

Fine structure of spermatogenesis in monopisthocotylean monogeneans (*Macrogyrodactylus polypteri*; *Pseudodactylogyrus bini*)

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Abstract. The development of spermatozoa in the monopisthocotylean fish gill flukes, *Macrogyrodactylus polypteri* Malmberg and *Pseudodactylogyrus bini* Kikuchi was investigated by means of light and transmission electron microscopy. In *M. polypteri* spermatogonia give rise by successive mitoses to primary spermatocytes clearly characterized by the presence of synaptonemal complexes. In *M. polypteri*, secondary spermatocytes are characterized by a fine, dense intranuclear inclusion connected by a small strand to the nuclear membrane, and by the appearance of several Golgi complexes. Both in *M. polypteri* and *P. bini* a syncytial mass of spermatids develops, which gives rise to 32 filiform spermatozoa. Mature spermatozoa of *M. polypteri* have two axonemes, whereas *P. bini* has only one. In contrast to *P. bini*, spermatozoa of *M. polypteri* have a region with a lateral protrusion, formed by a fold of the spermatozoan membrane. The outer edge of the protrusion surrounds the mitochondrion. The observation that in *M. polypteri*, as well as in *P. bini*, the immature spermatozoa are each situated within ring-shaped cytoplasmic canals in the syncytial spermatids is comparable to findings in other monopisthocotylean species.

Compared with the large number of taxonomic descriptions of new taxa of Monogenea, only relatively few attempts have been made to study the ultrastructure of their organs. Among the various tissues, specific attention has been paid to the structure of the mature spermatozoa, and, in part, to spermatogenesis (cf. Tuzet and Ktari 1971b; Halton and Hardcastle 1976, Kritsky 1976, Justine and Mattei 1982, 1983a, b, c, 1984a, b, 1986, 1987, Malmberg and Afzelius 1990, Tappenden and Kearn 1990, 1991, Schmahl and Obiekezie 1991).

Rohde (1980) and Justine et al. (1985) pointed out that two simple characteristics may be used to evaluate the relationships between the monogenean families as well as with other plathelminths, i.e. a) the number of axonemes, and b) the presence or absence of cortical microtubules.

The fine structure of the fertile spermatozoa in *Pseudodactylogyrus anguillae* has been described by Le Brun et al. (1986). The present study describes the maturation of the spermatozoa in two monopisthocotylean flukes, *Macrogyrodactylus polypteri* and *Pseudodactylogyrus bini*.

MATERIALS AND METHODS

Material. Specimens of *Macrogyrodactylus polypteri* were removed from the skin of *Polypterus senegalus* caught near Khartoum, Sudan. Mature specimens of *Pseudodactylogyrus bini* were collected from naturally infected European eels (*Anguilla anguilla*) obtained from commercial trade companies.

Methods. The parasites were fixed in 5% (v/v) glutaraldehyde buffered with 0.1 M sodium cacodylate, pH = 7.4 at 4 °C for 24 h. Postfixation was done for 1 h in 2% OsO₄ in the same buffer. For light and transmission electron microscopy, the specimens were dehydrated in graded ethanol, transferred to propylene oxide, and embedded in Araldite. Araldite sections were stained with methylene blue and Azur A (1:1) for light microscopy and with uranyl acetate and lead citrate for transmission electron microscopy (TEM). Ultrathin sections were examined under a ZEISS EM 9 S 2 electron microscope.

RESULTS

Light microscopic observations

In *M. polypteri* and in *P. bini*, the testis is situated in the midregion of the body. In sexually mature specimens of *M. polypteri* all developmental stages were detectable within the male system, whereas in *P. bini* differentiating spermatids and fully developed spermatozoa were observed in the testis only.

