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Ultrastructure of spermatogenesis of the anoplocephalid cestode *Gallegoides arfaai* (Mobedi et Ghadirian, 1977) Tenora et Mas-Coma, 1978

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Abstract

Spermatogenesis in *Gallegoides arfaai* is similar to that described for other cestode species. Six incomplete synchronic cytokineses occur: four mitotic and two meiotic cell divisions. The primary spermatogonium divides forming two secondary spermatogonia. All further divisions occur simultaneously, resulting in a rosette of four tertiary, then eight quaternary spermatogonia and sixteen primary spermatocytes. The first meiotic division forms thirty-two secondary spermatocytes and after the second meiotic division sixty-four spermatids are formed. Spermiogenesis begins with the formation of a differentiation zone in the form of a conical projection of cytoplasm delimited by a ring of arching membranes. Within this area there are two centrioles, a centriolar adjunct and vestigial striated rootlets. During spermiogenesis, only one of the centrioles develops an axoneme that grows directly into the cytoplasmic extension. The other centriole remains oriented in a cytoplasmic bud and posteriorly aborts. The nucleus elongates and moves into the cytoplasmic extension. Granular material present in each sperm originates from electron-dense material present in the periphery of the spermatid. In the final stage of spermiogenesis two crest-like bodies appear at the base of the spermatid. Finally, the ring of arching membranes constricts and the young spermatozoon detaches from the residual cytoplasm. In order to increase homogeneity in the designation of the non-typical striated rootlets previously described, in this study we propose to group them under the common designation of "vestigial striated rootlets" and its importance is discussed according to previous findings of related structures in other cyclophyllideans.

Key words

Ultrastructure, spermatogenesis, Cestoda, Anoplocephalidae, *Gallegoides arfaai*

Introduction

The ultrastructural characters of spermatogenesis and the spermatozoa of parasitic Platyhelminthes have been shown to be useful in the interpretation of the phylogenetic relationships within this group of parasites (Swiderski 1968, 1986; Euzet *et al*. 1981; Justine 1991, 1998, 2001; Bâ and Marchand 1995; Hoberg *et al*. 1997). Several ultrastructural characters involved in spermatogenesis have been established as synapomorphies for the major groups of cestodes. In fact, the so-called "proximodistal fusion" has been recognized as a synapomorphy for the Cercomeridea, a taxon that includes the parasitic flatworms (Justine 1991). Furthermore, both the absence of "typical striated rootlets" in the zone of differentiation and absence of "flagellar rotation" during spermiogenesis have been accepted as synapomorphies for the Cyclophyllidea (Justine 1998, 2001).

The family Anoplocephalidae Cholodkowsky, 1902 comprises four subfamilies: Anoplocephalinae Blanchard, 1891, Inermicapsiferinae López-Neyra, 1943, Linstowinae Fuhrmann, 1907 and Thysanosomatinae Skryabin, 1933. Ultrastructural studies on spermatology have been done on several species of these subfamilies, particularly in the Anoplocephalinae. Ultrastructural studies on spermiogenesis in the Anoplocephalidae cestodes have been done on only six species (Bâ *et al*. 1991, 2000; Bâ and Marchand 1994a, b; Miquel and Marchand 1998; Li *et al*. 2003). We have previously described the fine structure of the mature spermatozoon of *Gal-*

