3. Résultats

Nos résultats sont réunis dans six publications issues de l’étude ultrastructurale de la spermatogenèse de sept Plathelminthes (quatre trématodes et trois cestodes).

Chaque publication, parue ou à paraître, est précédée d’une page de garde en français avec titre, résumé et mots clés.

3.1. ULTRASTRUCTURE DE LA SPERMIOGENESE ET DU SPERMATOZOÏDE DE *SCAPHIOSTOMUM PALAEARCTICUM* MAS-COMA, ESTEBAN ET VALERO, 1986 (TREMATODA, DIGENEA, BRACHYLAIMIDAE)

Résumé :

Chez *Scaphiostomum palaearcticum*, la spermiogenèse commence par la formation d'une zone de différentiation qui comprend deux centrioles séparés par un corps intercentriolaire et associé chacun à une racine striée. Elle est caractérisée par une rotation flagellaire asynchrone, suivie d'une fusion proximo-distale des deux flagelles avec l'expansion cytoplasmique médiane. La migration du noyau vers l'expansion cytoplasmique médiane a lieu avant la fusion proximo-distale. Par contre, la migration de la mitochondrie vers l'expansion cytoplasmique médiane intervient avant la fusion du deuxième flagelle. Des zones d'attachement, disposées sur la face interne de la membrane cytoplasmique de l'expansion médiane sont également observées avant la fusion de cette dernière avec les flagelles.

Le spermatozoïde mature de *S. palaearcticum*, est filiforme, effilé à ses deux extrémités et présente toutes les caractéristiques décrites chez le gamète mâle des digènes : présence de deux axonèmes, de mitochondrie, d'un noyau et de deux rangées parallèles de microtubules corticaux. Cependant, le spermatozoïde de *S. palaearcticum* présente d'autres caractéristiques qui le distinguent du spermatozoïde des autres digènes.

Mots clés :

Ultrastructure, spermiogenèse, spermatozoïde, *Scaphiostomum palaearcticum*, Trematoda, Digenea, Brachylaimidae.

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Spermiogenesis and sperm ultrastructure of *Scaphiostomum palaearcticum* Mas-Coma, Esteban et Valero, 1986 (Trematoda, Digenea, Brachylaimidae)

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Abstract

Spermiogenesis in *Scaphiostomum palaearcticum* begins with the formation of a zone of differentiation, which comprises striated rootlets associated with the two centrioles and an intercentriolar body in-between. It is characterised by an asynchronic flagellar rotation and subsequent proximo-distal fusion with a median cytoplasmic process. The migration of the nucleus toward the median cytoplasmic process before its fusion with the free flagella is also described. However, in the case of *S. palaearcticum*, the mitochondrion migrates toward the median cytoplasmic process before the fusion of the second axoneme. Attachment zones are also formed before the fusion of the axonemes with the median cytoplasmic process. The mature spermatozoon of *S. palaearcticum* is filiform and tapered at both ends and presents all the features found in the Digenea gamete: two axonemes, mitochondrion, nucleus and two bundles of parallel cortical microtubules. Nevertheless, certain features allow us to distinguish *S. palaearcticum* from other digenetic trematodes.

Key words

Ultrastructure, spermiogenesis, spermatozoon, *Scaphiostomum palaearcticum*, Digenea, Brachylaimidae

Introduction

The use of ultrastructural characters of the reproduction of Platyhelminthes has shed some light on the knowledge of the phylogeny in several groups. In particular, the spermatozoon screens various useful characters for phylogeny, which have been used by most authors to elucidate the phylogenetic relationships between higher parasitic platyhelminths (Brooks *et al.* 1985; Justine 1991a, b, 1998, 2001; Bâ and Marchand 1994, 1995; Hoberg *et al.* 1997 and others). The majority of available studies deal with the Eucestoda, especially the Cyclophyllidea order, consisting of 15 families, 9 of which have been examined (Ndiaye *et al.* in press b). Nevertheless, in the Digenea, there is an absence of key data for establishing a correct phylogenetic model. Brachylaimidae phylogeny is also controversial. According to Mas-Coma and Gállego (1975), the family Brachylaemidae Joyeux et Foley, 1930 comprises three subfamilies: Brachylaeminae Joyeux et Foley, 1930, Ityogoniminae Yamaguti, 1958 and Panopistinae Yamaguti, 1958, and the first includes only two generally accepted genera: *Brachylaima* Dujardin, 1843 and *Scaphiostomum* Braun, 1901. The latter comprises four species, *S. illatabile* Braun, 1901 isolated from birds in South America, *S. pancreaticum* McIntosh, 1934 and *S. microti* (Kruidemier et Gallicchio, 1959), both from rodents in North America, and *S. palaearcticum* Mas-Coma, Esteban et Valero, 1986 from rodents in the Balearic Archipelago (Spain) (Mas-Coma *et al.* 1986). According to these authors, *Scaphiostomum* sp. reported by Lim (1967) from rodents in Malaysia probably constitutes a new species (see Mas-Coma *et al.* 1986).

Few ultrastructural descriptions of the sperm of *Brachylaimus aequans* (Zdarska *et al.* 1991) and *Brachylaima* sp. (Gracenea *et al.* 2000) are available. In the present study, we aimed to undertake an ultrastructural study of spermiogene-