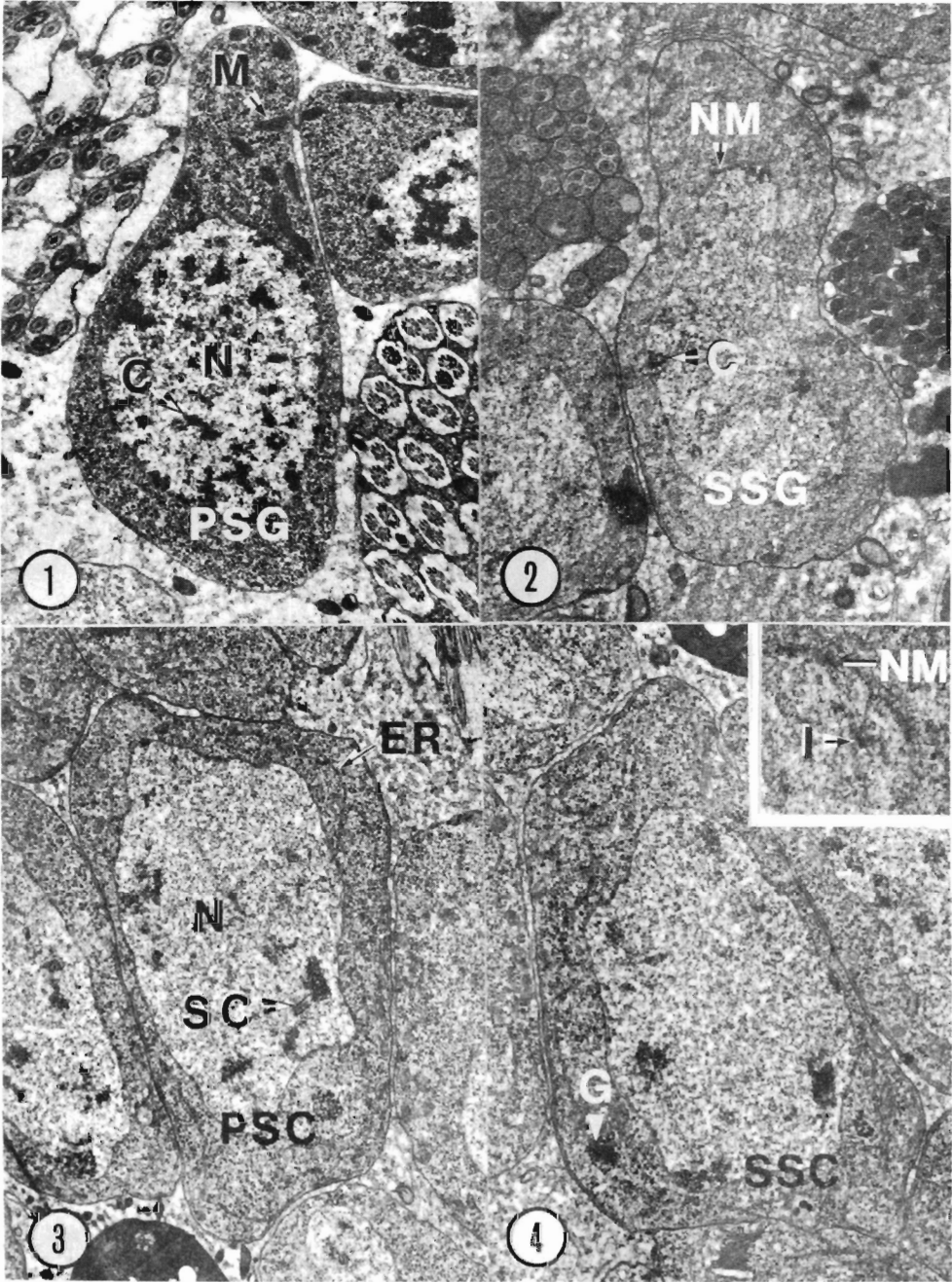
TEM observations

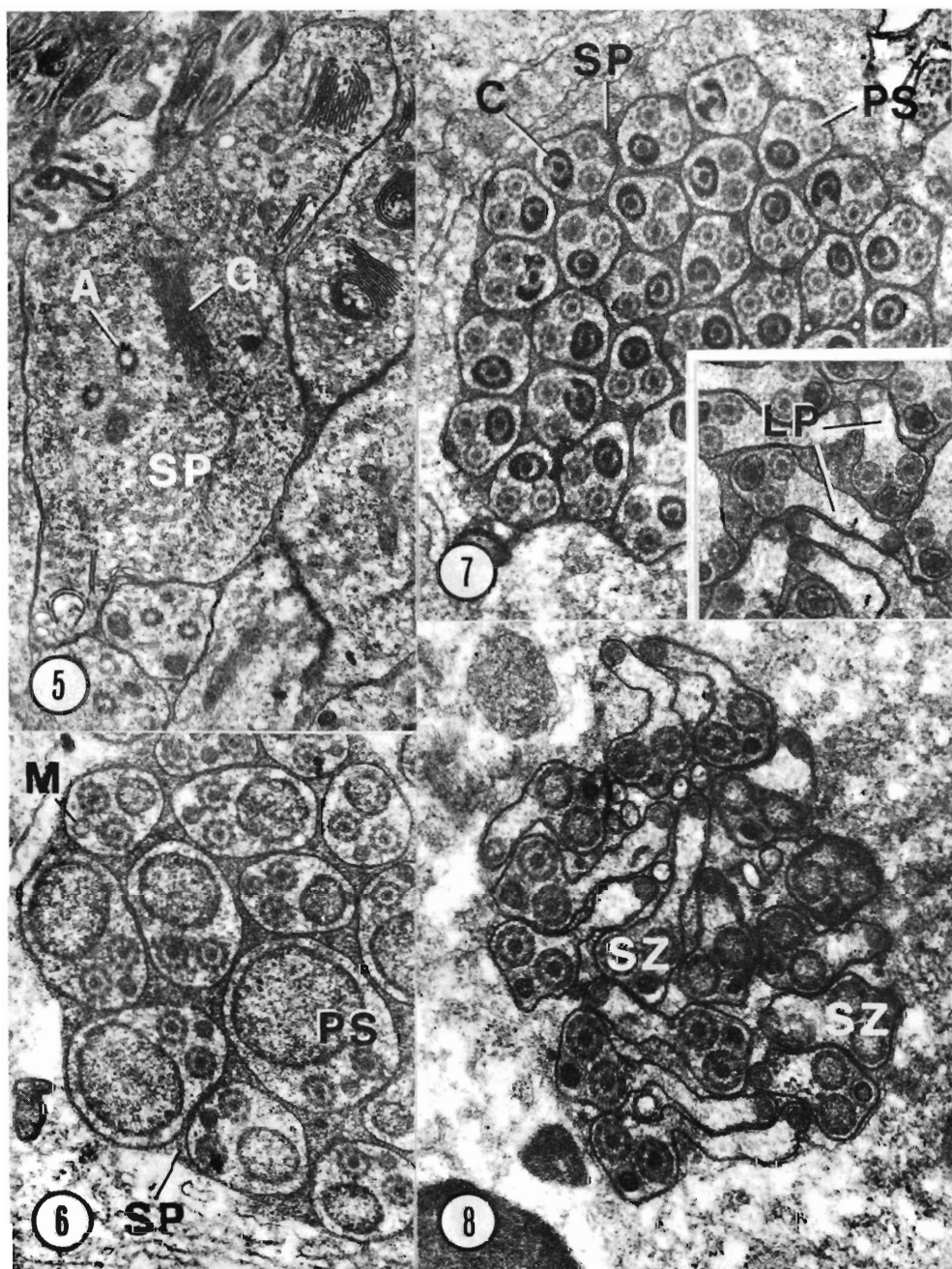
Spermatogonia. In *M. polypteri*, the primary spermatogonia (8 × 4 µm) are pyriform shaped cells often situated near the periphery of the testis (Fig. 1). The ratio of the volume of nucleus to that of the cytoplasm is high. Small aggregations of dense chromatin are frequently observed within the karyoplasm. The bulk of the cytoplasm is occupied by numerous free ribosomes, and mitochondria with small cristae are found at the small end of the spermatogonia. In the secondary spermatogonia of *M. polypteri*, small chromatin aggregations are found adjacent at the inner side of the nuclear membrane (Fig. 2).