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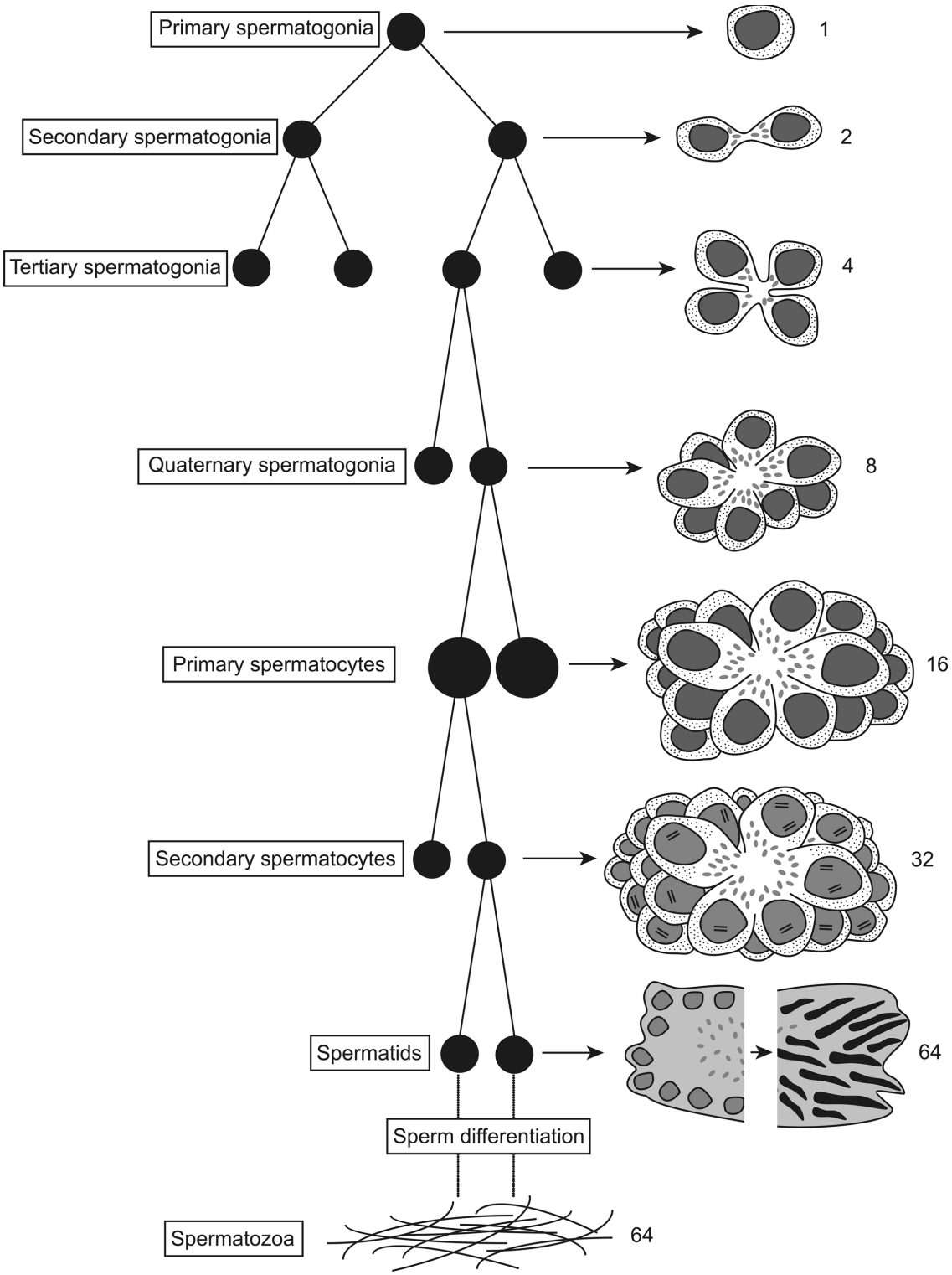
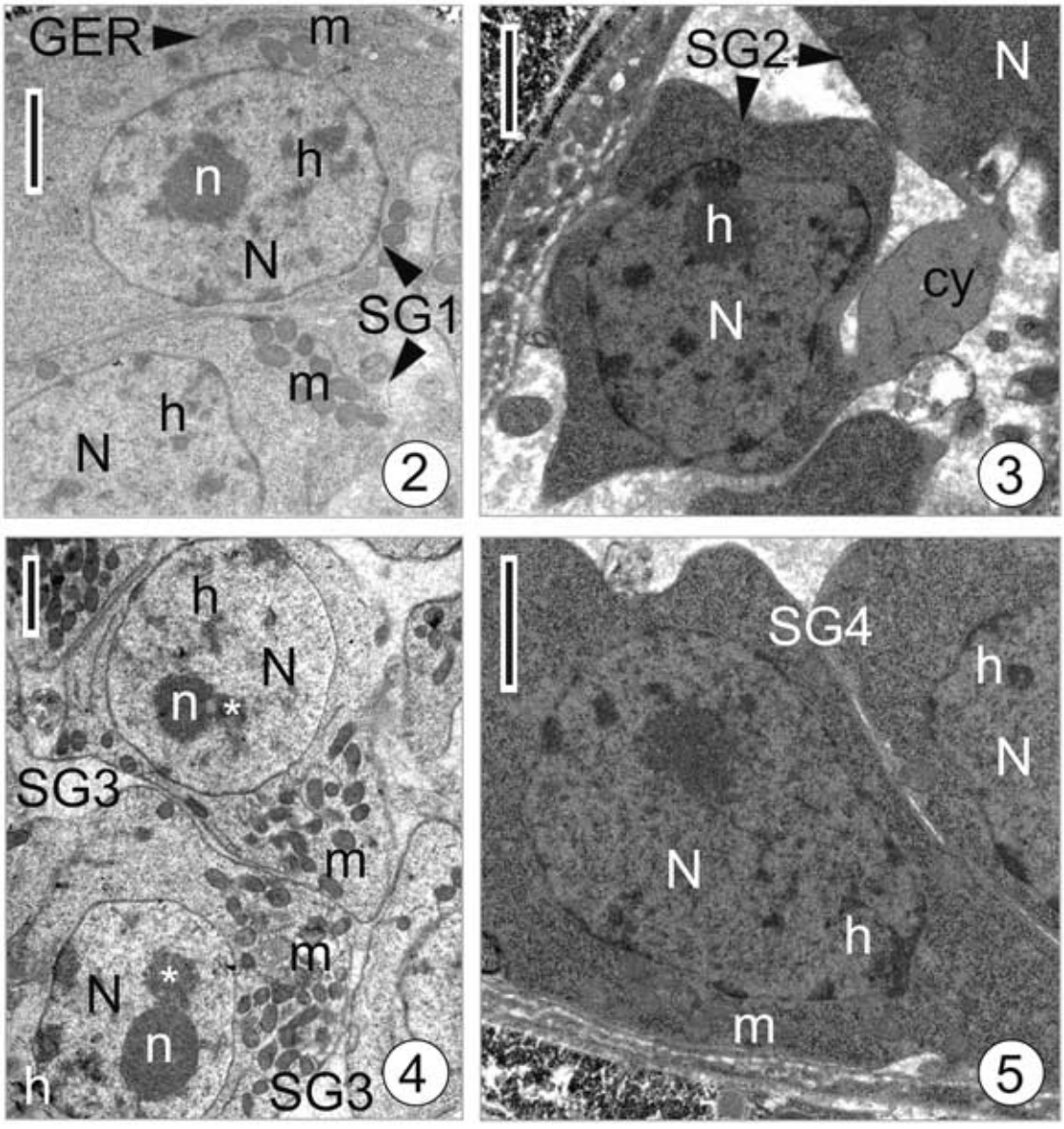


Fig. 1. Diagram illustrating the general pattern of the consecutive stages of spermatogenesis in *G. arfaai*. Number of nuclei of each stage originating from six incomplete synchronic divisions of a single primary spermatogonium is indicated on the right-hand margin. **Abbreviations to all figures:** am – arching membranes, ax – axoneme, b – cytoplasmic bud, c – centriole, ca – centriolar adjunct, cb – crest-like body, ce – cytoplasmic extension, cm – cortical microtubules, cy – cytophore, dm – dense material, fr – flagellar rotation, g – dense granules, GER – granular endoplasmic reticulum, h – heterochromatin islands, ib – intercentriolar body, m – mitochondria, N – nucleus, n – nucleolus, np – nuclear pores, pf – proximodistal fusion, pm – plasma membrane, SC1 – primary spermatocytes, SC2 – secondary spermatocytes, SG1 – primary spermatogonia, SG2 – secondary spermatogonia, SG3 – tertiary spermatogonia, SG4 – quaternary spermatogonia, sy – synaptonemal complexes, vsr – vestigial striated rootlets, *pars amorpha, **distal areas of spermatid

legoides arfaai (Miquel *et al*. 2004) while the process of fertilization in the same species was done by Świderski *et al*. (2004). The purpose of the present study is to describe the ultrastructural aspects of spermatogenesis in *G. arfaai*, thus providing additional new data on spermatology of anoplocephalids.

