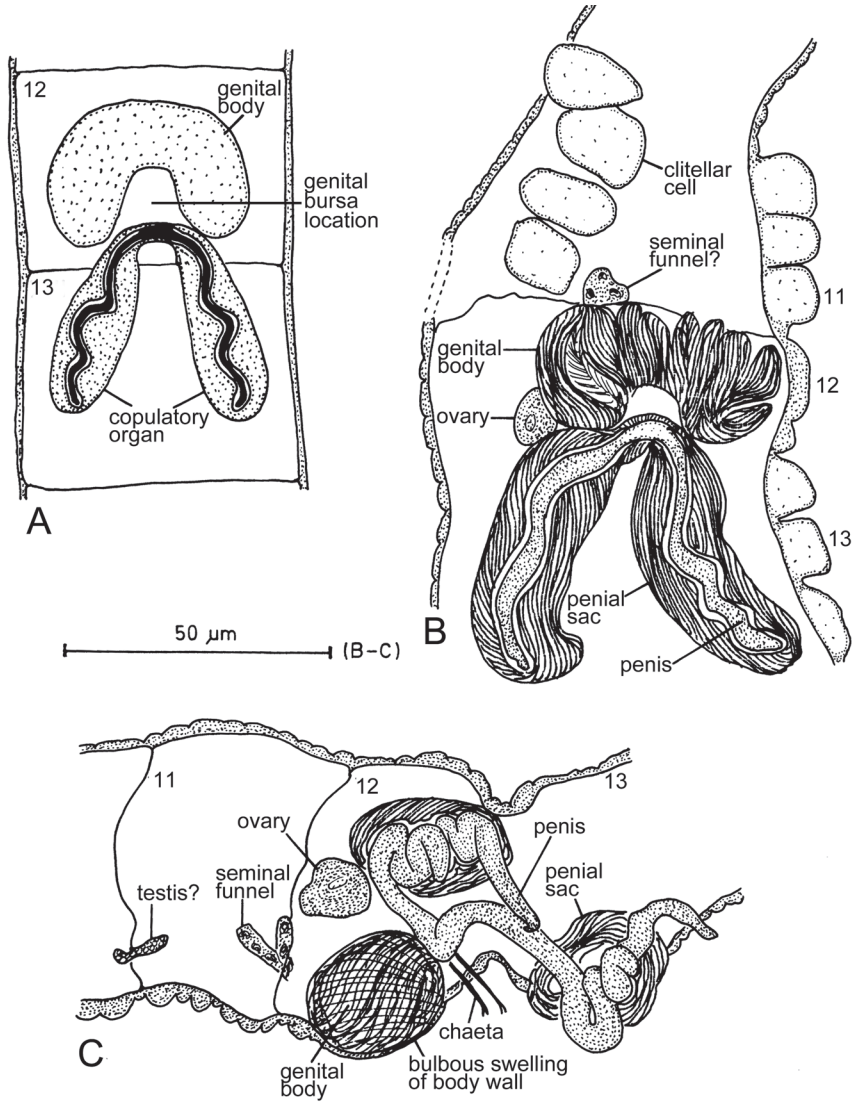


Fig. 8.23. *Parvidrilus strayeri* (Parvidrilidae). **A**. Schematic horizontal view of segments 12 and 13, showing general outline of 'genital body' and 'copulatory organ'. **B**. Somewhat horizontal view of segments 11-13 of a paratype. **C**. Somewhat lateral view of segments 11-13 of a further paratype. Relabeled after Erséus, C. 1999. Proceedings of the Biological Society of Washington 112(2): 327-337, Fig. 2.

species, *P. spelaeus*, suggest that the genital body and copulatory organ are respectively the atrium and spermathecae.

Narapidae. Narapidae, a monotypic family (Righi and Varela 1983), are plesioporous and resemble the Naidinae in having the testes in segment 5 but differ from these in having the spermathecae in the ovarian, not the testicular, segment (Fig. 8.24). Male pores and atria are in 6, and penes are present. The

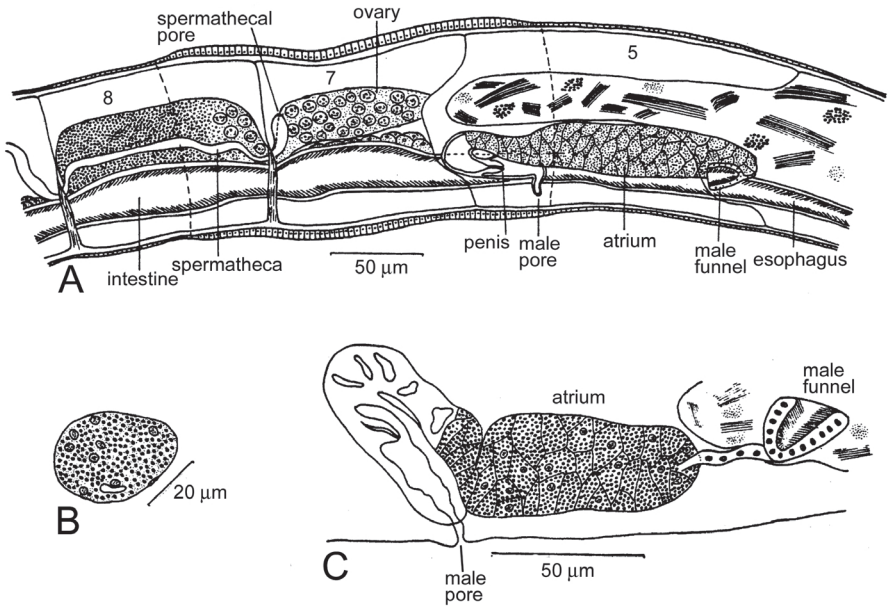


Fig. 8.24. *Narapa bonettoi* (Narapidae). **A**. Lateral view of segments 6-8. **B**. Transverse section of atrium. **C**. Lateral view of male ducts. Relabelled after Righi, G. and Varela, M. E. 1983. Revista De La Asociacion De Ciencias Naturales Del Litoral 14(1): 7-15, Figs. 4-6.

atria are covered by diffuse gland cells. Whereas the testes and efferent ducts are paired, the ovary, in 7, is unpaired. There is a pair of spermathecae in 7. Like the Randiellidae and Propappidae, there is a gonad-less segment between the testicular and ovarian segments. This might represent a proandric condition derived from former holandry.

8.2.11.3 Subclass Lumbriculata

We will here deal only with the oligochaetous members, the Lumbriculidae. Other taxa here included are the Branchiobdellida, Acanthobdellida and Euhirudinea which are discussed in Chapter **9**.

Lumbriculidae. The Lumbriculidae is an Holarctic family with extension into West Asia. Some species have become widely distributed, including the Southern Hemisphere. The reproductive system is very variable. There are one to four pairs of testes, in variable locations. Atria are one to four pairs, located between segments 7 and 15, paired or unpaired, always in a testis-bearing segment, each being associated with one or two pairs of testes (Figs. 8.4B, 8.25). There are commonly two pairs of testes in adjacent segments, both with funnels and vasa deferentia feeding a single pair of atria in the same segment as the posterior pair of testes. Sometimes the anterior testes and ducts are absent, leaving a single pair of atria, testes and vasa deferentia within one segment, and then often with this arrangement serially repeated. There are one or two pairs of ovaries beginning one, or rarely two, segments behind the most posterior testis-bearing segments. Spermathecae are variable

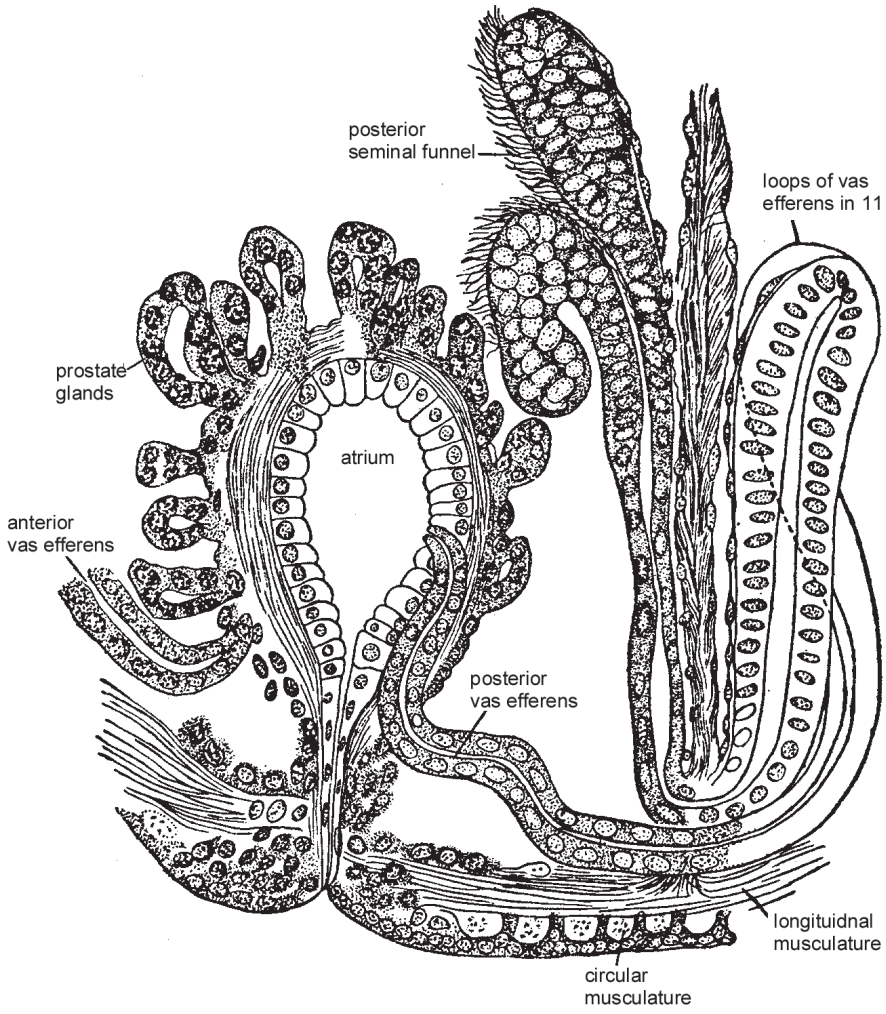


Fig. 8.25. *Bythonomus mirus* (Lumbriculidae). Atrium and posterior male gonoduct. After Chekanovskaya, O.V. 1981. Aquatic Oligochaeta of the USSR, United States Department of the Interior and the National Science Foundation, Washington, D.C., Amerind Publishing Co. Pvt. Ltd., New Delhi, pp. 513, Fig. 232.

in number and either anterior or posterior to the testicular segments (Pinder and Brinkhurst 1994).

Michaelsen (1928-32) (see Michaelsen 1928) brilliantly foreshadowed the findings of molecular phylogenetics when he illustrated (Fig. 8.26) a pathway from lumbriculid organization to that of hirudinid leeches. The progressive stages were exemplified by 1) the lumbriculid *Rhynchelmis*, with compact testes but long seminal vesicles, extending through several segments; 2) the lumbriculid *Agriodrilus vermivorus*, in which a chain of testes has developed within the elongate seminal vesicles, though still with a single pair of

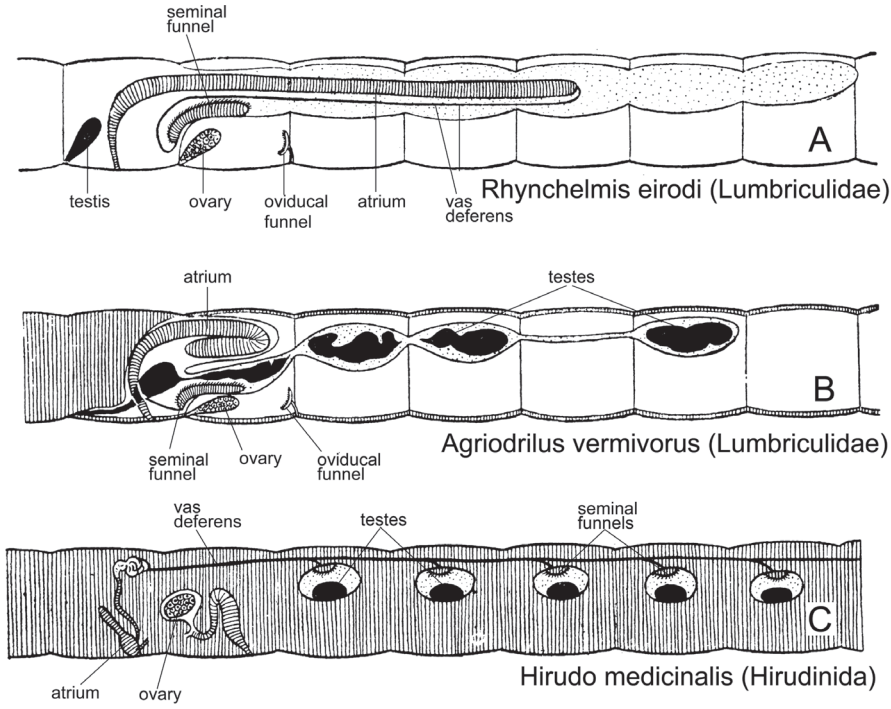


Fig. 8.26. Hypothetical scheme suggesting that the testicular sacs of leeches corrrespond to the seminal vesicles of lumbriculids. **A**. *Rhynchelmis*. **B**. *Agriodrilus vermivorus* offers an intermediate in which a long series of testicular portions has developed in the original seminal vesicles. In an abnormal condition one of these was seen to be isolated like a testis in the chain of testes occurring in leeches. **C**. The leech condition: a chain of postovarian testes. Modified from Michaelsen, W. 1928. Oligochaeta. In W. Kukenthal and T. Krumbach (eds). Handbuch der Zoologie 2, Fig. 93.

seminal funnels, and in which the coelom is constricted; 3) *Hirudo* in which each testicular chamber has acquired its own pair of seminal funnels and the coelom has been reduced to a system of sinuses. The leech distinction from oligochaetes, extension of testes posterior to the ovaries, was thus explained in terms of modification of pre-existing seminal vesicles.

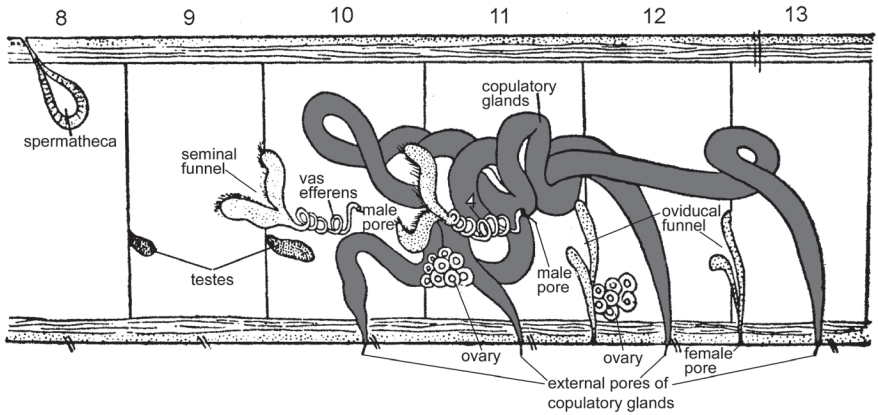
8.2.11.4 Subclass Diplotesticulata

The validity of recognizing the Diplotesticulata is discussed in 8.1.4 above.

Superorder Haplotaxidea. Order Haplotaxida *sensu stricto*. The haplotaxid reproductive system usually has two pairs of testes, in segments 10 and 11 (rarely in 9 and 10); the anterior pair is rarely absent. There are one or two pairs of ovaries in the segments following the testicular segments. The male ducts are simple and lead to ventrolateral or lateral male pores. However there is a large glandular mass between the male pores in *Hologynus hologynus*, in which both pairs of male pores lie in the same segment, the posterior vasa deferentia being reflexed forward. The two pairs of vasa deferentia also open into a single segment in *Pelodrilus violaceus* but

in that case the anterior vasa penetrate more than one segment, discharging near the posterior pair in segment 12.

In *Adenodrilus denticulatus* there are four pairs of large copulatory glands which open externally near the ventral setae and are not directly associated with the male ducts (Chekanovskaya 1981) (Fig. 8.27). These glands are reminiscent of those of *Sparganophilus*, a genus which has in the past been placed in the Haplotaxidae (Tétry 1934), but the molecular study (Jamieson *et al*. 2002) indicates that at least the type-species, *Haplotaxis gordioides*, is genetically distant from *Sparganophilus*. One species, *H. brinkhursti*, has lost the anterior pair of ovaries and therefore is unique in the known Haplotaxidae in having the metagynophoran condition.



8.27. *Adenodrilus denticulatus* (Haplotaxidae). Lateral view of genital organs, showing large copulatory glands. After Chekanovskaya, O. V. 1981. Aquatic Oligochaeta of the USSR, United States Department of the Interior and the National Science Foundation, Washington, D. C., Amerind Publishing Co. Pvt. Ltd, New Delhi. pp. 513, Fig. 204.

Tiguassuidae. The Tiguassuidae was recognized as a family by Jamieson (1988b) and by Brinkhurst (1988) in morphocladistic analyses for *Tiguassu reginae* which Righi *et al*. (1978) had placed in the Hapolotaxidae. In the analysis of Jamieson (1988b) *Tiguassu* proved paraphyletic relative to the Haplotaxidae *sensu lato* and formed the plesiomorphic sister-taxon of the Metagynophora. Its sole autapomorphy was restriction of the hearts to segment 10. The large proboscis-like prostomium (not computed) was a unique apomorphy in the entities included but is known homoplasically in the glossoscolecid *Enantiodrilus bilolleyi* Cognetti and is approached in some naids and lumbriculids. In its reproductive system (Fig. 8.28) *Tiguassu* provides evidence of reduction from two pairs of testes, and possibly from an ocotogonadal condition, in having two pairs of seminal funnels (in 10 and 11) of which those in 10 are vestigial in the absence of testes. Well developed testes are present in 11. The female system is progynous, as in most haplotaxids, with a single pair of ovaries in 12 immediately succeeding a testis-segment. There are no atria or other modifications of the male ducts, presumably as plesiomorphic conditions. There are two pairs of small,

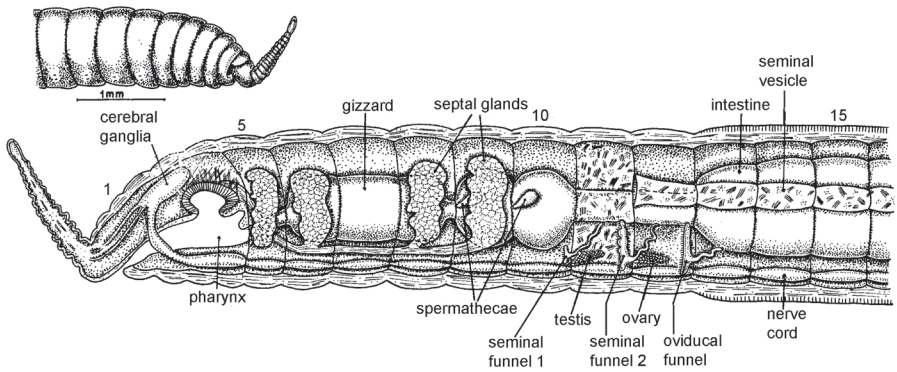


Fig. 8.28. *Tiguassu reginae* (Tiguassuidae). Reconstruction of the anterior 15 segments based on serial sections, showing the reproductive system with vestigial anterior seminal funnels. The smaller figure shows the proboscis-like prostomium. After Righi, G. *et al.* 1978. Acta Amazonica 8 (3 Supplement 1): 1-49, Figs. 3, 1.

adiverticulate spermathecae, in segments 9 and 10. The ova have a diameter of 40-50 μm (Righi *et al*. 1978). The co-occurrence of spermathecae with male funnels in 10 is a condition also seen in the Tubificidae, as is the great elongation of the seminal vesicle, but ovisacs were not found.

Superorder Metagynophora. Loss of the anterior ovaries of a hypothetical octogonadal set, with retention of ovaries in segment 13 so that a segment lacking gonads intervenes between the posterior testes and the ovaries, or two segments in proandric taxa such as alluroidids, diagnoses all oligochaetes above the tubificid-enchytraeid assemblage and the Lumbriculidae, i.e. from the Moniligastridae through the Megascolecidae, loosely termed 'megadriles'. This synapomorphy characterizes the Metagynophora of Jamieson (1988b) (Fig. 8.4). These are equivalent to the Lumbricida of Brinkhurst (1982). As the most plesiomorphic representatives, the Moniligastridae, Alluroididae and Syngenodrilidae, have not been sequenced for DNA, monophyly of the Metagynophora awaits confirmation from molecular analysis.

