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SPERMATOGENESIS OF *HAPLOPHARYNX ROSTRATUS* (PLATYHELMINTHES, HAPLOPHARYNGIDA)

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Abstract. During spermatogenesis, nuclei of spermatids are at first large with an irregular outline, containing chromatin granules; then condense to become round containing dense chromatin; then elongate containing loose fibrillar chromatin. Golgi bodies produce dense granules. Immature sperm in the testis are amoeboid, aflagellate, with numerous peripheral microtubules. Mature sperm in the sperm duct have a nucleated part with a narrow layer of cytoplasm drawn out into two flanges, and a thicker non-nucleated part. Peripheral microtubules and dense thickenings of the plasma membrane are present in both parts. Two symmetrically arranged blunt bristle-like structures, apparently modified large granules that do not reach the surface, are located in the nucleated part. Sperm structure supports the view that Haplopharyngida and Macrostomida are closely related.

Key words: Platyhelminthes, Haplopharyngida, *Haplopharynx rostratus*, sperm, spermatogenesis, ultrastructure, phylogeny

INTRODUCTION

The Haplopharyngida are usually considered a taxon separate from but close to the Macrostomida (*e.g.*, CANNON, 1986). EHLERS (1985) includes them in the Macrostomida. DOE (1986) found a matrix syncytium of the copulatory stylet in both taxa supporting their monophyly. Not a single electron-microscopic study of sperm and spermatogenesis of a haplopharyngid has been made (WATSON & ROHDE, 1995), whereas several such studies of macrostomids have been published (BEDINI & PAPI, 1970; NEWTON, 1980; ROHDE & WATSON, 1991; ROHDE & FAUBEL, 1997 a, b). In this paper we describe the ultrastructure of sperm and spermatogenesis of *Haplopharynx rostratus* MEIXNER, 1938 with the aim of contributing to a better understanding of the phylogeny of the Platyhelminthes in general and of the Haplopharyngida in particular.

MATERIAL AND METHODS

One specimen of *Haplopharynx rostratus* was collected from the beach near the old Research Station on the Island of Sylt, North Sea, and immediately fixed in 3 % glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2) for about one week at room temperature. It was washed for 40 minutes at 4° C in 0.1 sodium-cacodylate buffer (pH 7.2) made up with seawater, postfixed for 1 hr in 1 % OsO_4 in the same buffer and dehydrated in a graded series of ethanol. It was embedded in Spurr's resin. Serial longitudinal sections were stained with uranyl acetate and lead citrate and examined under a JEOL 1200EX electron microscope at 60 kV.

RESULTS

Primary spermatocytes, clearly characterised by their synaptonemal complexes (Fig. 1 A, B) have large nuclei and light cytoplasm. Cells with much more electron-dense nucleoplasm and distinctly aggregated chromatin, and with dense cytoplasm containing much endoplasmic reticulum (Fig. 1 A) are interpreted as spermatogonia, although the possibility cannot be excluded that they are secondary spermatocytes. The second alternative is not unlikely in view of the well developed endoplasmic reticulum, which is unusual for spermatogonia. Spermatids contain Golgi complexes producing many vacuoles (Fig. 1 C, D), their nuclei are at first large with light nucleoplasm containing many chromatin granules, but later condense and become round, containing many small electron-lucent areas (Fig. 2 A-C). Mitochondria aggregate around the nucleus (Fig. 2 B). Lack of membranes between some nuclei indicates that spermatids form syncytial clusters. At an even later stage of spermiogenesis, the chromatin becomes loosely fibrillar and the cells and nuclei elongate (Fig. 2 D). Sperm in the testis are amoeboid, with many deep invaginations and lobe-like processes (Figs 3 A-C, 4 A). They contain numerous electron-dense granules of a range of sizes (Fig. 5), many of them observed to be lined by distinct membranes (Figs 3 A-E, 4 A). They also contain mitochondria (Figs 3 E, 4 A), a nucleus with fibrillar chromatin (Fig. 3 C, D), aggregations of dense material and more or less electron-lucent vacuoles (Fig. 3 C), and their surface has a densely packed row of peripheral microtubules below the plasma membrane (Figs 3 B, E, 4 A). The plasma membrane has many dense thickenings, some of which at least are artefacts (*e.g.*, Fig. 3 E).

Mature or maturing sperm in the sperm duct have a narrow part containing the dense nucleus with very small lucent spaces, surrounded by a thin layer of cytoplasm with mitochondria and dense granules, and this narrow part is drawn out into two flanges (Figs 4 C, 6 A-E). A row of microtubules is found below the surface membrane (Figs 4 C, 6 A-D). Some microtubules are present below the peripheral row (Figs 4 A, 6 B). The non-nucleated parts of the sperm are much larger in cross-section, and they contain a much greater number of dense granules of various sizes; the surface membrane also has a row of microtubules (Fig. 4 B-D). Two symmetrically located curved and tapering structures (« bristles ») that do not reach the surface and apparently are modified granules, are located in the nucleated part of the sperm close to the nucleus (i.e., not at the tips of the flanges), in the region close to the beginning of the flanges (Figs 6 B, 7 A, B). Dense structures seen once at the tip of the flange (Fig. 6 B) are of unknown nature but may represent a disintegrating granule.

