

Ultrastructural Aspects of Spermatogenesis in *Metamicrocotyla macracantha* (Alexander, 1954) Koratha, 1955 (Monogenea; Microcotylidae)

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Ultrastructural aspects of spermatogenesis, spermiogenesis and of the mature spermatozoon of a microcotylid monogenean Metamicrocotyla macracantha parasite from Mugil liza, are described. The irregularly-shaped spermatogonia divide by successive mitoses, forming the primary spermatocytes, identified by the presence of synaptonemal complexes in their nuclei. The spermatids formed by meiotic cell divisions of the secondary spermatocytes, differentiate into a mature spermatozoon. Cross sections of the head and the middle region of mature spermatozoa show the nucleus with strong condensed chromatin, the mitochondria with short cristae, peripheral microtubules and two axonemes with a 9+1 pattern, confirming the characteristics of this genus.

Key words: ultrastructure - *Metamicrocotyla macracantha* - Monogenea - spermatogenesis - spermiogenesis - spermatozoa

The additional data obtained on spermiogenesis and the ultrastructural aspects of spermatozoa are complementary to the traditional methods of morphological analysis, even to molecular biology techniques. Associated with other characteristics, these data have been utilized for phylogenetic analysis of the monogenean by various authors (Lambert 1980, Justine et al. 1985b, 1991a,b, 1993a, Lebedev 1988, Malmberg 1990).

In the present study, spermatogenesis and ultrastructural aspects of the spermatozoa of the oligonchoinean, *Metamicrocotyla macracantha*, are examined for the first time by transmission electron microscopy.

MATERIALS AND METHODS

Mugil liza (Mugilidae) were collected by fishermen at the "Canal de Marapendi", coast of Rio de Janeiro, Atlantic Ocean. Infected gills were removed and examined in a saline medium under stereoscopic microscopy.

For transmission electron microscopy, the worms were fixed in 0.1M phosphate-buffered 2.5% glutaraldehyde, post-fixed for 1 hr in 1%

osmium tetroxide in the same buffer, dehydrated in an increasing graded ethanol series, and embedded in Epon (Luft 1961). Ultrathin sections were collected on copper grids, double-stained with 2% alcoholic uranyl acetate and lead citrate (Reynolds 1963) and examined in a Zeiss EM 900 electron microscope.

RESULTS

The morphology of *M. macracantha* by light and scanning electron microscopy was recently published (Kohn et al. 1994). The testes of *M. macracantha* is composed of numerous follicles, each of which includes different stages of cellular development of spermatogenesis.

The primary spermatogonia produce primary spermatocytes by mitotic divisions. The secondary spermatocytes are formed by the same divisions. By meiosis, numerous spermatids originate from the spermatocytes and each one differentiates into a mature spermatozoon.

Spermatogonia - The spermatogonia appear as irregularly-shaped cells (Fig. 1). By mitotic divisions, they divide twice, forming secondary and tertiary spermatogonial stages. The nucleus of these cells is almost spherical, located centrally in the cells and occupies most of the cell volume. It contains a single nucleolus and irregular clumps of heterochromatin scattered throughout a granular nucleoplasm. Few ribosomes are attached to the nuclear envelope.

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The cytoplasm of the spermatogonia contains few membranous elements. Many free ribosomes were observed as clusters or rosettes (polyribosomes). Small mitochondria have short cristae and weakly stained matrices. In the primary and secondary spermatogonia, the mitochondria are located at the cell periphery. In the tertiary stage the nucleus becomes displaced to the border of the cell, and the mitochondria become more numerous and accumulate at the opposite side (Fig. 2). A pair of centrioles near the nucleus was observed (Fig. 3).

Spermatocytes - Mitotic divisions of the tertiary spermatogonia results in the production of eight spermatocytes, joined at their bases around the central cell portion.

The spermatocytes are the largest cells in the process of spermatogenesis. The nucleus is proportionally smaller than that of the spermatogonia, with granular nucleoplasm containing sparse and condensed chromatin (Fig. 4). Synaptonemal complexes were observed within the chromatin mass of the primary spermatocytes, indicating the pairing of homologous chromosomes during meiosis (Fig. 5).

The spermatocytes possess a rough endoplasmic reticulum (Fig. 9), Golgi cisterns (Fig. 6), and numerous free ribosomes inside the cytoplasm. The mitochondria are similar to those of spermatogonia.

