ULTRASTRUCTURE OF SPERMIOGENESIS AND SPERMATOZOA OF *POLYLABROIDES AUSTRALIS* (PLATYHELMINTHES, MONOGENEA, POLYOPISTHOCOTYLEA, MICROCOTYLIDAE)

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Summary :
Testis follicles are surrounded by a sheath and contain several stages of spermatogenesis. Primary spermatocytes have large nuclei with a loose granular appearance and synaptonemal complexes, and their densely packed cell bodies are small and rich in mitochondria. Spermatids are joined to a central cytophore; their nuclei are at first spherical and located near the tips of the spermatids, later they elongate and coil. In each of the cytoplasmic protuberances (the "zones of differentiation") in the periphery of the cytophore, two flagella, each with a cross-striated rootlet, grow out from basal bodies separated by an intercentriolar body, and later fuse in a proximo-distal direction with the median cytoplasmic process which has developed at the tip of the zone of differentiation. The zones of differentiation and the median cytoplasmic processes possess a row of peripheral microtubules. Mature sperm have a single row of peripheral microtubules that is incomplete in some parts of the sperm. Number of peripheral microtubules decreases towards both ends from about 55 in the principal region. One axoneme terminates before the other in the nuclear region, and nucleus and mitochondrion overlap only in the middle regions. The proximal end contains the axonemes, and the distal end the nucleus.

KEY WORDS : Monogenea. Polyopisthocotylea. *Polylabroides australis*. ultrastructure. spermatozoa. spermiogenesis.

Résumé : ULTRASTRUCTURE DE LA SPERMIOGENÈSE ET DU SPERMATOZOIDE DE POLYLABROIDES AUSTRALIS (PLATYHELMINTHES, MONOGENEA, POLYOPISTHOCOTYLEA, MICROCOTYLIDAE).

Les follicules du testicule sont entourés par une gaine et contiennent différents stades de la spermiogenèse. Les spermatocytes I ont de grands noyaux clairs et des complexes synaptonémaux, et leurs corps cellulaires, très serrés, sont petits et riches en mitochondries. Les spermatides sont attachées à un cytophore central; leurs noyaux sont tout d'abord sphériques et localisés près des extrémités des spermatides, puis ensuite allongés et enroulés. Dans chacune des protubérances cytoplasmiques (les "zones de différenciation"), dans la périphérie du cytophore, deux flagelles, chacun avec une racine striée, grandissent à partir de corps basaux séparés par un corps intercentriolaire et ensuite fusionnent dans une direction proximo-distale avec l'expansion cytoplasmique médiane qui s'est développée à l'extrémité de la zone de différenciation. Les zones de différenciation et l'expansion cytoplasmique médiane possèdent une rangée de microtubules périphériques. Les spermatozoïdes mûrs ont une rangée simple de microtubules périphériques qui est incomplète dans certaines parties du spermatozoïde. Le nombre de microtubules périphériques décroît vers les deux extrémités à partir de 55 environ dans la région principale. Un des axonèmes se termine avant l'autre dans la région nucléaire, et le noyau et la mitochondrie se superposent seulement dans les régions moyennes. L'extrémité proximale contient les axonèmes, et l'extrémité distale contient le noyau.

MOTS CLES : *Monogenea. Polyopisthocotylea. Polylabroides australis. ultrastructure. spermatozoïdes. spermiogenèse.*

INTRODUCTION

The ultrastructure of sperm and spermatogensis of Monogenea has received much recent attention, mainly with the aim of contributing to a phylogenetic system of the group (reviews by Justine 1991a, 1993). Ultrastructure of sperm of Monogenea Polyopisthocotylea has been examined by Rohde (1971, 1975, 1980), Ktari (1971), Tuzet and Ktari (1971a,b), Bekkouche *et al.* (1974), MacDonald and Caley (1975), Halton and Hardcastle (1976), Justine and Mattei (1984, 1985a, b, c), Justine (1985, 1991), Justine *et al.* (1985a, b), and Schmahl and Obiekezie (1991). Spermiogenesis of Polyopisthocotylea has received much less attention (Tuzet and Ktari 1971a; Justine and Mattei 1983; Justine 1985, 1991a, b; Schmahl and Obiekezie 1991). In this paper, we describe the ultrastructure of sperm and spermiogenesis in the microcotylid *Polylabroides australis*.