Order Moniligastrida. Moniligastridae. Reproductive features among unambiguous synapormorphies for the Moniligastrida, as represented by *Desmogaster* and *Moniligaster*, are: ovaries in septal chambers; testis-sacs suspended on the posterior septum of the testicular segment; seminal vesicles absent; prostates capsular; spermathecae with non-seminal diverticula.

Brinkhurst and Jamieson (1971) had already recognized the Moniligastrida as a separate order. Jamieson (1977b) re-interpreted the long debated nature of the testis-sacs, showing that they were neither reduced segments, as proposed by Stephenson (1922, 1930), nor intraseptal cavities, as argued by Gates (1962), but that they were normal testis-sacs which, with their enclosed testes, had become detached from the original testis-bearing septa (Fig. 8.29). It was recognized that moniligastrids are extraordinarily primitive in retaining a plesiopore condition, the extremely plesiomorphic

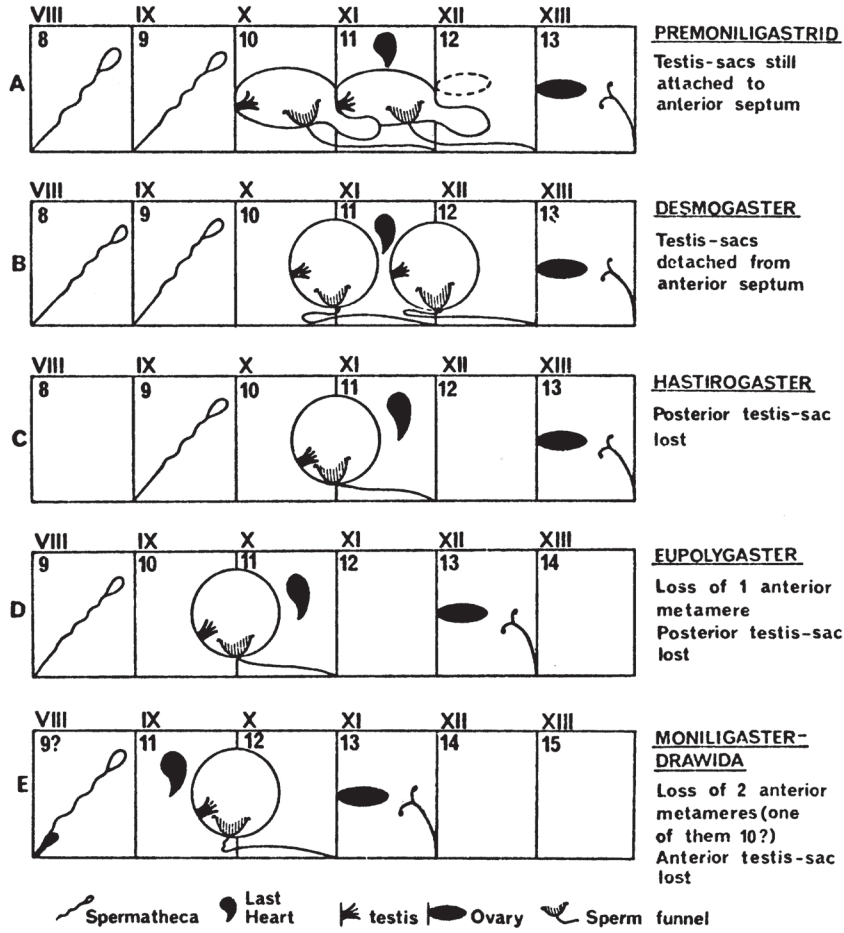


Fig. 8.29. Distribution of genital organs in relation to existing segmentation and hypothetical segmental homologues in Moniligastridae. Origin of the 'intraseptal' testis-sacs from premoniligastrid sacs attached, with their testes, to the anterior septa of segments 10 and 11, as in other metagynophorans, is hypothesized. From Jamieson, B. G. M. 1977. Evolutionary Theory 2: 95-114, Fig. 3.

state (in *Desmogaster*) of 2 pairs of male pores in consecutive segments (both conditions seen elsewhere only in the Haplotaxidae) and a single layered clitellum with large yolked eggs. The moniligastrid clitellum is here shown to consist of a single layer of tall, slender modified epidermal cells with basal nuclei and dense granular secretory contents which discharge at the outer surface of each cell (Fig. 8.30A,B). They contrast with the wider, more robust goblet cells (putative large orthochromatic mucous cells) which predominate in the general epidermis (Fig. 8.30C).

The reproductive system of a moniligastrid is here exemplified by that of *Moniligaster troyi* described by Jamieson (1977b). Details of the system are

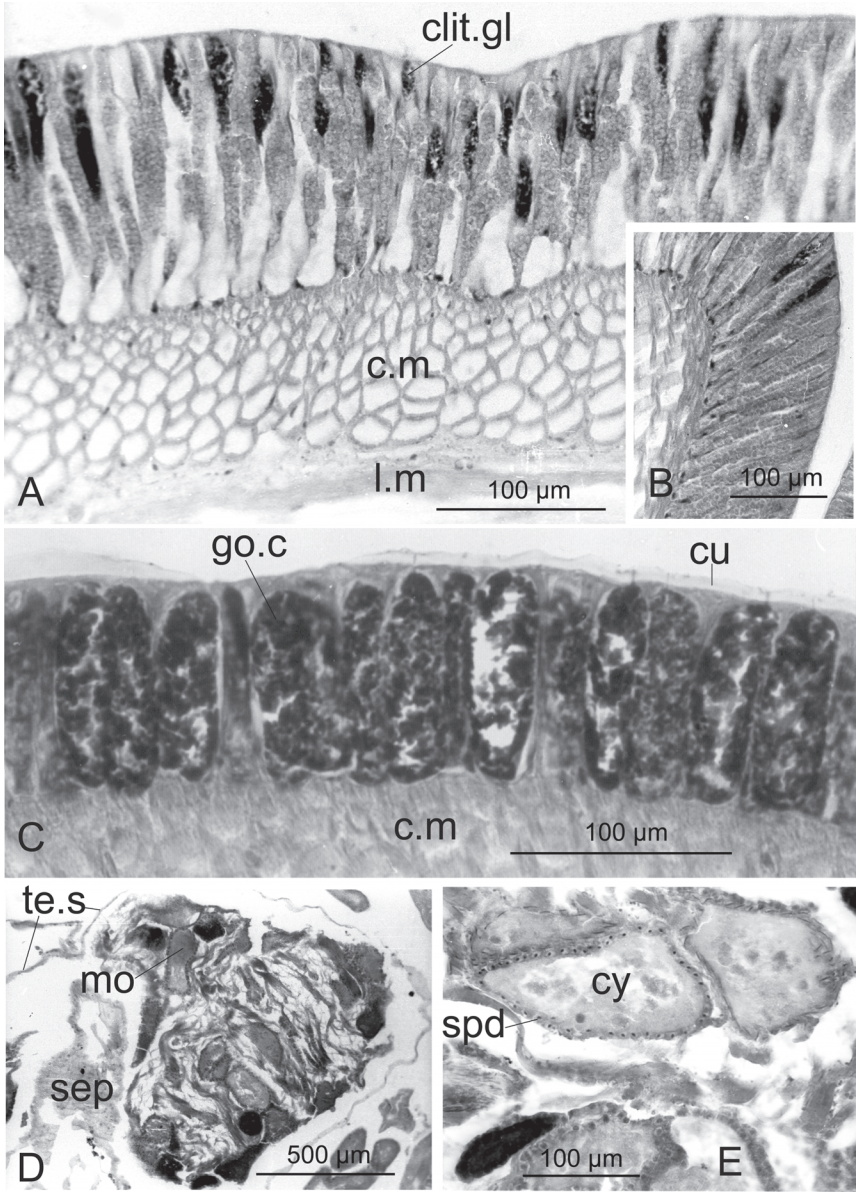


Fig. 8.30. Light micrographs of the reproductive anatomy of *Moniligaster troyi* (Moniligastridae). **A**. Longitudinal section of the clitellum confirming that is consists of a single layer of cells. **B**. Same, through an intersegmental furrow. **C**. Longitudinal section of the general epidermis, showing the goblet cells. **D**. Longitudinal section of a testis-sac showing that it has anterior and posterior portions suspended in a septum. **E**. Morulae of spermatids in a testis-sac; a spermatid is labeled in a layer of spermatids encircling and attached to the cytophore. Abbreviations: c.m, circular muscle; clit.gl, secretory contents of a cell of the clitellum; cu, cuticle; cy, cytophore; go.c, goblet cell; l.m, longitudinal muscle; mo, morula of spermatids; sep, septum; spd, spermatid (in a layer of spermatids encircling and attached to the cytophore); te.s, testis-sac wall. From Jamieson, unpublished.

illustrated from previously unpublished light micrographs (Figs. 8.30, 8.31). Testes and putative funnels are enclosed in a pair of diaphanous iridescent testis-sacs. Each sac is suspended in a septum so that it has pre- and post-septal portions (Figs. 8.29, 8.30D). The vas deferens from each testis-sac joins the sac ventrally at the anterior face of the supporting septum and passes into the anterior segment (segment 9) abutting the septum; it is very long and much coiled in this segment; numerous coils nearest the sac are narrow and iridescent but by far the greater length is wider and non-iridescent, with many hair-pin bends, and forms a large cluster. The vas deferens continues posteriorly to join the glandular portion of the prostates, in segment 11, considerably ectal of the ental end of the gland, and is straight in this segment. Immediately within the testis-sac the vas deferens gives rise to several iridescent ribbons which pass posteriorly for the entire length of the sac and were interpreted (Jamieson 1977b) as a backwardly directed sperm funnel. The testis-sac contains developmental stages of spermatozoa, including morulae of spermatids and free spermatozoa (Fig. 8.30D,E); it thus functions as a testis-sac and seminal vesicle.

Each prostate extends from its pore, at 10/11 to intersegment 13/14; it has a clavate, superficially slightly lobulated glandular portion and a shorter, narrow duct which is poorly differentiated from the gland; the duct forms a muscular swelling at the pore which houses the base of the combined male and prostatic porophore (Jamieson 1977b) (Figs. 8.15, 8.31H,J). The wall of the gandular portion of the prostate consists of an outer thick longitudinal muscle layers, a thinner, though still thick, intermediate circular muscle layer, and an inner epithelium which contains gland cells (Fig. 8.31G,I).

The ovary consists of folded (fan-like) laminae (Fig. 8.31F,H) on the anterior septum of its segment (11?). Oviducal funnels have yet to be recognized but large elongate ovisacs extend into segment 13 though arising from septum 11/12 against which septum 13/14 is adpressed; some lobules each contain a large-yolked egg (putative primary oocyte) with conspicuous nucleus.

Moniligaster troyi has one pair of spermathecae, each with a large, elongate-ovoid ampulla in segment 8, its duct is long and much coiled in this segment but almost straight (Fig. 8.31C) on passing into segment 7 where it joins the apex of the wide, muscular ectal spermathecal duct (Figs. 8.14, 8.31A,B). The latter duct has two branches or horns, one on each side of the apex, each of which bears a large lobulated gland, the dichotomous gland; with the ectal spermathecal duct this constitutes the spermathecal atrium, discharging at intersegment 7/8 on each side. The spermathecal ampulla, in its ectal half, and its duct are exceptional for oligochaetes in being internally ciliated (Fig. 8.31A,B). The dichotmous gland consists of many blind tubules, opening into a common lumen; each tubule consists of a tall, glandular epithelium (Fig. 8.31C-E).

Order Opisthopora. All remaining oligochaetes, above the Moniligastridae, from the Alluroididae to the Megascolecidae, form a convincing clade, the Opisthopora (see 8.1).

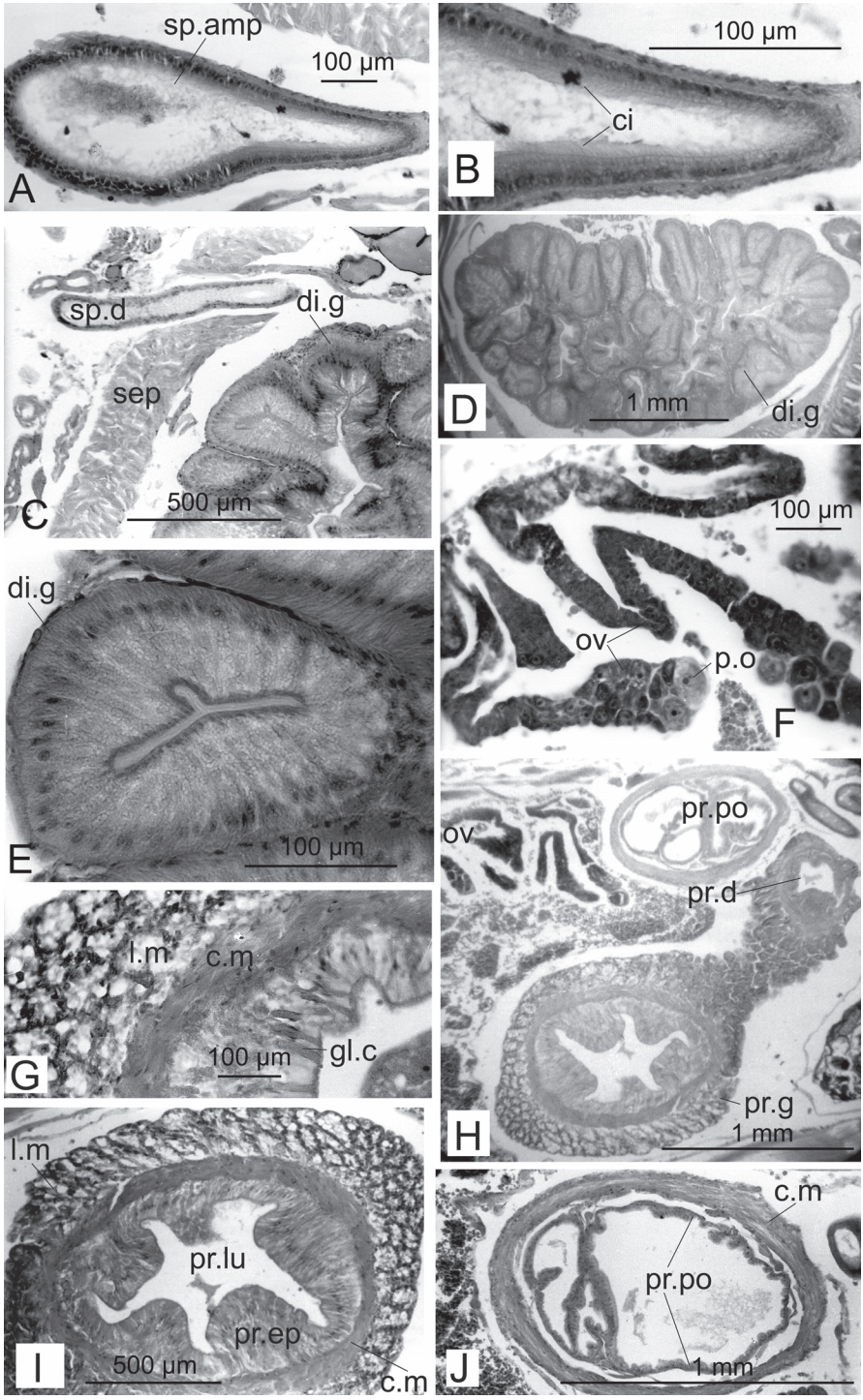


Fig. 8.31 contd

Suborder Alluroidina. **Superfamily Alluroidoidea. Syngenodrilidae**. Apomorphies of the syngenodrilid reproductive system include presence of longitudinal tubercula pubertatis, intrasegmental testis-sacs, tubular prostate-like glands, all of which occur in other taxa, and a unique location of prostate pores in segments 11, 12 and 13. The male pores are lateral, and separate from the prostate pores, in segment 13. Genital and penial chaetae are present or absent. The clitellum begins in segment 11 and intraclitellar tubercula are present. Female pores lie in segment 14. Spermathecal pores are two pairs, posteriorly in segments 7 and 8. Testes are two pairs in 10 and 11 (rarely on segment more posterior), enclosed in the testis-sacs. The seminal vesicles are of a microdrile type, extending posteriad within the ovisacs through several segments.

Alluroididae. Like the Syngenodrilidae, alluroidids represent an evolutionary transition in that they have the microdrile characteristic of a single layered clitellum but have attained the most plesiomorphic opisthoporan condition of male pores in segment 13, as in *Righiella jamiesoni* (Fig. 8.32A).

In alluroidids the unilayered clitellum (Fig. 8.32D) commences on segment 12 or 13. Male pores are ventral to lateral in the chaetal arc of segment 13 or 14. The pair of female pores lies at or near the anterior border of segment 14. Spermathecal pores are paired, lateral, or are single, mid-dorsal, in segments 6-9, maximally in three of these segments, never in line with the male pores. The male gonads are proandric, with testes in segment 10. In *Kathrynella guyanae*, all gonads are homeotically displaced one segment further posteriorly so that the testes are in 11. The sperm funnels have their mouths directed anterodorsally. Seminal vesicles project into the segment behind that of the testes or are absent; in the latter case spermatogenesis occurs in the testis-segment. Prostates (atria) are tubular or bulbous, receiving the male ducts, or discharging with the latter but separately from them, into a terminal chamber; they consist of an internal epithelium surrounded by a

Fig. 8.31 contd

Fig. 8.31. Light micrographs of the reproductive anatomy of *Moniligaster troyi* (Moniligastridae), continued. **A**. Longitudinal section (LS) of a spermathecal ampulla. **B**. Same, showing internal ciliation. **C**. Passage of the straight region of the spermathecal duct through septum 7/8 into segment 7, where it joins (not shown) the dichotomous gland. Note lobes of the gland. **D**. Cross section through the dichotomous gland. **E**. Cross section through a single tubule of the dichotomous gland. **F**. Longitudinal section of the ovary, showing stages of oogenesis with terminal putative primary oocytes. **G**. Section through the prostate gland, showing the three layers of its wall, with inner gland cells. **H**. Section showing all regions of the prostates in segment 11: glandular region, duct and muscular swelling containing the common prostatic and male porophore. The ovary is visible (top left) in the following segment. **I**. Section of the glandular part of the prostate. **J**. Approximately horizontal section of the muscular swelling containing the common prostatic and male porophore. Abbreviations: ci, ciliation; c.m, circular muscle; di.g, dichotomous gland; gl.c, gland cell; l.m, longitudinal muscle; ov, ovary; p.o, putative primary oocyte; pr.d, prostate duct; pr. ep, internal epithelium of prostate gland; pr.g, prostate gland; pr. lu, lumen of prostate gland; pr.po, common prostatic and male porophore; sep, septum; sp.amp, spermathecal ampulla; sp.d, straight part of spermathecal duct. From Jamieson, unpublished.

