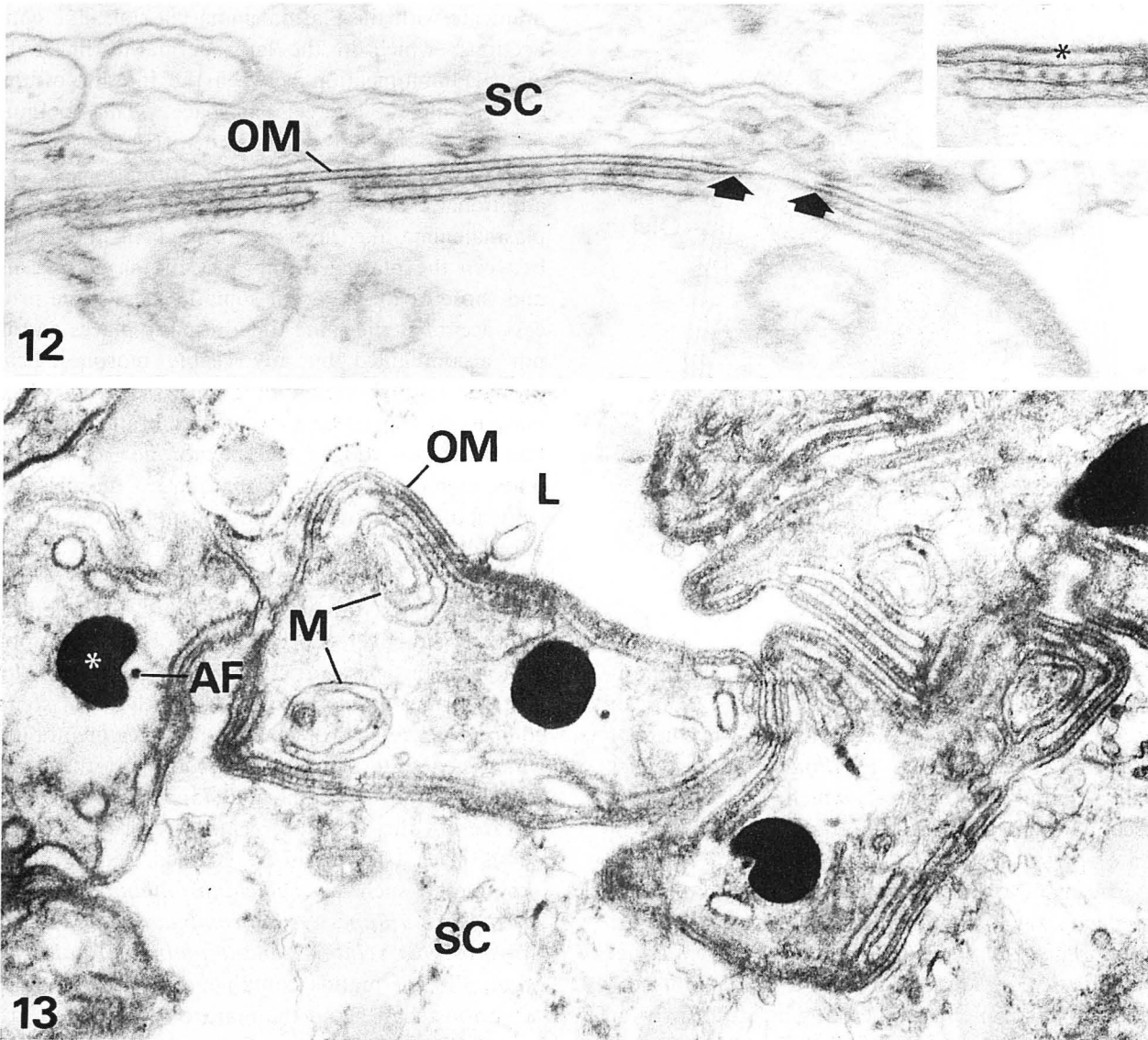
FIGS. 10-11 : *Erythraeus phalangoides* :

- 10) The acrosomal region of late spermatid. The nuclear material (white asterisk) and acrosomal filament (AF) adhere to the acrosomal cisterna (AC). Microtubules running along both sides of the nucleus (black asterisks) can be seen. SC, somatic cell. $\times 63,500$.
- 11) Group of late spermatids in cross section. An appendage of the nuclear material envelope the acrosomal filament (asterisk). M, mitochondria ; FC, flattened cisterna ; TC, tubular cisterna. $\times 17,200$.