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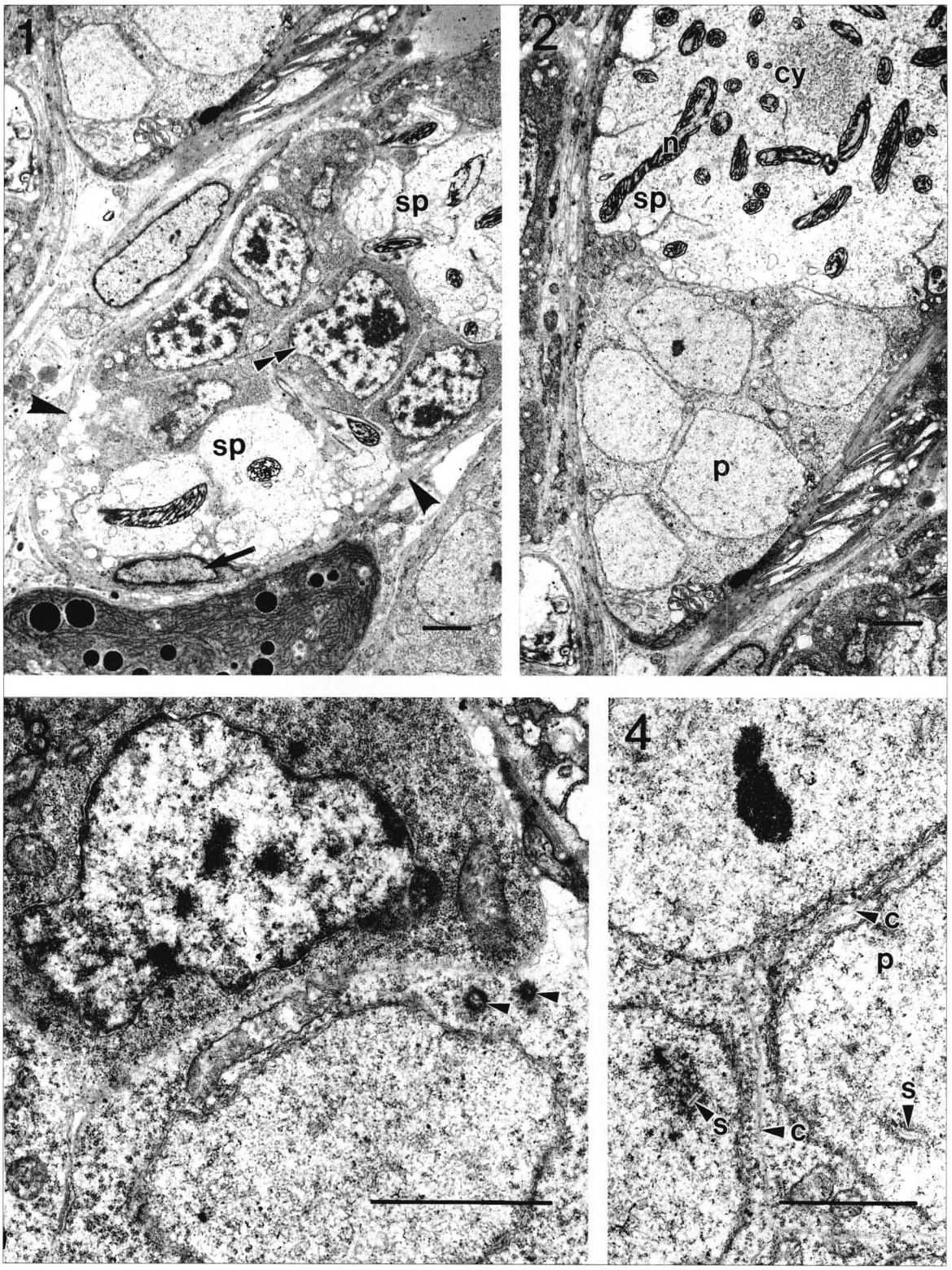