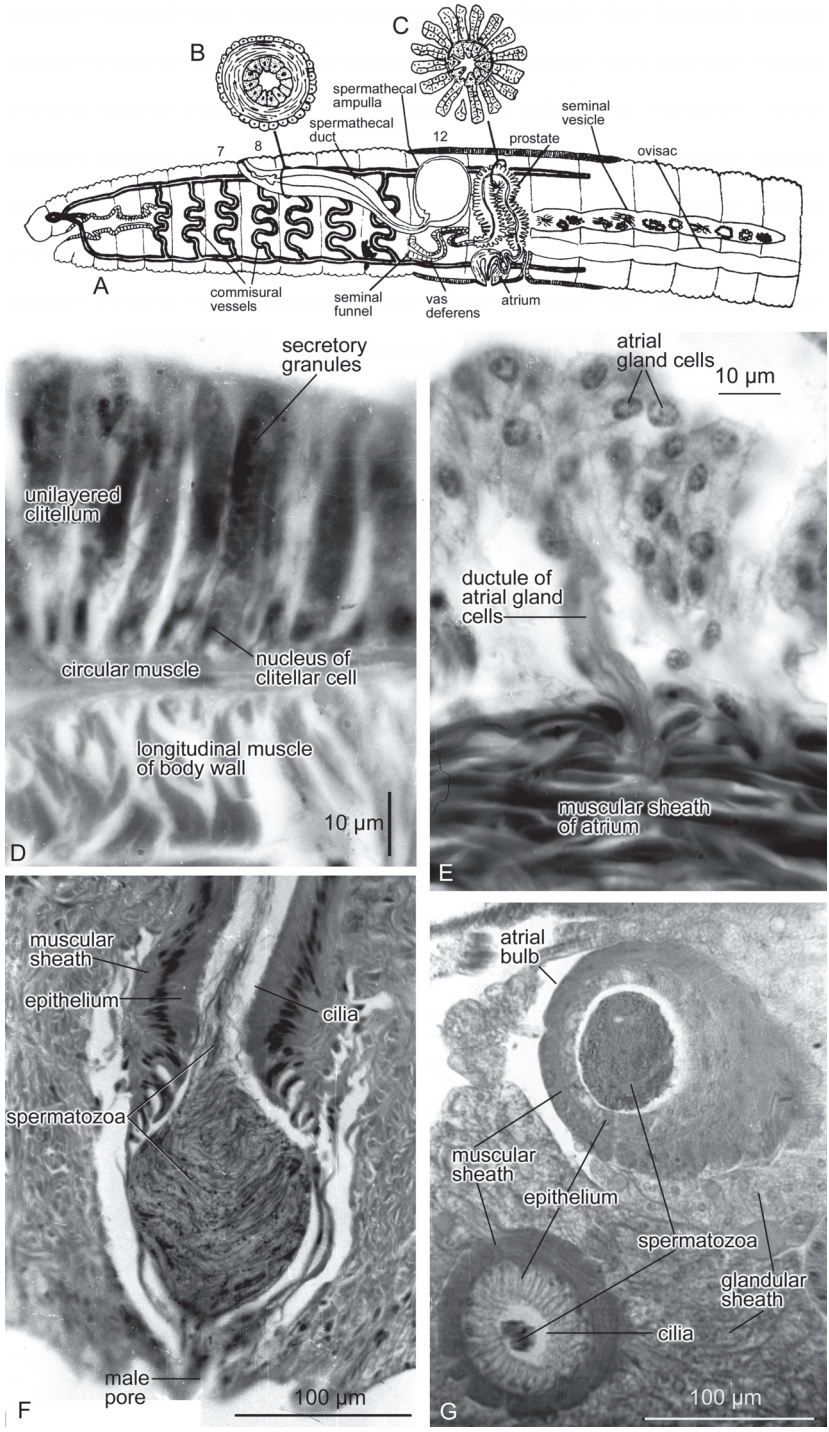


Fig. 8.32 contd

muscular sheath outside which prostatic (atrial gland) cells are usually present. Ductules from the atrial gland cells penetrate the muscular sheath of the atrium, as in *Alluroides brinkhursti brinkhursti* (Fig. 8.32E,G), to reach the atrial lumen; an elongate penis, terminally containing a spermatozoal mass, may be present (Fig. 8.32F). Genital or penial chaetae are present or absent. Ovisacs extend posteriorly from the ovarian segment through several segments.

Suborder Crassiclitellata. Crassiclitellate relationships are discussed under molecular phylogeny in 8.1.4 above (see also Fig. 8.4A,B).

Biwadrilidae.The reproductive apparatus of *Biwadrilus bathybates*, illustrated by Nagase and Nomura (1937) (Fig. 8.33) lacks spermathecae, an absence shared with *Criodrilus*, in the Almidae (*sensu* Jamieson 1988b) and with *Ocnerodrilus*. In the case of *Ocnerodrilus*, at least, this appears to be a homoplasy. The male system is holandric, with testes in 10 and 11; testis-sacs are absent; seminal vesicles are two pairs, in segments 11 and 12. Vasa deferentia are intraparietal for much of their lengths, uniting only at the base of the conical male porophore, on each side on segment 13. The ventral chaetae of 13 are replaced by bifid genital chaetae. Prostate glands consisting of numerous lobules with branched ducts, bundles of ducts, and common ducts open into a male slit just ventral to each male pore. A large single 'copulation gland', resembling the chaetal gland of *Microchaetus*, is present on each side in 13, opening into the male slit ventrally to the prostate pores and just external to the genital chaetae; each gland has a terminal duct and a glandular portion consisting of outer peritoneum, a middle muscular-vascular layer, an inner glandular layer with three types of cells, and a simple lumen. The lobed ovaries occupy the metagynophoran location of segment 13, with pores in 14. Ovisacs are restricted to segment 14 but an extensive subenteric (non-genital?) septal pouch (also seen in *Microchaetus*) may be present, arising further anteriorly.

The location of the male pores, in segment 13, in *Biwadrilus*, only one segment behind the plesioporous location, is the most plesiomorphic condition for the Opisthopora and for the Crassiclitellata. It is shared with

Fig. 8.32 contd

Fig. 8.32. *Righiell*a *jamiesoni* (Alluroididae). **A**. Diagram showing arrangement of genital organs and vascular commissures. **B**. Transverse section (TS) of spermthecal duct. **C**. TS of prostate. After Omodeo, P. and Coates, K.A. 2000. Hydrobiologia 463(39): 39-47, Fig. 6. **D-F**. *Alluroides brinkhursti brinkhursti*. **D**. Transverse section (TS) of clitellum, showing single cell layer; the cells with conspicuous secretory granules and each with a basal nucleus. **E**. TS through the wall of the atrium, showinga group of atrial gland cells with ductule penetrating the muscular sheath of the atrium. **F**. Longitudinal section through the male pore, showing the ectal end of the atrium, which forms a penis with muscular sheath, ciliated epithelium and rope of spermatozoa in the lumen, forming in the ectal chamber a sperm mass. **G**. *Alluroides pordagei*. Oblique section through the atrial bulb, containing a large sperm mass, and the associated atrium. D-G. From Jamieson, unpublished figures from the study of Jamieson, B. G. M. 1971a. Alluroididae. Pp. 708-722. In R. O. Brinkhurst and B. G. M. Jamieson (eds), Aquatic Oligochaeta of the World, Oliver and Boyd, Edinburgh.

the non-crassiclitellate Alluroididae and the male genital systems of the two families show considerable similarities. Testing of phylogenetic proximity from molecular sequences would be desirable.

Glossoscolecidae. The glossoscolecid clitellum is usually saddle-shaped and occupies as many as 15 segments, beginning near or shortly behind the female pores. Male pores are inconspicuous, one pair, rarely two pairs, intraclitellar or (*Opisthodrilus*) postclitellar. The female pores have the normal crassiclitellate location in segment 14 or exceptionally (*Enantiodrilus*) there are two pairs, in segments 13 and 14. The spermathecal pores are pretesticular, rarely extending into or behind the testis-segments; and in each intersegment occupied are usually a pair, though sometimes multiple, sometimes absent. Testes are one or two pairs, in segment 10 or segments 10 and 11; testis-sacs are present or absent. Copulatory sacs are present or absent. Spermathecae are absent (*Glossoscolex*, *Fimoscolex*, *Goiascolex*) or, usually, are present, when they extend freely into the coelom and are well differentiated into duct and ampulla or are intraparietal and poorly differentiated; they usually lack diverticula (Gates 1972; Jamieson 1971c; Righi 1995; Sims 1982).

Tumakidae. *Tumak hammeni* (Fig. 8.35C,D) has a saddle-shaped clitellum commencing in segment 14 and occupies 9 segments. Male and female pores are microscopic, the female in the usual crassiclitellate location of segment 14, the male in 18 on the tubercula. Genital papillae surround the ventral setae (*a* and *b* separately) in segment 12 and throughout the clitellar region except in 17-20 where there is one pair of rectangular, tumid glandular pads in each segment; the pads on each side collectively considered to probably be homologous with the puberal bands (here termed tubercula pubertatis) of glossocolecids. We may also note the striking resemblance of the genital field to that of the microchaetid *Michalakus* (Fig. 8.35A,B). Testes and male funnels occupy segments 10 and 11, lacking testis-sacs; seminal vesicles are paired in 11 and 12. *Tumak* differs from the Glossoscolecidae in having intraparietal male ducts. The ovaries are large, folded and fan-shaped. Prostates and copulatory chambers are absent. The spermathecae are post-testicular, simple, two pairs in each of segments 12-14, lacking diverticula or seminal chambers and opening by microscopic pores in the corresponding anterior intersegments (Righi 1995).

Eudrilidae. In eudrilids the male pores lie segment in 17, as is also typical of Ocnerodrilidae. Eudrilids differ from the Megascolecidae in having euprostates (Fig. 8.34), i.e. tubular prostates through which the male ducts discharge and which appear to be reflexed modifications of these ducts, thus more resembling the atria of lumbriculids and monilgastrids than the separate prostates (metaprostates) of megascolecids. However, the ectal ends of the vasa deferentia in some ocnerodriles are enlarged and somewhat resemble euprostates, though accompanied by tubular metaprostates. Eudrilids further differ from megascolecids, and ocnerodrilids, in migration of the spermathecae from the basic earthworm anterior location (in and/or anterior to segment 9) to the vicinity of the ovaries (in 13; sometimes posterior

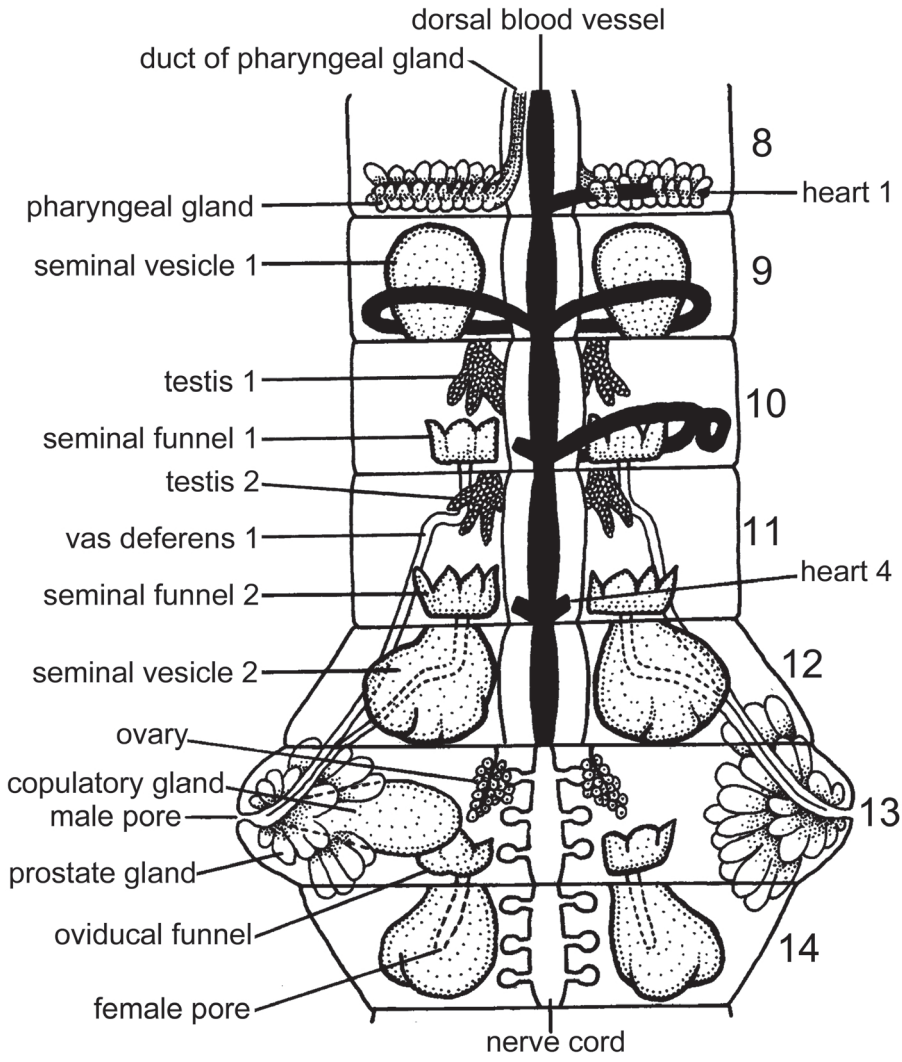


Fig. 8.33. *Biwadrilus bathybates* (Biwadrilidae). Diagrammatic dorsal view of genitalia. Relabelled from Jamieson 1971c. Glossoscolecidae. Pp. 147-199. In R. O. Brinkhurst and B. G. M. Jamieson (eds), The Aquatic Oligochaeta of the World, Oliver and Boyd, Edinburgh, Toronto, Fig. 15.12A, After Nagase and Nomura.

to the male pore) and in the development in many of internal fertilization, foreign sperm passing internally from the spermathecae to ovisacs, on the oviducts, internally (see, for instance, Jamieson 1967, 1969; Sims 1967; Zicsi 1997). Elsewhere in the Oligochaeta, only the Phreodrilidae are suspected of having internal fertilization.

The reproductive system in the Eudrilinae is more complex than that of the Pareudrilinae. In the latter transitions are seen in *Stuhlmannia* from

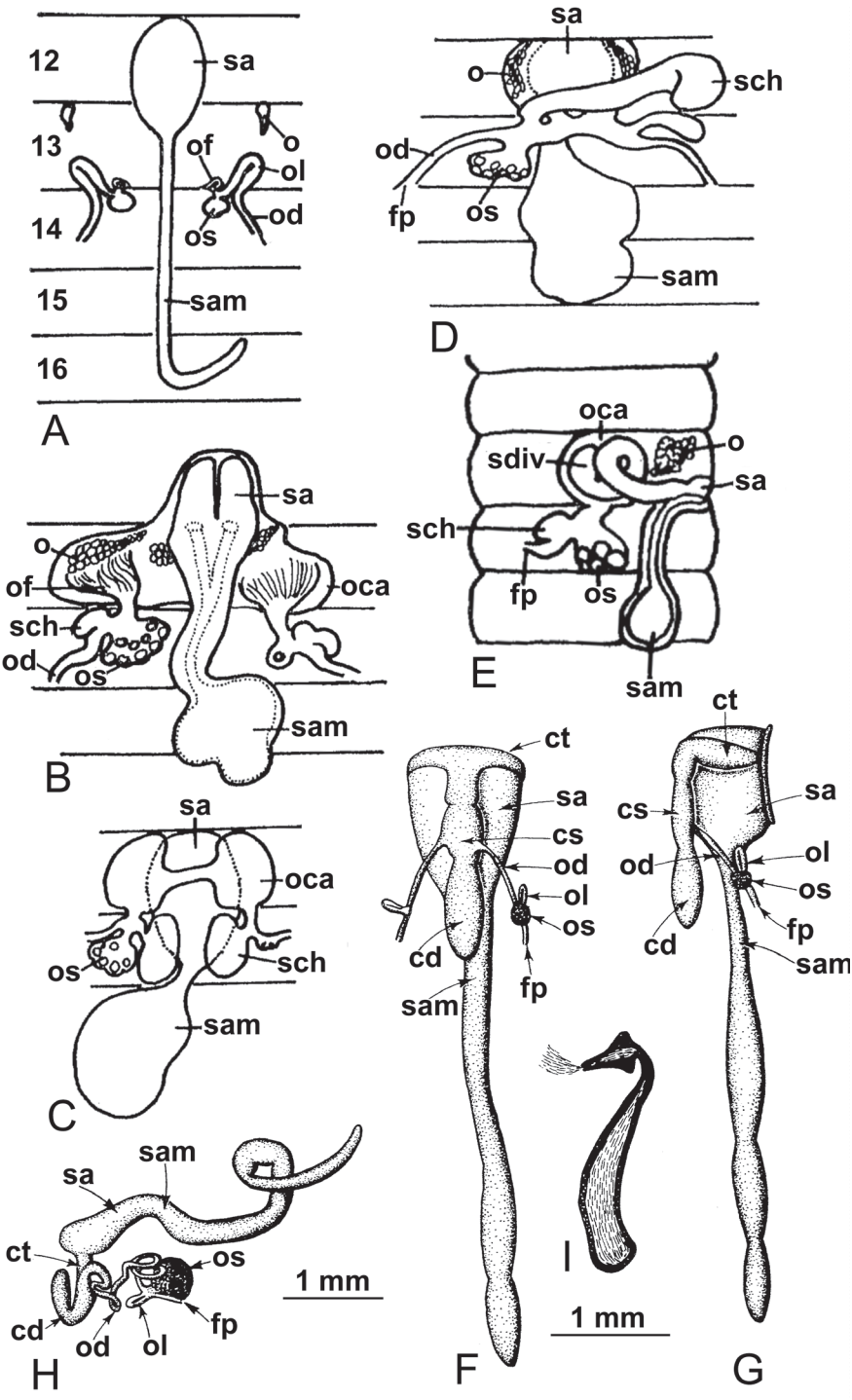


Fig. 8.34 contd

ovaries free in the ovarian segment, presumably with fertilization in the cocoon, to ovaries enclosed within the spermathecal system and with presumed internal fertilization. Penetration of the wall of the spermatheca by sperm from the partner, thus gaining access to the ovisacs has been demonstrated in *Stuhlmannia variabilis* by Jamieson (1958, 1967). Transition from free to enclosed ovaries is also seen in the pareudrilines *Chuniodrilus* and *Scolecillus* (see Jamieson 1969) (Fig. 8.34).

Microchaetidae. In microchaetids the single pair of male pores is intraclitellar, behind segment 16, and female pores are on segment 14. The clitellum is saddle-shaped, beginning on segment 11 to 14 and occupying as many as 44 segments though sometimes a more modest six segments. Spermathecal pores are immediately postesticular or also occupy the last testis segment and are paired or multiple in each intersegment. Testes are in segments 10 and 11 or 10 only, in testis-sacs. Copulatory sacs and prostates are absent. The spermathecae do not project far into the coelom but are sometimes sinuous tubes. Tubercula pubertatis and/or genital papillae are present and have been illustrated by Plisko in several papers (e.g. Plisko 1996a,b) (see, for instance, *Michalakus*, Fig. 8.35).

Lumbricidae. Lumbricidae, native in the Holarctic, are readily distinguished by location of the male pores, on 15, as in *Lumbricus terrestris* (Figs. 8.36, 8.53) or exceptionally 11, 12 or 13, well anterior to the clitellum. The clitellum is usually saddle-shaped, commencing between segments 17 and 52, and occupying 4-32 segments (Fig. 8.53). The spermathecal pores are preclitellar and usually paired, in two to eight of furrows 5/6-19/20, commonly in 9/10 and 10/11. There are two pairs of testes (Fig. 8.36, 8.37A) rarely one pair, in segments 10 and 11, usually free but occasionally in suboesophageal or perioesophageal testis-sacs. The vasa deferentia are

Fig. 8.34 contd

Fig. 8.34. Genital anatomy of some Eudrilidae (Pareudrilinae), showing transition from free to enclosed ovaries with internal fertilization. **A-E**. Spermathecal and female genital systems in *Chuniodrilus* and *Scolecillus* arranged in order of increasing modification. **A**. *C. ghabbouri*. **B**. *C. zielae*. **C**. *C. vuattouxi*. **D**. *C. compositus*. **E**. *S. tantillus*. Note, in B-E, asymmetry of the oviducal system, with the ovisac vestigial on the left side (contrast *Stuhlmannia*). **F, G**. *Stuhlmannia variabilis*. Dorsal and lateral view of female reproductive system, respectively. Ovaries in the 'coelomic tube' discharge eggs into the ovisac on the right side, that of the left side being vestigial. Allosperm received into the spermatheca pass through the wall of the spermathecal atrium into the oviducal system where they are presumed to effect internal fertilization. **H**. *Stuhlmannia asymmetrica*. Here the oviducal system is developed on the left side only, having been totally suppressed on the right side. Sperm do not have to penetrate the wall of the spermatheca to reach the oviducal system as there is a wide, ciliated portal between the two. **I**. *Stuhlmannia variabilis*. Spermatophore redrawn after Beddard. **A-E**. After Jamieson, B. G. M. 1969. Journal of Natural History 3: 41-51, Fig. 1, After Omodeo 1958. Mémoires de l'Institut Français d'Afrique Noire 53: 1-109, and Wasawo, D. and Omodeo, P. 1963. Memorie del Museo Civico di Storia Naturale di Verona 11: 211-223. **F-I**. After Jamieson, B. G. M. 1967. Journal of Zoology, London 152: 79-126, Figs. 2, 3, 7 and 4 respectively. Abbreviations: cd, coelomic diverticulum. cs, coelomic sac; ct, coelomic tube; fp, female pore; o, ovary; oca, ovarian capsule; od, oviduct; of, oviducal funnel; ol, oviducal loop; os, ovisac; sa, spermathecal atrium; sam, spermathecal ampulla; sch, seminal chamber; sdiv, spermathecal diverticulum.