Spermatocytes. In *M. polypteri* (Fig. 3) the primary spermatocytes are large cells (about 15 × 8 µm), and they exhibit a greatly increased cytoplasmic to nuclear volume ratio compared to that of the spermatogonia. The primary spermatocytes are clearly identified by the presence of synaptonemal complexes within their nuclei (Fig. 3). Condensed chromatin is scarcely found in small aggregations dispersed throughout the karyoplasm. The spermatocyte's cytoplasm is speckled with ribosomes. Large cavernae of rough endoplasmic reticulum are frequently seen. Small, spherical mitochondria with short cristae are detected in the periphery of the cytoplasm.

Figs. 1–4. Spermiogenesis in *M. polypteri*. Transmission electron micrographs. →

Fig. 1. Longitudinal section through a primary spermatogonium (PSG). Note the small chromatin aggregations (C) within the nucleus (N). Mitochondria (M) are located at one end of the spermatogonium. × 8,700. **Fig. 2.** Secondary spermatogonium (SSG). Note the small chromatin aggregations (C) at the inner side of the nuclear membrane (NM). × 5,400. **Fig. 3.** Primary spermatocyte (PSC). Note the synaptonemal complex (SC) within the nucleus (N); endoplasmic reticulum (ER). × 4,700. **Fig. 4.** Secondary spermatocyte (SSC). Note the Golgi complex (G). × 8,400. Inset: Note the small inclusion (I) within the nucleus. × 46,000.





In *M. polypteri*, clusters of secondary spermatocytes (Fig. 4) are found within the testicular lumen. In certain places, a dense inclusion is observed within the nucleus, which is connected by a small strand to the nuclear membrane (Fig. 4, inset). In contrast to the findings in the primary spermatocytes, Golgi complexes are frequently detected within the secondary spermatocytes. The other ultrastructural features of the secondary spermatocytes are similar to those within the primary ones.