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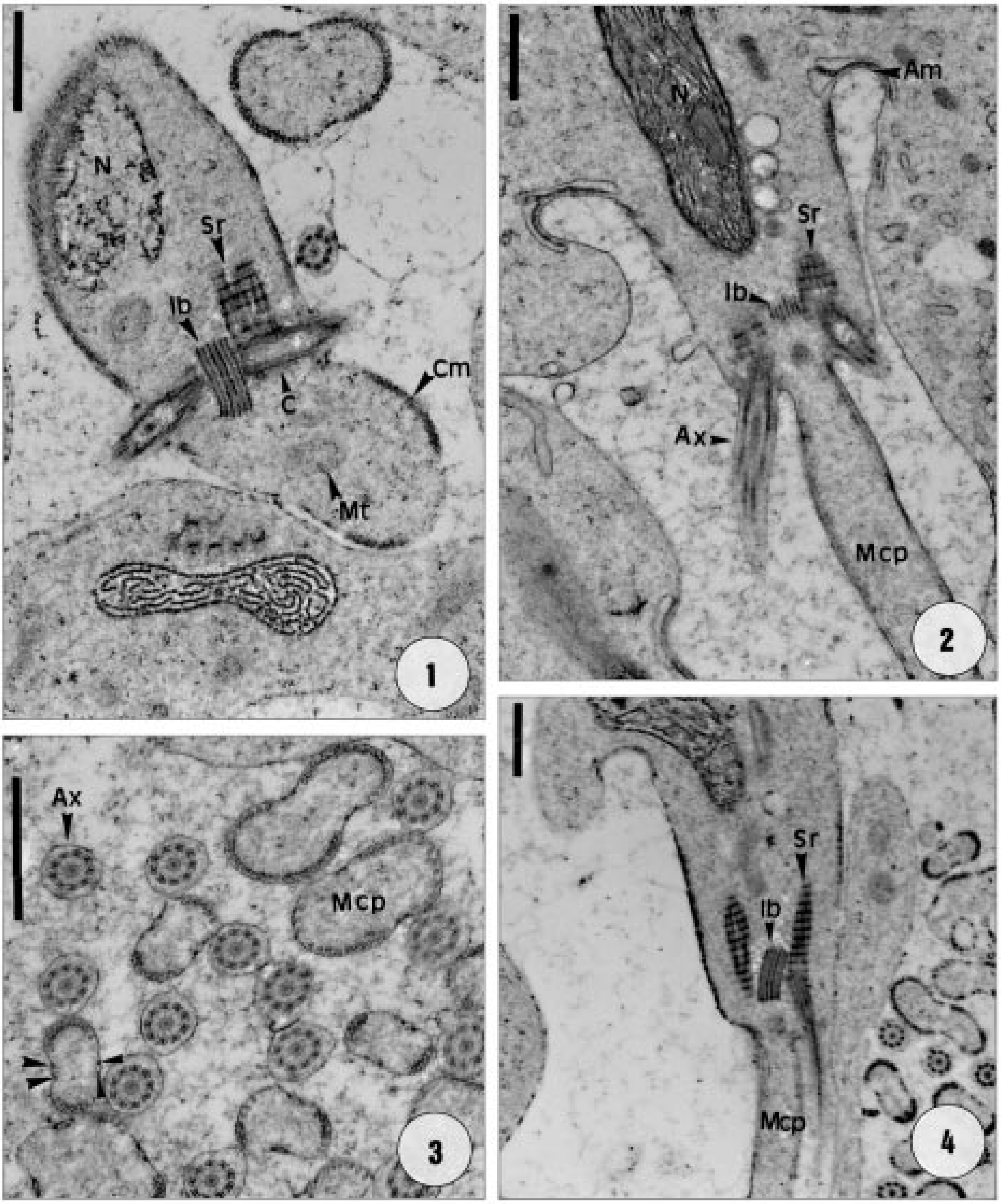


Figs. 1–4. Spermiogenesis of *S. palaearcticum*, TEM micrographs: **1.** Oblique section of a zone of differentiation showing an initial stage of spermiogenesis. Bar = 0.5 μm. **2.** Longitudinal section showing a spermatid after the flagellar rotation. The nucleus penetrates the ring of arched membranes. Bar = 0.5 μm. **3.** Cross-sections of spermatids showing the median cytoplasmic process previous to the proximo-distal fusion. Note the presence of attachment zones (arrowheads) at this moment. Bar = 0.5 μm. **4.** Longitudinal section of spermatid. Note the parallel disposition of flagellum previous to the proximo-distal fusion. Bar = 0.5 μm. **Abbreviations to all figures:** Am – arched membrane, Ase – anterior spermatozoon extremity, Ax – axoneme, Az – attachment zones, C – centriole, C1 – centriole 1, C2 – centriole 2, Cm – cortical microtubules, D – doublets, Eo – extramembranar ornamentation, F1 – flagellum 1, F2 – flagellum 2, Fr – flagellar rotation, G – granules of glycogen, Ib – intercentriolar body, Mcp – median cytoplasmic process, Mt – mitochondrion, N – nucleus, Pf – proximo-distal fusion, Pm – plasma membrane, Pse – posterior spermatozoon extremity, S – singlets, Sr – striated root

sis and the spermatozoon of *S. palaearcticum*, detected in *Mus spretus*, and also reported in other Muridae (Mas-Coma *et al.* 1986).