Materials and methods

Adult specimens of *Gallegoides arfaai* (Mobedi et Ghadirian, 1977) Tenora et Mas-Coma, 1978 were obtained from the small intestine of naturally infected wood mice, *Apodemus sylvaticus* Linnaeus, 1758 (Rodentia, Muridae) captured in Mosset and in the Natural Reserve of Py (Pyrenean Mountains, France). The living cestodes were placed in a 0.9% NaCl solution. Mature proglottids were routinely processed for TEM examination; they were dissected and fixed in cold (4°C) 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.2 for 2 h, rinsed in a 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 a 0.1 M sodium cacodylate buffer at pH 7.2, dehydrated in an ethanol series and propylene oxide, and finally embedded in Spurr's resin. Ultrathin sections were obtained using a Reichert-Jung Ultracut E ultramicrotome, placed on copper grids and double-stained with uranyl acetate and lead citrate. Ultrathin sections were examined using a JEOL 1010 transmission electron microscope.

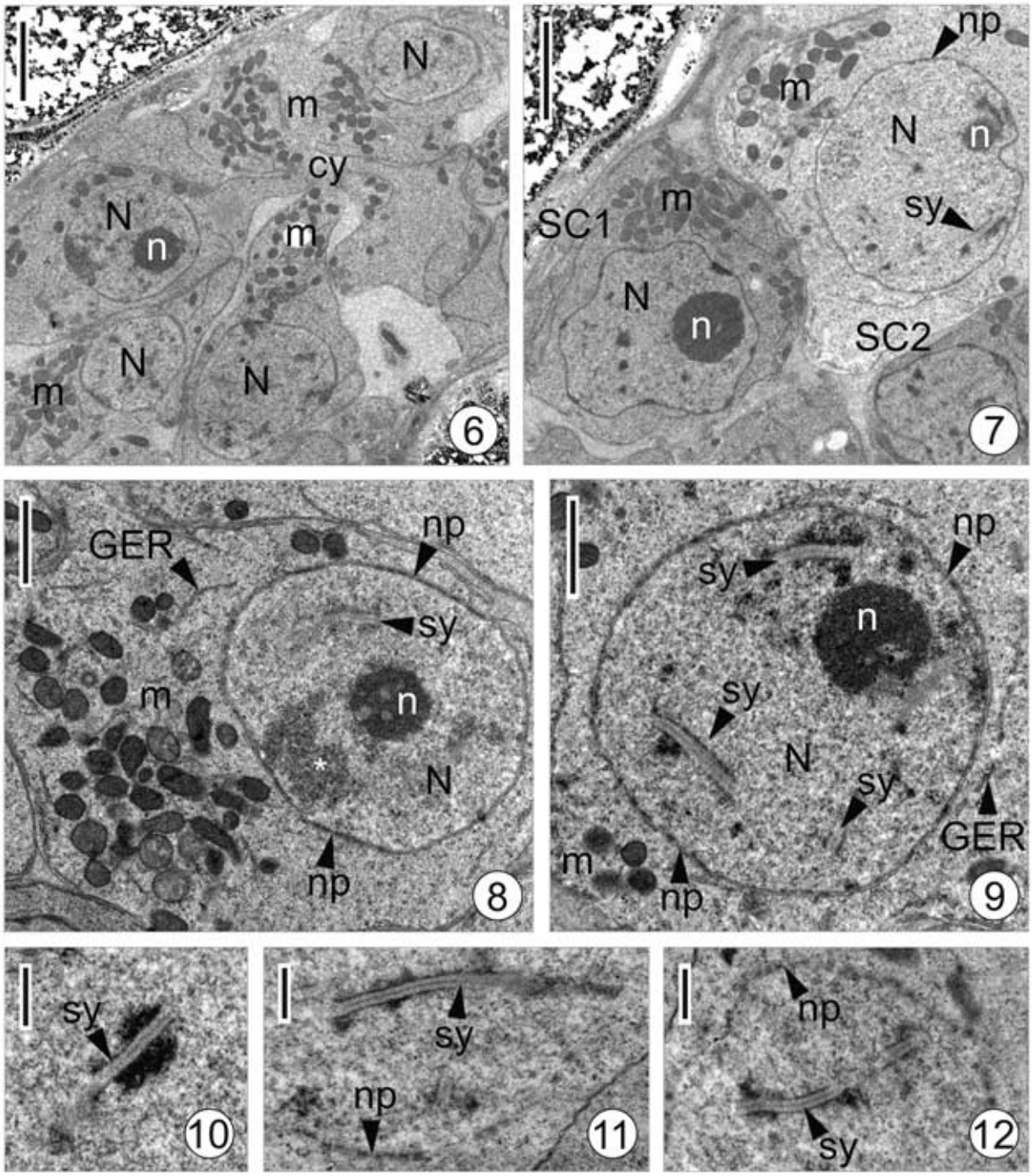


Figs 2–5. Ultrastructural details of four spermatogonial stages of *G. arfaai*. **Fig. 2.** Primary spermatogonia. **Fig. 3.** Two secondary spermatogonia. **Fig. 4.** Tertiary spermatogonia. **Fig. 5.** Quaternary spermatogonia. Scale bars = 1 μm

Results

General pattern of spermatogenesis

The testes follicles from mature proglottids of *Gallegoides arfaai* contain all consecutive stages of spermatogenesis. As in most parasitic Platyhelminthes, spermatogenesis in *G. arfaai* is of the rosette type (Fig. 1). During spermatogenesis six incomplete synchronic cytokineses occur: four mitotic and two meiotic. The primary spermatogonium (Figs 1 and 2), divides mitotically, producing two secondary spermatogonia



Figs 6–12. Ultrastructural details of the primary and secondary spermatocytes of *G. arfaai*. **Fig. 6.** Rosette of primary spermatocytes. **Fig. 7.** Comparison of primary and secondary spermatocytes. **Fig. 8.** Details of the cytoplasm and nucleus of the primary spermatocytes. **Fig. 9.** Details of the nucleus of the secondary spermatocytes. **Figs 10–12.** Details of synaptonemal complexes and nuclear pores in the secondary spermatocytes. Scale bars (Figs 6, 7) = 2 μm; (Figs 8, 9) = 1 μm; (Figs 10–12) = 0.5 μm