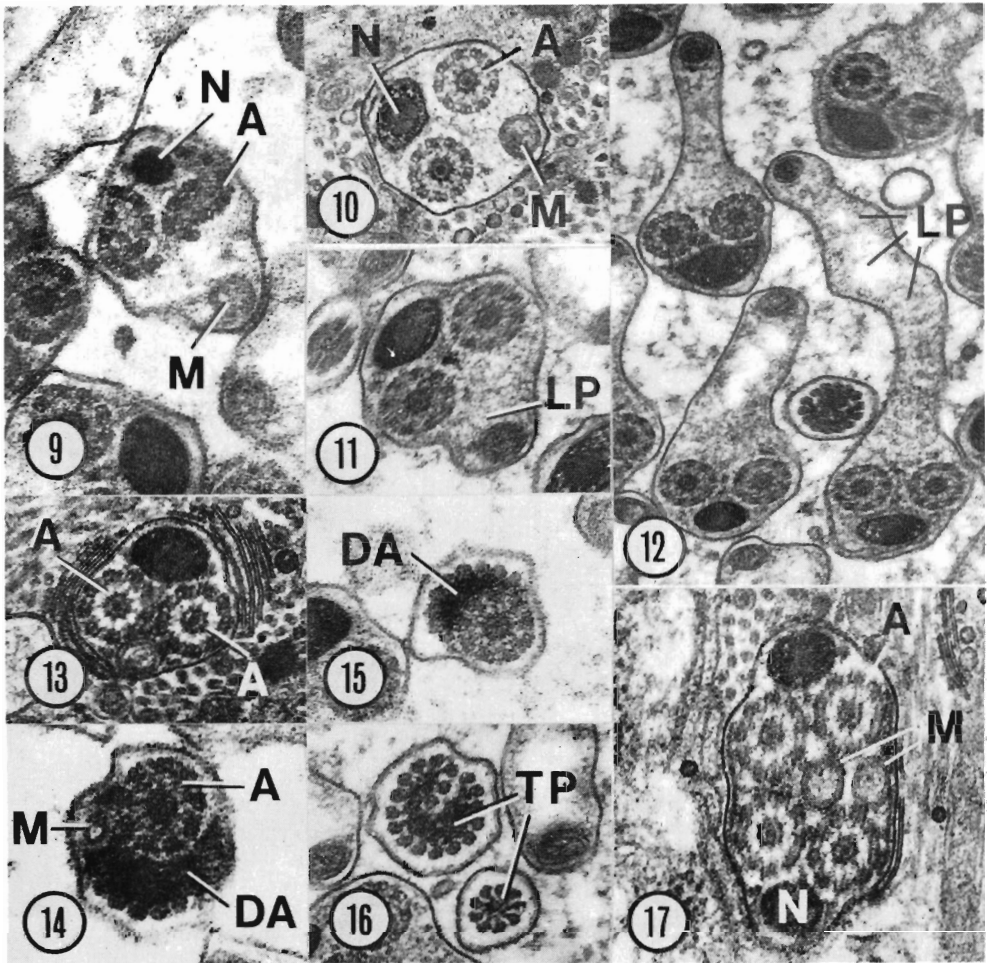
In *M. polypteri*, the early spermatids form a syncytial mass (Fig. 5). Moreover, in the early stage of differentiation, at certain places profiles of paired axonemes associated with one mitochondrion are observed within the syncytial cytoplasm. Strands of the rough endoplasmic reticulum are evident as clusters of free ribosomes. Several prominent Golgi complexes are also visible. Crescent-shaped profiles of ER-cisternae occur in the differentiation zone of the membrane which, in the proceeding development, limit the immature spermatozoa (see also Fig. 6).

In an advanced stage of differentiation, the immature spermatozoa appear as clusters of closely associated cells, which are in close contact with the residual cytoplasm of the spermatids (Fig. 6). That part of the spermatid cytoplasm, which is not incorporated into the future sperm cell, appears more electron dense compared to that of the immature spermatozoa (Figs. 6, 7). Cross sections through the anterior part of the immature spermatozoa reveal the presence of the nucleus, the mitochondrion and two axonemes (Fig. 6). In a more advanced stage, the chromatin becomes more condensed, and the diameter of the nuclei is reduced (Fig. 7). In the posterior region, the immature spermatozoa have a lateral protrusion, which includes at its edge the mitochondrion (Fig. 7, inset). Finally, the limiting membrane and the cytoplasm of the spermatid is disintegrated, the immature spermatozoa still remain in a cluster-like association (Fig. 8).

In *P. bini*, the spermatids form a spherical cytoplasmic mass (about $16 \times 10 \mu\text{m}$). The 32 nuclei, provided either with a clear and granular karyoplasm, or with an electron dense karyoplasm, can be found along the periphery of the spermatid (Fig. 18). Eighteen to twenty-four microtubules are closely associated to each nucleus. The association of a nucleus with a single mitochondrion indicates the start of spermatozoon differentiation. The syncytial cytoplasm of the spermatid is filled up with a large rough endoplasmic reticulum. Numerous cristate mitochondria are situated in the periphery of the spermatid cytoplasm.