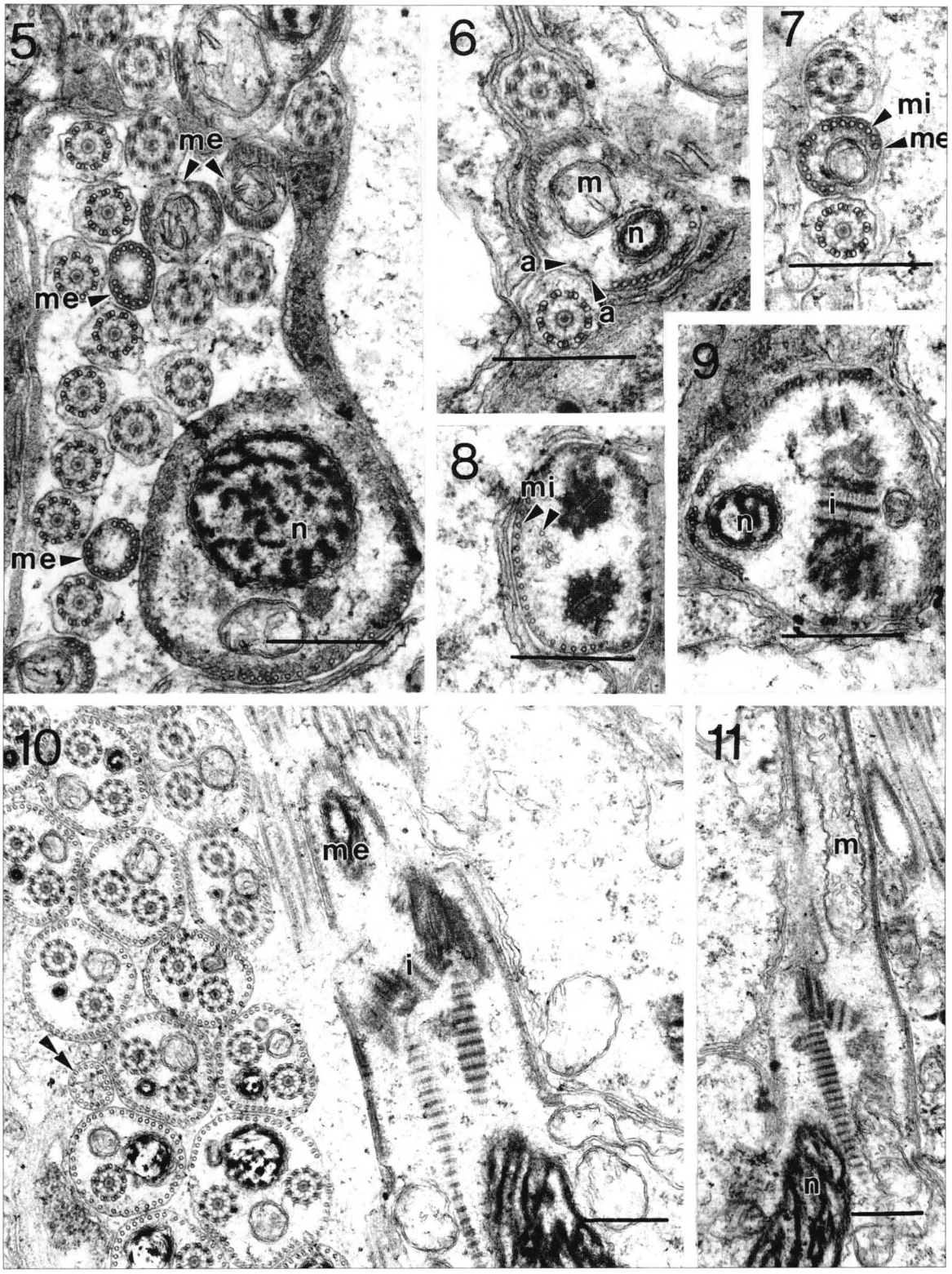
MATERIALS AND METHODS