Spermatids - The spermatids (Fig. 8) are formed by meiotic cell division of secondary spermatocytes. Each spermatid differentiates into a mature spermatozoon.

The nuclei of the spermatids are smaller than those of the spermatocytes. The cytoplasm contains mitochondria, endoplasmic reticulum and groups of free ribosomes.

Spermiogenesis - Spermiogenesis is characterized by the differentiation of the spermatids to spermatozoa.

A group of spermatids fuse forming an irregular cytoplasmic mass; all the spermatids of the same mass are at the same developmental stage (Fig. 8). This cytoplasmic mass produces elongated projections, known as the "zone of differentiation" (Fig. 12).

The spermatid nuclei become elongated, moving into the cytoplasmic projections, close to the plasma membrane, which is supported by a single row of microtubules.

Basal bodies and associated rootlet fibers (Fig. 7), intercentriolar bodies, numerous free ribosomes, and rough endoplasmic reticulum, were also observed in the zone of differentiation.

After the flagellar elements have rejoined the main central process, and the nucleus and mitochondria have migrated into the projection, the zone

of differentiation constricts and each spermatozoon is released from the general cell mass (Fig. 11).

Spermatozoa - The immature spermatozoa present a nucleus of low electron density, mitochondria not yet fused, axonemes and cortical peripheral microtubules (Fig. 13).

The mature spermatozoon is filiform, and can be divided in three regions: anterior, middle and terminal.

Observations on longitudinal sections show an elongated nucleus from the anterior to the middle region of the spermatozoa, and a single mitochondrion situated parallel to the nucleus (Fig. 10). Cross sections of the anterior and middle regions of mature spermatozoa show the nucleus with strongly condensed chromatin, the mitochondrion with short cristae and two axonemes (Fig. 10). Each axoneme presents a central unit surrounded by nine pairs of microtubules (9 + 1). The plasma membrane is supported by a single layer of microtubules, disposed parallel to the longitudinal axis of the spermatozoa (Fig. 15).

The number of peripheral microtubules reduces in the middle region (Fig. 14). Thereafter this layer of peripheral microtubules is reorganized in two rows: one opposite and the other near to the mitochondrion.

The axonemes remain joined at the proximal part of the terminal region (Fig. 16), and separate in two flagella at the distal part (Fig. 17).

DISCUSSION

Recently, Justine published the bibliographical data about the ultrastructure of monogeneans by scanning and transmission electron microscope observations and about spermiogenesis and spermatozoa of monogeneans (Justine 1993a,b).

Studies of spermiogenesis and spermatozoa on Microcotylidae had been published by several authors (Tuzet & Ktari 1971a,b, Yousif & Crane 1979, Justine & Mattei 1985, Schmahl & Obiekezie 1991, Justine 1992, Rohde & Watson 1994a,b).

The ultrastructural arrangement of the testis and the cytological changes during spermiogenesis in *M. macracantha* are in agreement with the patterns already established for the Monogenea.

Similar to other species of Oligonchoinea, as in *Diclidophora merlangi* (see Halton & Hardcastle 1976), *Protomicrocotyle ivoriensis* and *Gastrocotyle* sp. (see Schmal & Obiekezie 1991), spermatogonia in *M. macracantha* present the mitochondria and the free ribosomes as the main organelles found in the cytoplasm.

The Golgi complex occupies a typical position within the spermatocytes; its trans-face is directed to the plasma membrane. This pattern was also observed by Halton and Hardcastle (1976) in *D.*



Fig. 1: spermatogonium with well developed nucleus (N) and cytoplasm with mitochondria and large amount of free ribosomes (R). X 17000. Fig. 2: tertiary stage of spermatogonia with nucleus (N) displaced to the border of the cell and numerous mitochondria (arrow) assembled in the opposite side. Cytoplasm with free ribosomes (R). X 6000. Fig. 3: pair of centrioles (arrows) in the cytoplasm of spermatogonia. X 33000.