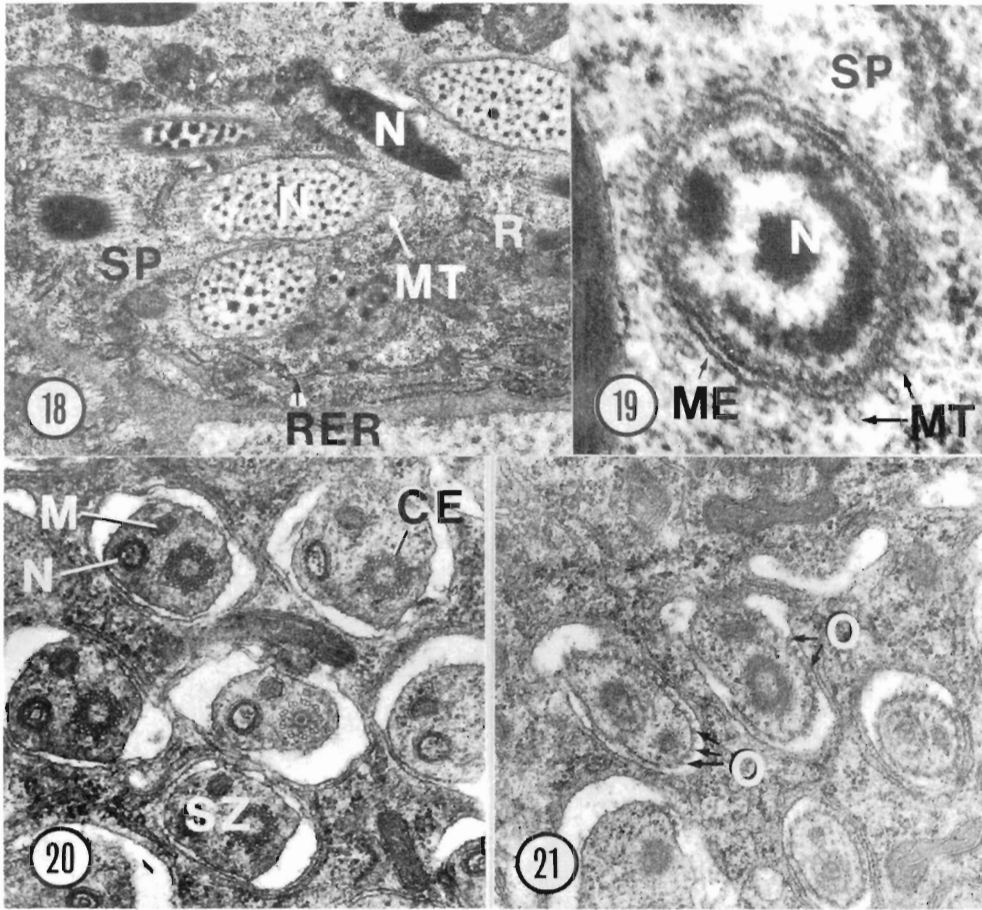
← Figs. 5–8. Spermiogenesis in *M. polypteri*. Transmission electron micrographs.

Fig. 5. Early spermatid (SP). Note the prominent Golgi complex (G), and the bases of the axonemes (A). $\times 16,900$. **Fig. 6.** Spermatid (SP) with differentiating immature spermatozoa (PS). Note the large nuclei, mitochondrion (M). $\times 19,500$. **Fig. 7.** Anterior region of late spermatids (SP). The chromatin (C) in the prestage spermatozoa (PS) is condensed. $\times 17,600$. Inset: Mid region of a late spermatid. Lateral protrusion (LP) of immature spermatozoa. $\times 15,500$. **Fig. 8.** Cluster of spermatozoa (SZ) freed of the surrounding cytoplasm of the spermatid. $\times 28,200$.



Figs. 9–17. Spermiogenesis in *M. polypteri*. Transmission electron micrographs.

Fig. 9. Mature spermatozoon. Anterior head region. Note the origins of the two axonemes (A), the nucleus (N), and the mitochondrion (M). $\times 39,200$. **Fig. 10.** Mature spermatozoon. Middle head region. Axoneme (A), mitochondrion (M), nucleus (N). $\times 69,000$. **Fig. 11.** Mature spermatozoon. Anterior part of the middle region. Note the small cone-shaped lateral protrusion (LP). $\times 40,000$. **Fig. 12.** Mature spermatozoa. Mid-part of the middle region. Note the large lateral protrusions (LP). $\times 35,300$. **Fig. 13.** Mature spermatozoon. Posterior part of the middle region. The axonemes (A) are situated close together. $\times 55,600$. **Fig. 14.** Mature spermatozoon. Anterior part of the terminal region. Note one complete axoneme (A), the disintegrated second axoneme (DA), and the tip of the mitochondrion (M). $\times 68,000$. **Fig. 15.** Mature spermatozoon. Middle part of the tail region. Note the disintegrated second axoneme (DA). $\times 48,300$. **Fig. 16.** Mature spermatozoon. Terminal part of the tail region. Note the terminal part (TP) of the axoneme. $\times 53,400$. **Fig. 17.** Abnormal spermatozoon with a double set of axonemes (A), mitochondria (M) and nuclei (N). $\times 63,200$.