Specimens of *Polylabroides australis* were collected from the gills of bream, *Acanthopagrus australis*, and fixed for 3 h at 4°C in 3% glutaraldehyde in 0.1M phosphate buffer at pH 7.4. They were washed in the same buffer at 4°C and postfixed for 1 h at room temperature in 1% OsO_4 in buffer, dehydrated in alcohol and embedded in Spurr resin. Ultrathin longitudinal sections through a mature individual were stained with uranyl acetate and lead citrate and examined under a JEOL 1200EX electron microscope at 60kV.



Figs. 1-4. – Spermiogenesis of *Polylabroides*. Fig. 1. Testis follicle containing spermatids and spermatocytes (double arrowhead). Note sheath (large arrowheads) around follicle and nucleus (arrow) in wall of follicle. Fig. 2. Part of testis follicle with spermatids connected to a central cytophore, and primary spermatocytes. Fig. 3. Probably primary spermatocyte with pair of centrioles (arrowheads). Fig. 4. Primary spermatocytes containing large nuclei surrounded by little cytoplasm. Note synaptonemal complexes and cell membranes between spermatocytes. Scale bars 2 µm.

Abbreviations : a, attachment zone; ax, axoneme; b, basal body; c, cell membrane; cy, cytophore; e, extension (?) of nucleus; i, intercentriolar body; m, mitochondrion; me, median cytoplasmic process; mi, microtubule; mu, muscle fibre; n, nucleus; p, primary spermatocyte; s, synaptonemal complex; sp, spermatid.



Figs 5-11. Spermiogenesis of *Polylabroides*. Fig. 5. Cross-sections through outgrowing spermatids at different levels: bottom right below level of flagella, note large nucleus and mitochondrion; others at level of median cytoplasmic process, note free flagella, mitochondrion in some sections through process, and incomplete row of peripheral microtubules. Fig. 6. Cross-section through median cytoplasmic process with nucleus and mitochondrion, incomplete row of peripheral microtubules and zones of attachment, and pair of free flagella. Fig. 7. Cross-section through median cytoplasmic process with mitochondrion and incomplete row of peripheral microtubules, and pair of free flagella. Fig. 8. Cross-section through median cytoplasmic process at level of ciliary rootlets, note peripheral microtubules, microtubules in periphery of rootlets possibly extensions of the basal bodies, and some microtubules in the interior of the median cytoplasmic process. Fig. 9. Cross-section through median cytoplasmic process at level of intercentriolar body and basal bodies, note nucleus on one and mitochondrion on the other side, and incomplete row of peripheral microtubules. Figs. 10, 11. Longitudinal sections through outgrowing spermatid. Note intercentriolar body between basal bodies of flagella, cross-striated rootlets, and median cytoplasmic process with nucleus and mitochondrion. Scale bars 0.5µm.

RESULTS

SPERMIOGENESIS

Testis follicles are surrounded by a sheath (Fig. 1) and nuclei, possibly belonging to the sheath, are located in their periphery (Fig. 1). The follicles examined contained several stages of spermatogenesis. Primary spermatocytes are small, densely packed and contain many mitochondria (Figs. 2, 4), and their nuclei have an electron-lucent, loosely granular appearance (Figs. 2, 4) and contain synaptonemal complexes (Fig. 4). Fig. 4 probably illustrates a spermatocyte with a pair of centrioles at the beginning of cell division and Fig. 13 illustrates two spermatocytes containing two nucleoli each. Clusters of cells, also densely packed but with denser cytoplasm and chromatin in distinct, dense patches are probably either secondary spermatocytes or primary spermatocytes with condensed chromatin (Fig. 1). Early spermatids are joined by a central cytophore (Fig. 2). Their nuclei are at first spherical and located near the tips of the spermatids (Fig. 12). Later, they elongate and coil, containing thin strands of dense chromatin (Figs. 1, 2). In the periphery of the cytophore, median cytoplasmic processes develop, into each of which a nucleus and an elongate mitochondrion (the result of fusion of many smaller mitochondria) migrate (Figs. 5-11). Comparison of many cross-sections through the median processes indicates that the mitochondrion migrates first (Fig. 5). Two flagella, each with a long cross-striated rootlet, grow out from basal bodies separated by an intercentriolar body (Figs. 9-11). In early spermatids, the flagella run parallel with the median cytoplasmic process (Figs. 5-7), which has an incomplete row of microtubules in its periphery (Figs. 5-8). In some sections, a few more internally located microtubules were seen (Fig. 8). Facing each flagellum are two attachment zones (Fig. 6). Flagellar rootlets have an irregular outline in cross-section and contain microtubules, possibly extensions of the basal bodies, in their periphery (Fig. 8).

At a later stage of spermiogenesis, flagella become incorporated in the sperm body (Fig. 10, 12, 14-20). Even in the sperm ducts, some sperm appeared large in cross-section, with a bulky electron-lucent cell body (Figs. 14-16), indicating that some sperm have not yet fully matured or are degenerating.