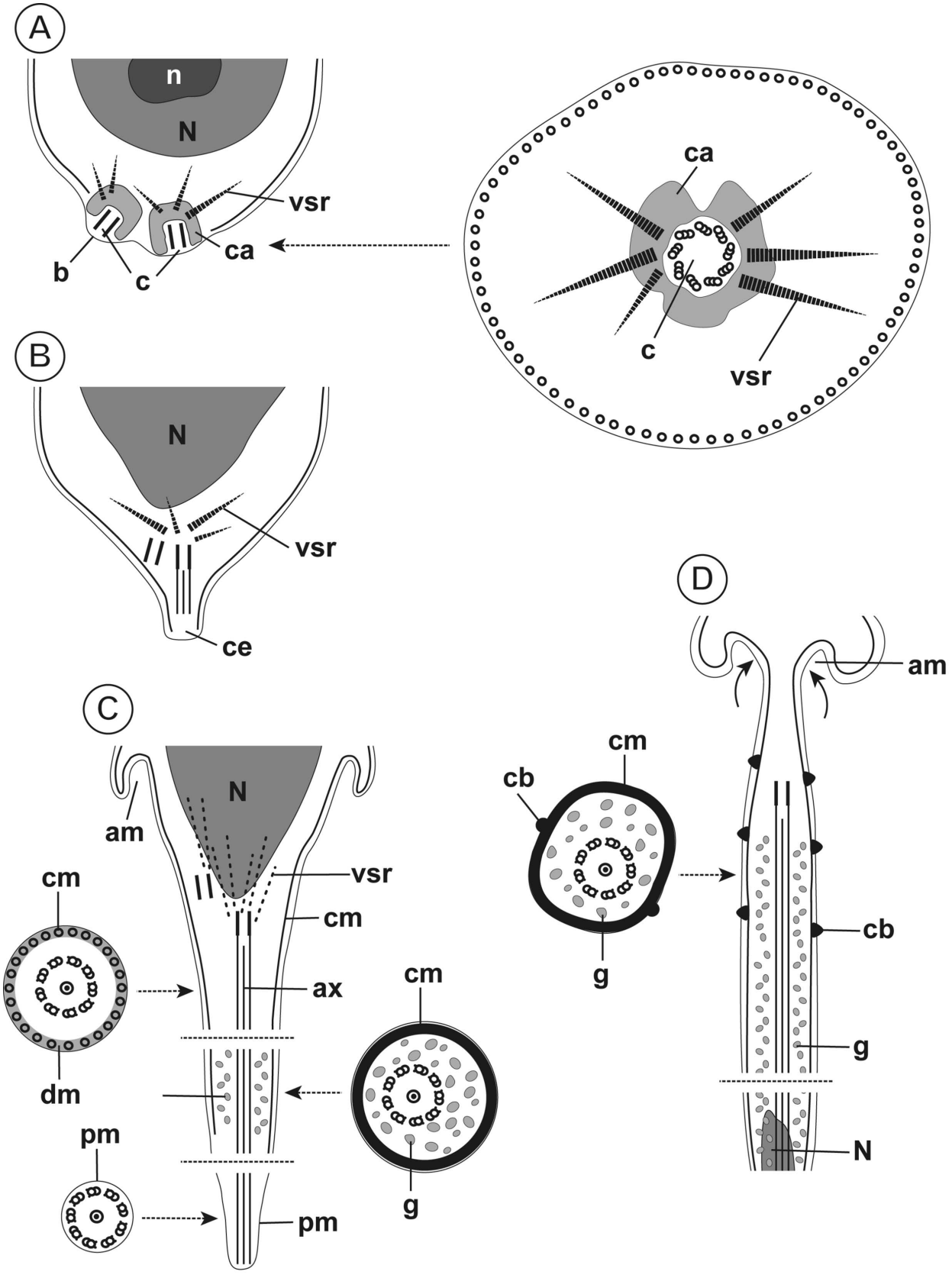
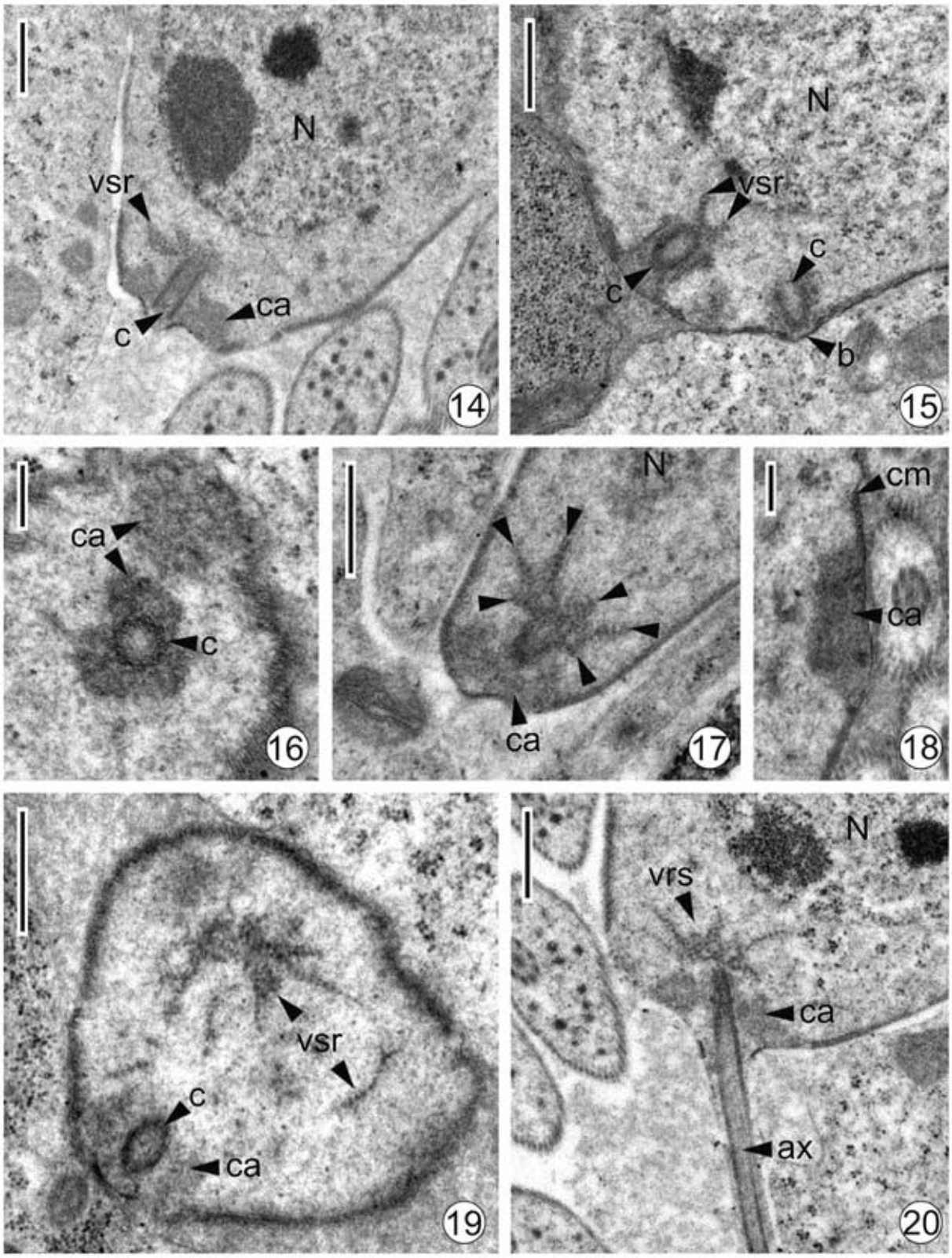
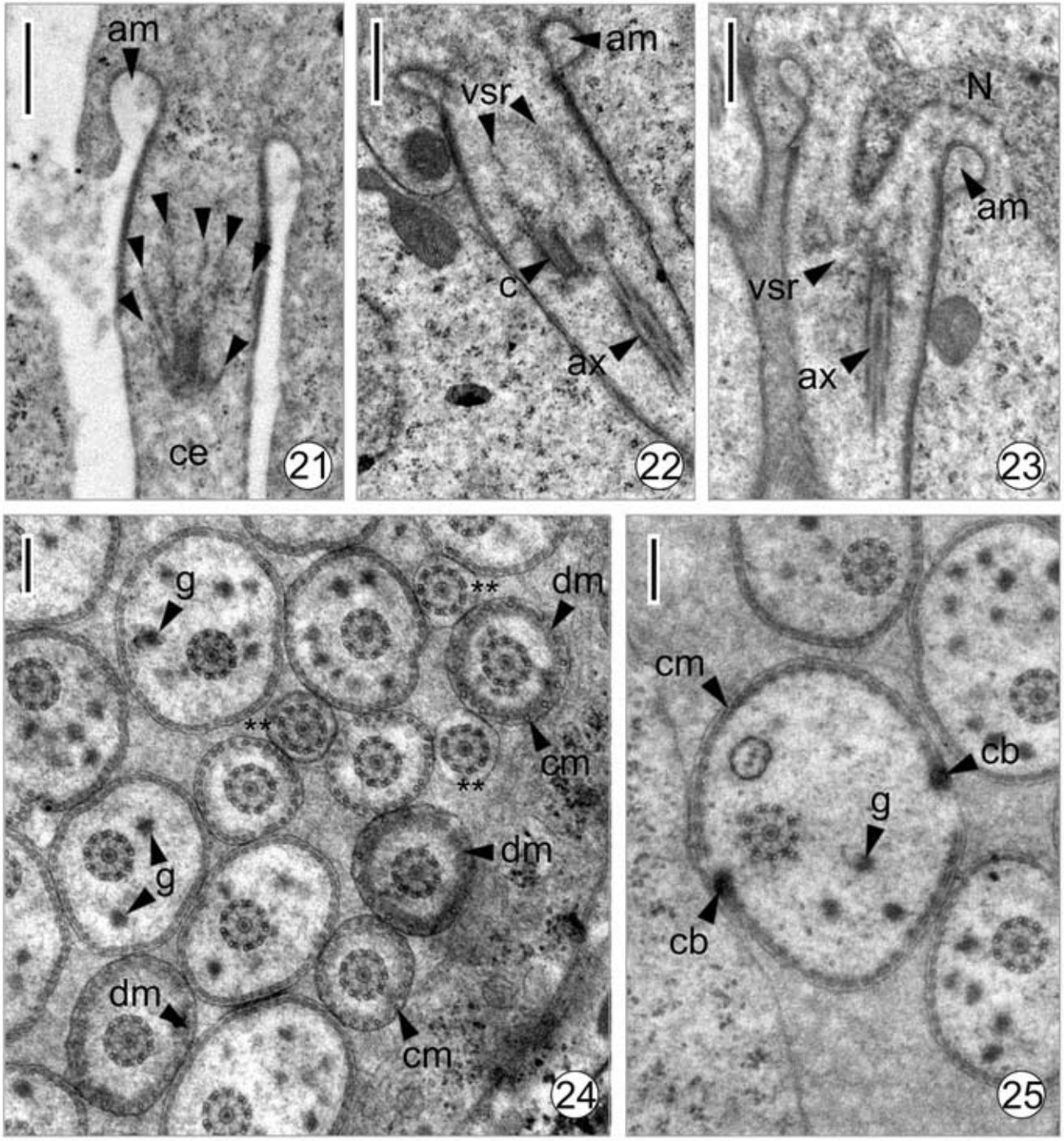