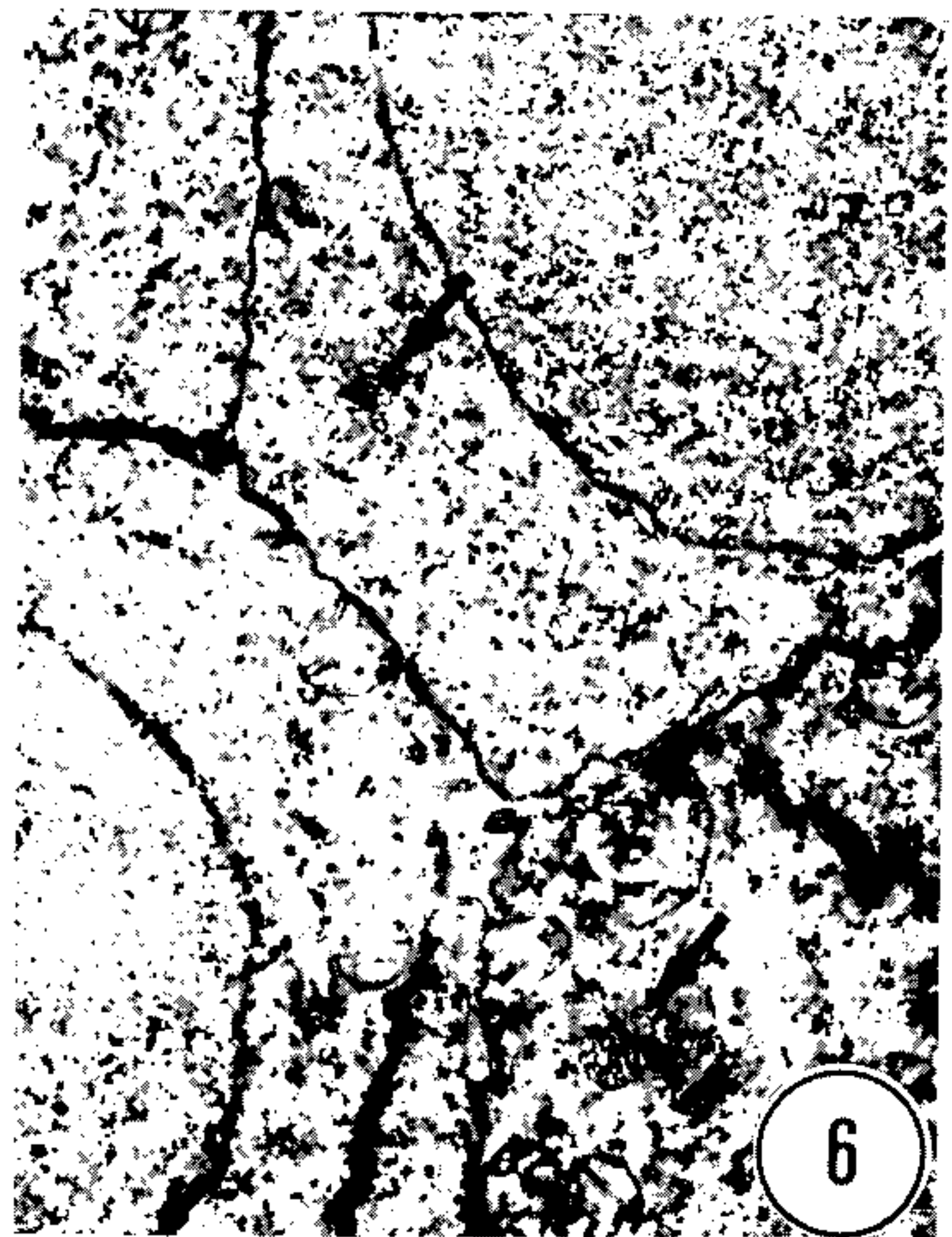
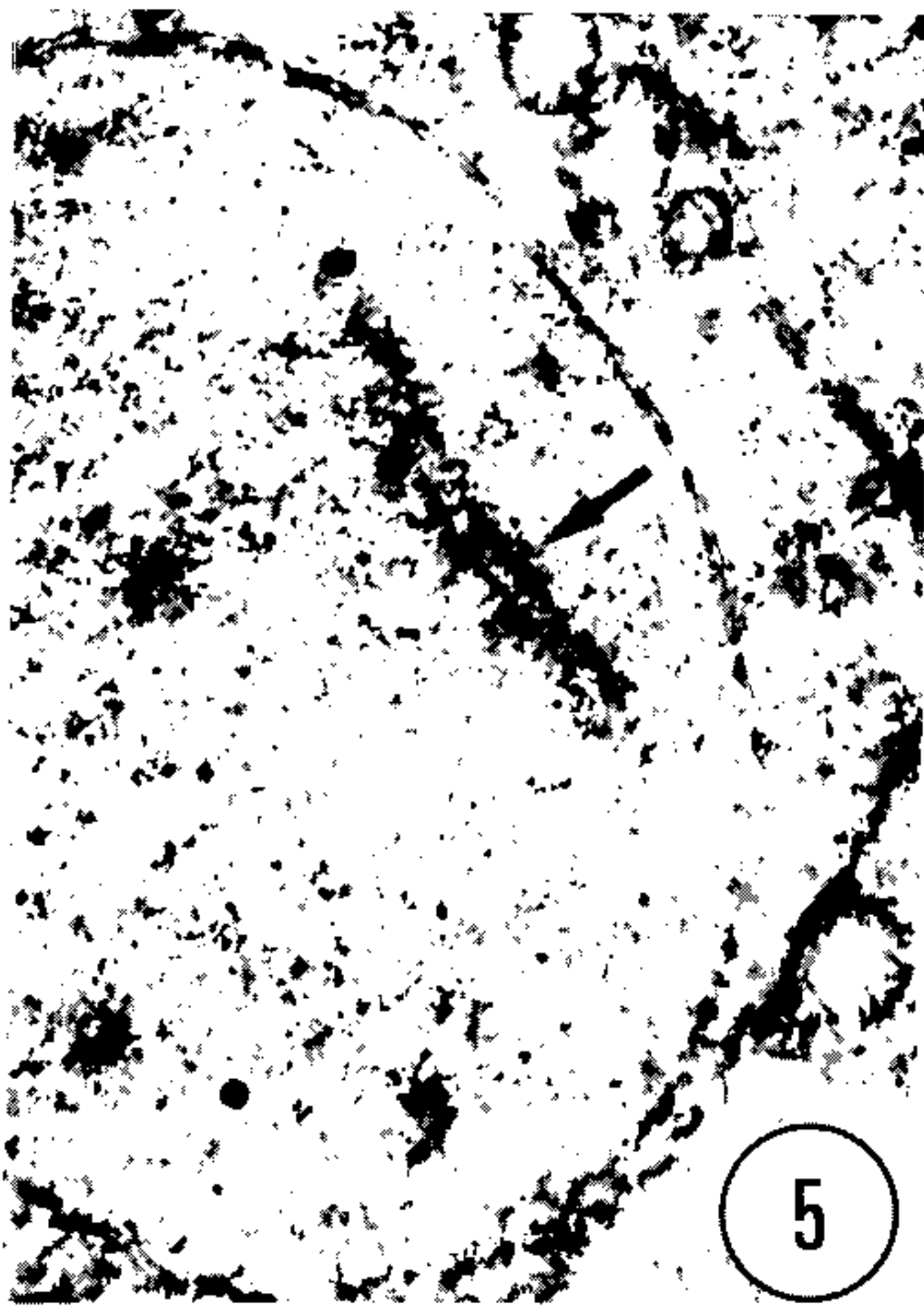