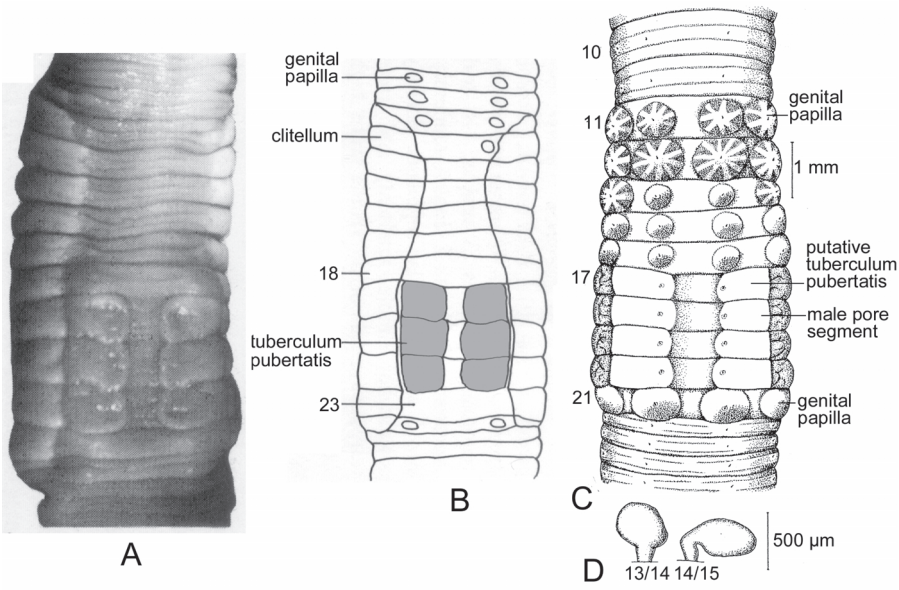


Fig. 8.35. A,**B**. Genital field of *Michalakus initus* (Microchaetidae). After Plisko, J. D. 1996. *Michalakus*, a remarkable new genus of microchaetid earthworm from South Africa (Oligochaeta: Microchaetidae). Annals of the Natal Museum 37: 287-293, Figs. 1,2. **C**,**D**. *Tumak hammeni*. **C**. Ventral view of segments 10 to 24, showing genital field. **D**. Spermathecae of segments 13 and 14. After Righi, G. 1995. Studies on Tropical Andean Ecosystems 4: 485-607, Fig. 201A,E.

extraparietal and sometimes coiled behind the seminal funnels to form epididymides. There are two to four pairs of seminal vesicles. Spermathecae are adiverticulate; they lack a distinct duct and are intraparietal, sessile or pedunculate. The ovaries, in segment 13, have a single egg string; each oviduct, discharging at a paired female pore in segment 14, bears a small ovisac (Bouché 1972; Gates 1976; Sims 1980).

Kynotidae. The clitellum is annular or saddle-shaped in the region of segments 18-47. Tubercula pubertatis are absent. Male pores (clasper pores) are preclitellar, very conspicuous, on segment 16 or, rarely, 15, on a flat area or, on erection, on everted copulatory sacs (Fig. 8.37B). The spermathecal pores are post-testicular in the region of intersegments 13/14-16/17 and multiple in each row. Distinctive tubular prostate-like glands are associated with the copulatory sacs and with the follicles of preclitellar genital chaetae (Fig. 8.37C). The adiverticulate spermathecae are spherical to tubular (see review in Jamieson 1971c).

Hormogastridae. In hormogastrids the male pores are intraclitellar, in the posterior half of segment 15, as in the type-species *Hormogster redii* (Fig. 8.39B), or, rarely, discharge on the tubercula pubertatis on 22 (as also in *Ailoscolex*). The clitellum is annular or saddle shaped, commencing on or near segments 12 or 14 and extends posteriorly for about 17 segments. The spermathecae are paired or multiple, in two to four intersegments, at the level

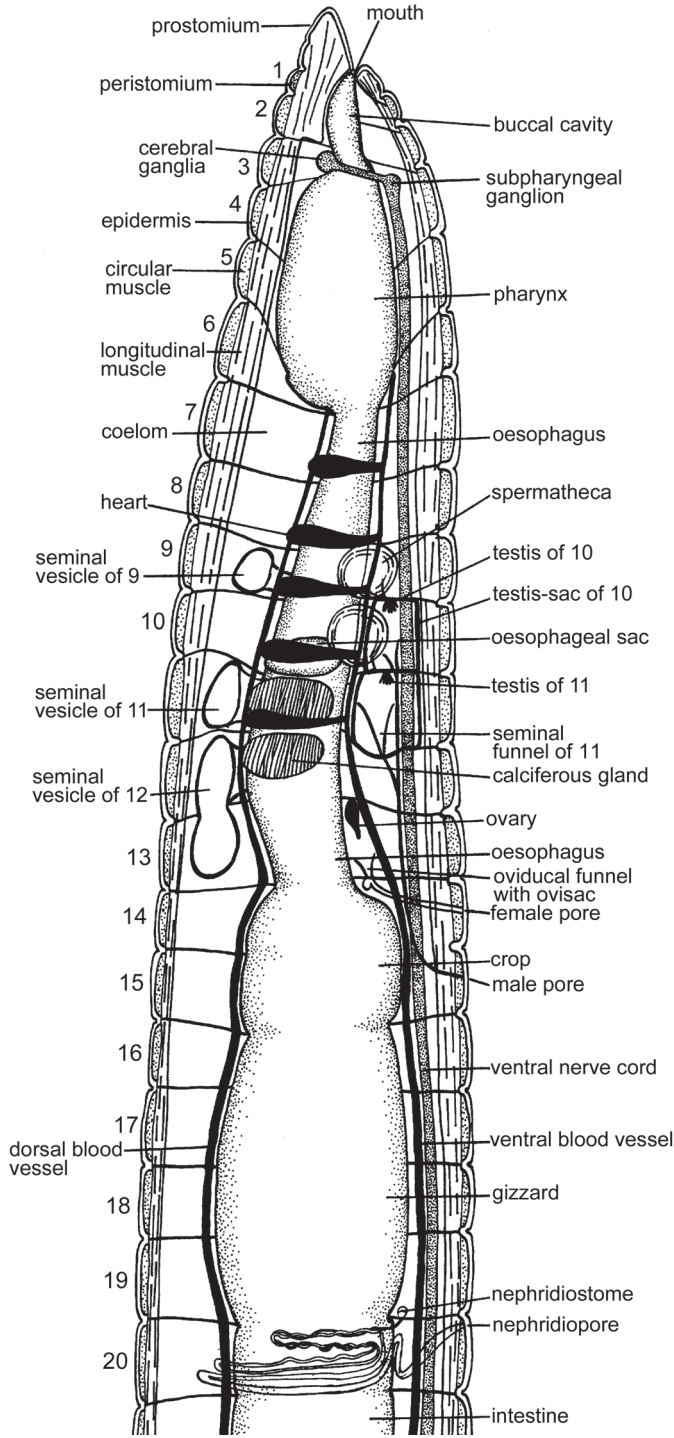


Fig. 8.36. *Lumbricus terrestris* (Lumbricidae). Anatomy revealed by sagittal bisection. Original.

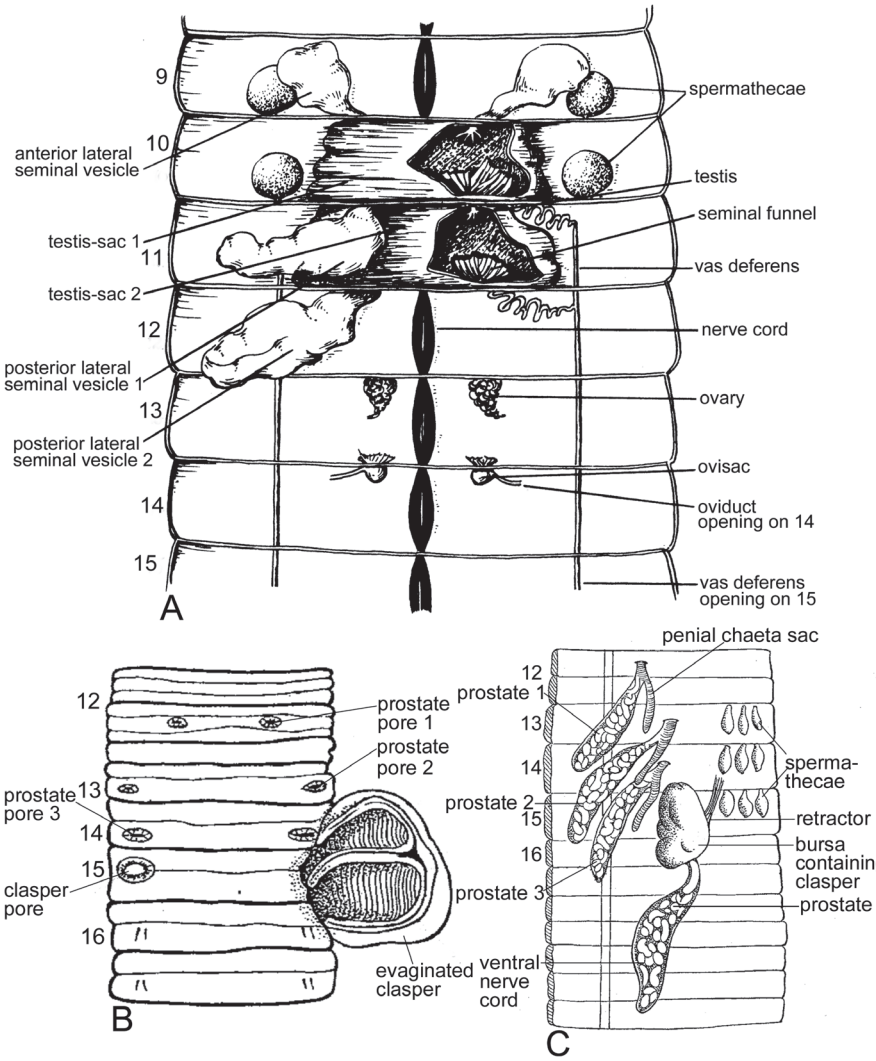


Fig. 8.37. A. *Lumbricus terrestris* (Lumbricidae). Diagram of the reproductive organs in dorsal view. Relabelled after Jepson, M. 1951. *Biological Drawings*. Part II. John Murray, London, p. 32. **B, C**. *Kynotus cingulatus* (Kynotidae). **B**. Ventral surface of segment 13-16, showing the pores of three pairs of prostates; a fourth pair discharges at the male pores. The clasper is shown evaginated through the left male pore (clasper pore). **C**. Internal view of the four pairs of prostates and the bursa propulsoria which contains the clasper. Each prostate is a convoluted tube enveloped in a sac. After Stephenson 1930. The Oligochaeta. Oxford. Figs. 145,146, from Benham.

of the genital segments. Testes are two pairs, in 10 and 11, or a pair in 11 only. Testis-sacs are absent but there are two pairs of seminal vesicles, in segments 11 and 12. Copulatory sacs and prostates are absent. Female pores lie in segment 14 (Bouché 1972; Sims 1980).

Lutodrilidae. *Lutodrilus multivesiculatus* is unique in the earthworms in having ten pairs of testes, in segments 12-21. It is considered to have interpolated ten segments, the last eight of them testicular, anterior to the normal megadrile location of testes in segments 10 and 11 (Jamieson 1978b). *Lutodrilus* stands apart from other almoids in having single-stringed ovaries, a feature clearly over-valued by Gates (1976) in aligning *Lutodrilus* with *Lumbricus* in his Lumbricoidea.

The male pores are in segment 32; the pores discharge on a tumescence that encloses both ventral chaetal couples on 32 and 33. There is one pair of female pores, on segment 24. The clitellum is annular, only slightly swollen, and covers 37-51 segments, between segments 20 to 71. Alae about 1.5-3 mm high, extend through 16-32 segments, through segments 22 to 53 (Fig. 8.38A). Similar alae are also seen in the almoids *Glyphidrilus* (Fig. 8.38B), and, as segmentally less extensive claspers, in *Drilocrius alfari* (Fig. 8.38C) and *Alma* (Fig. 8.11A,B). Genital tumescences surround the ventral chaetal pairs in some of segments 13-51. The male tumescence is an elevated flattened area on the ventrum of 32-33, sometimes also 31 and/or 34.

The ten pairs of testes are not enclosed in testis-sacs; each has several strings; vasa deferentia are intraparietal and prostates are absent. Seminal vesicles are largest in 14-22, attached to the posterior facesof their respective septa with the exception of the vesicles of 11 and 12 which attach to the anterior faces of septa 11/12 and 12/13 respectively. The ovaries are paired in segment 23, each with a single egg-string. The spermathecae are ovoidal and intraparietal in 2-5 of intersegments 15/16-25/26, that is, commencing in the gonadal region; they are multiple in each row; the external pores are not recognizable (McMahan 1976,1979). If the ten interpolated sections are deducted, comparison with other megadriles is facilitated and its closest relationship of seen to be with the Oriental *Glyphidrilus* and Ethiopian *Callidrilus*.

Almidae. The reproductive anatomy of the Alminae will here be considered separately from that of the Criodrilinae.

Alminae. In the Alminae genital chaetae, if present, are little if at all modified, except when on claspers. The male pores are one pair, on segments 15-30, always inconspicuous, intraclitellar or preclitellar. Female pores are on segment 14 but *Glyphidrilus kukenthali* is one of only three megadrile species known to have two pairs of female pores, in 13 and 14. Spermathecal pores are post-testicular (as in microchaetids), but are rarely continued into and anterior to the testis segments; they are sometimes (some *Alma* species) translocated into the hindbody; and are usually (with the spermathecae) multiple in an intersgement. Testes are paired in segments 10 and 11 or (*Areco*) 11 only. Prostate-like glands are rarely present. The paired intraceolomic parietal glands described by Righi *et al*. (1978) in some segments in *Areco*, although seen in some other almids, are reminiscent of the prostate-like glands of *Sparganophilus*. This endorses the view (Jamieson 1971b) that sparganophilids have a morphology close to that which might be attributed to proto-almids. A close relationship between *Sparganophilus*

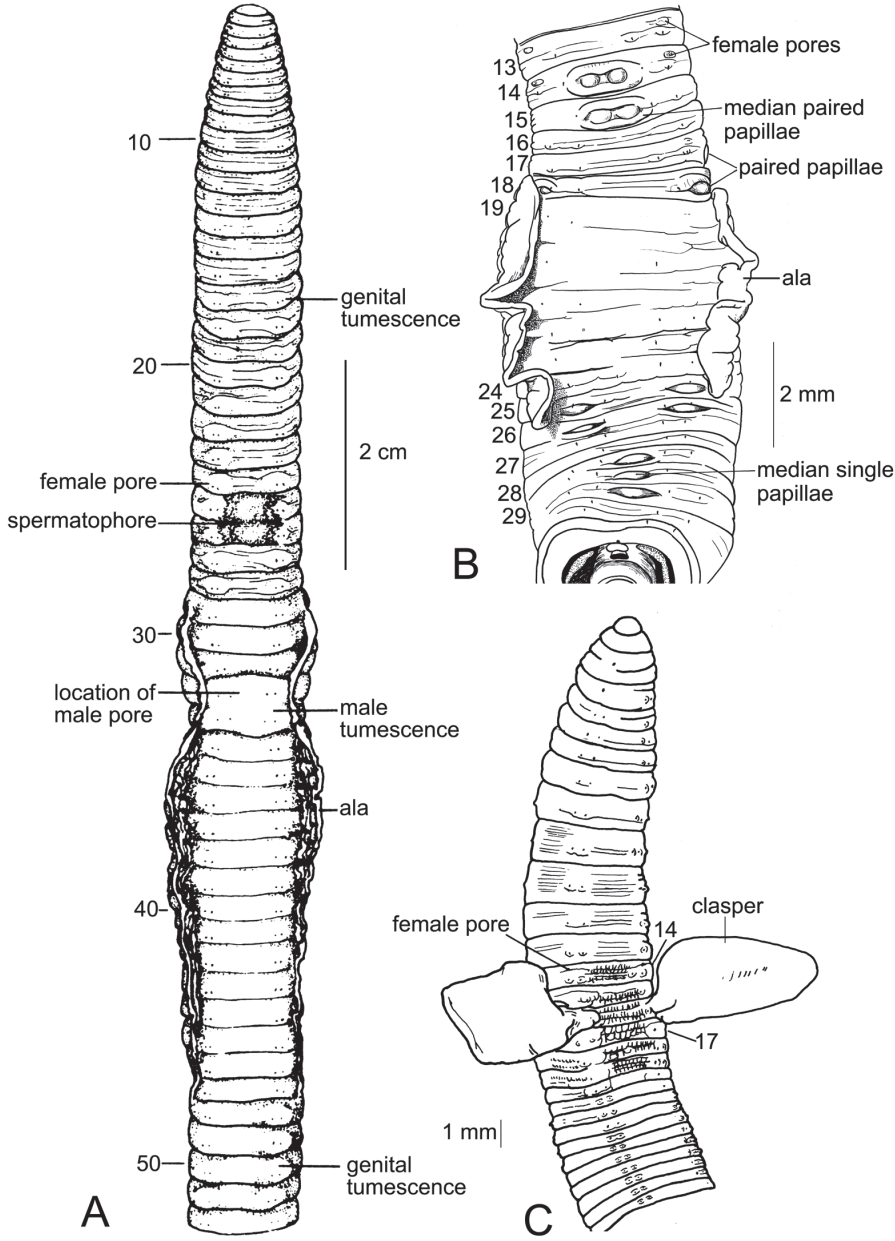


Fig. 8.38. Genital fields in Lutodrilidae and Almidae. **A**. *Lutodrilus multivesiculatus* (Lutodrilidae). Anterior end, with genital region, in ventral view, showing alae. After McMahan, M. L. 1979. Proceedings of the Biological Society of Washington 92(1): 84-97, Fig. 1. **B**. *Glyphidrilus kukenthali* (Almidae). Anterior end, with genital region, in ventral view, showing alae. **C**. *Drilocrius alfari* (Almidae). Anterior end, with genital region, in ventral view, showing claspers. C and D after Jamieson, B. G. M. 1971. Glossoscolecidae. Pp. 147-199. In R. O. Brinkhurst and B. G. M. Jamieson (eds), The Aquatic Oligochaeta of the World, Oliver and Boyd, Edinburgh, Toronto, Figs. 15.4B, 15.10A.

and almoids (represented by *Criodrilus* and *Lutodrilus*) is not refuted by molecular data (Figs. 8.1, 8.6).

Almines are notable for extensions of the body wall in the vicinity of or including the male pores. These extensions may be mere protuberances, as in some *Drilocrius* species; or involve a greater extent of the body wall, as in *Glyphidrilocrius*, or take the form of wing or keel-like structures (alae) in *Glyphidrilus* (Fig. 8.38B) or paddle-shaped claspers in *Drilocrius alfari* (Fig. 8.38C) and all species of *Alma* (Fig. 8.38A,B). In *D. alfari*, the male pores lie near the bases of the claspers but in *Alma* they are near the tips of the claspers which are furnished with genital chaetae and sucker-like structures (Figs. 8.11, 8.56).

The structure of the clitellum of *Alma emini* has been described by Grove (1931) (Fig. 8.8D) and corresponds closely with that observed by the same author in the glossoscolecid *Diachaeta exul*, in the almids, *Callidrilus ugandaensis* by Jamieson (1971b), *Glyphidrilus annandalei* by Nair (1938) and *Alma nilotica* (Fig. 8.8E) by Khalaf El Duweini (1951) and in the sparganophilid *Sparganophilus tamesis* by Jamieson (1971b) (Fig. 8.8B). The clitellum of the biwadrilid *Biwadrilus* is similar but has, in addition to the fine- and coarse-grained cells, club-shaped peripheral cells with fine or coarse granules (Nagase and Nomura, 1937). In *Criodrilus lacuum,* Benham (1887) observed only glandular cells with small spherical globules.

In *Alma nilotica* (Fig. 8.8E) the shortest (outermost) cells are normal epidermal supporting cells with a few sensory cells and mucin-secreting cells irregularly distributed amongst them. The cells that appear to make the middle layer are glandular cells which are fairly numerous and are irregularly distributed. They contain large granules and there is evidence that they secrete the cuticle and membrane of the cocoon. The apparent third layer is composed of cells appearing to form several tiers and arranged in groups that are separated from one another by thin lamellae of connective tissue. These contain fine granules of an albuminous secretion (Khalaf El Duweini 1951).

The latter author, as did Grove (1931) for *Alma emini* and Grove and Cowley (1927) for *Eisenia,* presents evidence that the fine-granule cells secrete the albuminous contents of the cocoon. Grove (1931) considered this relative abundance of mucin-secreting cells in the clitellum of *A. emini* to indicate secretion of a copulatory slime tube. Their paucity in the clitellum of *A. nilotica* corresponds with the absence of a slime tube in this species.