Inset : Tangential section through three flattened cisternae (asterisks). Inside the central and the lower cisternae sections through the stalks connecting the cisternae and plasmalemma (arrow-heads) can be seen. $\times 24,000$.

mites studied to date, the early stages of spermiogenesis proceed in a manner typical of the formation of flagellate spermatozoa. The early spermatid becomes polarized due to the localization of the acrosomal cisterna at one side of the nucleus and the centriole at the other. However, the axoneme is not formed. At late stages of spermioge-

nesis the centrioles are no longer visible. The presence of centrioles in the male germ cells of mites is a rather rare phenomenon. Apart from ticks (BREUCKER and HORSTMANN, 1972), some Gamasida possessing the same type of spermatozoa (ALBERTI, 1980a), and probably Opilioacarida, centrioles have been observed in only a few



FIGS. 12-13 : *Erythraeus phalangoides* :

12) Formation of the outer membrane (OM) on the surface of late spermatid. Part of plasmalemma not covered with the outer membrane is indicated by arrows. SC, somatic cell. $\times 50,700$.

Inset : Superficial fragment of spermatozoon with outer membrane (asterisk), plasmalemma, and flattened cisterna coated with particles. $\times 90,000$.

13) Spermatozoa in cross section. In the nearby late spermatid the nucleus (asterisk) and acrosomal filament (AF) are indicated. L, lumen of testis ; M, mitochondria ; OM, outer membrane of spermatozoon ; SC, somatic cell. $\times 25,200$.

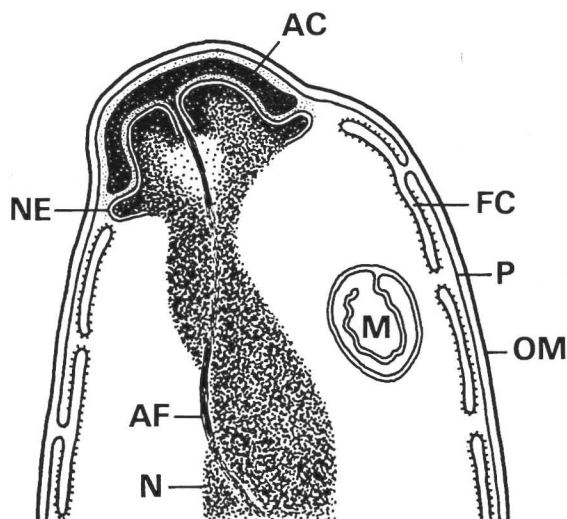


FIG. 14 : *Erythraeus phalangoides* :

A schematic drawing illustrating the distribution of cellular components in the acrosomal region of spermatozoon. AC, acrosomal cisterna ; AF, acrosomal filament ; FC, flattened peripheral cisterna ; M, mitochondrion ; N, nucleus ; NE, remnant of nuclear envelope ; OM, outer membrane ; P, plasmalemma.

cases, and in these only during spermiogenesis (ALBERTI, 1980b ; WITALIŃSKI, 1982). Thus, it seems justified to conclude that the structure of the early spermatid in *Erythraeus phalangoides* shows primitive features, which underlines the secondary character of the absence of flagellum in mite spermatozoa.