Fig. 4: group of spermatocytes joined at their bases, around the central cell region (asterisk), where mitochondrion (arrow) and free ribosomes (R) may be observed. X 6000. Fig. 5: synaptonemal complexes (arrow) in the spermatocyte nucleus. X 16000. Fig. 6: Golgi cisterns (arrow) in the spermatocyte cytoplasm. X 500.

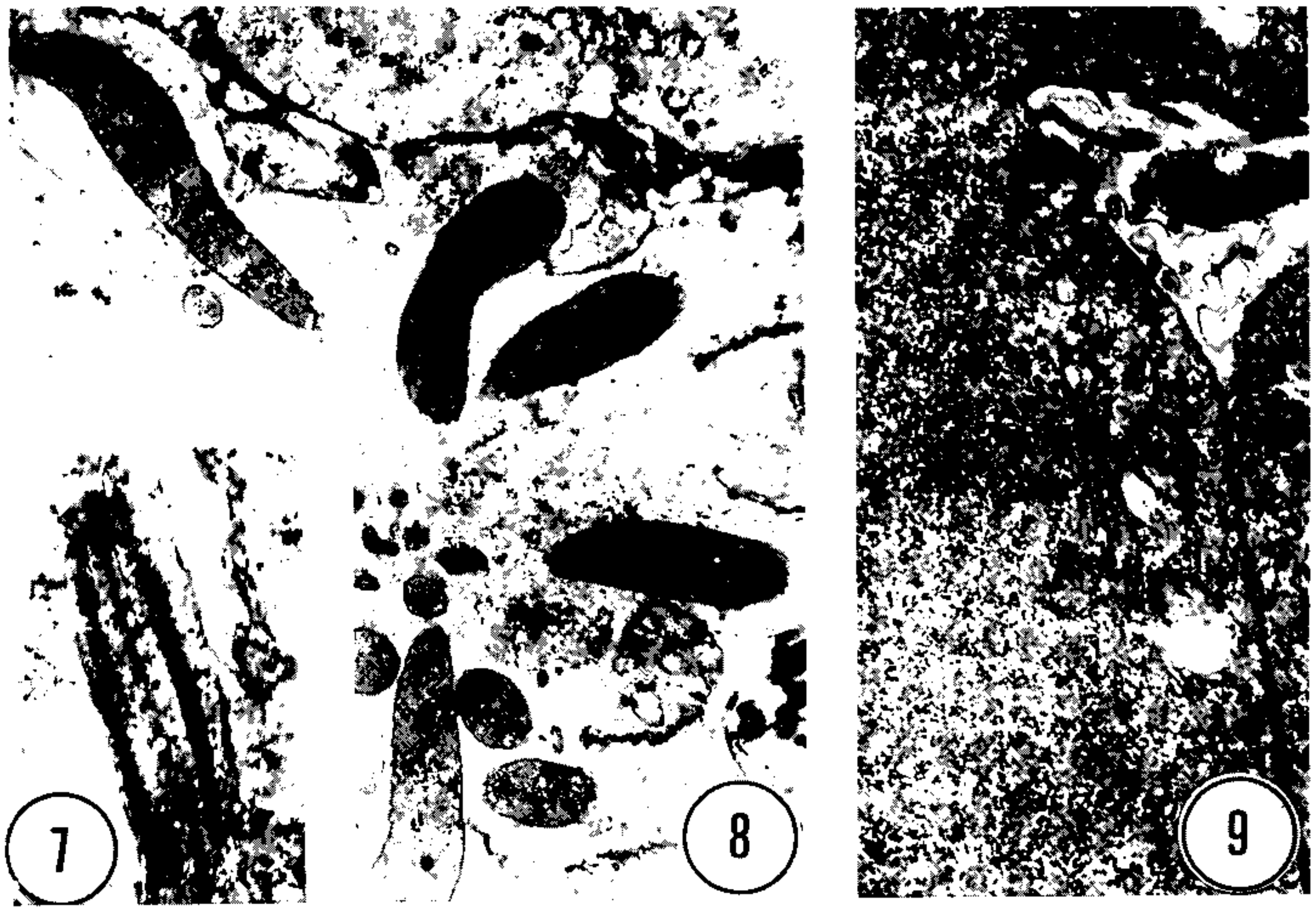


Fig. 7: rootlet fibers of the "zone of differentiation". Fig. 8: cytoplasmic mass with nuclei of spermatids moving to the projections of the mass. Degenerated mitochondria and spirally membranes in the residual mass (asterisk) may be observed. X 85000. Fig. 9: rough endoplasmatic reticulum (arrow) in the spermatocyte cytoplasm. X 12000. Fig. 10: longitudinal and cross sections of mature spermatozoa. An elongated nucleus (N), mitochondrion (M) and axonemes (arrow) are observed in a longitudinal section. X 14000.



Fig. 11: spermatid with nucleus (N) and the mitochondria (M) moving into the constriction region (arrow). X 11000. Fig. 12: spermatid in advanced stage of celular differentiation. The "zone of differentiation" can be noted. X 12000.