Criodrilinae. The Criodrilinae contain a single genus, *Criodrilus* (Fig. 8.19), including two species, the type-species *Criodrilus lacuum* and little know *species inquirendae*, *C. ochridensis*. In *C. lacuum* the ventralmost chaetae of at least segments 12, 13, 16-18 are modified as genital chaetae: terminally bearing four deep longitudinal grooves the proximal ends of which grade into irregular transverse jagged tooth-like ridges. The clitellum is indistinctly delimited anteriorly and posteriorly, annular, embracing 14, 15, 16 to 45, 47 (=30, 32, 34 segments). It consists histologically of an outer columnar epidermis continuous with that of the general body surface and three or four

layers of club-shaped glandular cells with basal nucleus and filled with highly refractive small spherical globules. Male porophores are very strongly protuberant, transversely placed, ellipsoidal mounds filling, and widening, segments 15 and 16 longitudinally. Each male pore is a transverse cleft deeply bisecting the summit of the male porophore. There may be one to several spermatophores: curved, horn-shaped, hard but flexible structures approximately 1 mm long and maximally about 0.4 mm wide, at the expanded base; attached in the vicinity of the genital field. The female pores are each a small transverse slit in intersegmental furrow 14/15. Spermathecal pores are absent.

The testes are free, digitate, or delicate, transversely slightly plicate lobes in segments 10 and 11; posterior to each is a much convoluted sperm funnel. Seminal vesicles are four pairs, in segments 9-12. The vasa deferentia are concealed deeply in the unusually thick body wall musculature, emerging in the coelom of segment 15 where that of each side of the body joins the antero-dorsal aspect of a large hemispherical male bursa or prostate gland which is restricted to that segment. The gland consisting of cells similar to and continuous with those forming the epidermis of the clitellum; the muscular layers of the body wall covering the inner surface of the gland are thin; the vas deferens is continuous through the substance of the gland to the male pore. Each ovary is a solid, tongue-like or paddle-shaped lobe showing few external indications of oocytes, almost filling the length of segment 13. Oviducal funnels form small rosettes. Ovisacs, in segment 14, at maturity are at least as large as the ovaries and contain large oocytes; they project into 14 from septum 13/14 and are closely associated with but apparently not directly connected with the funnels (Jamieson 1971c).

Ailoscolecidae. The male pores of *Ailoscolex* are intraclitellar, discharging on the tubercula pubertatis anteriorly in segment 22. The clitellum is annular, on segments 14-23 though incomplete ventrally in the first three segments. The tubercula pubertatis each consist of a gutter bordered dorsally by a pad and ventrally by the chaetal papillae, in segments 22-24. In *Ailoscolex lacteospumosus* the chaetal papillae form a row of contiguous tubercles from 14-24, of which the last three pairs are fused with the ventral aspect of the large tuberculum pubertatis (Bouché 1972) (Fig. 8.39A).

Testes are paired, in 10 and 11; testis-sacs are absent; seminal vesicles lie in 11 and 12. Spermathecae are simple, very large, intracoelomic, pedunculate and globose, in segment 9 and 10. Prostate-like glands occur on the body wall, associated with the tubercula pubertatis, and radiate about a point of maximum density situated on intersegments 21/22-23/24. Ovaries are in segment 13, and large ovisacs in 14 (Bouché 1972). *Ailoscolex* appears to have close affinities with the family Komarekionidae (see below), which was subsumed in it by Sims (1980, 1982) and with the Sparganophilidae.

Komarekionidae. This family is known from a single, terrestrial species, *Komarekiona eatoni* (Gates 1974), from North America. (Sims 1980, 1982) included *Komarekiona* in the Ailoscolecidae. There are striking similarities between the two entities, including the unusual location of male pores on

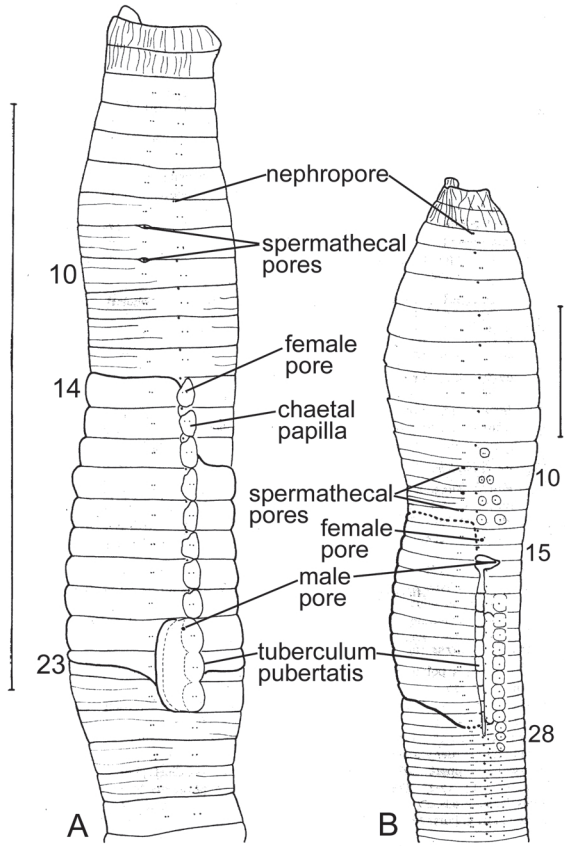


Fig. 8.39. Anterior ends, showing genital fields of **A**. *Ailoscolex lactospumosus* (Ailoscolecidae). **B**. *Hormogaster redii insularis* (Hormogastridae). After Bouché, M. B. 1972. Lombriciens de France: Écologie et Systématique, Institut National de la Recherche Agronomique, Vol. 72, Fig. 19.

segment 22; the long, saddle-shaped clitellum; tubercula pubertatis on the clitellum; the dorsolateral location of the spermathecal pores; the large number of tubular prostate-like glands associated with ventral chaetae; and the adiverticulate spermathecae. However, *Komarekiona* shows important differences from *Ailoscolex* which collectively are here considered to caution against synoymy in the Ailoscolecidae, although it must be admitted that variation of a similar magnitude occurs within other families, for instance the Megascolecidae. These differences are numbers of gizzards (single in segment 6, two, in 6-7 and 8-9, in *Ailoscolex*); absence of nephridial caeca and an intestinal typhlosole; a pretesticular (not testicular) location of the spermathecae; and presence of two pairs of latero-oesophgeal vessels which are not seen in *Ailoscolex*.

In *Komarekiona* the clitellum is saddle-shaped, in segments 19-25 or 26, and bears ridge-like tubecula pubertatis. The male pores are inconspicuous, near the equator of segment 22. Spermathecae are adiverticulate, with pores

in 6/7-8/9. There are two pairs of testes, in segments 10 and 11. The vasa deferentia are supraparietal. Prostates are absent but prostate-like tubular glands, resembling those of *Sparganophilus*, are associated with the ventral chaetae in any of segments 7 to 26, those in 9-11 are larger. Additional, intraclitellar paired glands occur between the ventral chaetal pairs in some or all of segments 20-26. The ovaries have a single, terminal egg-string.

Sparganophilidae. The male pores in *Sparganophilus* are one pair, inconspicuous in intersegmental furrow 18/19 or anteriorly in segment 19 (Fig. 8.40). The saddle-shaped clitellum is extensive, occupying eight to twelve segments in the region of segments 15-19. Tubercula pubertatis, in the

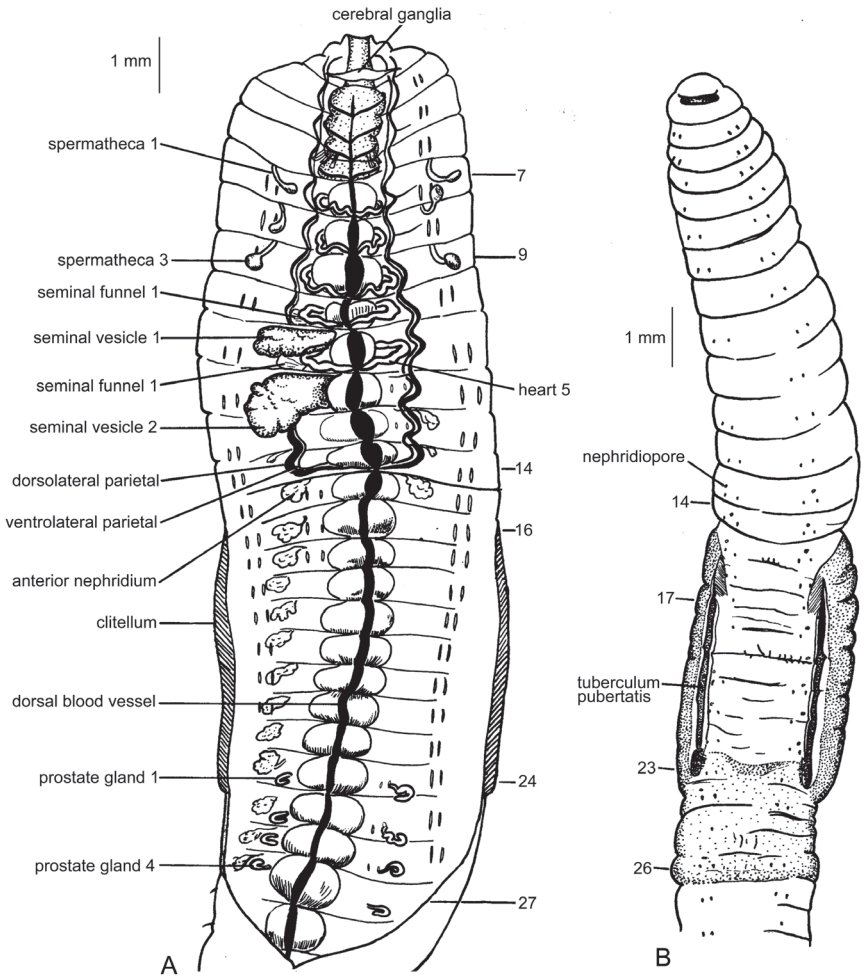


Fig. 8.40. *Sparganophilus tamesis* (=*eiseni*) (Sparganophilidae). **A**. Dorsal dissection. **B**. Anterior end, with genital region, in ventral view. After Jamieson, B. G. M. 1971. Glossoscolecidae. Pp. 147-199. In R. O. Brinkhurst and B. G. M. Jamieson (eds), The Aquatic Oligochaeta of the World, Oliver and Boyd, Edinburgh, Toronto, Figs.15.13C and B.

clitellar region, are ridge like or a series of paillae, lateral to the ventral chaetal couples. Female pores are inconspicuous, in front of the ventral chaetal couples of segment 14. Spermathecal pores are inconspicuous, and dorsolateral, in 6/7-8/9, or 5/6 also, a single pair or four pairs per intersegment. Pores of postate-like glands, if these are present, are minute in the vicinity of the ventral chaetae in several segments in the clitellar region and sometimes in a variable number of more anterior segments. Testes and funnels are free in segments 10 and 11; seminal vesicles two pairs, in 11 and 12. Vasa deferentia are intraparietal. Ovaries are of the lumbricid type, i.e. with a single egg string, in 14; small ovisacs are present. Spermathecae are adiverticulate, paired or multiple, and extend far into the coelom (Jamieson 1971c). Resemblances to the Ailoscolecidae are noted under that family, above.

Megascolecoidea. **Families Ocnerodrilidae and Megascolecidae**. This grouping is strongly supported by molecular data (Jamieson *et al*. 2002) (Fig. 8.1).

Ocnerodrilidae. Relationship of ocnerodriles to the Megascolecidae has been widely accepted but they have been given subfamilial or familial status or even dispersed within the Megascolecidae (see Jamieson 1971d). Molecular analyses (Jamieson 2000; Jamieson *et al*. 2002) indicate that they are the plesiomorph sister-group of the Megascolecidae (Acanthodriliinae + Megascolecinae) (Fig. 8.1). They are divisible into two groups, ranking as subfamilies if ocnerodriles are given familial rank: the Ocnerodrilinae and a small group, the Malabarinae. The Ocnerodrilinae have extramural calciferous glands (esophageal diverticula) in segment 9; they occur from near the Tropic of Cancer in western North America through Central America and some Caribbean Islands into South America near the Tropic of Capricorn and throughout Africa from the Nile Valley and south of the Sahara, into Madagascar and the Seychelles. The Malabarinae lack extramural calciferous glands; they occur in the Indian subcontinent and Burma (Jamieson 1971d; Sims 1980, 1982).

Ocnerodriles closely resemble Megascolecidae but differ from these in that calciferous glands, which are frequently absent from megascolecid species, are restricted to segment 9, or, in Malabarinae, 9 and 10. They are plesiomorphic relative to megascolecids in origin of the intestine in segment 12 (sometimes 13 or 14) and in not having added hearts behind segment 11 (Jamieson 1971d).

With regard to reproductive anatomy, there are one to three pairs of tubular prostates with pores in the region of segments 16-21, of which one or two pairs are sometimes united with the male pores. Penial chaetae if, rarely, present are little modified. In some genera, including *Eukerria* (Fig. 8.9) (Jamieson 1970) the male pores and prostates are in the acanthodrilin arrangement which is typical of the megascolecid subfamily Acanthodrilinae. This genus alone was represented in the molecular analysis which confirmed sister-group relationship with the Megascolecidae (Jamieson 2000; Jamieson *et al*. 2002) (Fig. 8.6) and it would be desirable in

further analyses to include species with the typical ocnerodrile arrangement of a pair of united prostatic and male pores on segment 17.

The clitellum in Ocnerodrilidae usually occupies up to seven segments, between 12 to 18, but in *Nematogenia* it is 13 segments long and extends to segment 26. Spermathecal pores are, as in megascolecids, pretesticular but, unlike the latter, rarely bear diverticula. Whereas in *Pygmaeodrilus nabugaboensis* the spermathecal diverticula are inseminated (Jamieson 1957), in *P. montiskenyae* the ampulla receives the sperm (Jamieson 1965) (Fig. 8.17).

Megascolecidae. Megascolecids usually have male pores on segment 18, fused with or near a pair of prostate glands, or prostates in 17 and 19 with male pores intermediate or fused with one pair of prostate pores. Different arrangements are shown and named in Fig. 8.8 *Hoplochaetella* is exceptional in having two pairs of male pores. Prostate glands are tubular to racemose (the latter with branched internal ducts, as in *Pheretima*). The vasa deferentia do not usually enter the glandular part of the prostate and they are therefore metaprostates and not euprostates. Spermathecae are usually diverticulate, rarely (Fig. 8.18) multiple.

The evidence of the Ocnerodrilidae, which may have from one to three or more pairs of prostates, suggests that more than one pair of prostates were present in ancestral megascolecids. In the Megascolecidae, two pairs are still seen in the acanthodrilin condition, in which two pairs of prostate pores lie on segments 17 and 19, and the male pores are on segment 18, or the homeotic equivalent of these segments. Correspondingly, there are usually two pairs of spermathecal pores, at intersegments 7/8 and 8/9. This condition of the male terminalia is typical, though not constant, for the Acanthodrilinae (Fig. 8.8) and is seen, and probably of common derivation, in the Ocnerodrilidae such as *Eukerria* (Figs. 8.9, 8.54A). It is well exemplified by the genus *Diplotrema*, in which, as is usual for the acanthodrilin condition, the two prostate pores of each side communicate with the male pores by a seminal groove. Michaelsen (e.g. 1928) may well have been correct in proposing the acanthodrilin arrangement as basic to the Acanthodrilinae and that the microscolecin condition (Figs. 8.8, 8.54B) resulted by loss of the posterior prostates (in segment 19) and migration of the male pores into the vicinity of the anterior prostate pores (on segment 17), but a sexprostatic (with prostates in segment 18 also, as in *Dichogaster damonis*) or multiprostatic precursor (as in some ocnerodriles, see Jamieson 1958) cannot be ruled out. The microscolecin condition is seen, for instance, in *Rhododrilus* and, with complete fusion of male and prostate pores on segment 17, in *Kayarmacia* and in the circummundane parthenogenetic *Microscolex dubius*.

The less common balantin condition (Figs. 8.8, 8.54C) was putatively derived by migration of the male pores onto segment 19 where they approached the single remaining, posterior, pair of prostate pores. This condition with male pores at intersegment 18/19 (and correspondingly a single pair of spermathecal pores, in 7/8) is seen in the Yucatan acanthodrile *Balanteodrilus* and was so derived by Pickford (1937) and is also seen in the New Zealand genus *Sylvodrilus*, in which the male pore remain on segment

18 (Lee 1959). The most extreme balantin reduction is seen in the acanthodrile *Torresiella* from Horn Island, Torres Strait, Australia, in which the male pores and those of the single pair of prostates are united on segment 19 (with spermathecal pores in 7/8) and, as a further profound apomorphy, the nephridia are wholly meronephric (Dyne 1997).

The strongly protuberant nature of the male pores in some acanthodriles suggests that in the acanthodrilin arrangement, the seminal grooves serve to pass prostatic secretion to the male pores rather than sperm to the prostate pores. However, the usual correlation of the number of pairs of spermathecal pores with the number of prostate pores might suggest the latter, commonly accepted, alternative.

The megascolecin condition of the male pores (Figs. 8.8, 8.54D) is characteristic of the subfamily Megascolecinae. In the megascolecin condition, the male pores are united with the pores of a single pair of prostates on segment 18 or, in a presumably more plesiomorphic condition seen only in the New Caledonian genus *Eudiplotrema*, are near but not fused with the prostate pores (Jamieson and Bennett 1979). Michaelsen (1913) debated, and in Michaelsen (1928) remained equivocal, as to whether the megascolecin condition was acquired by migration of the remaining anterior or posterior pair of prostates of a former acanthodrilin condition onto segment 18. However, the possibility exists that the prostates of the megascolecin arrangement are plesiomorphically and intrinsically of that segment and, though not necessitated by this proposition, that they are persistent from a longer segmental series of prostates, possibly from a sexprostatic condition, with a pair of prostates in each of 17, 18 and 19. The latter, sexprostatic condition, though exceedingly rare, is known in the ocnerodrile, *Diaphorodrilus doriae* Cognetti (1910), the acanthodrile *Pickfordia magnisetosa* Omodeo (1958) and supposedly in the inadequately described *Dichogaster damonis* Beddard (1889b), from Fiji, the type species of *Dichogaster*, and has been reported in other Fijian and also in Caribbean Dichogasters (James, pers. com.; Jamieson *et al*., 2002) (Figs. 8.1, 8.6).

The prostates are predominantly tubular in the Acanthodrilinae but they are tubuloracemose or even racemose in *Dipotrema scheltingai* and are racemose in *Exxus* Gates 1959.

8.3 OOGENESIS

Oogenesis in oligochaetes is intraovarian (Jamieson 1988a) (though considered extraovarian by Eckelbarger 1988) in that the germ cells are not released from the ovary into the coelom or a diverticulum of this, the ovisac, until they are ripe eggs (metaphase primary oocytes) and have completed vitellogenesis. Here they remain in metaphase of the first meiotic division and are released in this state from the female pores into the cocoon in which they are fertilized (see review by Jamieson 1981c). The size of the egg differs greatly between microdriles and crassiclitellates. Thus in microdriles the primary oocyte is very large, ranging from 300 μm to 1 mm, well within the

size range for so-called lecithotrophic eggs of polychaetes and other invertebrates, whereas in lumbricid earthworms primary oocytes reach 120 µm (see references in Jamieson 1988a), roughly in the range of planktotrophic eggs of polychaetes. The smaller size in megadriles is correlated with what is considered to be secondary acquisition of a multilayered clitellum, secretions of which, in the cocoon, reduce the necessity for reserves in the egg (Jamieson 1971b, 1988b).

Intercellular bridges. Whereas connections between oocytes are rare in polychaetes (Eckelbarger 1988), in earthworms (*Eisenia fetida*) the oogonia and premeiotic primary oocytes are interconnected, each group developing from a single oogonium. The bridges each have the form of a fuzzy-coated zonula collaris as seen in spermatogenesis and at their confluence they constitute the cytophore. Homology of such structures between developing eggs and sperm is attributed to the hermaphroditic condition. It is inferred that the bridges permit synchronization of development of the gametes but the mechanism of information exchange is unknown (Jamieson 1988a). As shown for *Enchytraeus* and *Tubifex,* when the primary oocyte of an oligochaete is released into the coelom it is detached from the cytophore (see review by Jamieson 1981c).