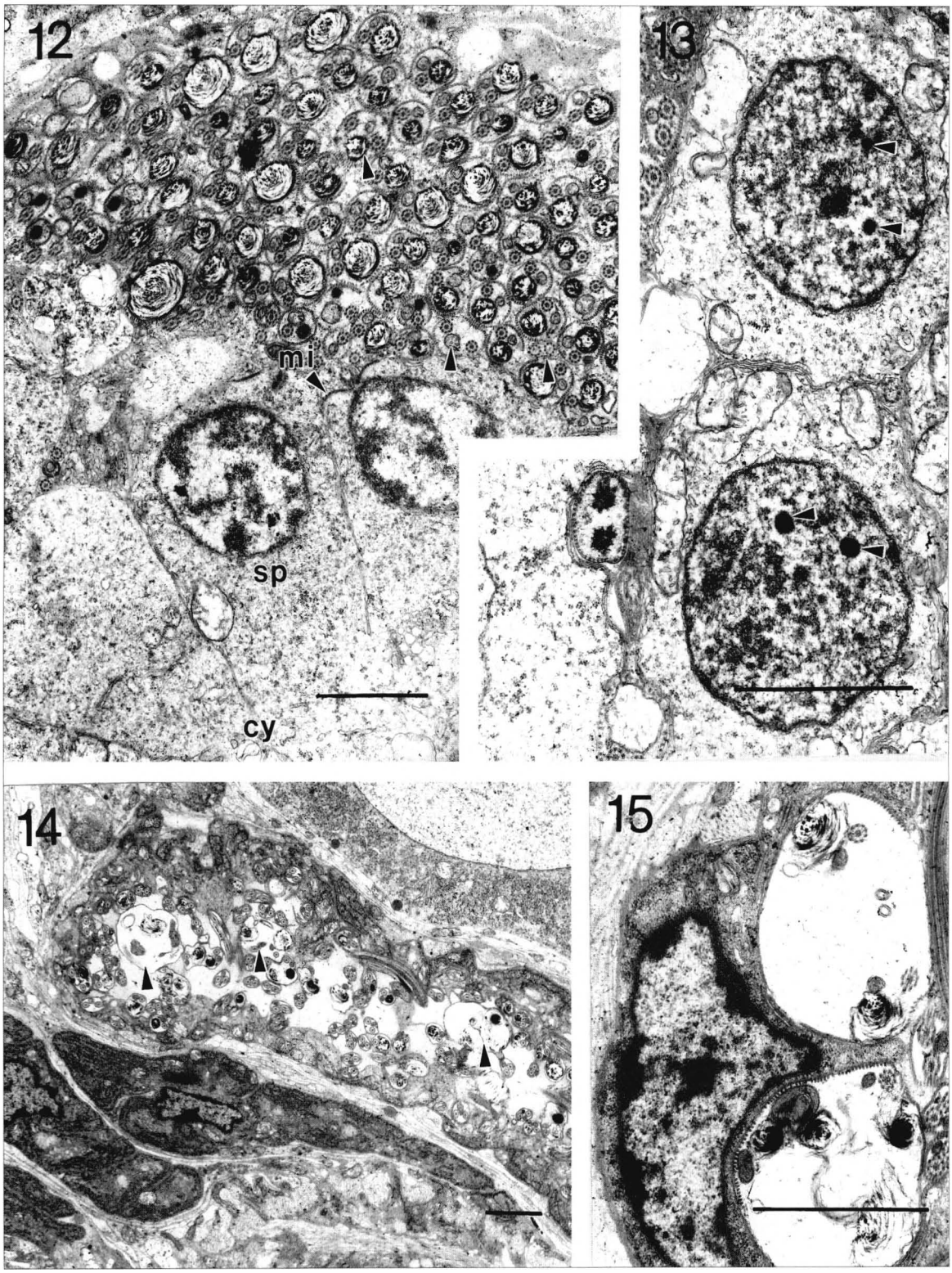
SPERMATOZOA

Spermatozoa were examined from various parts of the sperm ducts. Mature sperm has a single row of peripheral microtubules that is incomplete in some sections (Figs. 18, 19), and two incorporated axonemes (Figs. 14, 16-20). Nucleus and mitochondrion, in those parts of the sperm where both are present, are either close to each other or separated by the axonemes (Figs. 17-20). A small elongate body, probably an extension of the nucleus, was observed only in those parts of the sperm lacking the nucleus proper (Figs. 19, 20). Aggregations of electron-dense material, probably glycogen, are present usually close to the nucleus and the elongate body (Fig. 19). Once, a lamellate body was observed in addition to nucleus and mitochondrion (Fig. 17), and occasionally, sections through two mitochondria (?) or a single coiled mitochondrion were seen. In part of the middle region, only one axoneme is present in addition to the nucleus and mitochondrion (Figs. 12, 18, 19). Some sections contained