The wall of the sperm duct is formed by cells in contact by septate junctions. Cilia are not densely packed but occur all around the duct (Fig. 4 C, D).

Diagrams of mature sperm are given in Fig. 8.

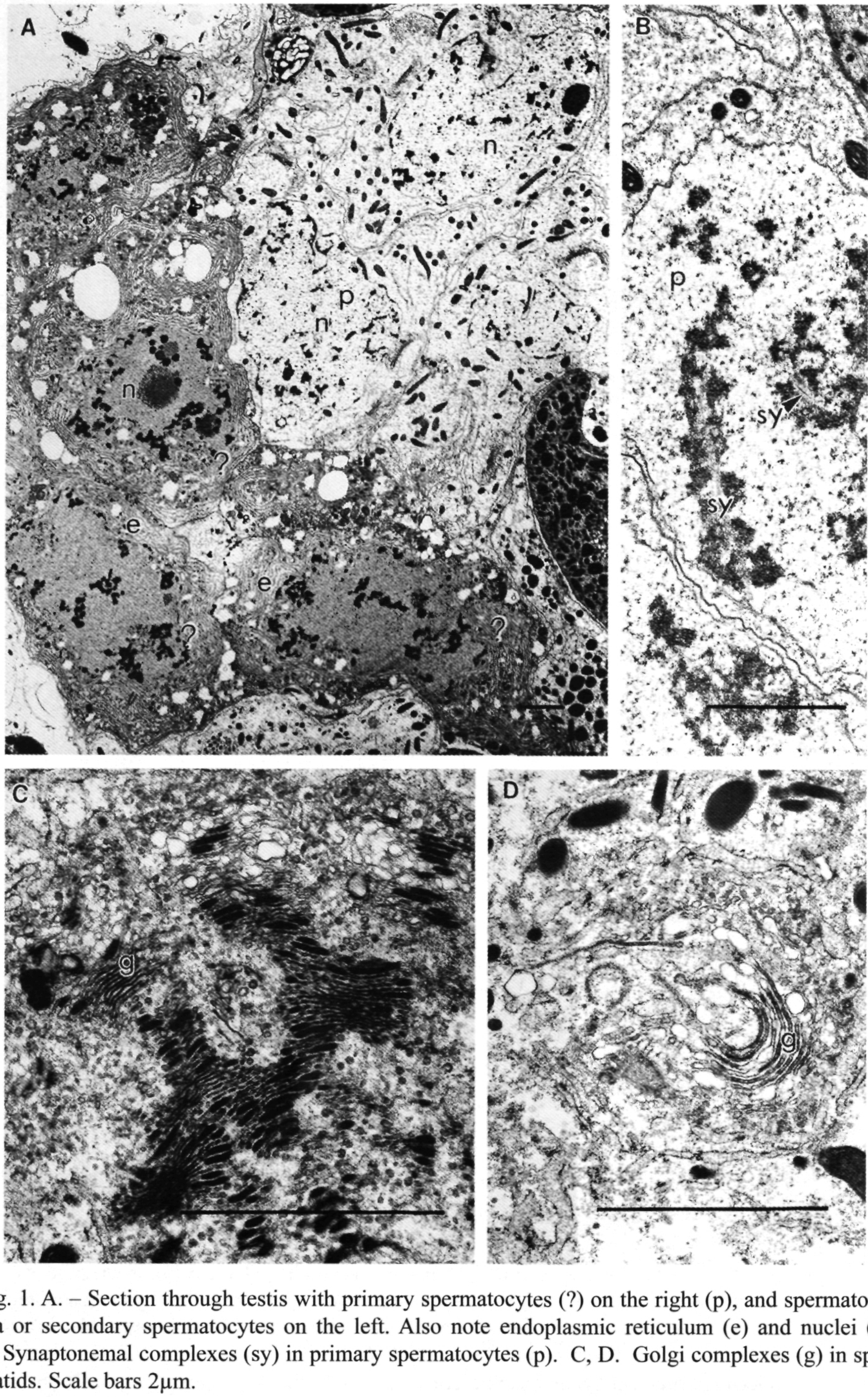


Fig. 1. A. – Section through testis with primary spermatocytes (?) on the right (p), and spermatogonia or secondary spermatocytes on the left. Also note endoplasmic reticulum (e) and nuclei (n). B. Synaptonemal complexes (sy) in primary spermatocytes (p). C, D. Golgi complexes (g) in spermatids. Scale bars 2µm.