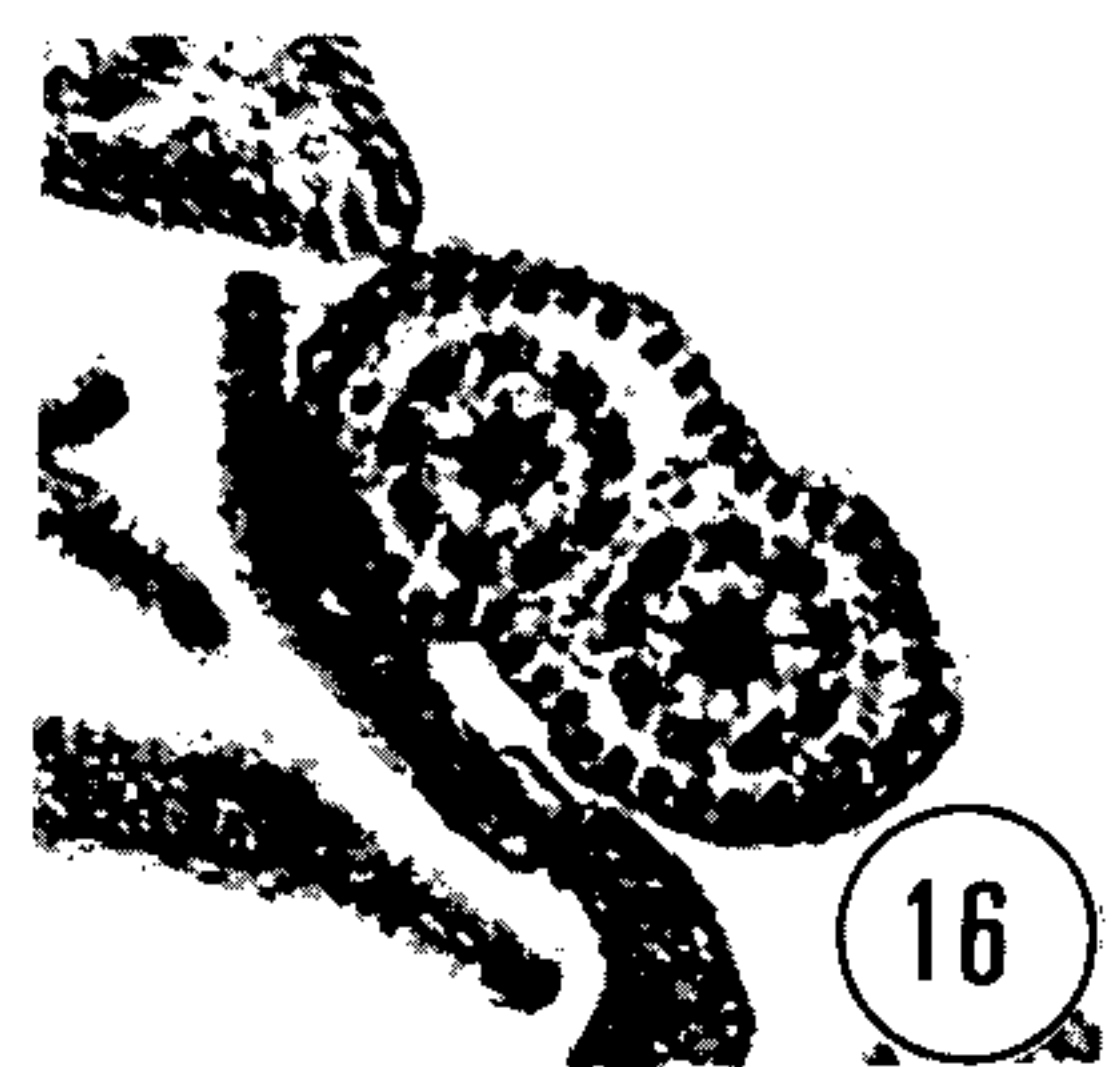