Vitellogenic Phase. Oligochaete vitellogenesis is already underway in the primary oocytes and in *Enchytraeus albidus* is said to begin in the third stage oogonium (Dumont 1969). It appears to be both autosynthetic and heterosynthetic; the distinction between the two being somewhat arbitrarily defined by the size of imported molecules. Heterosynthesis involves endocytosis (pinocytosis) as in polychaete eggs but some transference of yolk to the egg by chloragocytes occurs in enchytraeids (references in Jamieson 1981c). Such mechanisms for rapid incorporation of yolk precursors are characteristic of species having semi-continuous reproductive periods with short periods of oogenesis and frequent egg-laying (Eckelbarger 1988), as is true of oligochaetes, as opposed to monotelic species. For evidence for auto- and hetero-synthesis of vitelline materials see (Jamieson 1981c, 1988a; Siekierska 2003).

Cortical granules. Conspicuous cortical granules seen in some polychaete eggs, are not characteristic of oligochaete eggs. The cortical zone of the *Tubifex* primary oocyte in the ovisac is 2-3 µm thick, containing mitochondria and RER, ribosomes, minute vesicles, multivesicular bodies (often open to the surface) and other components in a finely particulate matrix devoid of granules and lipid droplets. Yolk granules and lipid droplets are confined to the endoplasm (Shimizu 1976).

Egg envelopes. Oolemmal microvilli first appear in *Eisenia* in the primary oocytes where they project into a newly acquired acellular sheath, the zona pellucida (ZP). The ZP is regarded as a thickened oocyte membrane, the chorion, by Lechenault (1968) and, with the microvilli, would therefore constitute a primary envelope *sensu* Eckelbarger (1988). However, the origin of the ZP requires further investigation. The ZP and microvilli may jointly be termed the vitelline envelope. The microvilli in the mature ovarian oocytes are

much shorter in microdriles *(Enchytraeus albidus, Tubifex tubifex)* than in crassiclitellates (Fig. 8.41). Their length has been shown in lumbricids to be strongly correlated with the length of the acrosome. They are illustrated for *Allolobophora chlorotica* in Fig. 8.41F,G and for *Lumbricus rubellus* in Figs. 8.41H. In tangential section of the ZP of *A. chlorotica* (Fig. 8.41F) it is seen that the microvilli are interrupted by circular fenestrae (Jamieson *et al*. 1983); these are perhaps equivalent to multiple micropyles. There is no ZP in tubificid oocytes but an indistinct fibrillar 'vitelline membrane' is traversed by the short microvilli. After fertilization the vitelline membrane of the *Tubifex* egg, becomes a trilaminar fertilization membrane overlying a perivitelline space. The oolemma loses its microvilli. The detached microvilli reach outwards only to the middle layer, suggesting (unless they have retracted) that the outer layer of the fertilization membrane is added from extrinsic sources, possibly from the cocoon fluid (Shimizu 1976, see Jamieson 1981c).

Nurse cells (as sterilized oocytes), seen in polychaetes (Eckelbarger 1988), have not been reported in oligochaetes, excepting in a recent paper by Siekierska (2003). As only one or two embryos commonly develop in earthworm cocoons with sixteen or more eggs, the infertile eggs are effectively vitelline cells equivalent to those of neoophoran plathyhelminthes (Jamieson 1981c). Siekierska (2003), for the lumbricid *Dendrobaena veneta* reported the presence of nurse cells (trophocytes): the ovarian stroma is composed of somatic cells, the processes of which are connected to each other via numerous desmosomes; the somatic cells (identified as follicle cells *sensu* Jamieson) and their processes envelop the germ cells tightly and play a supportive role; oogonia, oocytes and trophocytes are arranged in distinct zones in the ovary; trophocytes form chains of cells, which are interconnected by intercellular bridges and contain numerous microtubules; the oocytes are distally arranged in the ovary. The function of the nurse cells in *D. veneta* has not been elucidated. There is evidence that trophocytes are mainly responsible for RNA (mainly rRNA) synthesis (references in Siekierska 2003), RNA being partly or fully synthesized in these cells and then transported to oocytes. They do not seem to be involved in vitellogenesis in *D. veneta*; the connections between oocytes and trophocytes no longer exist in ovarian zone III (mature oocytes) as the trophocytes degenerate (Siekierska 2003). That these trophocytes are distinct from follicle cells requires further investigation.

Follicle cells. In *Enchytraeus* several layers of squamous epithelial cells (termed follicle cells by Jamieson 1981c but presumed to be modified peritoneal cells) cover the distal surfaces of the stage I and stage II oogonia. The stage III oogonium becomes covered distally and laterally by a thin, electron-dense layer derived from this epithelium by attenuation. Pillars of epithelial cytoplasm project towards the oogonial surface.

Follicle cells also occur in lumbricids. In *Eisenia fetida,* they surround the primary oocyte at least while it retains its connection to the ovary. They are very much branched, with long slender processes forming several beds around the ZP, the more internal projecting into the latter. The projections in *Enchytraeus* and *Eisenia* do not establish connections with the egg. As

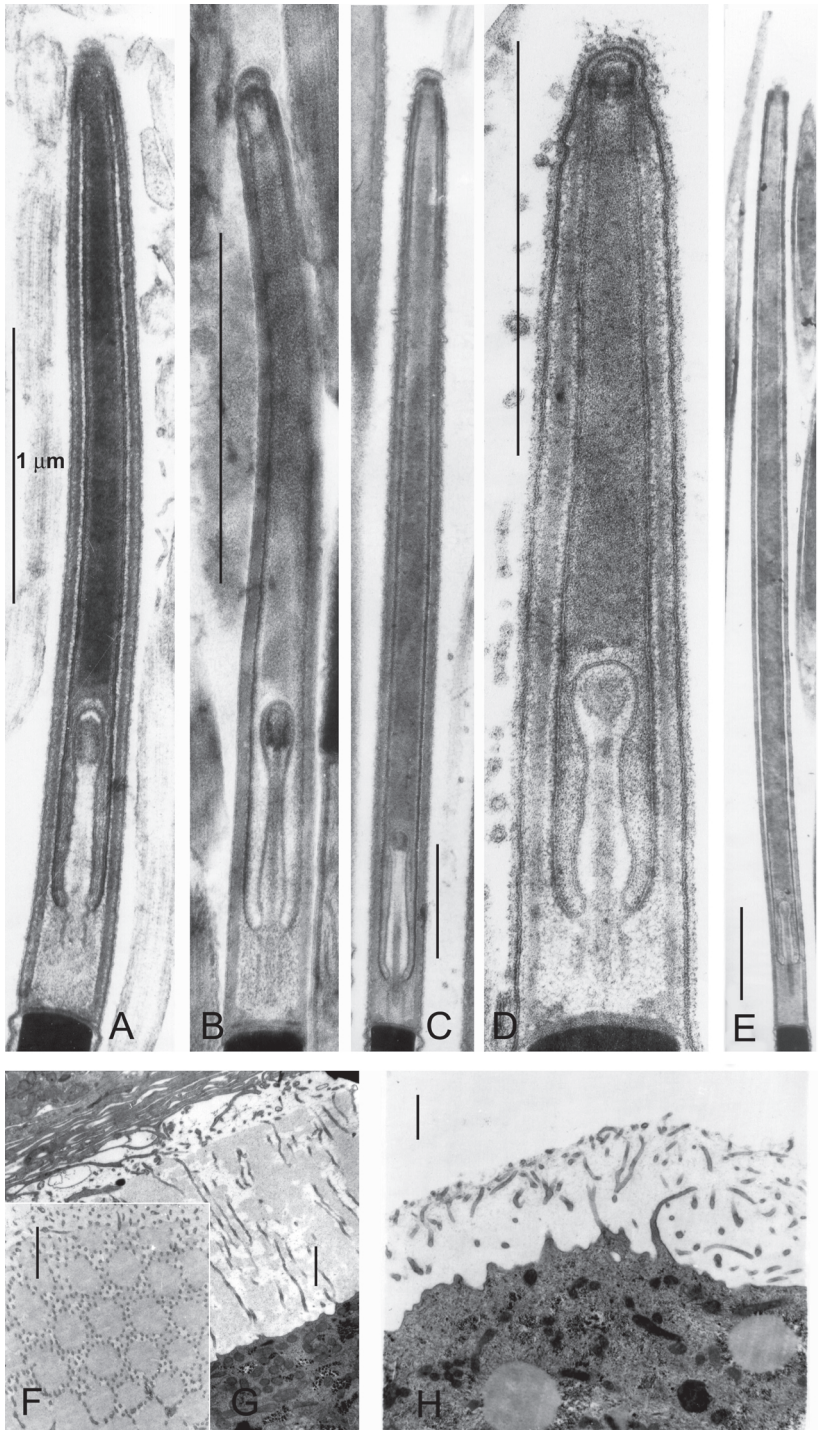


Fig. 8.41 contd

observed by Eckelbarger (1988) such follicle cells may have a supportive rather than nutritive role. In *Enchytraeus*, when the oocyte has entered the ovisac it has lost its follicle cells and this is presumably the case for the lumbricid egg (see reviews by Jamieson 1981c, 1988a, 1992).

Oogenesis and phylogeny. The modes of oogenesis and vitellogenesis are of little value in determining the phylogenetic position of the Oligochaeta within the Annelida but they do not appear to show specializations which exclude regarding oligochaetes as being near the stem of the Annelida (Jamieson 1988a).

8.4 SPERMATOGENESIS AND SPERMATOZOAL ULTRASTRUCTURE (MARCO FERRAGUTI AND BARRIE G.M. JAMIESON)

8.4.1 Spermatogenesis

Spermatogenesis refers to the process of cell division and differentiation that commences with the primordial germ cells (protogonia) and ends with production of the mature spermatozoa. The latter stage of the process, whereby the spermatids differentiate without division to produce spermatozoa, is distinguished as spermiogenesis. The first spermatogonial divisions occur in the testis. Division is synchronous and results in the development of groups of cells interconnected by cytoplasmic bridges (collars in Ferraguti and Lanzavecchia 1971; bridges in Martinucci *et al*. 1977; zonulae collaris in Jamieson 1978a) to form morulae (e.g. Jamieson 1981c, 1992), also termed cysts (Ferraguti 1999). Collars surrounding the bridges are illustrated for mature spermatids of *Haplotaxis ornamentus* (Fig. 8.42H,I) and *Eudrilus eugeniae* (Fig. 8.43D). Further spermatogonial divisions, and spermiogenesis, may be limited to the coelom of the testis segment, as in Phreodrilidae, but usually occur in diverticula of the septa which project into adjacent segments and constitute the seminal vesicles. Development may also occur in specialized compartments of the testis segments, termed testis-sacs, present in combination with seminal vesicles, as in lumbricids and many megascolecids. The many variations on these themes are beyond the scope of this volume but details are given by Jamieson (1981c, 1992). Light microscopical observations on spermatogenesis in the megascolecids *Amynthas hawayanus*, *A. morrisi* and *Metaphire californica* are given by de Majo (2002a,b).

The spermatogonia differ from oogonia in lacking smooth ER (present, however, in spermatids, see Boi *et al*. 2001), which is mostly situated

Fig. 8.41 contd

Fig. 8.41. A-E. Acrosome and proximal portion of the nucleus of spematozoa of Lumbricidae in longitudinal section. **A**. *Eisenia fetida*. **B**. *Lumbricus castaneus*. **C**. *Allolobophora longa*. **D**. *L. rubellus*. **E**. *A. chlorotica*. **F-H**. Zona pellucida of unfertilized primary oocytes of Lumbricidae. **F**. *Allolobophora chlorotica.* In near-tangential section, showing fenestrae between the microvilli of the ZP. **G**, **H**. In vertical section, showing microvilli and portion of adjacent oocyte. **G**. *Allolobophora chlorotica.* **H**. *Lumbricus rubellus.* Based on the study of Jamieson B. G. M. *et al.* 1983. Gamete Research 8: 149-169.

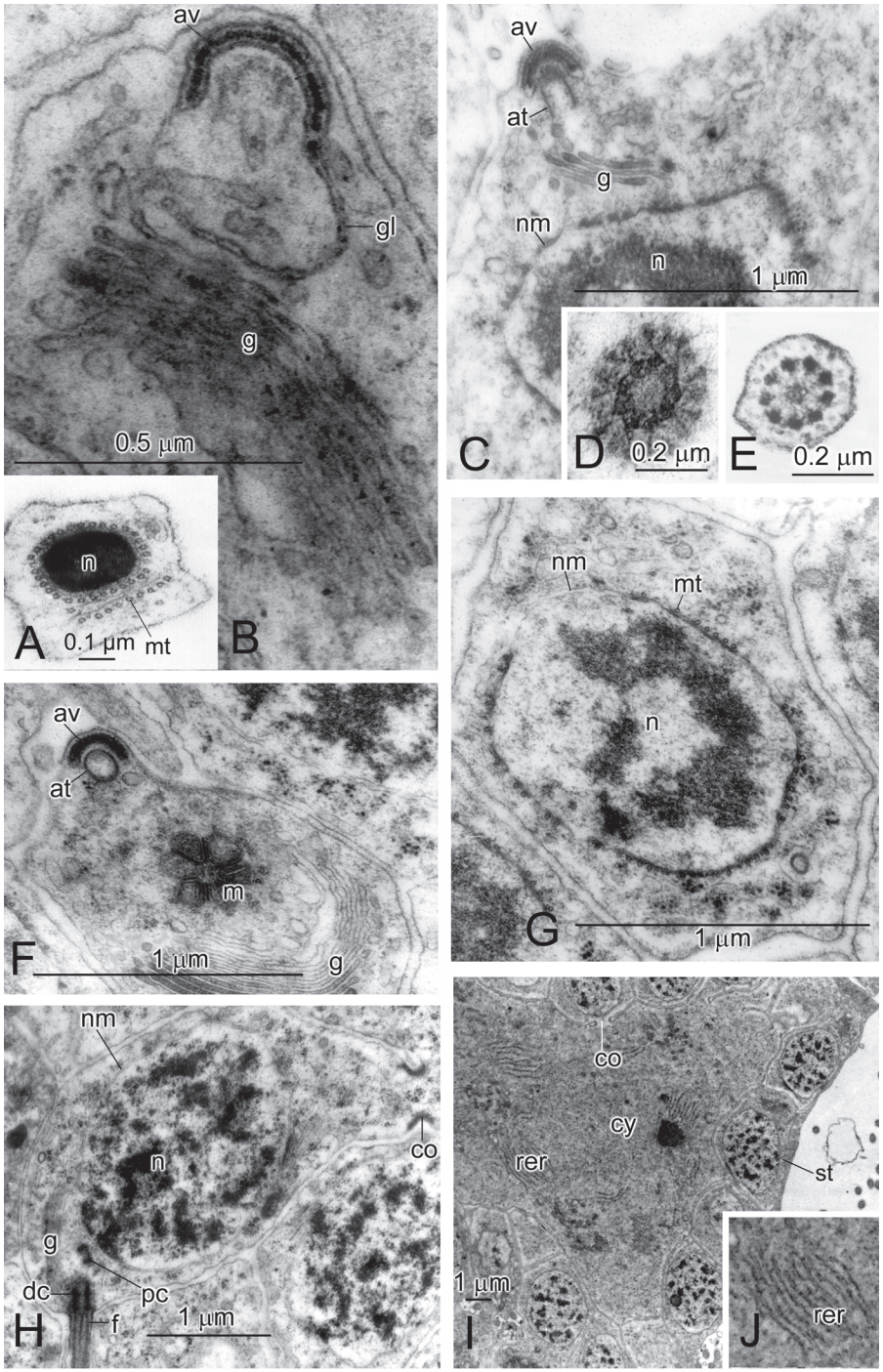


Fig. 8.42 contd

peripherally in the oogonia. The number of nuclei will increase stage by stage following powers of two. Thus morulae with 4, 8, 16, 32, 64, 128 (sometimes more) cells will be found. When the spermatogonial morula or follicle has reached the 8-32 cell stage, the region of confluence becomes a common cytoplasmic mass, the early cytophore. The spermatogonial morulae released from the testes into the coelom, or into modifications of this such as testis-sacs or seminal vesicles, possess only small cytophores (see *Eudrilus eugeniae*, Fig. 8.43A,B). In microdriles (*Branchiura sowerbyi*) (Hirao 1973) and earthworms (*Lumbricus terrestris*) (Walsh 1954) division of spermatogonia of the 16 cell morula gives the 32 primary spermatocytes. These are distinguished from spermatogonia, whose divisions are mitotic, in subsequently undergoing a meiotic reductional division, the first maturation division. In oligochaetes spermatocytes are smaller than spermatogonia, as there is a precocious enlargement of late spermatogonia. The products of reductional division of the diploid primary spermatocytes (usually 32) are haploid secondary spermatocytes (usually 64) (Hirao 1973). The cytophore further enlarges.

The second non-reductional meiotic division of the haploid secondary spermatocytes produces similarly haploid spermatids, 128 in most of the oligochaete species studied (Jamieson 1981c), but 256 in two different lumbriculids (Ferraguti 1999) and more than 400 in some cysts, considered atypical, of the phreodrilid *Astacopsidrilus* (=*Phreodrilus*) (Jamieson 1981b). The spermatids transform into spermatozoa without further divisions (details in Jamieson 1981c, 1992; Ferraguti 1999). After detachment of the spermatozoa (Martinucci *et al*. 1977) or shortly before this in the tubificid *Limnodriloides* (Jamieson and Daddow 1979) and in *Astacopsidrilus* (Jamieson 1981b), the cytophore becomes globular (see *Eudrilus eugeniae*, Fig. 8.43C) and disintegrates.

Functions of the cytophore. These are deduced to include support and synchronization of germinal cells through the interconnecting bridges. Boi *et al.* (2001) have shown that the bridges are kept open by actin rings. When the actin is de-polymerized by the end of spermiogenesis, some nuclei slip

Fig. 8.42 contd

Fig. 8.42. *Haplotaxis ornamentus.* Spermiogenesis by transmission electron microscopy. **A**. Transverse section (TS) through condensing nucleus of spermatid, surrounded by the micrtoubular manchette. **B**. Golgi apparatus of spermatid, showing a Golgi lamella contributing to the developing acrosome vesicle.**C**. Acrosome developing an acrosome tube. **D**. TS distal centriole with satellite rays of anchoring apparatus. **E**. TS axoneme showing tetragon arrangement at central singlets. **F**. Developing acrosome and midpiece accompanied by Golgi apparatus. **G**. Spermatid nucleus surrounded by microtubular manchette. **H**. Longitudinal section of a younjg spermatid, showing collar attaching it to cytophore, Golgi apparatus, undondensed nucleus, proximal and distal centrioles, and flagellum. **I**. Part of a cytophore, containing rough endoplasmic reticulum (RER) stacks, and attached spermatids. **J**. Detail of RER. Abbreviations: at, acrosome tube; av, acrosome vesicle; co, collar attaching spermatid to cytophore; cy, cytophore; dc, distal centriole; f, flagellum; g, Golgi apparatus; gl, Golgi lamella; m, mitochondria of midpiece; mt, microtubules; n, nucleus; nm, nuclear membrane; pc, proximal centriole; rer, rough endoplasmic reticulum. From Jamieson, unpublished.