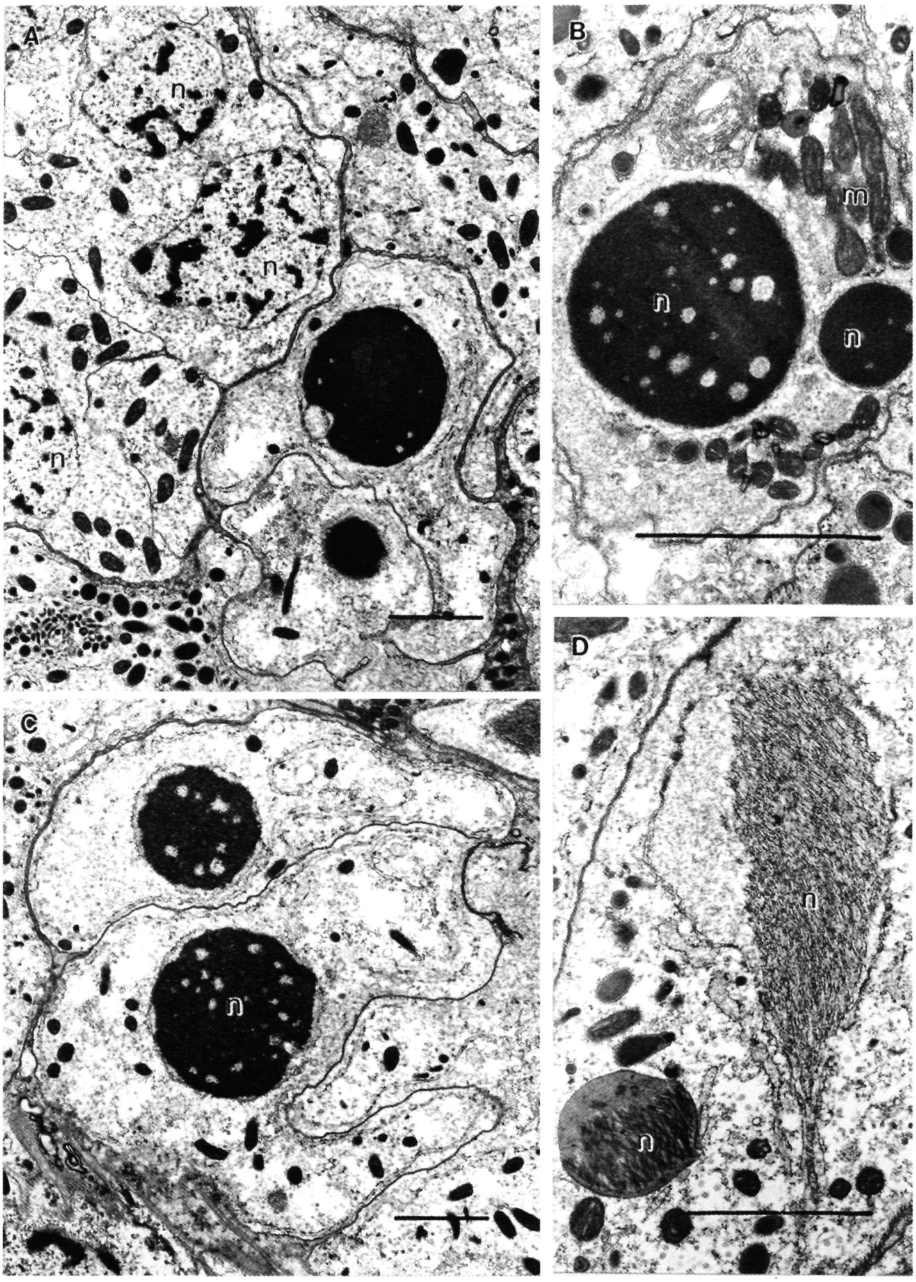


Fig. 2. A. – Section through testis with spermatids containing uncondensed nuclei (n). Note rounded compact nuclei of spermatids on the right. B. Spermatids with rounded nuclei (n) and many mitochondria (m) near them. Note lack of membrane between nuclei. C. Two spermatids with rounded nuclei (n). Note dense chromatin with some electron-lucent areas in B and C. D. Slightly later stage of spermatid. Note nuclei (n) with loose-fibrillar configuration, elongating on right. Scale bars 10μm (A), 2μm (B-D).