only two axonemes surrounded by an incomplete or complete row of peripheral microtubules (Fig. 18), some contained an axoneme, nucleus and mitochondrion surrounded by the peripheral microtubules (Fig. 17), some contained only peripheral and some internal microtubules (Fig. 17). Basal bodies were found in the proximal end of sperm from the sperm ducts (Fig. 18 inset) and some sections showed a single axoneme surrounded by peripheral microtubules (Fig. 18 inset).

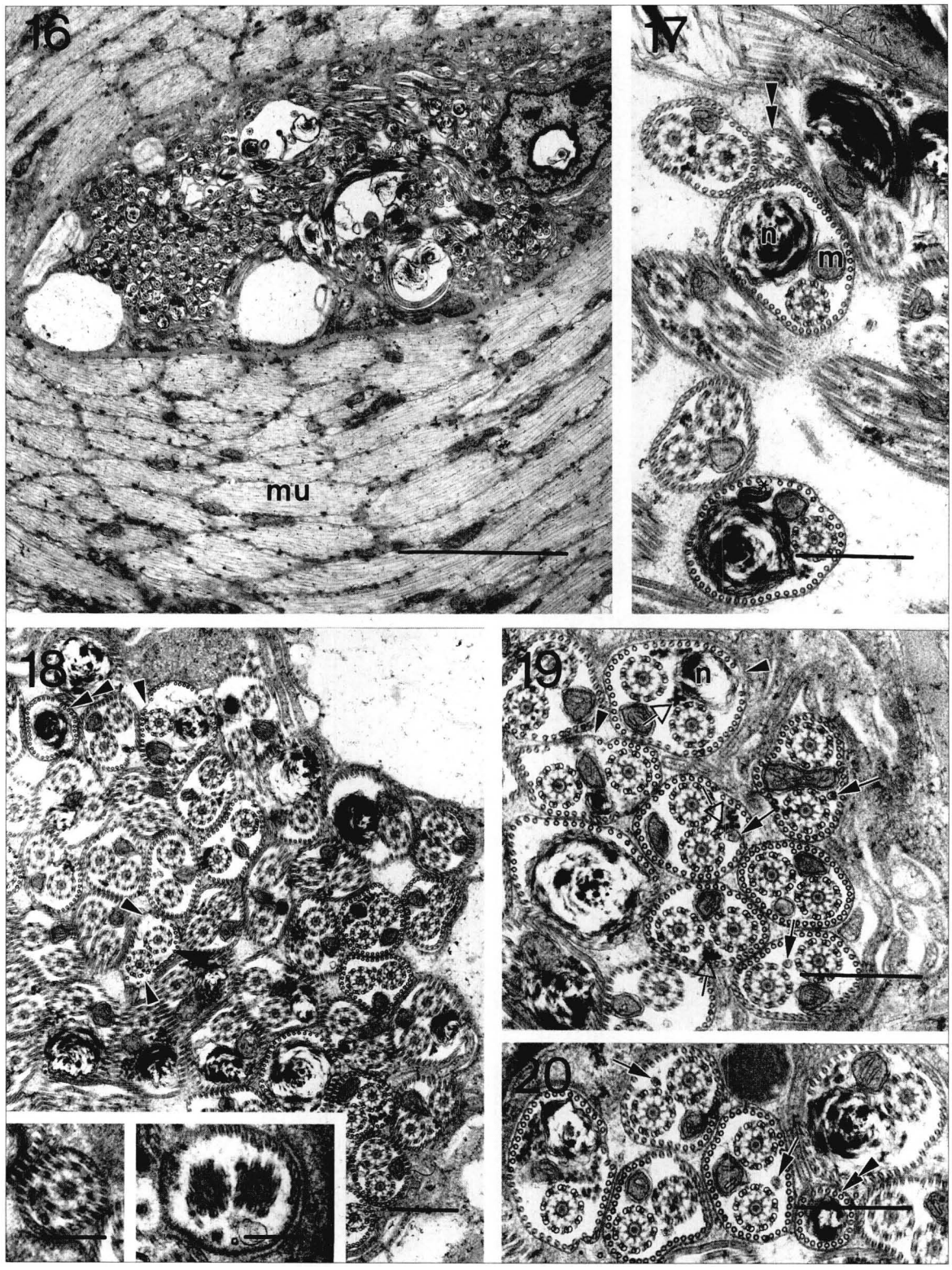
Numbers of peripheral microtubules were 48 and 55 in two middle sections (1 axoneme, mitochondrion, nucleus), 44 and 48 in two middle sections (2 axonemes, mitochondrion, nucleus), 23 in a section containing only the nucleus, 16 peripheral and 6 internal ones in a section without axonemes, nucleus and mitochondrion, 40 in a section with 2 axonemes and mitochondrion, and 22 in a section with 2 axonemes only. A reconstruction of the mature spermatozoan based on cross-sections through the various regions is illustrated in Fig. 21.