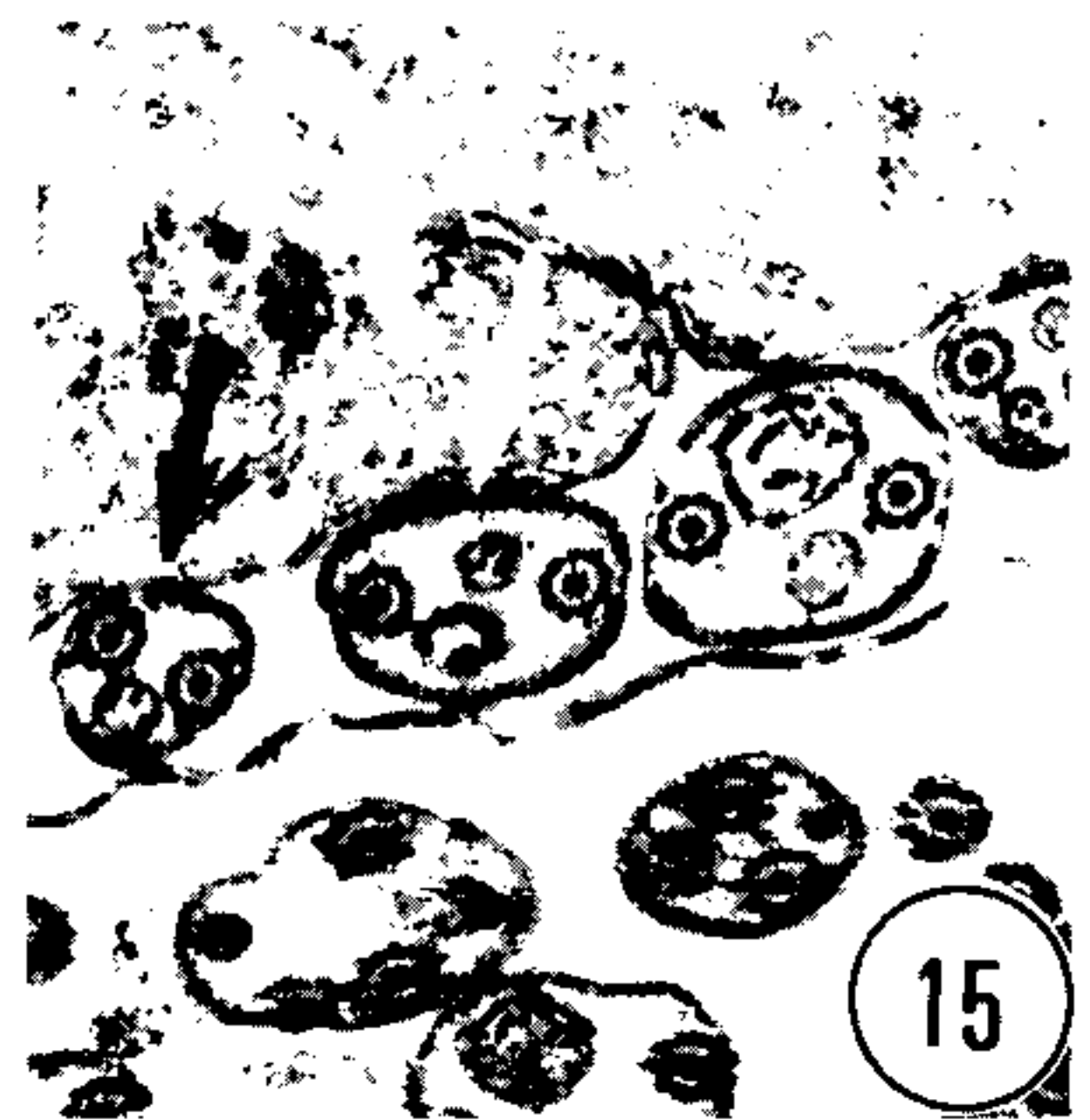