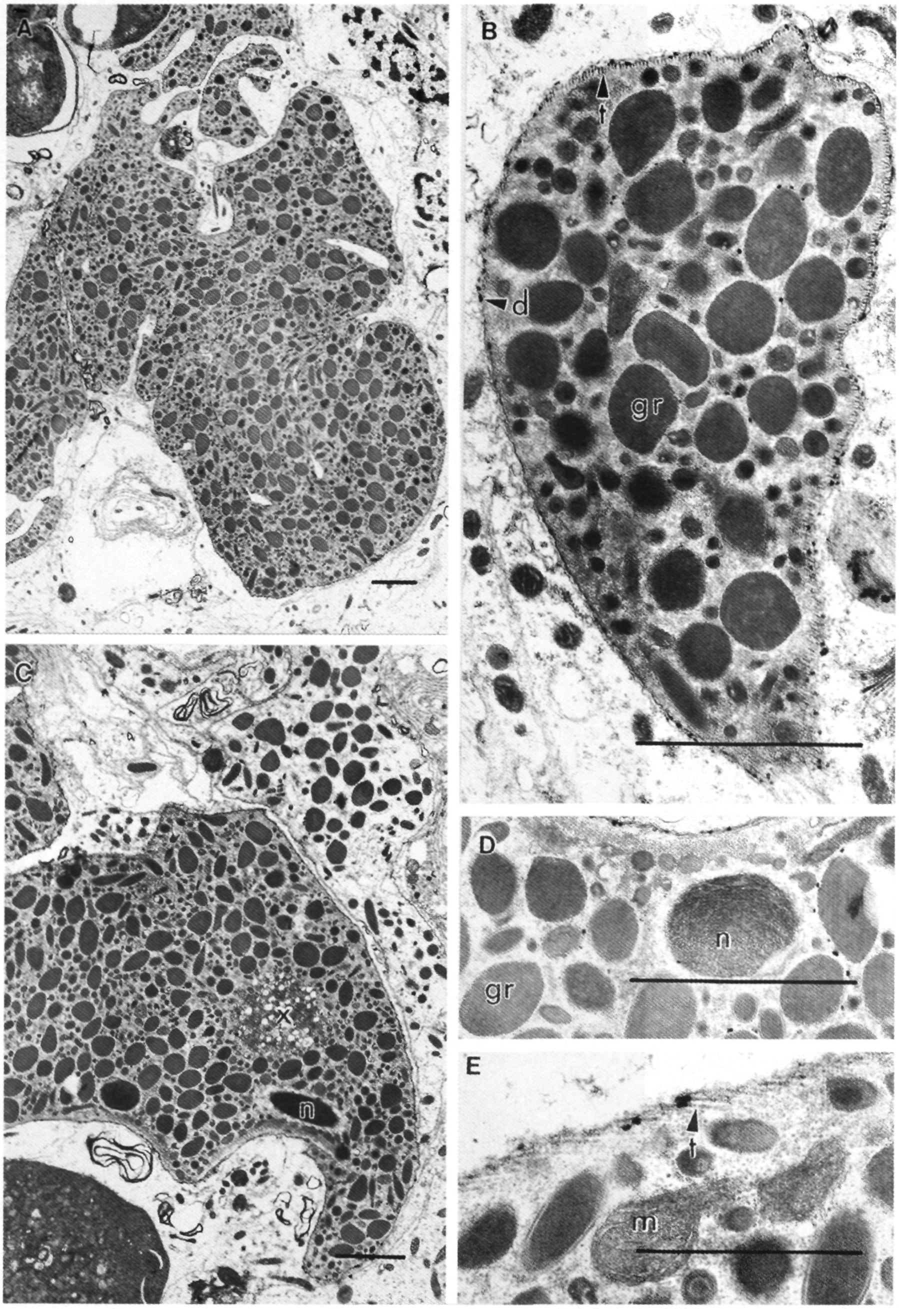
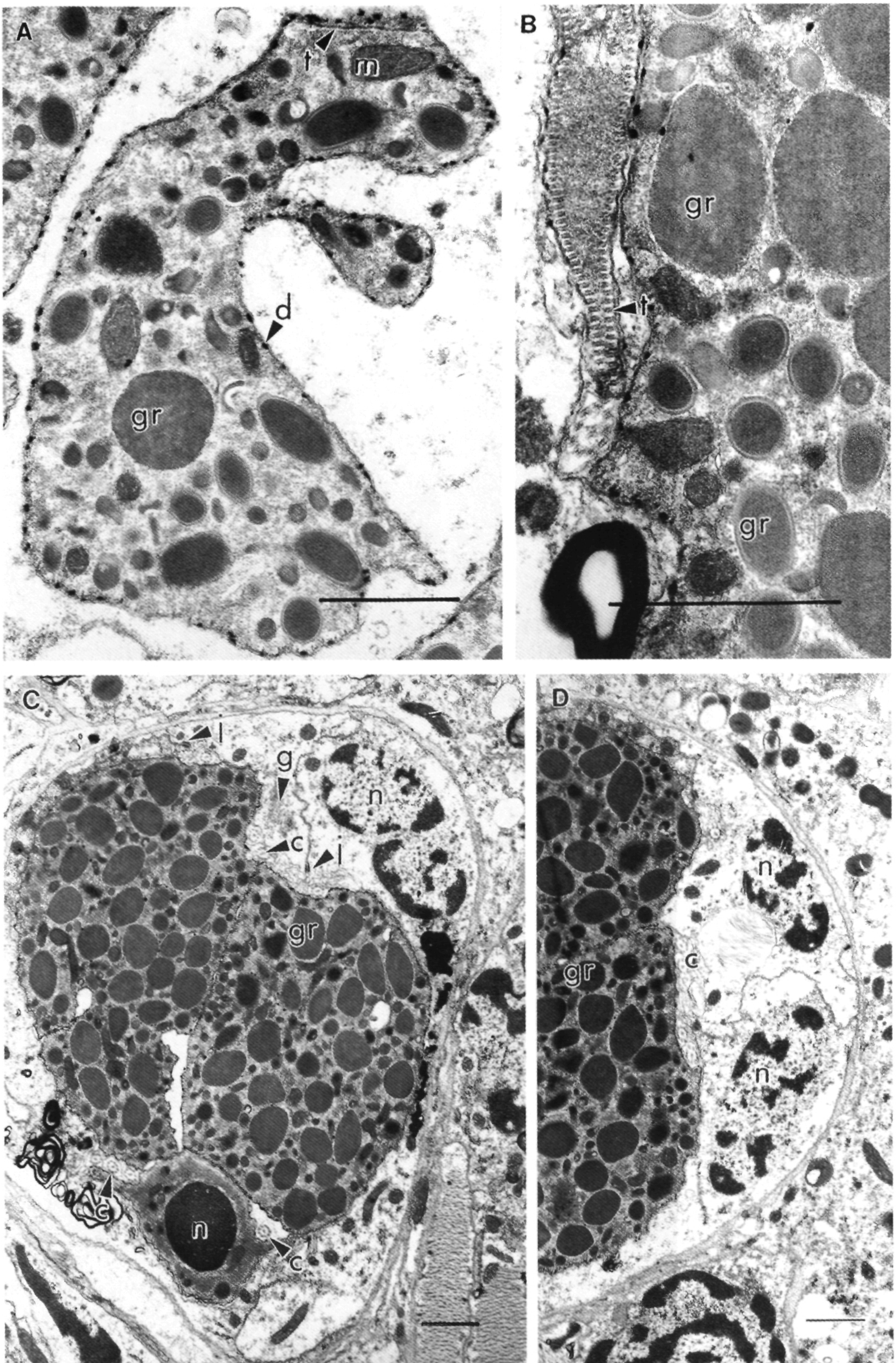