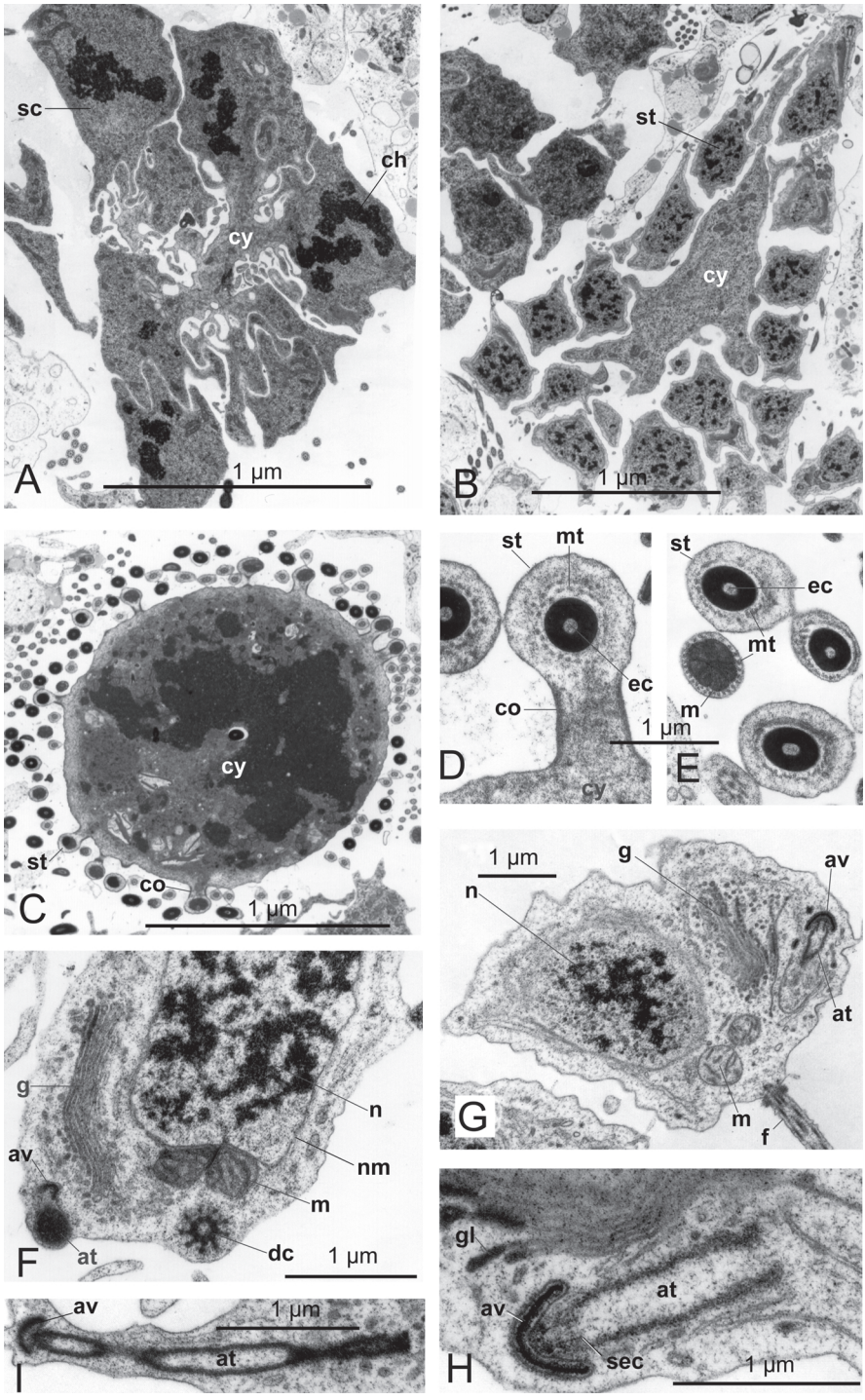


Fig. 8.43 contd

inside the cytophore. The bridges possibly also play an active role in migration of cell elements into the cytophore as evidenced by the abundant cytoskeletal elements and mitochondria, as energy transducers, on the cytophore side. This includes selective intake of cell organelles not required for further spermiohistogenesis such as ribosomes, chromatoid bodies, fibrillar clumps, smooth endoplasmic reticulum, the Golgi body after it has completed its secretory phase, multivesicular and lysosomal bodies, and mitochondria other than the midpiece set. Nuclear projections into the cytophore may provide a means of discarding nucleoplasm or, it is speculated (Jamieson 1981c), transmitting genetic instructions into the cytophore. The large number of mitochondria in the cytophore suggests that it has an important function in providing energy to the developing spermatids as it seems unlikely that the mitochondria of the midpiece precursor fulfill the total energy requirements of these cells. The cytophore at the second meiotic division has a volume which is at least twenty-two times that of the spermatogonial stage (*Eudrilus eugeniae*, Fig. 8.43C). This is taken as evidence of much endogenous synthesis of materials. Organelles are, clearly, synthesized. Some of them, including RER, remain in the cytophore but it has been suggested that materials involved in cell morphogenesis (precursors of manchette microtubules and of sperm tails) and in energy production (glycogen of sperm tail) probably countermigrate into the spermatids. It is also suggested that the great increase in size, and synthesis of materials, of the cytophore are mediated by transcription products emitted from the nucleus in the cell cytoplasm at earlier, protogonial and spermatogonial, stages (Martinucci and Felluga 1975; Martinucci *et al*. 1977). The transcription products are possibly represented by the chromatoid bodies and fibrillar mitochondria-associated material

Fig. 8.43 contd

Fig. 8.43. *Eudrilus eugeniae* (Eudrilidae). Stages in spermatogenesis by TEM. **A**. Morula of primary spermatocytes in metaphase. **B**. Morula of young spermatids, with some enlargement of the cytophore. **C**. Morula of elongated spermatids, with rounded cytophore (penetrated by a spermatozoon); spermatids with condensed nuclei in each of which an endonuclear canal has developed. **D**. Detail of late morula showing spermatid attached to the cytophore by a collar, microtubules surrounding the nucleus with its endonuclear canal. **E**. Transverse sections of nuclei of advanced spermatids and of a midpiece, both structures surrounded by microtubules. **F**. Longitudinal section (LS) of an elongating spermatid. The developing acrosome, being secreted by the Golgi apparatus, is still basal, near the distal centriole which has satellite rays. The mitochondria are assembling at the base of the nucleus. **G**. LS of an earlier spermatid. The acrosome rudiment shows a short acrosome tube and, within this, a shorter secondary tube. Mitochondria are assembling as the midpiece. The satellite apparatus of the distal centriole is visible as a 'muff' at the base of the axoneme. **H**. Later acrosome adjacent to the Golgi apparatus which has dense secretions at the ends of its lamellae. Beneath the dome-shaped acrosome vesicle there are two dense granules and a slender secondary acrosome tube. Outside the latter is the elongating acrosome tube. **I**. More advanced acrosome with more elongated acrosome tube. Abbreviations: at, acrosome tube; av, acrosome vesicle; ch, chromosomes; co, collar; cy, cytophore; dc, distal centriole; ec, endonuclear canal; f, flagellum; g, Golgi apparatus; m, mitochondria of midpiece; mt, manchette; n, nucleus; nm, nuclear membrane; sc, primary spermatocyte; sec, secondary tube; st, spermatid. From Jamieson, unpublished.

(Martinucci *et al*. 1977). Finally, the cytophore may play a part in separation of the spermatozoa and its own lysis (see reviews by Jamieson 1981c; 1992; Ferraguti 1999).

Origin of early acrosome rudiments. The history of the complex and sometimes confused terminology of the components of the acrosome and of their development is treated in detail by Jamieson (1981c). Only a brief outline can be given here.

The earliest anlage of the acrosome vesicle in oligochaetes originates from the Golgi apparatus of the spermatid, as the proacrosome. It becomes a small cap-shaped (concavoconvex) vesicle containing electron-dense material, the acrosome vesicle, as in *Haplotaxis ornamentus* (Fig. 8.42B,C,F). In all species studied it moves to the outer side of the Golgi under the plasmalemma, the latter becoming convex as a ''bleb'', over it. Additional Golgi vesicles may fuse with it during this migration (see review by Jamieson 1981c). Figure 8.42B is unique among published micrographs in showing an entire Golgi lamella fusing with the developing acrosome vesicle in *H. ornamentus*; this probably involves active movement of the lamellae.

The second early anlage of the acrosome consists of one to several electron densities or granules, which develop immediately below the acrosome vesicle.

A third structure which appears very early in development of the acrosome is the acrosome tube, as in *Haplotaxis ornamentus* (Fig. 8.42C,F) and *Eudrilus eugeniae* (Fig. 8.43G,H,I). This is a diagnostic autapomorphy of clitellates. It develops below the acrosome vesicle and, while growing in length, migrates with the vesicle to the tip of the nucleus which has undergone elongation and condensation. Its origin has been attributed, from micrographs, to the subacrosome granule(s), and/or directly to lamellae or vesicles originating from the Golgi apparatus (as in *H. ornamentus*, Fig. 8.42) and *Limnodriloides* or, questionably, to the proximal centriole (for a detailed discussion see Jamieson 1981c).

A fourth major component of the acrosome complex termed the secondary tube (periaxial sheath), appears shortly before the axial rod makes its appearance. This is a short sleeve-like tube which appears in *Lumbricus* to develop from the base of the dense subacrosomal granule. It is attached as its apical end to the encircling rim formed by the bounding membrane of the primary acrosome vesicle and lies within the acrosome tube.

Jamieson (1981c) proposed that in oligochaetes the acrosome vesicle, the dense granules, the acrosome tube and (indirectly) the secondary tube are all products of the Golgi apparatus but that the sequence of development of the granules and tube is variable.

In the final stages of acrosome morphogenesis the acrosome vesicle is withdrawn except for its terminal domed tip, into the acrosome tube, or in enchytraeids, capilloventrids, and some tubificid species remains external. Differentiation of a fifth major component, the acrosome rod (axial rod), the putative perforatorium also occurs. This rod is already present inside the basal invagination of the vesicle in the earthworms but in the lumbriculid

oligochaete *Bythonomus lemani* (see description below) differentiation of the rod occurs when the vesicle is already withdrawn. The rod probably develops from the dense granule(s) in all oligochaetes (see Jamieson 1981c, 1992; Ferraguti 1983, 1999).

Migration of the acrosome from its origin at the Golgi apparatus to its emplacement on the tip of the nucleus occurs outside the microtubular manchette and without any association with microtubules (MTs). However, after its emplacement on the nucleus the manchette, which surrounds the nucleus, extends anteriorly to ensheath the acrosome tube and elongation of the acrosome occurs within the manchette. It seems possible that the MTs play a part in the later morphogenesis of the acrosome (see Jamieson 1981c).

Some details of acrosome structure in the investigated oligochaete families are given below in 8.4.2.

Nuclear morphogenesis. The nucleus of clitellate male germ cells is a long, filiform structure in the mature spermatozoa, but is rounded, as is the cell, in early, so-called isodiametric spermatids. Nuclear morphogenesis consists in the transformation of the nucleus from the spheroidal to the elongate, cylindroid form, and in the condensation of its chromatin, accompanied by major modifications of the microtubules present in the spermatid cytoplasm. The microtubules first appear, during early spermiogenesis in the vicinity of the distal centriole and later surround the nucleus as the manchette which is at first circumferentially discontinuous but becomes continuous (see *Haplotaxis ornamentus*, Fig. 8.42A,G; *Eudrilus eugeniae*, Fig. 8.43D,E). That the manchette controls nuclear elongation and chromatin condensation has been the subject of much debate (see, for instance, Fawcett *et al*. 1971; Ferraguti and Lanzavecchia 1971; Lora Lamia Donin and Lanzavecchia 1974; Webster and Richards 1977; Jamieson and Daddow 1979; Martinucci and Felluga 1979; Troyer 1980; Jamieson 1981c; Ferraguti and Ruprecht 1992; Ferraguti 1999). This subject cannot be revisited here. Suffice it to say that with the progress of spermiogenesis, the diameter of the nucleus decreases, as does the number of microtubules forming the manchette (Jamieson and Daddow 1979; Hodgson and Jamieson 1992). The microtubules discarded from the manchette are found in the cytoplasm of the spermatids, and are later discarded with the residual cytoplasm.

Origin of the midpiece. Posterior to the nucleus, and approximately as wide as its base, are the mitochondria of the midpiece. The mitochondria are cristate but are radially adpressed; where several occur, they form a cartwheel configuration in cross section (*Haplotaxis ornamentus*, Fig. 8.42F; *Eudrilus eugeniae*, Fig. 8.43E). The fact that in ontogeny of the spermatozoon the midpiece originates as two to 11 separate, rounded mitochondria and that little elongation occurs in most tubificids suggests that a short untorted midpiece is plesiomorphic. There are seven mitochondria in the spermatozoon of *Branchiobdella*, and there is one in that of leeches. As four is the most common number in "primitive" sperm this has been somewhat arbitrarily assumed to be the basic number for oligochaete sperm, with divergence to two in *Tubifex* and a maximum of 11 in *Capilloventer* (see

Jamieson 1981c; Ferraguti 1999). However, as *Capilloventer* appears to be the most basal oligochaete it is possible that many parallel mitochondria is the plesiomophric condition.

Transformation of the centrioles. As reveiwed by Ferraguti (1999), two centrioles with the conventional ultrastructure of nine microtubular triplets are present only in the very early euclitellate spermatids (Jamieson 1981c), as here shown for *Haplotaxis ornamentus* (Fig. 8.42H). Soon after, the proximal centriole disappears. A remnant of the proximal centriole is said to make contact with the base of the nucleus just before the clustering of the midpiece mitochondria (Gatenby and Dalton 1959). The fate of the proximal centriole has never been followed in detail, but its topographical, if not causational, importance as a center for aggregation of the midpiece mitochondria has been repeatedly demonstrated (Ferraguti and Jamieson 1984).

Very early in spermiogenesis the distal centriole produces the flagellum when it is connected to the plasma membrane of the spermatid through a complex nine-rayed structure, as in *Haplotaxis ornamentus* (Fig. 8.42D) and *Eudrilus eugeniae* (Fig. 8.43F), identical with the anchoring apparatus of 'primitive' spermatozoa, i.e. those fertilizing in sea water. The anchoring apparatus disappears progressively as spermiogenesis continues. It has been supposed that the anchoring apparatus in mature spermatozoa loses its function, since the basal body is constrained by the midpiece (Ferraguti 1984a,b).

In mature sperm the remnants of the anchoring apparatus assume the shape of a ring or a cylinder of dense material placed under the plasma membrane (annuloid in Jamieson 1982) which may involve the basal body microtubules, as in tubificid oligochaetes and in branchiobdellids or be discontinuous and irregular as in leeches or in megascolecid oligochaetes (Jamieson 1978a).

The basal body never shows a conventional triplet appearance in the mature oligochaete spermatozoon. Tannic acid treatment reveals microtubular doublets surrounded by dense material (Ferraguti and Gelder 1991). In the oligochaetes the basal cylinder is a structure with a diameter ranging from 60 to 100 nm and a length from 0.1 to 0.3 μm from which the central apparatus of the flagellum emerges (Ferraguti 1999). It makes its appearance in the early spermiogenetic stages and elongates to fill, to a variable extent, the basal body (Ferraguti 1984a). In branchiobdellids, hirudineans, and acanthobdellids, all lacking a basal cylinder, the basal body is progressively penetrated by the central apparatus of the axoneme from the early spermiogenetic stages. The central apparatus reaches the mitochondrion in acanthobdellids and leeches, or even penetrates into the midpiece axis, as in branchiobdellids.

Modifications of the flagellum. The flagellum of the early euclitellate spermatids has a conventional 9+2 appearance. During spermiogenesis, the basic structure is modified by the appearance of glycogen granules surrounding the axonemal doublets, and by the transformations of the central apparatus, as in *Haplotaxis ornamentatus* (Fig. 8.42E). The glycogen

granules appear in the mid-spermatids to surround the axoneme for almost its complete length, so that only the terminal portion of the euclitellate flagella has the conventional 9+2 appearance. The central apparatus of the flagellum may be modified by 'tetragon fibers' (two fibers at right angles to the two central singlets) (Fig. 8.42E) or by the prominent central sheath. In some oligochaete species, the tetragon fibers grow into the central apparatus of mid-spermatids (Jamieson 1981a); in others, the tetragon fibers are replaced (or embedded?) in later stages by the prominent central sheath. This process has been followed in detail in the tubificid *Monopylephorus limosus* (Ferraguti 1999) and proceeds from the basal towards the distal portion of the flagellum. In some oligochaete species (for instance in the tubificid *Coralliodrilus rugosus*, see Erséus and Ferraguti 1995) a mixture of the two modifications of the central apparatus is present in the mature spermatozoa: the basal portion of the flagellum shows a prominent central sheath appearance, whereas a more distal portion shows the tetragon fibers, and a short final tract has a 9+2 appearance without any modification of the central apparatus. The branchiobdellids, acanthobdellids, and leeches, show the progressive involvement of the central tubules by the prominent central sheath (Ferraguti and Lanzavecchia 1977).

8.4.2 Mature Spermatozoa

The Clitellata (Oligochaeta *sensu lato*) possess only introsperm (terminology of Rouse and Jamieson 1987), i.e., sperm that are not introduced into the ambient water if the species is aquatic and are usually (obligatorily in leeches) internally fertilizing, though in most oligochaetes fertilization occurs external to the body, in the cocoon.

The spermatozoa of the Clitellata are filiform cells characterized by (1) the presence of an acrosome tube containing and/or supporting the acrosome vesicle which is often withdrawn into it; (2) interpolation of the mitochondria between the nucleus and the basal body of the flagellum; (3) peculiar modifications of the central apparatus of the axoneme and (4) two glycogen granules per doublet cross section (Jamieson 1981c). The oligochaetes *sensu stricto* are diagnosed by the presence of a basal cylinder situated within the basal body which is lost in brachiobdellids and leeches (Ferraguti 1984a,b; Ferraguti and Erséus 1999); the branchiobdellidans by an apical, conical indentation of the nucleus and by a helical marginal fiber coiled around the tail; the acanthobdellids by a dense sheath and accessory fibers surrounding the axoneme; the euhirudineans by an anterior prolongation of the acrosome tube (Jamieson 1978c, 1981b,c, 1982, 1983a,b,c, 1984, 1986, 1987, 1988a ,b, 1992; Jamieson *et al*. 1978, 1982, 1987; Ferraguti 1983, 1999; Ferraguti and Erséus 1999). Some caveats apply to these characteristic features. The acrosome tube though highly distinctive, being unknown in polychaetes, occurs convergently, though only superficially similar, in nematomorphs (Valvassori *et al*. 1999). It is reduced or possibly absent in *Bathydrilus formosus*, a phallodriline tubificid (Ferraguti *et al*. 1989). Interpolated mitochondria also occur in Onychophora, with possible

phylogenetic implications (Jamieson 1986) and, with no phylogenetic significance, in Chondrichthyes (Jamieson 1991) and in some gastrotrichs (Ferraguti *et al*. 1995). Similarly distributed glycogen is seen, convergently, in the polychaete *Micromaldane* (Rouse and Jamieson 1987).

The clitellate spermatozoal synapomorphies of possession of an acrosome tube and interpolation of the midpiece between nucleus and distal centriole do not occur in *Aeolosoma* and *Potamodrilus*, thus confirming that aphanoneurans are not clitellates (Bunke 1985, 1986). A combined immunohistochemical and ultrastructural investigation of the central nervous system and the sense organs in *Aeolosoma hemprichi* (Hessling and Purschke 2000) also indicated that aphanoneurans could not be included in the Clitellata. Some molecular cladograms based on three combined genes, two nuclear (18S, 28S) and one mitochondrial (COI), gees placed *Aeolosoma* as the sister-taxon of the Clitellata (Hugall *et al*. unpublished) (Fig. 8.5). However, Struck *et al*. (2002) found that 18S rDNA sequences did not unequivocally support a sister-group relationship of *Aeolosoma* sp. and the Clitellata. Instead, depending on the algorithms applied, *Aeolosoma* clustered in various clades within the polychaetes, for instance, together with eunicidan species, the Dinophilidae, *Harmothoe impar* or *Nereis limbata*.

Although spermaozoal ultrastructure asserts monophyly of the Clitellata, it does not prove monophyly of the oligochaetes *sensu stricto* and, though not conflicting with monophyly of the latter, it could equally support the view that oligochaetes *sensu stricto* are a paraphyletic congeries, as indicated by molecular data (see 8.1.2 above). The constantly present basal cylinder, though distinctive of oligochaetes, is presumably plesiomorphic. A generalized, plesiomorphic oligochaete spermatozoon is illustrated in Fig. 8.44 to aid in understanding of the components of a mature spermatozoon.

Regarding major departures from general oligochaete sperm characteristics, only the eudrilid *Eudrilus eugeniae* (Jamieson and Daddow 1992) and the tubiificid *Rhizodrilus russus* (Ferraguti *et al*. 1994) are known to have an endonuclear canal. In members of the subfamily Tubificinae, and some Limnodriloidinae, a double sperm line produces euspermatozoa and paraspermatozoa (Braidotti *et al*. 1980; Braidotti and Ferraguti 1982, 1983; Ferraguti *et al*. 1983, 1988, 1989, 1994, 2002b; Ferraguti and Ruprecht 1992; Boi *et al*. 2001; Marotta *et al*. 2003).

An account follows on the ultrastructure of spermatozoa in those oligochaete families for which it has been investigated.