Fig. 13A-D. Diagram showing the main stages of spermiogenesis in *G. arfaai*



Figs 14–20. Spermiogenesis in *G. arfaai*. **Fig. 14.** Differentiating zone showing vestigial striated rootlets and centriolar adjunct associated with centriole. **Fig. 15.** Differentiating zone with centrioles. **Fig. 16.** Detail of centriole with centriolar adjunct. **Fig. 17.** Oblique section of differentiating zone showing six vestigial striated rootlets. **Fig. 18.** Detail of centriolar adjunct. **Fig. 19.** Several vestigial striated rootlets in a cross-section of a differentiation zone. **Fig. 20.** Longitudinal section of a differentiation zone showing the growth of the axoneme. Scale bars (Figs 14, 15, 17, 19, 20) = 0.5 μm; (Figs 16, 18) = 0.2 μm

(Figs 1 and 3), but the two daughter cells remain connected with each other by a cytoplasmic bridge. All further divisions occur simultaneously, resulting in a rosette of four tertiary (Figs 1 and 4), then eight quaternary spermatogonia (Figs 1 and 5); subsequently sixteen primary spermatocytes are formed (Figs 1, 6 and 7). Those enlarge, their nuclei move to the periphery and the syncitium takes on the form of a cluster. After the first meiotic division, a cluster of thirty-two secondary spermatocytes is formed (Figs 1 and 7–9). The latter present smaller nuclei, less granular cytoplasm (Fig. 7) and the cell membranes near the centre of the cluster become indistinct as the displacement of the nuclei toward the periphery continues.