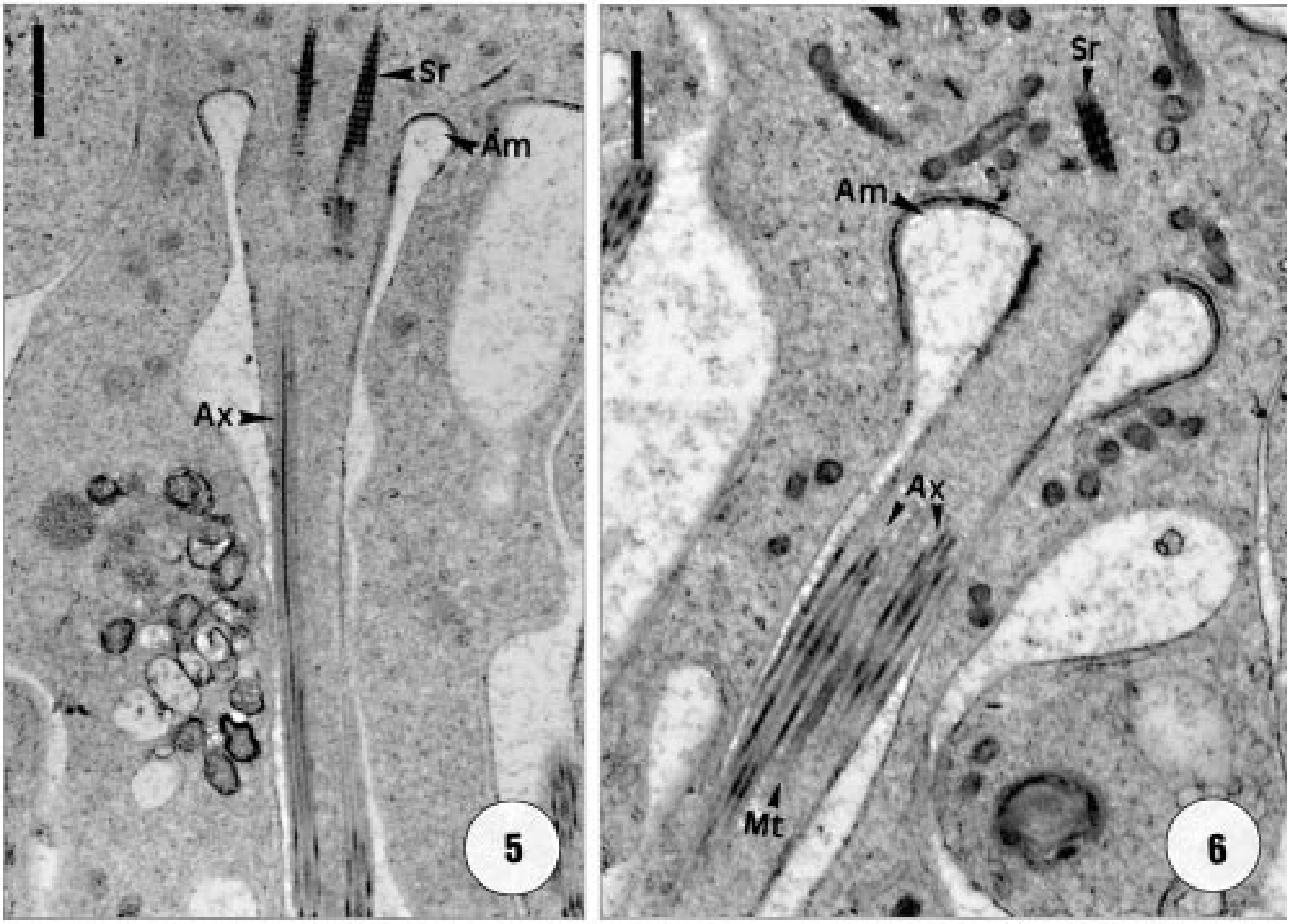
Materials and methods

Live specimens of *S. palaearcticum* were collected from the pancreatic ducts of *Mus spretus* in Petra (Mallorca Island, Balearic Archipelago, Spain). Adult trematodes were fixed in cold (4°C) 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2 for 2 h, rinsed in 0.1 M sodium cacodylate buffer at pH 7.2, postfixed in cold (4°C) 1% osmium tetroxide in the same buffer for 1 h, rinsed in 0.1 M sodium cacodylate buffer at pH 7.2, dehydrated in ethanol solutions and propylene oxide, embedded in Spurr and polymerised at 60°C for 48 h. Ultrathin sections (60–90 nm) of various tissues (testes and seminal ducts) were obtained using a Reichert-Jung Ultracut E ultramicrotome, placed on 200-mesh copper grids and double-stained with uranyl acetate (30 min) and lead citrate (15 min) following Reynolds (1963) methodology. Sections were examined using a Hitachi H-600 electron microscope at 75 kV in the Barcelona and Corsica Universities.

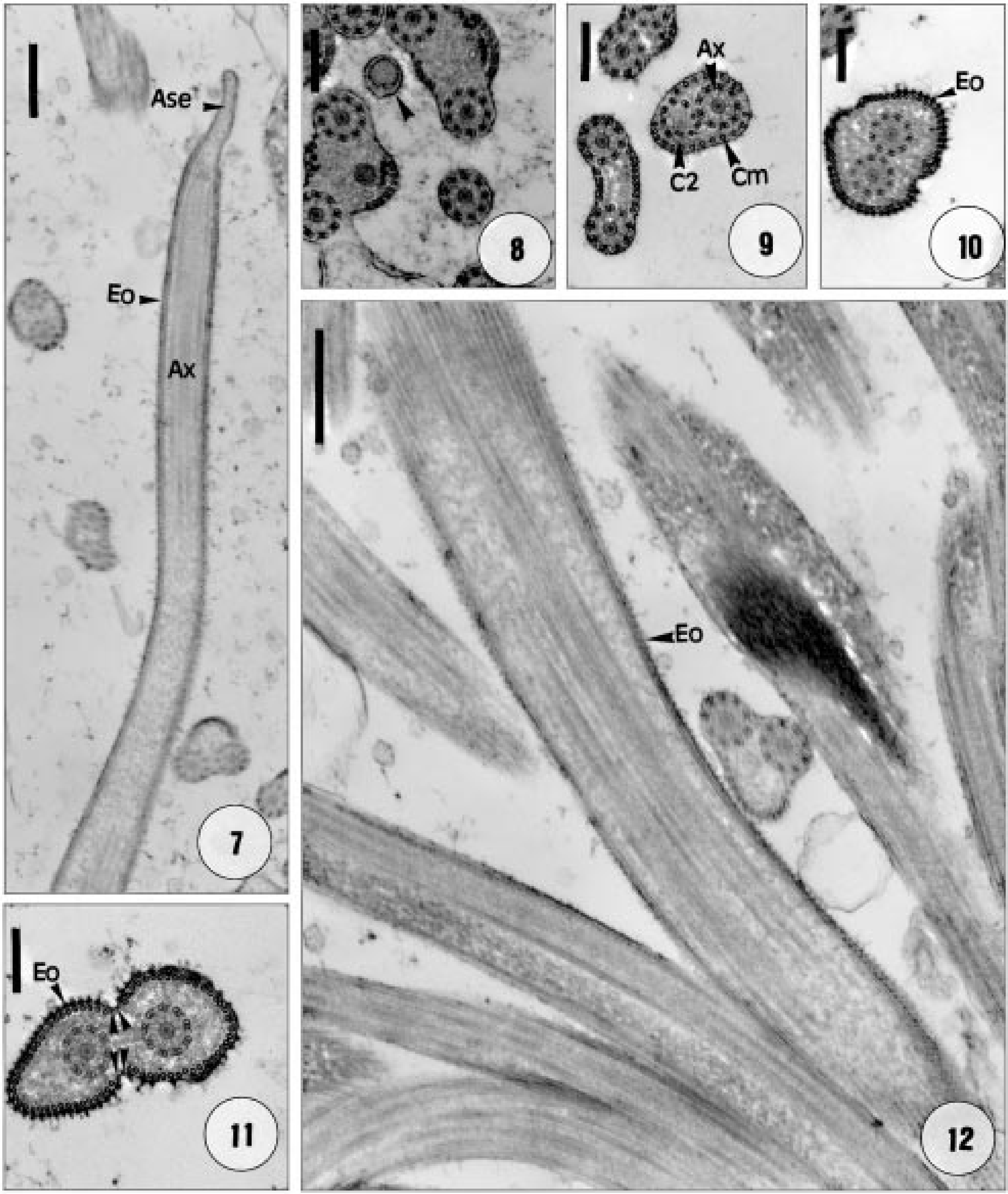
Results

Spermiogenesis (Figs. 1–6, 23A-E)

Following the general pattern in Digenea, spermiogenesis in *S. palaearcticum* begins with the formation of a differentiation zone in the spermatid. This is a conical area, characterised by the presence of arched membranes and bordered by a layer of cortical microtubules. It also contains two centrioles separated by an intercentriolar body, striated rootlets associated with the centrioles and an emerging median cytoplasmic process