Fig. 3. – Sperm in testis. Note amoeboid shape (A-C), numerous electron-dense granules (gr) of different sizes, some with distinct membranes, nucleus (n), and mitochondria (m). Also note the vesicular regions (x) in C, the peripheral microtubules (t) and dense thickenings (d) (some of them apparently artefacts)... Scale bars 2μm (A-D), 1μm (E).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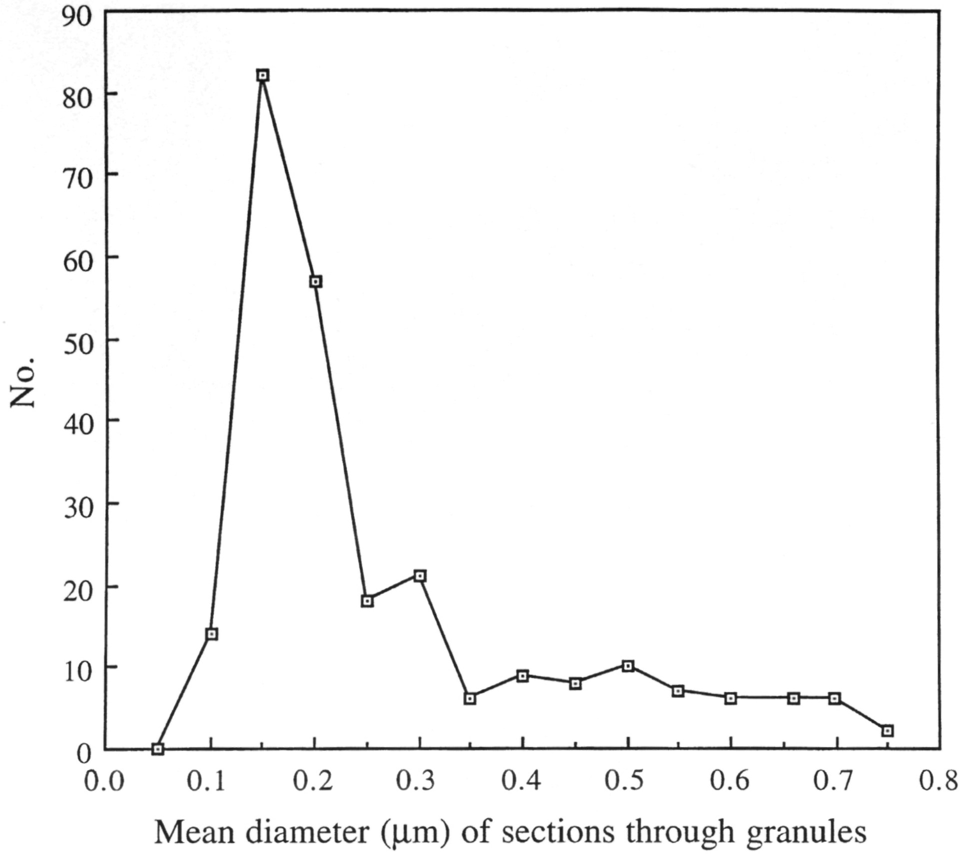

Fig. 5 . – Frequency distribution of mean diameters of sections through granules in sperm from the testis.

Legend to the figure (see page 182)

Fig. 4. – A. Sperm in testis. Note peripheral microtubules (t) and dense thickenings (d), mitochondria (m) and granules (gr) of different sizes, some with distinct membranes. B. Sperm in sperm duct. Note marginal section through sperm on left, showing densely packed microtubules (t). C, D. Sperm in sperm duct. Note dense-fibrillar nucleus (n) with some lucent spaces, granules (gr), mitochondria (m), peripheral microtubules (t) and dense thickenings (d). Also note that nucleated part of sperm has only a thin layer of cytoplasm drawn out into two flanges, the non-nucleated parts are thicker and packed with granules. The wall of the sperm duct consists of cells in contact by septate junctions (j); cilia (c) are not densely packed but occur all around the duct. Note nucleus (n) of sperm duct in C, and two nuclei (n) in D. Scale bars 1 µm.