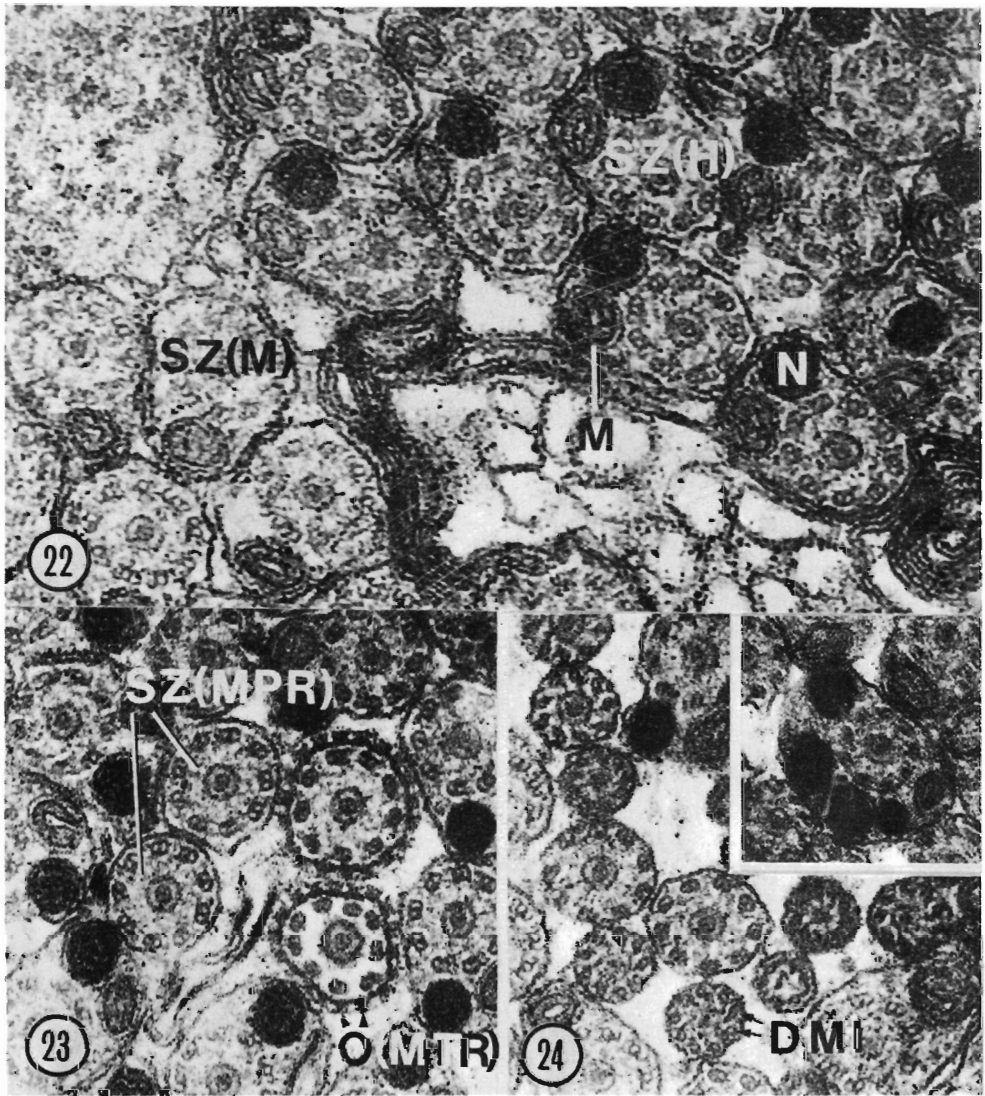


Figs. 18–21. Spermiogenesis in *P. bini*. Transmission electron micrographs.

Fig. 18. Cross section through an early spermatid (SP) of *P. bini*. Note the nuclei (N) with the adjacent microtubules (MT), the rough endoplasmic reticulum (RER), and the numerous ribosomes (R). $\times 16,500$. **Fig. 19.** Early spermatid (SP). Note the nucleus situated in an invagination limited by a membrane (ME). Note also the microtubules (MT) adjacent to the membrane. $\times 78,000$. **Fig. 20.** Late spermatid. Cross section through the anterior region. The immature spermatozoa (SZ) are situated in deep invaginations. Centriole (CE), mitochondrion (M), nucleus (N). $\times 19,800$. **Fig. 21.** Late spermatid. Cross section through a more posterior region. The membrane lining the microtubules bears ornamental structures (O). $\times 34,500$.

In the early phase of differentiation of the cytoplasmic canals, within which the immature spermatozoa are included, the membrane limiting the canal has several protuberances which each faces one microtubule. Within the canal the nucleus is visible (Fig. 19).

In a later stage of differentiation, the immature spermatozoa are each situated in ring-shaped cytoplasmic canals (Fig. 20). Large electron lucent interspaces separate the immature spermatozoa from the border of the cytoplasmic canals.



Figs. 22-24. Spermiogenesis in *P. bini*. Transmission electron micrographs.

Fig. 22. Mature spermatozoa (SZ). Head region, SZ(H): Each spermatozoon has one axoneme one nucleus (N), and a single mitochondrion (M). - Anterior middle region, SZ(M): The mitochondrion is longer than the nucleus. $\times 48,500$. **Fig. 23.** Mature spermatozoa. Cross sections at various levels. In the mid-part of the tail region (MTR), ornamentations (O) were found in the limiting membrane of the spermatozoa. In the posterior region (MPR), the limiting membrane lacks the ornamentations. $\times 47,400$. **Fig. 24.** Mature spermatozoa. Posterior part of the terminal region. Note the nine microtubule doublets (DMI). $\times 48,000$.