The mature spermatozoa of *Erythraeus phalangoides* show subplasmalemmal cisternae, their membranes communicate with the cell membrane. Such structures are also present in other representatives of Parasitengonae-Trombidia studied so far (ALBERTI, 1980b ; WITTE and STORCH, 1973) and probably are a permanent component of the spermatozoa in this group of mites. In *Erythraeus phalangoides* there are two types of peripheral cisternae : a) large and flattened ones and b) tubular ones. Both types appear at about the same time in the late spermatid. They are formed at the end of the intermediate spermatid stage as tubular invaginations of the plasmalemma. Later, the tubules become wider and undergo transformation into the peripheral cisternae, which occupy the

characteristic subplasmalemmal position in spermatid.

The membranes of the peripheral cisternae communicate with the plasmalemma via stalk-like connections, which in the late spermatids probably allow communication between the interior of the cisterna and the intercellular space. This communication would, however, be cut off in the mature spermatozoon because of the formation of an additional, continuous outer membrane. This plasmalemma-like layer is formed in the space between the plasma membranes of the germ cells and those of the adjacent somatic cells. The process occurs at the same time in many places and is not accompanied by any visible morphological changes in the vicinity. The presence of free margins in the nascent outer membrane indicates that it is not a true cell membrane. Since the outer membrane is strictly parallel to the plasmalemma of the spermatozoon and not to the undulate cell membrane of the somatic cells, it seems likely that it is the spermatid which plays the principal role in its formation. Possibly, the peripheral cisternae covered by regularly distributed ribosome-like particles participate in the process. The particles may be involved in the synthesis of some components (e.g. proteins) of the outer membrane which penetrate into the cisternae and then, released via the communicating apertures onto the surface of the cell, aggregate to form a continuous layer. It should be stressed that in other Trombidia, such as *Dolichothrombium borceai*, *Abrolophus rubipes*, *Allothrombium fuliginosum*, *Calypstoma velutinus* and *Trombidium holosericeum*, the spermatids contain peripheral cisternae of various shapes and the mature spermatozoa are surrounded by a layer of electron-dense material more or less similar to the cell membrane (ALBERTI, 1980b ; WITTE and STORCH, 1973).

The occurrence of plasmalemmal invaginations, sometimes complex in structure, is a common feature of the spermatozoa in other representatives of Acari as well. These invaginations may have the form of either simple, regularly distributed, more or less deep invaginations, as in Bdellidae, Halacaridae and Tetranychidae (ALBERTI, 1980b ; ALBERTI and STORCH, 1976 ; MOTHES and SEITZ,

1981), or infoldings of the plasmalemma that eventually form long microvilli described in *Speleorchestes poduroides* (ALBERTI, 1980b). The most elaborate systems of plasmalemmal infoldings occur in the "ribbon-type" spermatozoa of Anactinotrichida, where the plasmalemma forms complex infoldings called "stiff bands" (WITALIŃSKI, 1975; WITALIŃSKI, 1979), or "Langsbänder" (ALBERTI, 1980a). The interior of those structures communicates with the extracellular environment due to the absence of any additional outer membrane in both the spermatids and the mature spermatozoa. It has been found in a few mite species studied so far (MOTHES and SEITZ, 1981; WITALIŃSKI, in preparation) that after copulation the spermatozoa present in the female genital tract undergo considerable structural transformation which also involves the plasmalemma. In *Tetranychus urticae* the spermatozoa increase their volume (MOTHES and SEITZ, 1981) and, consequently, their surface area; thus, the numerous infoldings might represent a reservoir of the plasmalemma. In *Pergamasus barbarus* (WITALIŃSKI, in preparation) the stalks of the bodies constituting the "stiff bands" become markedly dilated in the spermatozoa located in the spermatheca and ovary. In the latter case, these complex structures do not seem to be source of the cell membrane, but may instead play some other, so far unspecified, role in the fertilization process.

To sum up, the subplasmalemmal membrane specializations commonly found in the spermatozoa of different mite groups usually originate from the spermatid plasmalemma. They may perform various functions: *a*) participate in the formation of an additional outer membrane, *b*) constitute a reservoir of plasmalemma or *c*) play an unspecified role during fertilization.

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