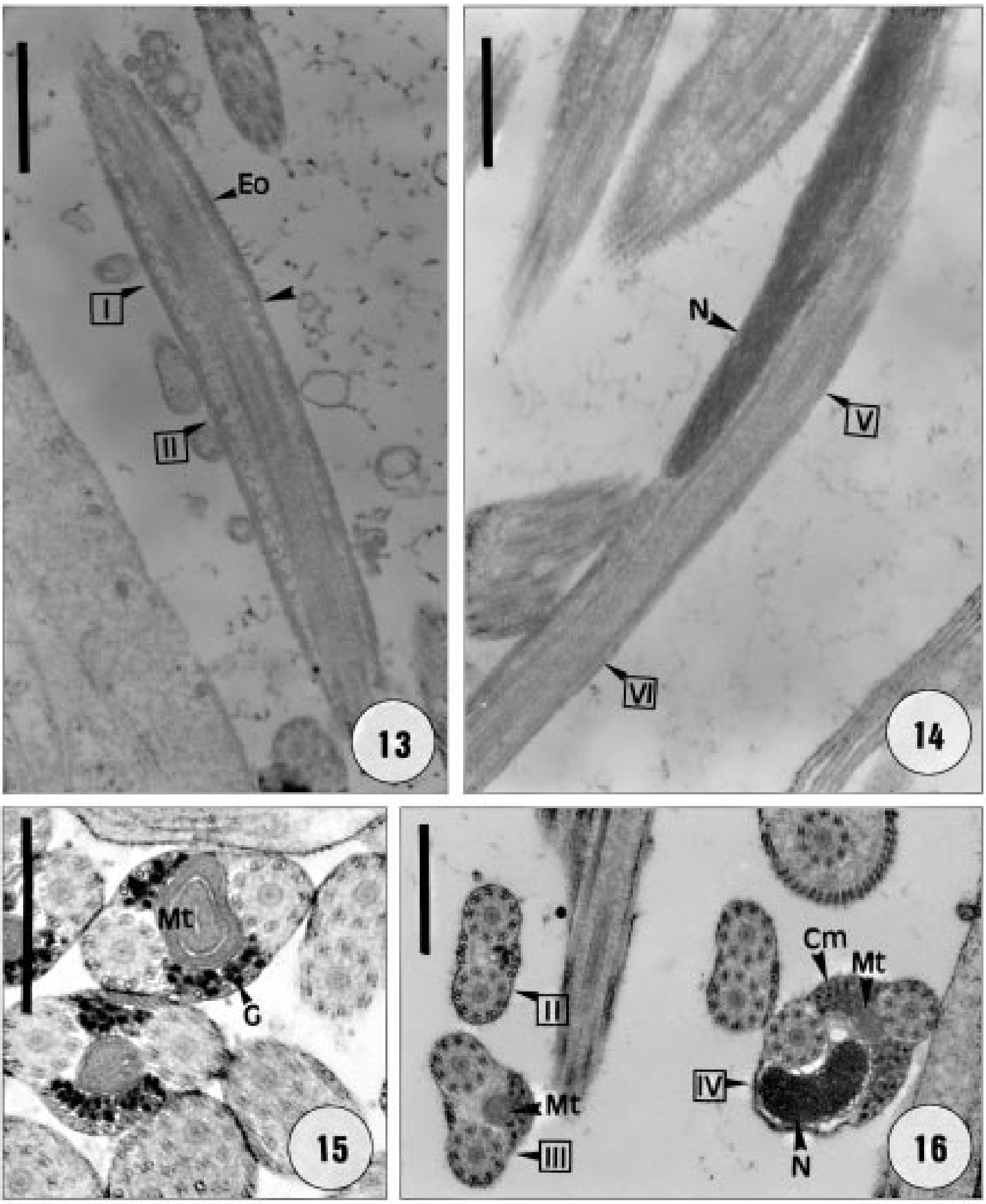


Figs. 5 and **6.** Spermiogenesis of *S. palaearcticum*, TEM micrographs: **5.** Longitudinal section of a spermatid with the striated roots at the level of the ring of arched membranes. Bar = 1 µm. **6.** Longitudinal section of a spermatid previous to the strangulation of the ring of arched membranes. Note the striated root in the residual cytoplasm. Bar = 1 µm



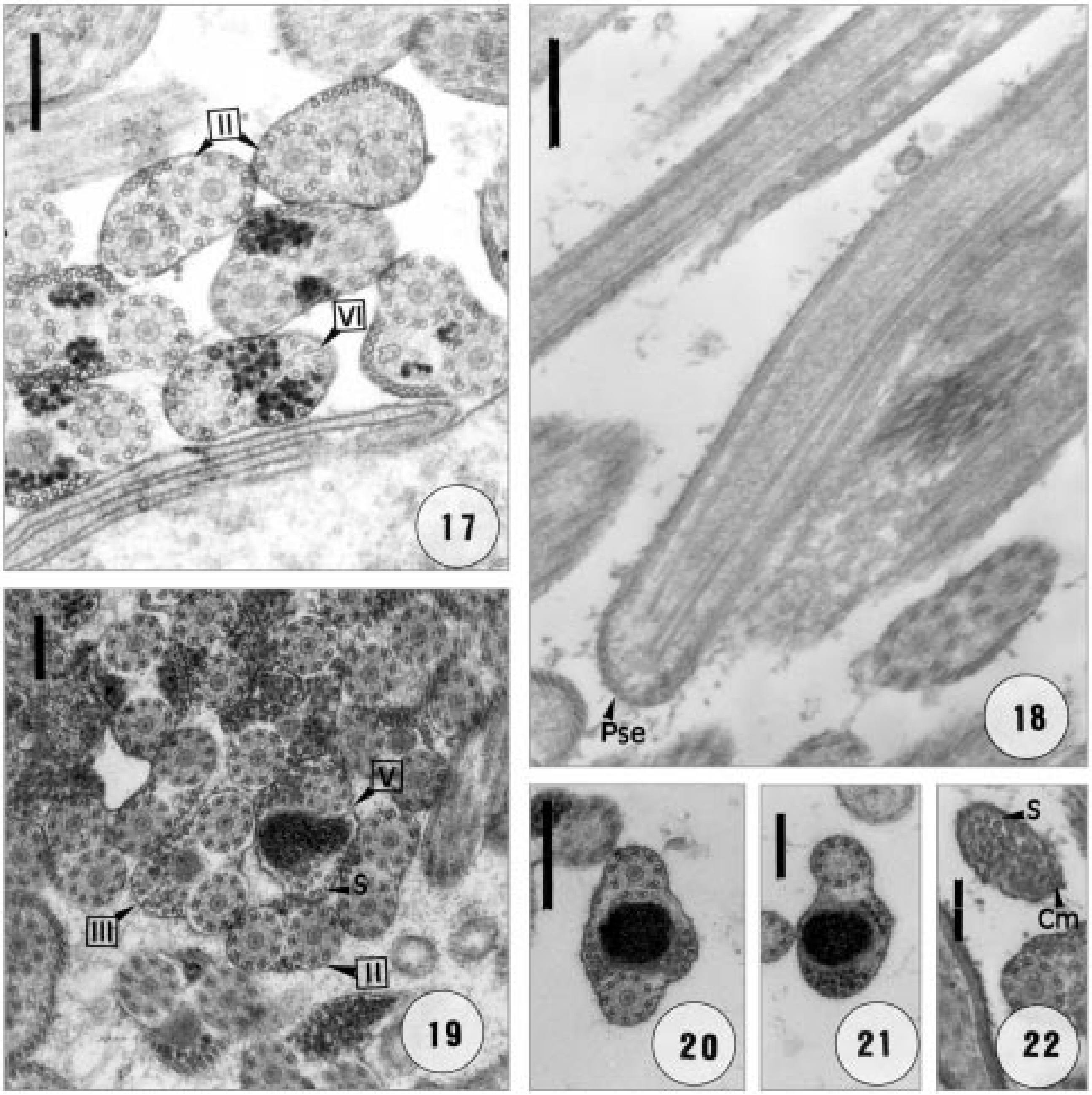
Figs. 7–12. Spermatozoon of *S. palaearcticum*, TEM micrographs: **7.** Longitudinal section of the anterior extremity of spermatozoon. Bar = 0.5 µm. **8.** Two cross-sections of region I at the level of anterior spermatozoon extremity (arrowhead) and at the level of areas with a single axoneme. Bar = 0.25 µm. **9.** Cross-section of region I at the level of the centriole (C2) of the second axoneme. Bar = 0.25 µm. **10.** Cross-section of region I at the level of the external ornamentation of the plasma membrane. Bar = 0.25 µm. **11.** Another cross-section of region I at the level of the external ornamentation of the plasma membrane. Note the location of attachment zones (arrowheads). Bar = 0.25 µm. **12.** Longitudinal section of region I at the level of the extramembranar ornamentation. Bar = 0.5 µm