Fig. 13: cross section of an immature spermatozoon with nucleus (N), mitochondria (M), axonemes (A) and cortical microtubules (arrow). X 40000. Fig. 14: cross section of mature spermatozoa, showing nuclei, mitochondria, axonemes and the cortical microtubules. X 50000. Fig. 15: cross sections at different levels of mature spermatozoa, showing the decrease of peripheric microtubules (white arrow) and the absence of nucleus and decrease of peripheric microtubules (black arrow). X 19000. Fig. 16: terminal region of a spermatozoon; note the axonemes joined by the external membrane and cortical microtubules. X 60000. Fig. 17: terminal extremity of a spermatozoon showing the separated axonemes, forming two flagella. X 30000.

merlangi. The presence of two axonemes with the 9 + 1 configuration, the nucleus, mitochondria and cortical longitudinal microtubules can be observed at transversal sections and constitutes a typical pattern for the majority of parasites of this subclass. The cortical microtubules are absent in almost all Polyonchoinea, whereas in Oligonchoinea, they occur in most of the species studied (Rohde 1971, 1980, Tuzet & Ktari 1971b,

Halton & Hardcastle 1976, Justine & Mattei 1984), except in *D. gracile* which present aflagellate spermatozoa (Justine et al. 1985a).

The cortical microtubules of *M. macracantha* may be considered as reinforcement elements which develop at the beginning of the transformation process.

Justine et al. (1985b) have proposed the use of spermatozoa ultrastructure for phylogeny studies

of Monogenea; they use two spermatozoan characters to classify the group: the number of axonemes and the presence or absence of cortical microtubules. The spermatozoa of *M. macracantha* clearly belongs to the type I referred by these authors.

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