Figs 21–25. Spermiogenesis in *G. arfaai*. **Figs 21–23.** Longitudinal sections of spermatids showing vestigial striated rootlets, abortive centriole and elongation of nucleus, respectively. **Fig. 24.** Cross-sections of different areas of spermatids showing the formation of dense granules. **Fig. 25.** Cross-section of a spermatid with two crest-like bodies. Scale bars (Figs 21–23) = 0.5 μm; (Figs 24 and 25) = 0.2 μm

The second maturation division results in sixty-four spermatids (Fig. 1), the nuclei of which subsequently elongate and differentiate into spermatozoa (Figs 1 and 13A-D). The mature spermatozoa are released, leaving behind a large, deeply stained body as a residual mass of cytoplasm in the testis.

Ultrastructural details of the consecutive stages of spermatogenesis

The primary spermatogonia (Fig. 2), approximately 7 to 8 μm in diameter, are oval in shape with a large nucleus and a thin layer of cytoplasm, thus showing a low cytoplasm:nucleus ratio. The two secondary spermatogonia share a common cytoplasm (Fig. 3), whereas the four tertiary (Fig. 4) and the eight quaternary spermatogonia (Fig. 5) are linked by narrow cytoplasmic processes, indicating incomplete cytokineses. The nuclei of tertiary spermatogonia are euchromatic; they contain a large nucleolus of heterogeneous type composed of electron-dense nucleolonema and a network of pars amorpha (Fig. 4). Their nucleoplasm shows peripheral islands of heterochromatin of different size and electron density, usually adjacent to the nuclear envelope. A thin layer of their cytoplasm contains numerous ribosomes, few narrow cisternae of granular endoplasmic reticulum (GER) and several small mitochondria (Fig. 4). The ultrastructure of secondary, tertiary and quaternary spermatogonia (Figs 3–5) generally resembles the primary spermatogonia in many features, but they are arranged in groups of two, four and eight, respectively. The nuclei of spermatogonia are at the periphery of the rosettes and are connected by cytoplasmic bridges to the central cytoplasm, the cytophore (Figs 1 and 3). The cytophore is usually less electron-dense than the rest of the rosette (Fig. 3).

The spermatocytes of *G. arfaai* and particularly secondary spermatocytes (Fig. 7) are less electron-dense than the spermatogonia (compare Figs 2–5 and 7–9) and primary spermatocytes (Figs 6 and 7). Another characteristic of secondary spermatocytes is the large nuclear pores (Figs 7–9, 11 and 12). The presence of numerous synaptonemal complexes (Figs 7–12) confirms the first division of meiosis. Each synaptonemal complex (Figs 7–12) consists of a pair of coarse filaments separated by a clear space, through which runs a dense central element or medial complex. Transverse fibres cross the central region and connect with lateral filaments on each side of the complex (Figs 10–12).

The spermatid nuclei (Figs 1, 13A, 14 and 15) are smaller than those of spermatogonia and spermatocytes and are situated at the periphery of the cluster. The plasma membrane of the cluster invaginates into its cytoplasm, encircling partially or completely the nuclei, forming pockets or the so-called differentiation zones from which the spermatozoa develop (Figs 1, 13A-D and 14–25).

Spermiogenesis

The general pattern of spermiogenesis in *G. arfaai* is schematically illustrated in Figure 13A-D. The initial stage of spermiogenesis in *G. arfaai* begins with: (1) the formation of an arching membrane-bound cleft; (2) delimitation of a differentiation zone in the form of a cone-shaped cytoplasmic projection supported by a ring of peripheral microtubules and (3) a change in the shape and density of the spermatid nucleus. Within the differentiation zone there are two centrioles associated both with an electron-dense material, the so-called centriolar adjunct, and with remnants of striated rootlets for which the term "vestigial striated rootlets" is proposed (Figs 13A-C, 14, 15, 17, 19 and 20–23). During spermiogenesis only one of the centrioles forms an axoneme that grows directly into the cytoplasmic extension (Figs 13B and 20). The other centriole remains oriented in a cytoplasmic bud and aborts in a later stage of spermiogenesis after its incorporation into the cytoplasmic extension (Figs 13A-C, 15 and 22). The nucleus elongates and moves into the cytoplasmic extension (Figs 13C and 23). The granular material present in sperm probably originates from an electron-dense material present in the periphery of the early spermatids (Figs 13C and 24). Such electron-dense material is observed when cortical microtubules are still parallel (Figs 13C and 24). Later on, when cortical microtubules become twisted, this material transforms into electron-dense granules (Figs 13C and 24). The axoneme reaches distal areas of spermatids ahead of the cortical microtubules (Figs 13C and 24). In the final stage of spermiogenesis two crest-like bodies appear at the base of the spermatid near the arching membranes (Figs 13D and 25). Finally, the ring of arching membranes constricts and the young spermatozoon detaches from the residual cytoplasm (Fig. 13D).

Discussion

Spermatogenesis

The general pattern of spermatogenesis observed in *Gallegoides arfaai* is similar to that described for other cestode species (for review see: Rybicka 1966, Świderski and Mackiewicz 2002). This pattern differs from that described in trematodes, where there are only three mitotic divisions of spermatogonia, resulting in 32 spermatozoa in one cluster originated from a single primary spermatogonium (Rybicka 1966, Świderski and Mackiewicz 2002). The formation of multicellular rosettes and clusters is similar to that described in other cestodes and appears typical for parasitic Platyhelminthes. However, some exceptions from the rosette-type spermatogenesis in parasitic flatworms do occur. For example, the gametogenic cells remain separated throughout their development and differentiation into spermatozoa in some schistosome species such as *Schistosoma bovis* (Justine 1980) and *S. mattheei* (Swiderski and Tsinonis 1986). On the other hand, in other schistosome species such as *Schistosomatium douthitti* (Nez and Short 1957) and *Schistosoma magrebowiei* (Awad and Probert 1989) there is the rosette-type spermatogenesis.

As in most of other Platyhelminthes, all cell divisions within rosettes in *G. arfaai* are synchronic and allow for the

production of a large number of spermatozoa. There remains some controversy regarding the nature of rosette formation and the precise timing of this process. According to Taneya (1973), a true rosette appears at the primary spermatocyte stage only as a result of secondary fusion of initially separated cells. However, our results on *G. arfaai* support the interpretation presented in most of the previous studies on parasitic Platyhelminthes, which consider incomplete cytokineses as the major mechanism leading to rosette formation.