(Fig. 1). Each centriole develops a flagellum that grows externally. The cortical microtubules initiate their migration along this median process (Fig. 1). At the beginning of their development, the two flagella grow orthogonal to the median cytoplasmic process (Fig. 1). Thereafter, they undergo rotation, become parallel to the cytoplasmic extension and fuse with it (Figs. 2–4). The rotation and proximo-distal fusion of the free flagella are asynchronous: one flagellum fuses before the other. Attachment zones of the axonemes to the median cytoplasmic extension were observed before the proximo-distal



Figs. 13–16. Spermatozoon of *S. palaearcticum*, TEM micrographs: **13.** Longitudinal section of regions I and II. Arrowheads indicate the end of the extramembranar ornamentation. Bar = 0.5 µm. **14.** Longitudinal section of regions V and VI. Bar = 0.5 µm. **15.** Cross-section of region III. Bar = 0.5 µm. **16.** Cross-sections of regions II, III and V. Bar = 0.5 µm

fusion of the free flagella (Fig. 3). The fusion of the flagella with the median cytoplasmic process determines the appearance of two sets of cortical microtubules which show a parallel disposition related to the hypothetical longitudinal axis of the spermatid (Fig. 3). The nucleus migrates toward the median cytoplasmic process before the proximo-distal fusion of the flagella. However, a longitudinal section of the differentiation zone revealed that nuclear migration takes place after flagellar rotation (Figs. 2 and 4). The mitochondria migrate along the spermatid body after the fusion of the first flagella with the median cytoplasmic process (Fig. 6). Striated rootlets are progressively displaced towards the base of the spermatid (Figs.



Figs. 17–**22.** Spermatozoon of *S. palaearcticum*, TEM micrographs: **17.** Cross-sections of regions II and VI. Bar = 0.25 µm. **18.** Longitudinal section of region VI showing the posterior extremity of the spermatozoon. Bar = 0.5 µm. **19.** Cross-sections of regions II, III and V. The last one shows the disorganization of the first axoneme. Bar = 0.25 µm. **20.** Cross-section of region V. Bar = 0.5 µm. **21.** Another cross-section of region V after the disappearance of the first axoneme. Bar = 0.25 µm. **22.** Cross-section of region VI showing axonemal singlets and cortical microtubules. Bar = 0.25 µm