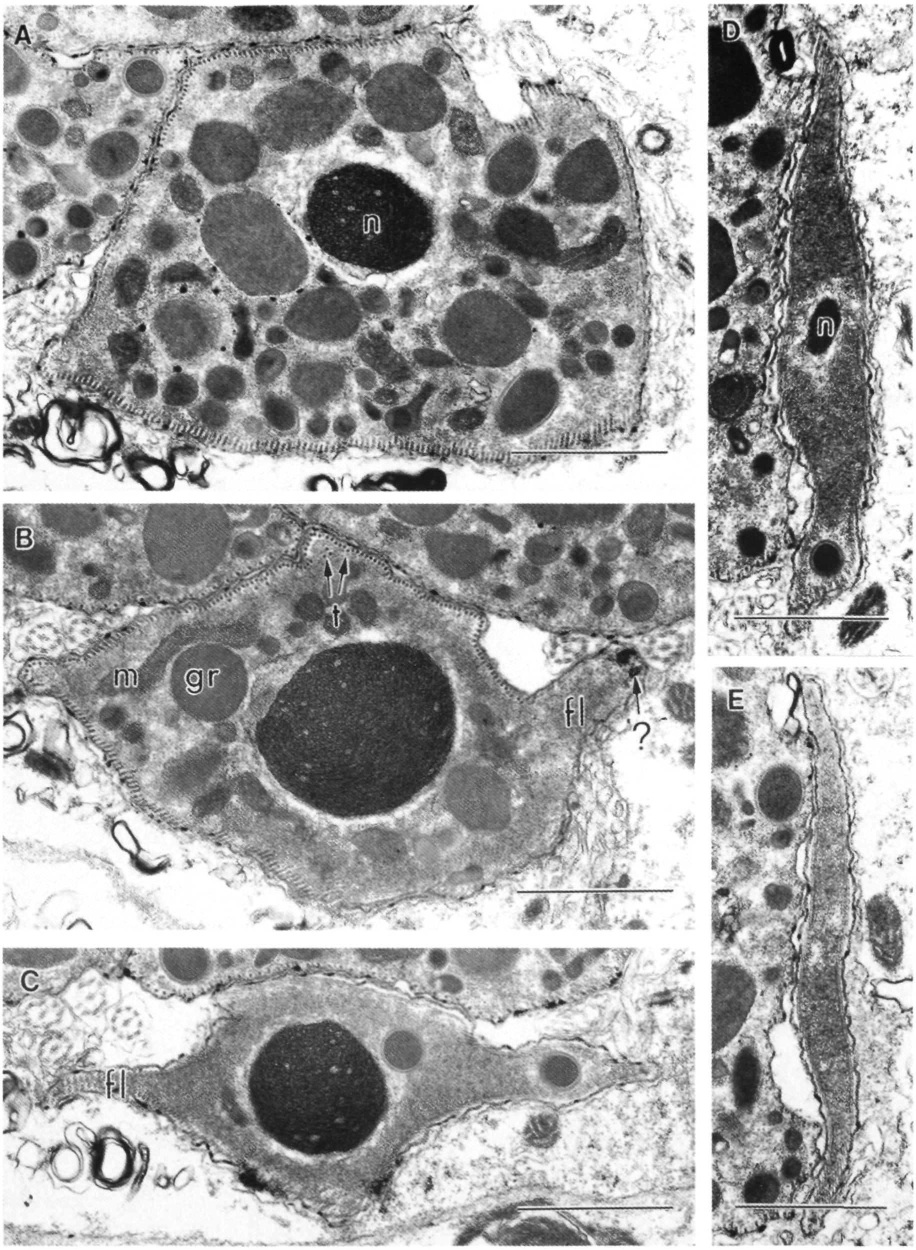

Fig. 6. – Transverse sections through nucleated part of one spermatozoon in sperm duct. A. Section through wide part with many granules and end of nucleus (n). B. Section through part at beginning of flanges (fl), with dense structures of unknown nature (?). Note peripheral row of microtubules (t), and some microtubules below the peripheral row, granules (gr) and mitochondrion (m). C. Section through part with flanges (fl). D. Section through part near the end of nucleus (n). E. Section through tip without nucleus. Scale bar 1μm.