Inset: aberrant spermatozoon with four nuclei. $\times 38,000$.

Cross sections through the immature spermatozoa reveal a nucleus with an electron lucent central zone bordered by a dark core which is surrounded by another lucent ring. This ring alters again with a dark outer zone. A single mitochondrion and a single axoneme showing the 9 + "1" pattern are recognizable (Fig. 20). In the periphery of the spermatid cytoplasm cristate mitochondria and Golgi complexes are apparent. Towards the zone of differentiation, 18–22 microtubules are found closely associated with the membrane of the immature spermatozoa (Fig. 21). The membrane lining the microtubules has ornamentations at its outer surface.

Spermatozoa. In both monopisthocotyleans the mature spermatozoa can be subdivided into the head region, the middle region and the terminal region.

Head region. In *M. polypteri*, cross sections immediately posterior to the apex of mature spermatozoa reveal the origin of both axonemes, which consist of nine pairs of microtubules. The paired microtubules of each axoneme are arranged in a S-shaped configuration and encircle an electron-dense area filled up with fuzzy particles (Fig. 9). A single mitochondrion is situated opposite to the nucleus. In the posterior region, two fully developed parallel axonemes become visible (Fig. 10). The oval-shaped nucleus has an electron dense central zone (probably of condensed chromatin) and a moderate electron light peripheral zone. The spherical mitochondrion shows only a single cristate infolding. In *P. bini*, cross sections through the head region of the spermatozoa revealed the presence of a single axoneme, the mitochondrion and the nucleus (Fig. 22). The mitochondrion and the nucleus are situated close together. In both species the axonemes observed belong to the 9 + "1" type, which is typical for the Trepaxonemata.

Middle region. In the more anterior part of the middle region, in *M. polypteri* a lateral protrusion bearing the mitochondrion at its edge becomes visible (Fig. 11). More posterior, this protrusion is enlarged about three times compared to the diameter of the remaining spherical portion of the spermatozoon (Fig. 12). At the end of the middle region, the protrusion is reduced. Finally, cross sections through the most posterior part of the middle region closely resemble to that of the head region, however, the two axonemes are closely adjacent to each other (Fig. 12). In *P. bini*, the mitochondrion extends for a longer distance into the anterior middle region than the nucleus (Fig. 22).

Terminal region. In the anterior part of the terminal region, one can observe in *M. polypteri* that one axoneme is complete. Also, remnants of the disintegrated second one are arranged in a crescent-like configuration (Fig. 14). The mitochondrion is still visible as a tip. In the posterior region, the complete axoneme and the remnants of the disintegrated second one are only recognizable in cross sections (Fig. 15). Very close to the terminal tip of the spermatozoon's tail is where elements of the terminal part of the axoneme can be seen (Fig. 16). In *M. polypteri*, aberrant mature spermatozoa which have a double set of nuclei, as well as mitochondria and axial filaments are occasionally observed (Fig. 17).

In *P. bini*, ornamentations occur along the limiting membrane of the spermatozoon in the anterior part of the terminal region (Fig. 23). The axoneme is still

recognizable, but the mitochondrion has disappeared. In the posterior part of the terminal region, the diameter of the spermatozoon's tail is reduced (Fig. 24). Nine doublet microtubules make up the terminal tip of the axoneme (Fig. 24). Finally, at places where aberrant spermatozoa containing 4 nuclei can be found, only one axoneme and one mitochondrion (Fig. 24, inset).

A diagrammatic interpretation of the mature spermatozoa of *M. polypteri* and *P. bini* is given in Fig. 25.

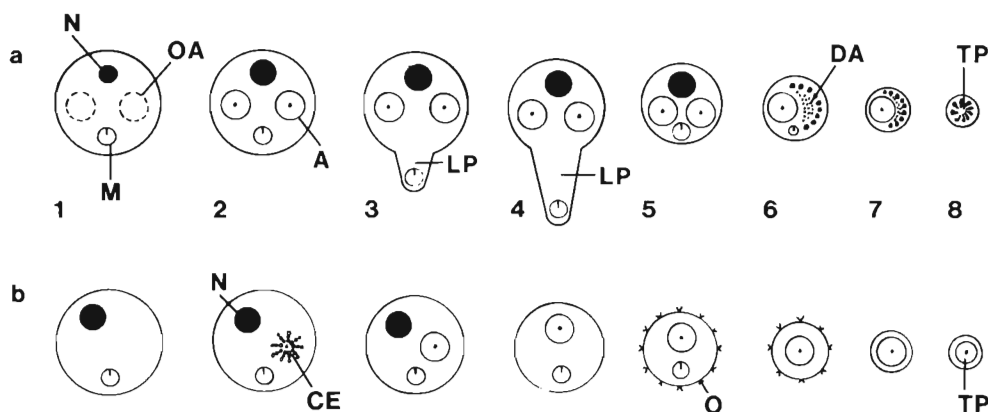


Fig. 25a, b. Diagrammatic interpretation of the mature spermatozoa of a) *Macrogyrodactylus polypteri* and b) *Pseudodactylogyrus bini* (1), Anterior head region; (2), Middle head region; (3), Anterior part of the middle region; (4), Mid-part of the middle region; (5), Posterior part of the middle region; (6), Anterior part of the terminal region; (7), Middle part of the tail region; (8), Terminal part of the tail region.