Mitotic figures are common features of the testes of caryophyllideans *Hunterella nodulosa* (Kazacos and Mackiewicz 1972) and *Glaridacris catostomi* (Świderski and Mackiewicz 2002), but they were never observed in *G. arfaai*.

The initial stages of spermiogenesis in *G. arfaai* are similar to those of other cestode species examined so far, and are characterized by the formation of an arching membrane-bound cleft, zone of differentiation supported by cortical microtubules and a change in the density of the spermatid nucleus.

Type of spermiogenesis

Swiderski (1986), based on the data available at the time, recognized three patterns of spermiogenesis in the cestodes: (1) the "pseudophyllidean type" based on sperm development in the Tetraphyllidea-Onchobothriidae *Acanthobothrium filicolle benedeni*; (2) the "caryophyllidean type" with the example of spermiogenesis in the Caryophyllidea *Glaridacris catostomi*; and (3) the "cyclophyllidean type" illustrated by spermiogenesis in the Cyclophyllidea *Rodentolepis microstoma* (= *Hymenolepis microstoma*). The three types of spermiogenesis were proposed based on the formation of one or two flagella from the zone of differentiation and on the growth of the flagellum inside or outside the cytoplasmic extension. The pseudophyllidean type is characterized by the orthogonal growth of two free flagella externally to the cytoplasmic extension, the so-called "median cytoplasmic process", as well as by the posterior "flagellar rotation" and "proximodistal fusion" of these flagella. The caryophyllidean type is characterized by the orthogonal and external growth of a single flagellum. Spermiogenesis finishes with the flagellar rotation and proximodistal fusion of the free flagellum with the cytoplasmic extension. Finally, according to Swiderski (1986), the cyclophyllidean type is characterized by the growth of a single axoneme directly into the cytoplasmic extension. Therefore, neither flagellar rotation nor proximodistal fusion occurs in the cyclophyllidean pattern of spermiogenesis.

Bâ and Marchand (1995), based on further ultrastructural studies on spermatology of cestodes, established four patterns of spermiogenesis. Type I occurs in the Tetraphyllidea-Onchobothriidae, Proteocephalidea, Trypanorhyncha (= Tetrarhynchidea) and Pseudophyllidea. Type II is typical of Tetraphyllidea-Phyllobothriidae and Caryophyllidea. Type II spermiogenesis has also been described in the Tetrabothriidea *Tetrabothrius erostris* (Stoitsova *et al*. 1995) and the cyclophyllidean Mesocestoididae *Mesocestoides litteratus* (Miquel *et al*. 1999). Moreover, Bâ and Marchand (1995) added a second type of spermiogenesis for the cyclophyllideans and thus cyclophyllideans present either type III or type IV. Type III is characterized by an external but parallel growth of a single flagellum. In this type, a proximodistal fusion occurs. On the other hand, type IV also gives origin to a spermatozoon with a single axoneme, but in this case the axoneme grows directly into the cytoplasmic extension and, consequently, the proximodistal fusion does not occur. Bâ and Marchand (1995) described the presence of an electron-dense material associated with the centrioles, the so-called centriolar adjunct. This structure was also observed during spermiogenesis of *G. arfaai*.

Gallegoides arfaai, as most of the anoplocephalids studied to date, follows a type IV spermiogenesis according to Bâ and Marchand (1995) (see Table I). These authors have established this apomorphic type based on the absence of both fla-

Table I. Present state of knowledge on the ultrastructure of spermiogenesis in Anoplocephalidae species

Anoplocephalidae subfamilies and species	Character						Reference
	type	pf	fr	vsr	ib	ca	
Anoplocephalinae							
Anoplocephaloides dentata	IV	–	–	+*	–	–	Miquel and Marchand (1998)
Aporina delafondi	IV	–	–	–	–	–	Bâ and Marchand (1994b)
Gallegoides arfaai	IV	–	–	+	–	+	present study
Moniezia expansa	IV	–	–	+**	–	–	Li *et al*. (2003)
Sudarikovina taterae	IV	–	–	–	–	+***	Bâ *et al*. (2000)
Inermicapsiferinae ****							
Linstowinae							
Mathevotaenia herpestis	III	+	–	–	–	–	Bâ and Marchand (1994a)
Thysanosomatinae							
Thysaniezia ovilla	IV	–	–	–	–	+	Bâ *et al*. (1991)

ca – centriolar adjunct, fr – flagellar rotation, ib – intercentriolar body, pf – proximodistal fusion, vsr – vestigial striated rootlets, + presence of the character, – absence of the character, *thin striated rootlets, **spiral rootlets, ***intercentriolar dense material, ****no data.

gellar rotation and proximodistal fusion. Within the anoplocephalids, only *Mathevotaenia herpestis* (Linstowinae) (Bâ and Marchand 1994a) differs from this pattern; it presents type III spermiogenesis. All the Anoplocephalinae and Thysanosomatinae species follow type IV. No data exist for Inermicapsiferinae (Table I). Apart from the anoplocephalids, within the Cyclophyllidea, type IV spermiogenesis has been found only for the representatives of the family Hymenolepididae: *Dicranotaenia coronula* (Chomicz and Swiderski 1992), *Hymenolepis diminuta* (Kelsoe *et al*. 1977), *Monorcholepis dujardini* (Swiderski and Tkach 1996), *Rodentolepis nana* (= *H. nana*) (Bâ and Marchand 1992) and *R. microstoma* (= *Vampirolepis microstoma*) (Bâ and Marchand 1998). Species belonging to the other examined families of Cyclophyllidea follow type III spermiogenesis. This is the case for *Catenotaenia pusilla* (Catenotaeniidae) (Hidalgo *et al*. 2000); *Raillietina tunetensis* (Davaineidae) (Bâ and Marchand 1994c); *Dipylidium caninum*, *Joyeuxiella echinorhyncoides* and *J. pasqualei* (Dipylidiidae) (Miquel *et al*. 1998, 2005; Ndiaye *et al*. 2003a); *Nematotaenia chantalae* (Nematotaeniidae) (Mokhtar-Maamouri and Azzouz-Draoui 1990); and *Taenia hydatigena*, *T. parva*, *T. solium* and *T. crassiceps* (Featherston 1971; Ndiaye *et al*. 2003b; Willms *et al*. 2003, 2004).