DISCUSSION

The process of spermiogenesis, characterized by the outgrowth of a cytoplasmic protuberance, the zone of differentiation, containing two basal bodies separated by an intercentriolar body from which flagella grow out that later fuse with the median process, corresponds to that described for other polyopisthocotylean monogeneans (Tuzet and Ktari 1971a, Halton and Hardcastle 1976; Justine 1985, 1991a, b, 1992, 1993; Schmahl and Obiekezie 1991). Justine (1991a,b) has called the fusion of flagella with the median process beginning near the basal bodies and proceeding towards the flagellar tips, proximo-distal fusion. It is synapomorphic for the original Neodermata (Aspidogastrea, Digenea, Monogenea Polyopisthocotylea). The nucleus suppo-



Figs. 12-15. Spermiogenesis of *Polylabroides*. Fig. 12. Spermatids connected to a central cytophore at bottom and cross-sections through spermatozoa or spermatids at the top. Note that almost all sections through the sperm contain only a single incorporated axoneme; the only sections with two axonemes (one containing a nucleus and mitochondrion, one a mitochondrion, and one only the axonemes) are indicated by arrowheads. Fig. 13. Early spermatids or spermatocytes with two nucleoli (arrowheads) each. Fig. 14. Sperm duct containing sperm, note two incorporated axonemes visible in most sections, some sperm very large in cross-section indicating that even in the sperm duct some sperm are not fully mature, or that they degenerate. Fig. 15. Part of sperm duct with abnormally large sperm and nucleus in duct wall. Scale bars 2 µm.



Figs. 16-20. Sperm of *Polylabroides*. Fig. 16. Sperm duct surrounded by many muscle fibres; note that some sperm are abnormally large in cross-section (degenerate, artefact or not fully mature). Fig. 17. Cross-sections through sperm in sperm duct; note nucleus, mitochondrion, one or two incorporated axonemes, and complete row of peripheral microtubules; also note tip of sperm (double arrowhead) with peripheral and some internal microtubules, and lamellate body (asterisk). Scale bars 0.5 µm. Figs. 18-20. Cross-sections through sperm in sperm duct. Note row of peripheral microtubules continuous in most but discontinuous in some sections of nuclear region (arrowheads); two axonemes in most but a single axoneme in some sections; parts of sperm with nucleus and without axonemes (double arrowheads); parts of sperm without nucleus and mitochondrion but containing two axonemes (large arrowhead); parts of sperm with two axonemes, a mitochondrion and a small elongate body (arrows) opposite the mitochondrion or displaced to one side by it (Fig. 19 right), sometimes beside a cluster of electron-dense particles (glycogen?) which also extends along part of the nucleus (open arrows); part of sperm with two basal bodies (Fig. 18 inset) and with a single axoneme surrounded by peripheral microtubules (Fig. 18 inset). Scale bars 0.5 µm.