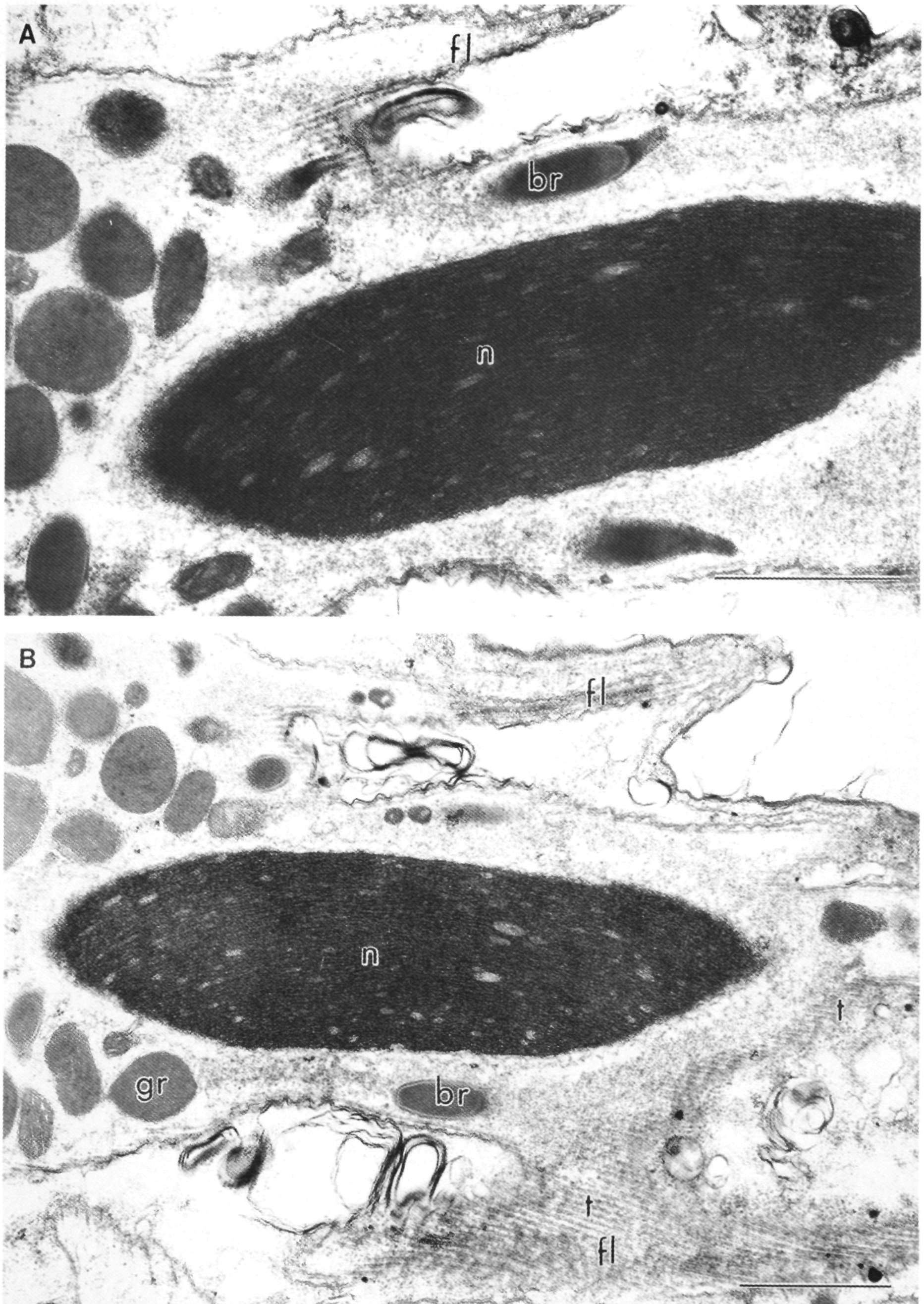

Fig. 7. – Longitudinal sections through sperm at level of beginning of flanges (fl), with nucleus (n), granules (gr), and two symmetrically located «bristles» (br). Also note longitudinal sections through peripheral microtubules. Scale bar 1 μm.