(A), Axoneme; (CE), Centriole; (DA), Disintegrated axoneme; (LP), Lateral protrusion; (M), Mitochondrion; (N), Nucleus; (O), Ornamentation in the limiting membrane of the spermatozoon; (OA), Base of axoneme; (TP), Terminal part of the axoneme.

DISCUSSION

The results in the present study reveal that the cytological events and the development in spermiogenesis of both monopisthocotylean species, *Pseudodactylogyrus bini* and *Macrogyrodactylus polypteri*, are in agreement with the general pattern so far known from other monopisthocotyleans, but are clearly different from that in polyopisthocotyleans. In *M. polypteri*, the spermatogonium gives rise by 2 mitoses to 4 spermatogonia II, which divide to form 8 primary spermatocytes. These undergo the first meiotic division to produce 16 secondary spermatocytes. By the second part of meiosis 32 spermatids are formed, where each develops into a fertile spermatozoon. The spermatogonia of *M. polypteri* have no peculiar cytoplasmic structures, and are thus similar to other monogenean species (Halton and Hardcastle 1976). In the spermatid stage, Golgi complexes are apparent in both species.

The occurrence of such Golgi complexes has been reported in similar stages of the polyopisthocotylean species *Microcotyle mormyri*, *Diclidophora merlangi* and *Protomicrocotyle ivoriensis* (Tuzet and Ktari 1971; Halton and Hardcastle 1976; Schmahl and Obiekezie 1991). The finding that in *M. polypteri* and in *P. bini* each immature spermatozoon was situated deeply within canals of the spermatid syncytium corresponds to observations in other monopisthocotyleans such as *Heterocotyle* sp., *Megalocotyle* sp., *Diplectanum* sp. and *Calceostoma* (Justine and Mattei 1983a, b; 1984b; 1986). In digeneans, the maturing zone of the spermatid juts out from the cytoplasmic mass (Sato et al. 1967; Burton 1972; Grant et al. 1976; Fujino et al. 1977; Rees 1979; Justine and Mattei 1982a, b) as it does also in the polyopisthocotyleans *M. mormyri*, *D. merlangi*, *P. ivoriensis*, and *Gastrocotyle* sp. (Tuzet and Ktari 1971a; Halton and Hardcastle 1976; Schmahl and Obiekezie 1991). In *P. bini*, neither inter-centriolar bodies nor striated rootlets are found within the differentiation zone of the spermatid. This is also the case in the monogenean *Megalocotyle* sp. (Justine and Mattei 1983a).

In contrast to *P. bini*, in *M. polypteri* the developing immature spermatozoa are not separated by interspaces from the spermatid cytoplasm. An interesting peculiarity in the immature spermatozoa as well as in the mature spermatozoa of *M. polypteri* is the lateral protrusion seen in cross sections. The function of this structure remains unclear. A similar structure but with cortical microtubules and acting as an undulating membrane has been reported in the polyopisthocotylean *Gotocotyla acanthura* (Justine and Mattei 1985).

Cross sections of mature spermatozoa of *P. bini* revealed the presence of one nucleus, one mitochondrion and one axoneme of the 9 + "1" type, similarly as it has been described for *P. anguillae* by Le Brun et al. (1986). In *M. polypteri*, two axonemes were found. Both species did not possess cortical microtubules within their spermatozoa. Spermatozoa with one axoneme and without cortical microtubules have been reported for members of the monopisthocotylean families Ancyrocephalidae, Calceostomatidae, Diplectanidae and Amphibdellatidae (Justine et al. 1985). Spermatozoa with two axonemes and without cortical microtubules have been found in Dionchidae, Capsalidae, Udonellidae, Gyrodactylidae, Acanthocotylidae, and *Euzetrema* sp. (Justine et al. 1985; Malmberg and Afzelius 1990).

In *P. bini* and in *M. polypteri* the axonemes show the 9 + "1" a typical pattern for the Trepaxonemata (sensu Ehlers 1985), i.e. in turbellarians (Silveira and Porter 1964), in digenetic trematodes (Shapiro et al. 1961; Hershenov et al. 1966; Burton 1967), in aspidogastrid trematodes (Rohde 1971), in monogeneans (Tuzet and Ktari 1971a, b; Halton and Hardcastle 1976; Justine et al. 1985; Malmberg and Afzelius 1990; Tappenden and Kearn 1990, 1991; Schmahl and Obiekezie 1991), and also in cestodes (Rosario 1964; Morseth 1969).

The sperm patterns found in both species that were investigated confer with the patterns described for the monopisthocotylean monogeneans, but they are significantly different from the standard pattern in polyopisthocotyleans, where two axonemes and cortical microtubules are the rule.

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