sedly lies in the distal end of sperm, whereas the mitochondrion supposedly lies in the proximal end. Our observations have shown that the mitochondrion migrates first and is located in the distal tip of the median cytoplasmic process before migration of the nucleus begins. Justine (1992) refers to migration of mitochondrion and nucleus without mentioning which migrates first in the polyopisthocotylean *Atriaster* sp., although he illustrates cross-sections through median cytoplasmic processes that contain only a mitochondrion. During its migration into the median process, the nucleus apparently "overtakes" the mitochondrion which accounts for the more distal location of the nucleus in mature sperm, as indicated by our observation that basal bodies of the axonemes in sperm from sperm ducts are located at the end which does not contain the nucleus. The spermatozoon of *Polylabroides* corresponds to that of most other polyopisthocotylean monogeneans examined in its main features: two axonemes incorporated in the sperm body and a more or less complete row of peripheral microtubules. Differences are the lack of lateral microtubules in *Axine* and *Pterinotrema*, presence of an undulating membrane in *Gotocotyla*, lack of axonemes and presence of many internal microtubules and mitochondria in *Diplozoon* (Justine 1991a), presence of two separate tips each with one axoneme in *Protomicrocotyle* (Schmahl and Obiekezie 1991), and presence of surface ornamentations for instance in *Pseudomazocraes* and *Microcotyle* (Justine and Mattei 1985a; Schmahl and Obiekezie 1991). In spite of these relatively minor differences, our study confirms again that the ultrastructure of sperm and spermiogenesis in the Monogenea Polyopisthocotylea is remarkably uniform, lending strong support to the view that the group is monophyletic and distinctly separated from the Monogenea Monopisthocotylea. The aberrant sperm structure and spermiogenesis of *Diplozoon* must be considered to be autapomorphic for the genus, a consequence of its unique reproductive biology: permanent fusion of two worms and exchange of spermatozoa through the fused genital ducts (Justine et al. 1985b).

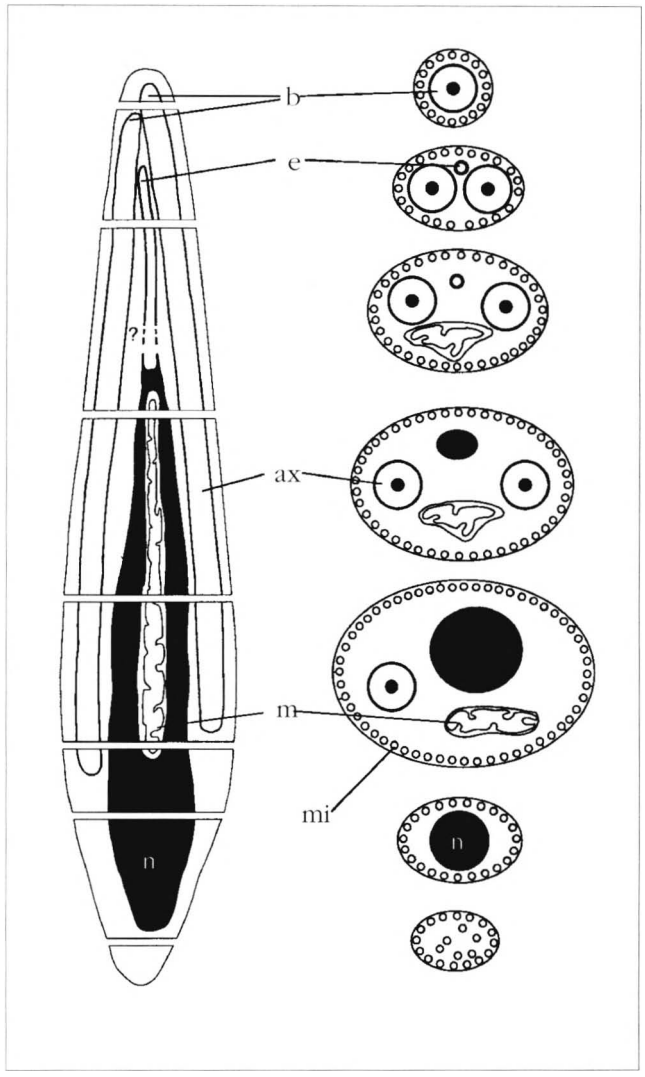


Fig. 21. Diagrams of cross-sections through mature sperm at different levels. Note: we could not find longitudinal sections that show the transition of the nucleus proper to the "extension", but the finding that sections through the "extension" were observed only in those without the nucleus proper, suggests that "extension" and nucleus proper are indeed continuous.

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