Striated rootlets and other rootlet-like structures

All anoplocephalid species lack "typical striated rootlets". In fact, this has been postulated as a synapomorphy for the cyclophyllideans (Justine 2001), although the mesocestoidid *M. litteratus* (Miquel *et al*. 1999) presents typical striated rootlets associated with their centrioles. However, the controversial situation of mesocestoidids within the cyclophyllideans has been discussed by several authors (see Rausch 1994, Mariaux 1998, Miquel *et al*. 1999, Justine 2001). Concerning the Anoplocephalidae species, *Anoplocephaloides dentata* shows "thin striated rootlets" associated with the centrioles in its zone of differentiation (Miquel and Marchand 1998). The study of spermiogenesis in the dipylidiid *D. caninum* (Miquel *et al*. 1998, 2005) has also demonstrated the presence of thin striated rootlets associated with the centrioles. These two findings have motivated the recodification of the character "absence of striated roots" to "absence of typical striated roots" in order to consider the synapomorphy for the Cyclophyllidea (see Justine 2001). Additionally, recent studies of Ndiaye *et al*. (2003a) and Miquel *et al*. (2005) demonstrate the presence of well-developed striated rootlets in two dipylidiids, *J. echinorhyncoides* and *J. pasqualei*. The family Dipylidiidae has only three genera, all parasites of carnivores (*Dipylidium*, *Diplopylidium* and *Joyeuxiella*, see Jones 1994). Thus, two of the three genera of Dipylidiidae show striated rootlets associated with the centrioles: thin striated rootlets in *D. caninum* (Miquel *et al*. 1998, 2005) and typical striated rootlets in *Joyeuxiella* spp. (Ndiaye *et al*. 2003a, Miquel *et al*. 2005). Therefore, the study of spermiogenesis and the spermatozoon of the third genus of the family Dipylidiidae (*Diplopylidium*) would be of great interest. Moreover, recently Li *et al*. (2003) have examined sperm development in the anoplocephalid *Moniezia expansa* and described a new character named "spiral rootlets". These spiral rootlets are very different from typical well-developed striated rootlets and thin striated rootlets. They may represent an intermediate state between these two rootlet conditions. In our opinion, the plesiomorphic striated rootlets may have undergone a progressive reduction in the cyclophyllideans, leading towards the total absence of this feature in the more evolved cestodes. In the present study, we observed up to six thin striated rootlets together in groups of three situated at each side of the centriole. We believe that the typical striated rootlets, in certain species of cestodes are degenerated and divided forming several thin striated rootlets arranged in groups. This arrangement may represent an intermediate state between the typical well-developed striated rootlets in digeneans and non-cyclophyllidean cestodes (see Justine 2001) and its absence in cyclophyllideans, which are considered the most evolved cestodes (except for the mesocestoidid *M. litteratus* and for the dipylidiids *J. echinorhyncoides* and *J. pasqualei*, see Miquel *et al*. 1999, 2005; Ndiaye *et al*. 2003a). In order to increase homogeneity in the designation of the previously described non-typical striated rootlets, in this study we propose to group them under the common designation of "vestigial striated rootlets".

Flagellar rotation

Similar variations in the range of expression occur with other characters such as "flagellar rotation". Taking into account the recent observations of flagellar rotations of up to 120° in certain digeneans (Levron *et al*. 2003, 2004; Ndiaye *et al*. 2003c), it appears that there may be a gradual reduction of the rotation angle until there is no rotation, a state that is considered the apomorphic condition of this character (Justine 1998, 2001). In fact, two cyclophyllideans, the catenotaeniid *C. pusilla* (Hidalgo *et al*. 2000) and the taeniid *T. parva* (Ndiaye *et al*. 2003b) show a flagellar rotation of about 45° before the proximodistal fusion of the free flagellum with the cytoplasmic extension.

Intercentriolar body

The case of the intercentriolar body is quite similar. In fact, the mesocestoidid *M. litteratus* (Miquel *et al*. 1999) and the proteocephalideans *Nomimoscolex* sp. (Sène *et al*. 1997), *Proteocephalus torulosus* (Bruňanská *et al*. 2003b), *P. longicollis* (Bruňanská *et al*. 2004a) and *Corallobothrium solidum* (Bruňanská *et al*. 2005) present a "reduced intercentriolar body" as an intermediate state between the well-developed intercentriolar body in the more primitive cestode orders and its absence in the orders Tetrabothriidea and Cyclophyllidea (see Justine 2001). In this regard, it would be very interesting to elucidate the status of the intercentriolar body and other aspects of spermiogenesis in *Sandonella sandoni* (Proteocephalidea), species for which the ultrastructure of the spermatozoon is already known (Bâ and Marchand 1994d). This species also

exhibits spiralled cortical microtubules, considered the apomorphic condition in contrast with the plesiomorphic condition of parallel cortical microtubules found in the spermatozoon of other proteocephalideans (*Nomimoscolex* sp., Sène *et al*. 1997; *P. longicollis* and *P. torulosus*, Bruňanská *et al*. 2003a, c; and *Electrotaenia malopteruri*, Bruňanská *et al*. 2004b). However, Bruňanská *et al*. (2004c) described recently a spiralled pattern of cortical microtubules in certain areas of sperm in another proteocephalid (*Corallobothrium solidum*). In addition, the "intercentriolar body" of the Tetraphyllidea-Onchobothriidae, *Acanthobothrium filicolle filicolle* (Mokhtar-Maamouri 1982), probably represents a reduced intercentriolar body.

Concluding remarks

The results of the above-mentioned analysis indicate the need for further ultrastructural studies on cestode spermiogenesis, particularly in the anoplocephalids, in order to obtain a better picture of their phylogenetic relationship among the cestodes. Within the family Anoplocephalidae, the study of representatives of the subfamily Inermicapsiferinae would be particularly desirable. Results obtained so far indicate that type IV spermiogenesis is the most frequent in the Anoplocephalidae and appears constant in all the Anoplocephalinae species subject to ultrastructural studies.

In spite of the recent advances in research on anoplocephalid spermiogenesis, the variability among ultrastructural characters remains to be assessed, mainly in reference to the striated rootlets and to other structures associated with the centrioles, such as the centriolar adjunct.

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