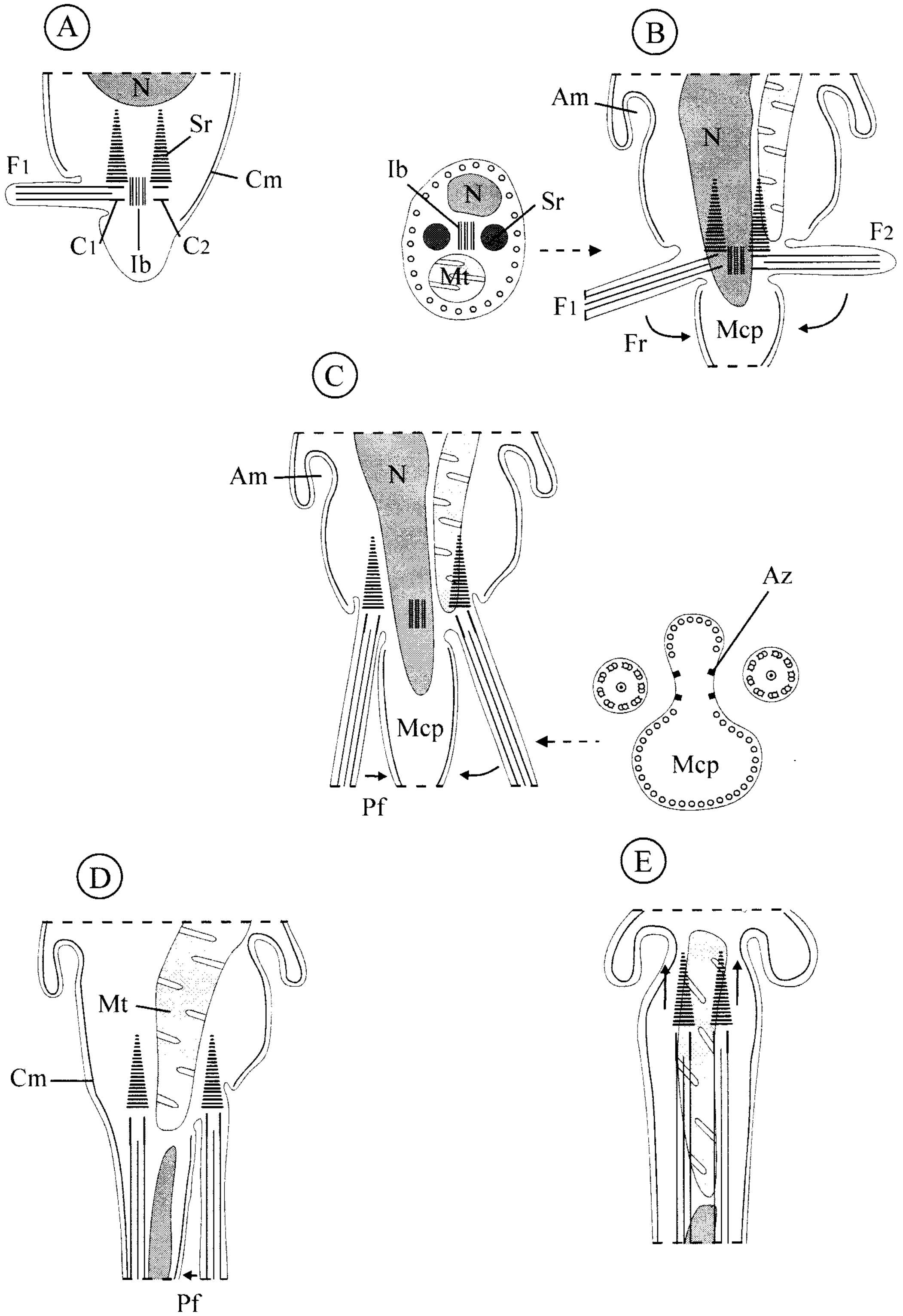


Fig. 23A-E. Diagram showing the main stages of spermiogenesis in *S. palaearcticum*: **A** – the initial stage; note one centriole forming already the first flagellum, **B** – differentiation zone with the two flagella oriented still perpendicularly to the median cytoplasmic process, **C** – differentiation zone after the flagellar rotation; note the nucleus entering already into the median cytoplasmic process and the initial phase of mitochondrial migration, **D** – differentiation zone before the fusion of the second flagellum with already fused median cytoplasmic process and the first flagellum, **E** – the final stage of spermiogenesis; note a fusion of both flagella with the median cytoplasmic process and the advanced phase of mitochondrial migration

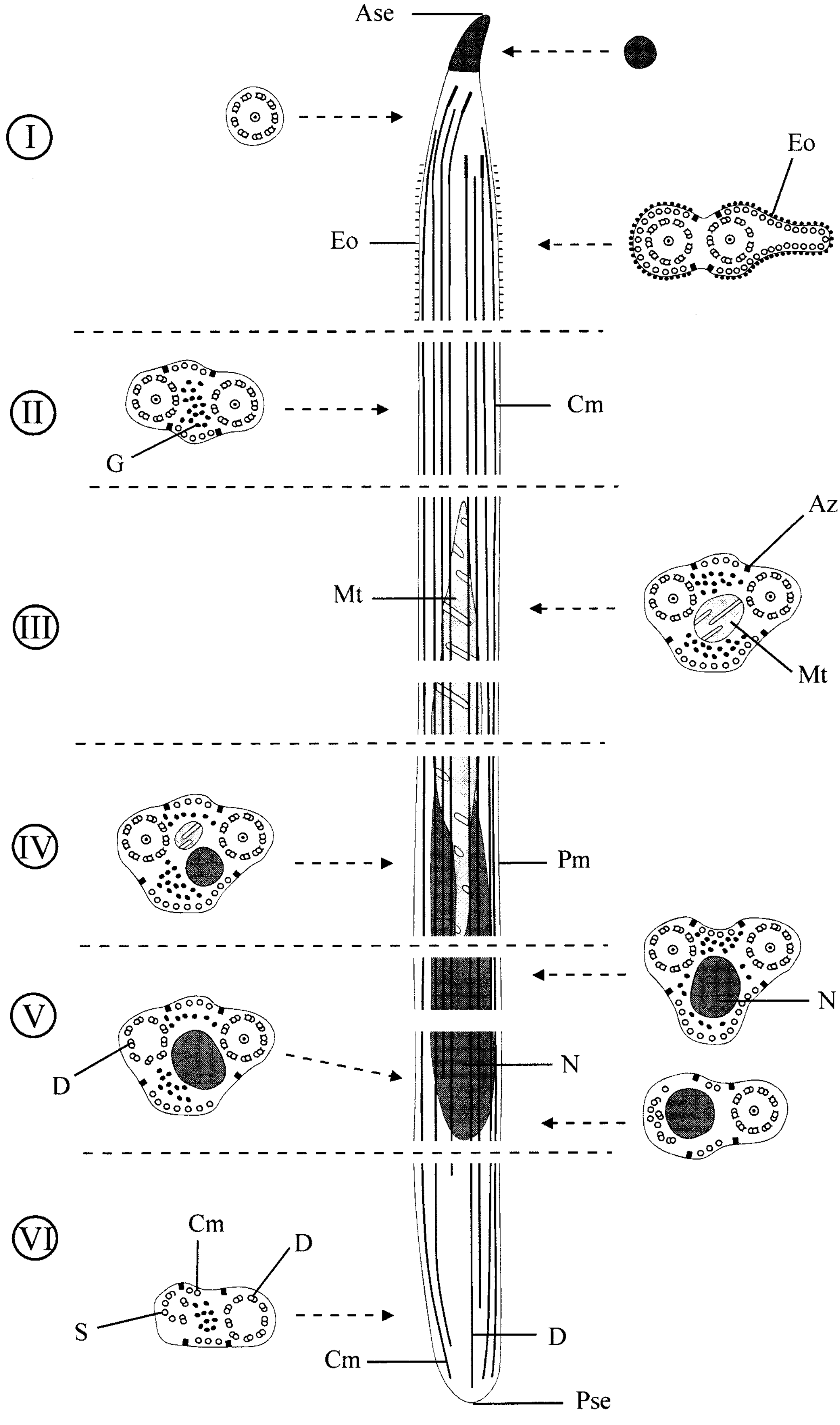


Fig. 24 I–VI. Diagram showing the ultrastructural organization of the mature sperm of *S. palaearcticum*. To make the diagram clearer, the granules of glycogen are not shown on the longitudinally oriented diagram of spermatozoon

5 and 6). Finally, the ring of arched membranes is constricted and the young spermatozoon detaches from the residual cytoplasm (Fig. 6).

Spermatozoon (Figs. 7–22 and 24 I–VI)

The mature spermatozoon *S. palaearcticum* is filiform and tapered at both ends. It is characterised by the presence of two axonemes, one mitochondrion, nucleus and two sets of cortical microtubules parallel to the longitudinal sperm axis. External ornamentation was also observed. After examining a large series of sections, we distinguished five regions (I–V) from the anterior to posterior extremities of the spermatozoon according to the following ultrastructural features:

Region I (Figs. 7–13 and 24 I). This corresponds to the anterior end of the spermatozoon. It is characterised by the presence of two axonemes of 9+'1' pattern of Trepaxonemata and cortical microtubules. At the beginning of the process, it is sharp (Fig. 7), shows a single axoneme (Figs. 7 and 8) and lacks cortical microtubules (Fig. 8). A second axoneme and cortical microtubules soon appear (Figs. 9–11). Most transverse sections of the posterior part of region I reveal that the plasma membrane is devoid of submembranous cortical microtubules only in a small area where four attach points are observed (Figs. 10 and 11). External ornamentation of the plasma membrane was also observed in this region (Figs. 10–13).