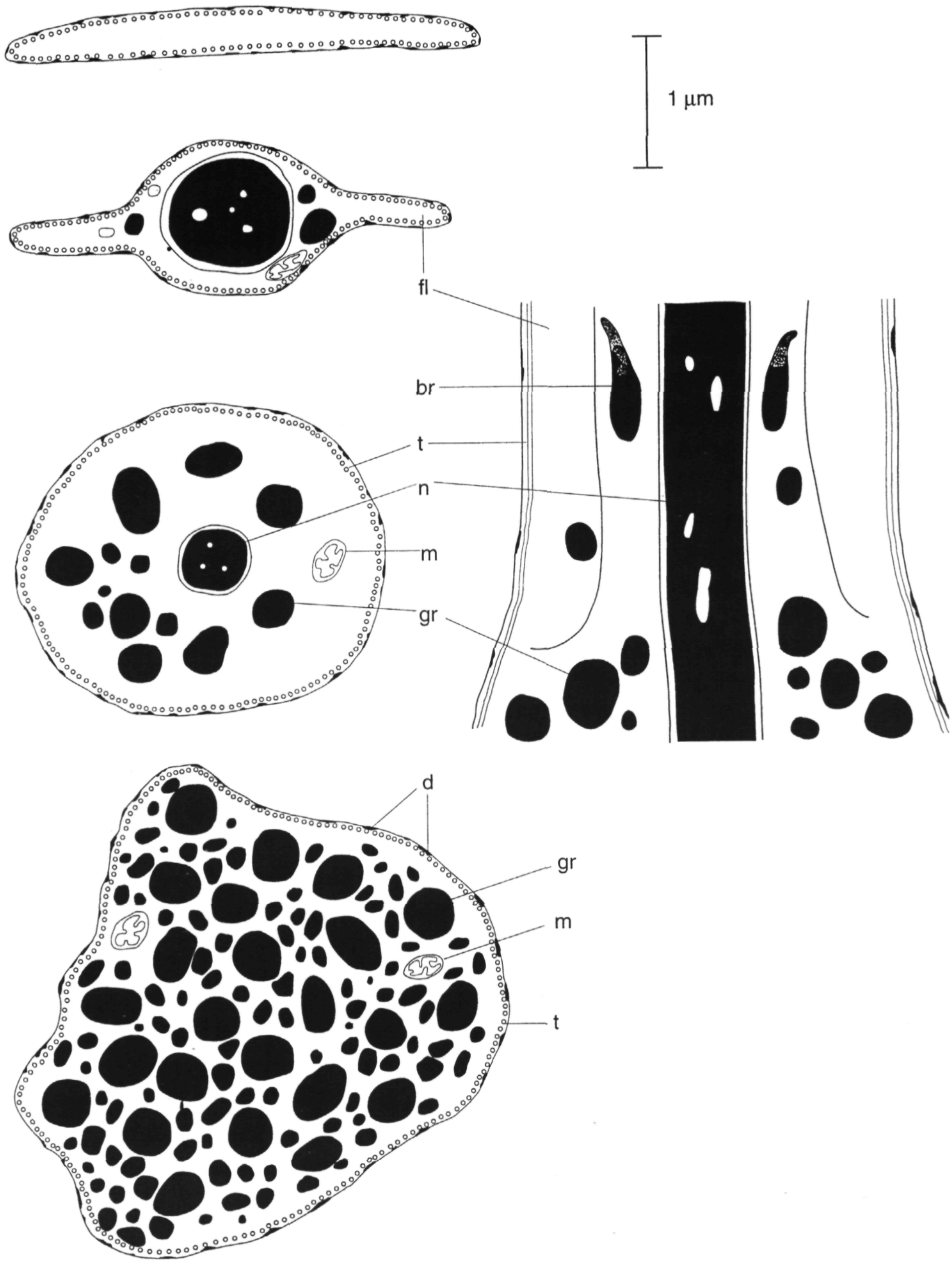

Fig. 8. – Diagrams of cross-sections through sperm (left), and of longitudinal section in the region of the flanges and bristles. Note «bristles» (br), dense thickenings (d), flanges (fl), granules (gr), mitochondria (m), nucleus (n), and microtubules (t).

DISCUSSION

Spermatozoa of *Haplopharynx rostratus* resemble all macrostomids examined in the lack of flagella, and most macrostomids in the presence of bristle-like structures, which are absent in *Paromalostomum fusculum*. They are apparently modified granules, which do not reach the surface of sperm in *Haplopharynx rostratum*. Differences between the two taxa are the number and kind of symmetry of the peripheral microtubules. In *Macrostomum tuba* Graff, 1882 and *M. pusillum* Ax, 1954 (Macrostomatidae), microtubules are arranged in two contralateral rows (ROHDE & WATSON, 1991 ; ROHDE & FAUBEL, 1997b). In *Paromalastomum fusculum* Ax, 1952 (Dolichomacrostamidae), the number of microtubules in each row is small (usually four), and a conclusion concerning the kind of symmetry is not possible (ROHDE & FAUBEL, 1997a). Furthermore, centrioles were found during spermiogenesis, apparently resorbed by the cytoplasm during sperm formation (ROHDE & FAUBEL, 1997a).

Sperm of *Haplopharynx rostratum* differ from those of the macrostomids in the much larger number of peripheral microtubules. Immature sperm from the testis differ from those of macrostomids in the distinctly amoeboid shape. Other aspects of spermiogenesis have no peculiar features.

Altogether, similarities of sperm structure in *Haplopharynx rostratum* and macrostomids support the view that haplopharyngids and macrostomids are closely related, although one of the synapomorphies, lack of flagella in mature sperm, is a purely negative characteristic due to secondary reduction (as indicated by the presence of centrioles during certain stages of spermiogenesis in *P. fusculum*), also found in some other platyhelminths (WATSON & ROHDE, 1995). Large bristles are present in *M. tuba* (ROHDE & WATSON, 1991), and rudimentary ones reaching the surface of sperm in *M. pusillum* (ROHDE & FAUBEL, 1997b). *P. fusculum* lacks bristles (ROHDE & FAUBEL, 1997a). The bristles of *M. pusillum* and *H. rostratus* clearly are not flagellar derivatives, whereas the much larger bristles of *M. tuba* have been interpreted as modified flagella. It is possible that dense granules in all three species contribute to bristle formation, but that the large size and more complex structure of bristles in *M. tuba* are due to a flagellar component. However, evidence for this assumption is lacking. The possibility must also be considered that the very small bristles of *M. pusillum* and *H. rostratus* are not homologous with the much larger and more complex ones of *M. tuba*. Also, there is a certain similarity between the bristles of *M. pusillum* and the crested bodies of cestodes (BÂ & MARCHAND, 1995), but homology is unlikely in view of the different shape (spiralling around the anterior end of sperm at least in some cestodes). The function of the bristles is unknown. That the curved dense structures in *H. rostratus* may act as « bristles » is indicated by their tapering tips close to the surface. Conceivably, the soft surface membrane, when pushed against a hard or elastic surface, permits the tips to find a hold and act as « bristles ».

The observation that many granules (from very small to medium large) in sperm of *H. rostratus* are lined by a distinct membrane, whereas many others of the same size are not, may indicate that loss of a membrane is a fixation artefact. The frequency distribution of diameters of sections through the granules suggests that there are a size class of small granules (average diameter approximately 0.2 µm, slightly greater than the peak of measurements of sections because most sections will not be exactly through the middle of the granules) and many larger granules which probably belong to a size class of their own. The observation that some curved structures occur among the granules in the interior of sperm from the testis, resembling the subsurface "bristles" in mature sperm, suggests that the «bristles» are formed in the interior and migrate to the surface during maturation of the sperm.

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