Region II (Figs. 13, 16, 17, 19 and 24 II). It is characterised by the presence of only two axonemes, cortical microtubules and glycogen granules. Cortical microtubules are disposed on two bundles and absent in the lateral faces along the axonemes (Figs. 16, 17 and 19). The external ornamentation of the plasma membrane observed in region I disappeared.

Region III (Figs. 15, 16, 19 and 24 III). This region presents, in addition to the mitochondrion, the same structures as region II. Cortical microtubules are also absent in the lateral faces along the axonemes. Transverse sections of the spermatozoon show abundant glycogen granules in contact with cortical microtubules (Figs. 15, 16 and 19).

Region IV (Figs. 16 and 24 IV). This is characterised by the simultaneous presence of two axonemes, mitochondrion and nucleus. In a transverse section, we also observed ventral and dorsal bundles of cortical microtubules, granules of β-glycogen and the four attach points (Fig. 16).

Region V (Figs. 14, 19–21 and 24 V). This corresponds to the nuclear area of the mature spermatozoon. In addition to a large nucleus, this region presents two axonemes, the two bundles of cortical microtubules and a reduced quantity of glycogen granules. The mitochondrion has disappeared. In the posterior areas of this region, one axoneme disorganizes and disappears (Figs. 19 and 21). In some transverse sections of this region, we detected only one axoneme and the nucleus (Figs. 14 and 21).

Region VI (Figs. 18, 22 and 24 VI). This corresponds to the posterior end of the spermatozoon (Fig. 18). It is characterised by the presence of a single axoneme. The cortical and axonemal microtubules reach the posterior extremity of the spermatozoon (Figs. 18 and 22).

Discussion

Spermiogenesis in *S. palaearcticum* follows the general pattern found in all the digenetic trematodes studied to date (e.g. Burton 1972, Erwin and Halton 1983, Gracenea *et al.* 1997, Miquel *et al.* 2000, Baptista-Farias *et al.* 2001). Thus, two free flagella arise from the differentiation zone perpendicular to a median cytoplasmic process. Thereafter, they undergo a rotation of 90°, become parallel to the median cytoplasmic extension and fuse with it. Flagellar rotation and proximo-distal fusion are asynchronic processes, as reported for other species of Digenea, e.g. the frog lung fluke *Haematoloechus medioplexus* (Plagiorchiidae) (Burton 1972), *Opecoeloides furcatus* (Opecoelidae) (Miquel *et al.* 2000) and *Saccocoelioides godoyi* (Haploporidae) (Baptista-Farias *et al.* 2001). In *O. furcatus*, Miquel *et al.* (2000) have described an asynchronic process of proximo-distal fusion and the first fused flagellum migrates to distal areas of the spermatid after its fusion with the median cytoplasmic process but before the complete proximo-distal fusion of the second flagellum. This may account for the origin of the anterior extremity of the future spermatozoa with a single axoneme. In the anterior extremity of the *S. palaearcticum* spermatozoon, we also observed a single axoneme both in cross and longitudinal sections, as a result of the displacement of one of the axonemes with respect to the other. Unexpectedly, we detected the formation of attachments zones before the proximo-distal fusion of the axonemes in *S. palaearcticum*. The position of attachment zones with respect to the axonemes (see transverse sections of region I of the spermatozoon) suggests a displacement of the axonemes after their proximo-distal fusion with the median cytoplasmic process.

Regarding the nucleus, the present study and most published reports (Burton 1972, Erwin and Halton 1983, Hendow and James 1988, Iomini and Justine 1997, Miquel *et al.* 2000, Baptista-Farias *et al.* 2001) describe its migration toward the median cytoplasmic process before the migration of the mitochondrion. Only Gracenea *et al.* (1997) describe nuclear migration after the migration of the mitochondrion in *Postorchigenes gymnesicus* (Lecithodendriidae). In *Notocotylus neyrai*, nuclear migration seems to begin in a relatively initial stage of spermiogenesis and the mitochondria migrate in a more advanced stage of spermiogenesis than the nucleus, after the complete proximo-distal fusion of the two flagella (Ndiaye *et al.* in press a).

The basal bodies, striated rootlets and intercentriolar body have never been described in the mature spermatozoon. Both in trematodes and cestodes, it is thus assumed that these structures remain in the residual cytoplasm and degenerate (Burton 1972; Mokhtar-Maamouri and Swiderski 1975; Swiderski and

Table I. Available data on the ultrastructure of spermatozoon of digenean trematodes

Families and species of Digenea	Eo	Position	References
Brachylaimidae			
Brachylaimus aequans	–		Zdarska *et al.* (1991)
Scaphiostomum palaearcticum	+	ant	present study
Bucephalidae			
Bucephaloides gracilescens	+	mit	Erwin and Halton (1983)
Pseudorhipidocotyle elpichthys	+	ant	Tang *et al.* (1998)
Cryptogonimidae			
Neochasmus sp.	+	mit	Jamieson and Daddow (1982)
Dicrocoeliidae			
Dicrocoelium dendriticum	–		Cifrián *et al.* (1993), Morseth (1969)
Dicrocoelium chinensis	–		Tang (1996), Tang and Li (1996)
Corrigia vitta	–		Robinson and Halton (1982)
Didymozoidae			
Didymozoon sp.	–		Justine and Mattei (1983, 1984a)
Gonapodasmius sp.	+	ant	Justine and Mattei (1982a,1984b)
Didymocystis wedli	–		Pamplona-Basilio *et al.* (2001)
Diplostomatidae			
Pharyngostomoides procyonis	–		Grant *et al.* (1976)
Echinostomatidae			
Echinostoma caproni	+	mit	Iomini and Justine (1997), Justine (1995)
Fasciolidae			
Fasciola hepatica	–	*	Stitt and Fairweather (1990)
Fasciola gigantica	+	ant, mit	Ndiaye *et al.* (2001)
Fellodistomidae			
Proctoeces maculatus	+	mit	Justine (1995)
Haematoloechidae			
Haematoloechus medioplexus	+	ant	Justine (1995), Justine and Mattei (1982b)
Haploporidae			
Saccocoelioides godoyi	–		Baptista-Farias *et al.* (2001)
Heterophyidae			
Cryptocotyle lingua	–		Rees (1979)
Lecithodendriidae			
Postorchigenes gymnesicus	+	mit	Gracenea *et al.* (1997)
Ganeo tigrinum	–		Sharma and Rai (1995)
Mesocoelidae			
Mesocoelium monas	–		Iomini *et al.* (1997)
Microphallidae			
Maritrema linguilla	–		Hendow and James (1988)
Notocotylidae			
Notocotylus neyrai	+	ant	Ndiaye *et al.* (in press a)
Opecoelidae			
Opecoeloides furcatus	+	mit	Miquel *et al.* (2000)
Opisthorchiidae			
Aphalloides coelomicola	+	ant	Justine (1995)
Paragonimidae			
Paragonimus miyazakii	–		Sato *et al.* (1967)
Paragonimus pulmonalis	–		Fujino and Ishii (1982)
Paragonimus westermani	–		Fujino *et al.* (1977), Orido (1988)
Paragonimus ohirai	+	mit	Hirai and Tada (1991)
Paramphistomidae			
Ceylonocotyle scoliocoelium	–		Li and Wang (1997)
Schistosomatidae			
Schistosoma curassoni	–		Justine *et al.* (1993)
Schistosoma rodhaini	–		Justine *et al.* (1993)
Schistosoma intercalatum	–		Justine *et al.* (1993)
Schistosoma bovis	–		Justine *et al.* (1993)
Schistosoma mansoni	–		Justine *et al.* (1993), Justine and Mattei (1981), Kitajima *et al.* (1976)
Schistosoma margrebowiei	–		Justine *et al.* (1993), Justine and Mattei (1981)
Schistosoma mattheei	–		Swiderski and Tsinonis (1986)
Schistosoma japonicum	–		Justine and Mattei (1981), Yang *et al.* (1998)

Eo – extramembranous ornamentation, +/– presence/absence of considered character, ant – anterior extremity of sperm, mit – mitochondrial area of sperm, *unpublished data show the presence of extramembranous ornamentation both in anterior and mitochondrial areas of the mature sperm.

Eklu-Natey 1978; Rees 1979; Swiderski and Mokhtar-Maamouri 1980; Erwin and Halton 1983; Swiderski 1985, 1986a, b, c, 1994; Swiderski and Mackiewicz 2002). In the pseudophyllidean cestode *Eubothrium crassum*, after the proximo-distal fusion of the two flagella with the median cytoplasmic process, the basal bodies and rootlets detach from the flagella and move towards the proximal extremity of the zone of differentiation (Brunanska *et al.* 2001), as described for the digenean trematode *N. neyrai* (Ndiaye *et al.* in press a). The intercentriolar body is destroyed. According to Brunanska *et al.* (2001), the striated roots participate in flagellar rotation, which occurs earlier than the proximo-distal fusion between the flagella and the median cytoplasmic process.

The spermatozoon of *S. palaearcticum* exhibits the usual structure observed in Digenea so far: two axonemes of 9+'1' pattern of Trepaxonemata (Ehlers 1984), mitochondrion, nucleus and parallel cortical microtubules (Burton 1972, Jamieson and Daddow 1982, Miquel *et al.* 2000, Ndiaye *et al.* in press a). However, it presents some ultrastructural particularities. We thus distinguished six regions from its anterior to posterior extremities. In contrast, Zdarska *et al.* (1991) distinguished only three regions in the mature spermatozoon of *Brachylaimus aequans*, a species belonging to the same family: head region with nucleus and two axial filaments, middle region with mitochondrion and two axial filaments and the tail with only two axial filaments. These authors argue that the nuclear region is anterior to the mitochondrial region, as in the spermatozoon of the vertebrates. However, in agreement with Justine (1991b), we regard the nuclear portion of platyhelminth spermatozoa as the posterior region of the spermatozoon, as reported elsewhere (e.g. Stitt and Fairweather 1990, Iomini and Justine 1997, Miquel *et al.* 2000).

The *S. palaearcticum* mature sperm is characterised by an anterior extremity with only one axoneme and an external ornamentation membrane, disrupted in some parts and lacking spine-like bodies. Miquel *et al.* (2000) and Ndiaye *et al.* (in press a) have described spine-like bodies associated with the external ornamentation in *O. furcatus* and *N. neyrai*, respectively. According to these authors, spine-like bodies were inserted to the ornamentation, forming a discontinuity of this. In the beginning of the anterior part of the spermatozoon, there is a small area with only an electron-dense material. On the other hand, we described for the first time an external ornamentation associated with the membrane in Brachylaimidae, although this has been reported in eleven other Digenea species (Table I). Nevertheless, the location of this ornamentation along the spermatozoon seems to differ in these species. It has been described in the anterior part of the spermatozoon only in three species: *Gonapodasmius* sp. (Justine and Mattei 1982a), *Haematoloechus medioplexus* (Justine and Mattei 1982b) and *S. palaearcticum* (present study). Moreover, in the anterior region there is usually a continuous circle of peripheral microtubules associated with the external ornamentation of the membrane, as described for *S. palaearcticum*. Extramembranous ornamentation has been described in the mitochondrial region of the mature sperm in most species, e.g. *Paragonimus ohirai* (Orido 1988), *Neochasmus* sp. (Jamieson and Daddow 1982), *Echinostoma caproni* (Iomini and Justine 1997), *P. gymnesicus* (Gracenea *et al.* 1997), *Bucephaloides gracilescens* (Erwin and Halton 1983) and *O. furcatus* (Miquel *et al.* 2000). Finally, we would like to highlight that Stitt and Fairweather (1990) did not find external ornamentation in *Fasciola hepatica*, but personal unpublished observations of the liver fluke reveal external ornamentation of the plasma membrane both in anterior and mitochondrial areas. According to Justine and Mattei (1982b) and Miquel *et al.* (2000), the external ornamentation of the plasma membrane is formed during spermiogenesis, after the proximo-distal fusion, probably in the final stage of spermiogenesis. Accordingly, parts of the differentiation zone were persistent in the mature spermatozoon. As a result of the proximo-distal fusion there are dorsal and ventral microtubules in the nuclear region of the spermatozoon. These microtubules come from the microtubules of the median cytoplasmic process.

Attachment zones in mature spermatozoa locate the area of fusion of flagella with the median cytoplasmic process. These electron-dense and submembranous marks are present in several sections of the mature spermatozoon and have been reported elsewhere by Justine and Mattei (1982b) in the genus *Haematoloechus*, Miquel *et al.* (2000) in *O. furcatus*, Tang and Li (1996) in *Dicrocoelium chinensis*, Robinson and Halton (1982) in *Corrigia vitta*, Ndiaye *et al.* (in press a) in *N. neyrai*, and in the present study in *S. palaearcticum*. However, we observed the formation of attachment zones before the fusion of the flagella.

According to the present knowledge of the ultrastructure of spermiogenesis and the mature sperm, the expansions and ornamentation of the plasma membrane may offer great potential for phylogenetic applications. Nevertheless, more accurate studies are required to shed light on this issue. On the other hand, spermiogenesis appears as a very constant process in all digeneans examined thus far.

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