Enchytraeidae. *Lumbricillus rivalis* has a simple, short acrosome, with the vesicle external to the tube, a well developed secondary tube, a stout rod, a basal chamber, and a limen (Webster and Richards 1977) (Fig. 8.45A). These characters have been considered to make the enchytraeid acrosome the most primitive oligochaete spermatozoon examined (Jamieson 1983a), though this status is now questionable in view of molecular phylogeny discussed above in 8.1.4. The nucleus is apically flanged and basally straight; the four mitochondria (six in one *Mesenchytraeus* species, Ferraguti and Fender unpublished) are twisted; the flagellum has a long basal cylinder and an

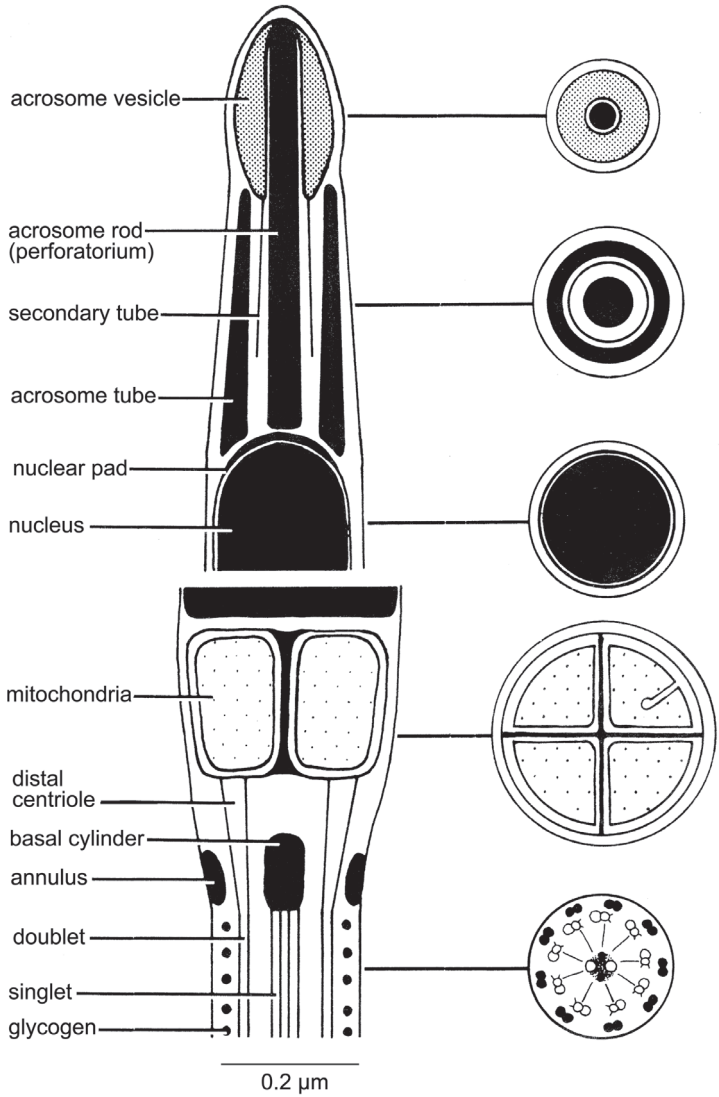


Fig. 8.44. Diagrammatic longitudinal section of generalized, plesiomorphic oligochaete sperm to illustrate chief components. From Jamieson, B. G. M. *et al.* 1987. Cladistics 3(2): 145-155.Fig. 3.

axoneme with a tract with a prominent central sheath followed by one with tetragon fibers (Fig. 9 in Webster and Richards 1977).

A profound comparative study on 19 different populations and species of *Enchytraeus* (Westheide *et al*. 1991) has confirmed the above description, except for the absence or extreme reduction of the basal chamber in the acrosome and for the nucleus being flanged (or corkscrew-shaped) for the whole length. Significant metric differences allowed the identification of the various populations and species.

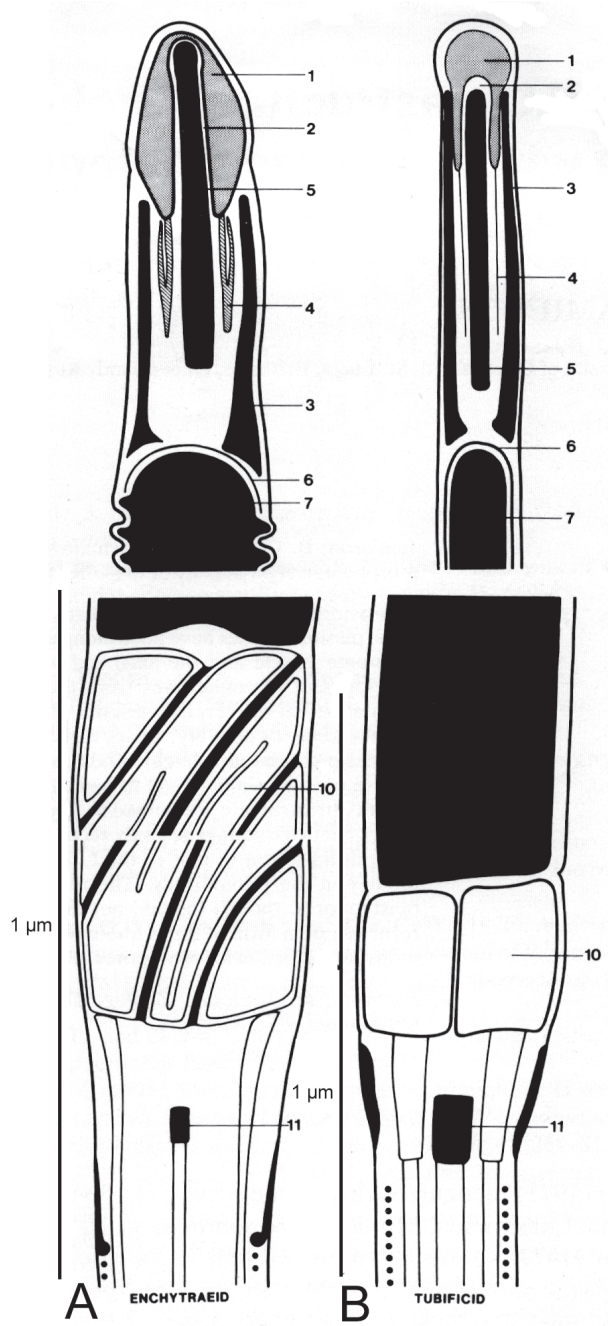


Fig. 8.45. Longitudinal section of a spermatozoon by TEM of **A**. An enchytraeid (*Lumbricillus rivalis*). **B**. A tubificid (*Rhyacodrilus arthingtonae*). 1. acrosome vesicle, 2. subvesicular space, 3. acrosome tube, 4. secondary tube, 5. axial rod (perforatorium), 6. nuclear pad, 7. nucleus, 8. capitulum, 9. connective, 10. midpiece, 11. proximal core of axoneme. From Jamieson, B.G. M. 1983. Zoologica Scripta 12: 107-14, Fig. 1.

The morphometric and qualitative spermatozoal data were used (Westheide *et al*. 1991) as morphologic taxonomic characters, as discussed in 8.1.5. Their analysis confirmed the value of sperm ultrastructure for solving taxonomical problems at the species level (Westheide *et al*. 1991).

Capilloventridae. The acrosome of the spermatozoon of the Australian freshwater *Capilloventer australis* (Fig. 8.46) is similar to that of enchytraeids, and putatively plesiomorphic, in having the vesicle completely external to the tube, but differs in lacking a secondary tube and a limen. The nucleus is again similar to that of enchytraeids, but *C. australis* has the condition, known in no other clitellate, of eleven mitochondria arranged longitudinally in parallel (not radially) in the midpiece. It is uncertain whether this is autapomorphic or plesiomorphic. The basal cylinder is very long, as in Enchytraeidae, but the tail shows only the prominent central sheath pattern (Ferraguti *et al*. 1996).

Phreodrilidae. The spermatozoon (Fig. 8.47A) of *Astacopsidrilus* (=*Phreodrilus*) *jamiesoni*, epizoic on an Australian freshwater crayfish (Jamieson 1981b), has a long undulating acrosome tube, with the vesicle only partly withdrawn, a small acrosome rod (perforatorium), a short secondary tube, a large basal chamber, a putative nuclear pad, an undulating nucleus, six helical mitochondria forming the unusually long (9 μm) midpiece, and a tail with prominent central sheath basally and tetragon fibers distally. The acrosome resembles that of some crassiclitellates, thus falling among them in the phylogenetic analysis. The sperm of *Insulodrilus bifidus* is similar in most respects: even longer midpiece; nuclear shape; six mitochondria; prominent central sheath and and tetragon fibres but the acrosome tube is less that 1 μm long, slightly bent to one side, has a limen, and a short basal chamber and the vesicle is completely withdrawn (Marotta, pers. comm.). Phreodrilids are suspected of having internal fertilization, which is rare among oligochaetes, occurring elsewhere in Eudrilidae (Jamieson *et al*. 1987), and this possibly accounts for the unusual acrosomal morphology in both taxa.

Tubificidae. This account is drawn from the review of Ferraguti (1999). The Tubificidae is the most speciose microdrile family (about 600 species) and shows the greatest diversity in sperm morphologies. Variations concerning all the characters were reviewed in detail for 17 species by Erséus and Ferraguti (1995) and Ferraguti *et al*. (1994). A list of all examined species is given in Ferraguti (1999).

A generalized tubificid sperm based on *Limnodriloides australis* is illustrated in Fig. 8.45B. The acrosome tube may be straight as in Tubificinae and Limnodriloidinae, or bent, as in the rhyacodriline *Monopylephorus limosus* and in the gutless phallodrilines, or twisted, as in the phallodriline *Thalassodrilus prostatus*. The acrosome tube has, in *M. limosus*, a crystalline appearance in negative staining (period of 4.7 nm). The vesicle can be completely external, as in *Thalassodrilus prostatus* or in *Limnodriloides* sp., partly withdrawn, as in *Rhyacodrilus arthingtonae* (Jamieson *et al*. 1978) or in the Tubificinae, or completely withdrawn, as in *M. limosus*. A large basal chamber is present, as in *Thalassodrilus prostatus* or absent, as in *M. limosus*.

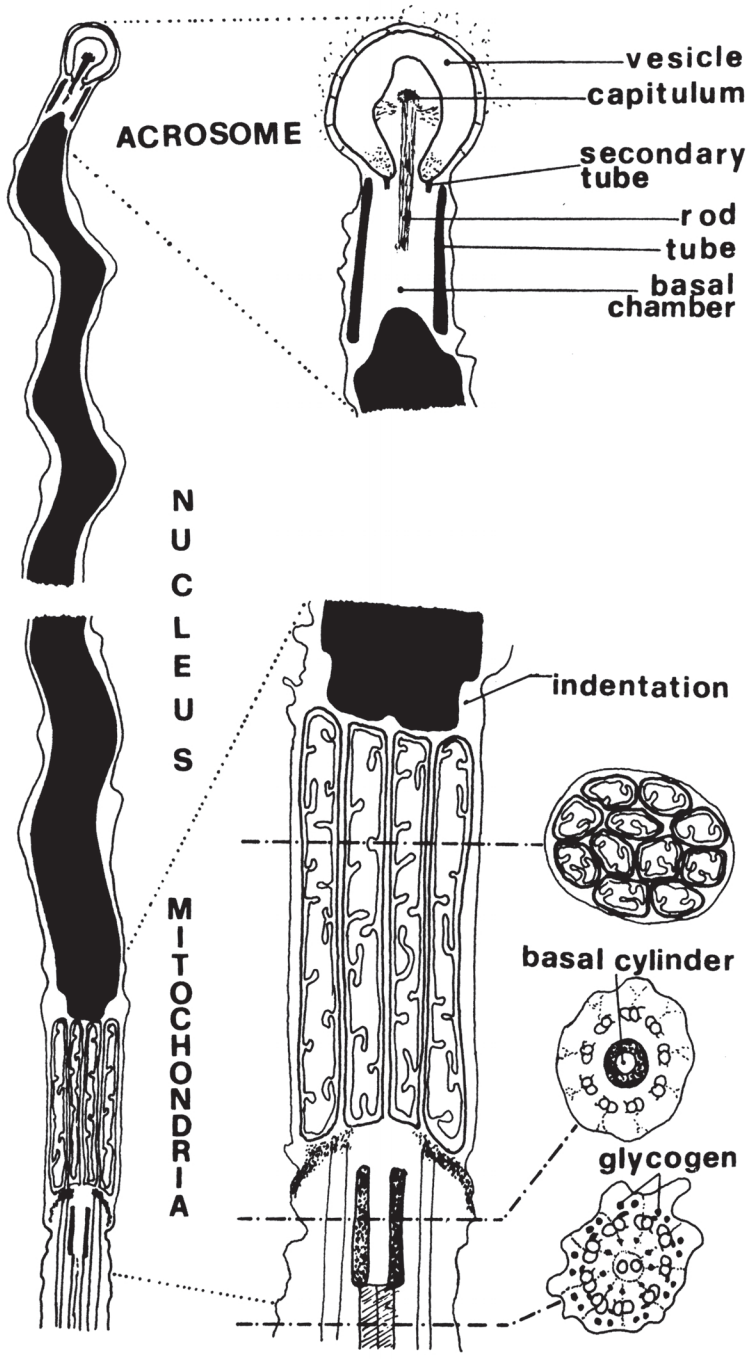


Fig. 8.46. *Capilloventer australis* (Capilloventridae). Ultrastructure of the spermatozoon. From Ferraguti *et al*. 1996. The spermatozoon of *Capilloventer australis* and the systematic position of the Capilloventridae (Annelida: Oligochaeta). Australian Journal of Zoology 44(5): 469-478, Fig. 1.

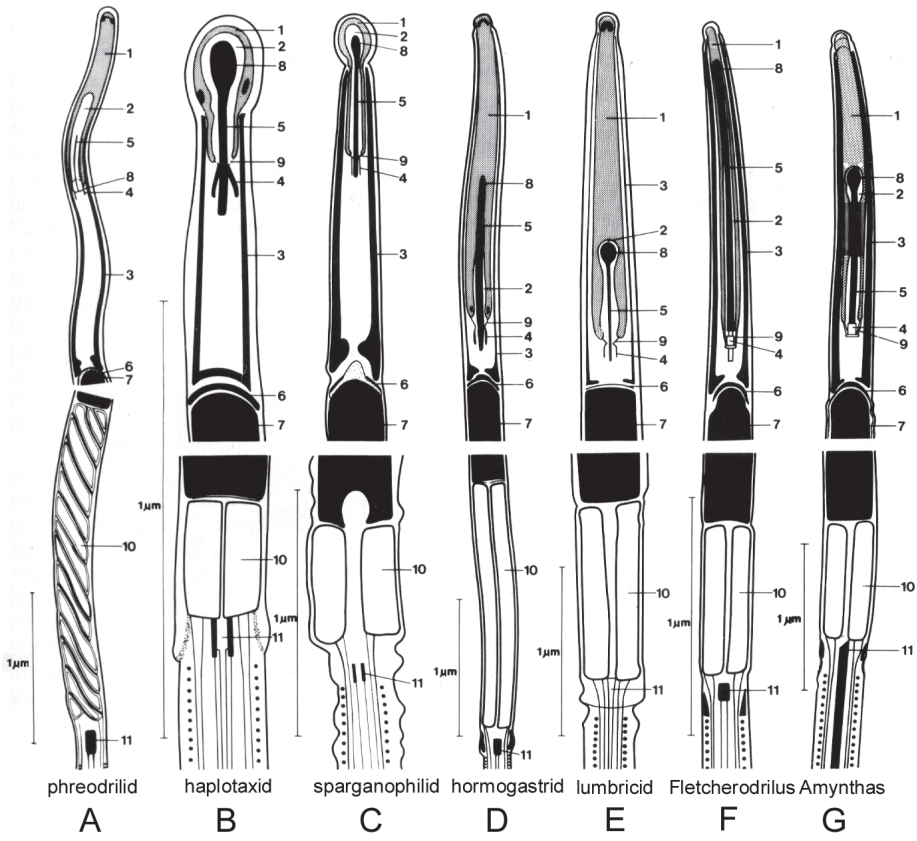


Fig. 8.47. Longitudinal section of a spermatozoon by TEM of **A**. A phreodrilid (*Astacopsidrilus (=Phreodrilus) jamiesoni*). **B**. A haplotaxid (*Haplotaxis ornamentus*). **C**. A sparganophilid (*Sparganophilus tamesis*). **D**. A hormogastrid (*Hormogaster redii*). **E**. A lumbricid (*Lumbricus rubellus*). **F**. A megascoelcid (*Fletcherodrilus unicus*). **G**. A megascolecid (*Amynthas gracilis*). 1. acrosome vesicle, 2. subvesicular space, 3. acrosome tube, 4. secondary tube, 5. axial rod (perforatorium), 6. nuclear pad, 7. nucleus, 8. capitulum, 9. connective, 10. midpiece, 11. proximal core of axoneme. From Jamieson, B. G. M. 1983. Zoologica Scripta 12: 107-14, Fig. 2.

Some acrosome tubes have a limen, others are simple. In *Tubifex tubifex* eusperm, and in *M. limosus*, the plasma membrane at the level of the tube shows a characteristic array of particles.

In tubificid sperm, the nucleus is never straight for its whole length, but can be in part straight, or twisted, or corkscrew-shaped, or flanged , or with regions with different shapes. The midpiece mitochondria vary in number from two (*Tubifex tubifex*) to five (all the Phallodrilinae), and in shape from hemispherical (in *T. tubifex*) to radial sectors of a cylinder, as in *Clitellio arenarius*. When elongated, the mitochondria can also be helical, as in *Pectinodrilus molestus* or in *Coralliodrilus rugosus*. The axoneme can have the prominent central sheath, or the tetragon fibers, or both in sequence (Erséus

and Ferraguti 1995). The plasma membrane of tubificid sperm tails is characterized by the possession of zipper lines, i.e. nine parallel double rows of particles at the level of the axonemal doublets (Ferraguti *et al*. 1991). Besides the zipper lines all rhyachodriline sperm tails, except in *Rhizodrilus russus*, also have "muffs" (Ferraguti *et al*. 1991, and Marotta, unpublished), double rows of particles running perpendicular to the zipper lines. The interplay between zipper lines, muffs, and other membrane structures gives rise to a complex model of the periaxonemal area.

The considerable variation of sperm models within tubificids may be the consequence of the great species differentiation within the family. However, no other microdrile family has been analyzed in such detail. A high amount of structural homoplasy was detected in a cladistic analysis (Erséus and Ferraguti 1995).

Naidinae. The spermatozoa of the naidines *Paranais litoralis*, *Nais communis* and *Slavina appendiculata* (Gluzman 1998, 1999), *Stylaria lacustris* and two species of *Paranais* (Ferraguti *et al*. 1999) have the usual oligochaete sperm components *Paranais litoralis* has a straight nucleus, five midpiece mitochondria and a complex flagellum with prominent central sheath. *Nais communis*, on the other hand, has a twisted nucleus and, questionably, only one mitochondrion. The spermatozoon of *Slavina appendiculata* has a 'corkscrew' nucleus, 5 or 6 mitochondria in the usual radial arrangement. The axonemes have the tetragon fiber configuration (Gluzman 1998, 1999).

In *Stylaria lacustris* (and two species of *Paranais*, see Ferraguti *et al*. 1999) the acrosome is short and straight, with the vesicle withdrawn, no secondary tube, and the acrosome rod barely visible. The nucleus is twisted for most of its length, but basally straight. There are five parallel mitochondria, and a tail with prominent central sheath basally and tetragon fibers distally. This description is confirmed for *Unicnais uncinata* (Marotta, pers. comm.). The ultrastructure of the naid spermatozoon is consistent with inclusion of Naididae within the Tubificidae (Erséus 1990), as the Naidinae (Erséus and Gustavsson 2002).

Lumbriculidae. Ultrastructural descriptions exist for the spermatozoa of *Bythonomus lemani* (Ferraguti and Jamieson 1987) (Fig. 8.48), *Kinkaidiana* sp., *Rhynchelmis limosella*, *R. alyonae* (Martin *et al*. 1998), *R. brachicephala* (Ferraguti *et al*. 1999) and an undetermined species from Lake Baikal (Ferraguti 1999). The acrosome is short, bent to one side, with the vesicle deeply withdrawn, well developed rod, secondary tube with possible connections to the rod, no basal chamber, no limen, and possibly the tube closed at its base (or a nuclear pad fused with the tube?). The nucleus is twisted for the whole length (*Bythonomus* and *Rhynchelmis alyonae*) or apically corkscrew-shaped and basally straight (*Kinkaidiana*), or completely straight as in *R. brachicephala*. In all *Rhynchelmis* species examined so far there is a deep apical concavity at the apex of the nucleus. In *Kinkaidiana* sp. there is also an apical concavity reminiscent of that present in most Branchiobdellidae and, possibly, in some leeches, a feature consistent with molecular phylogeny. There are six, highly twisted mitochondria in the lumbriculid midpiece, and a prominent central

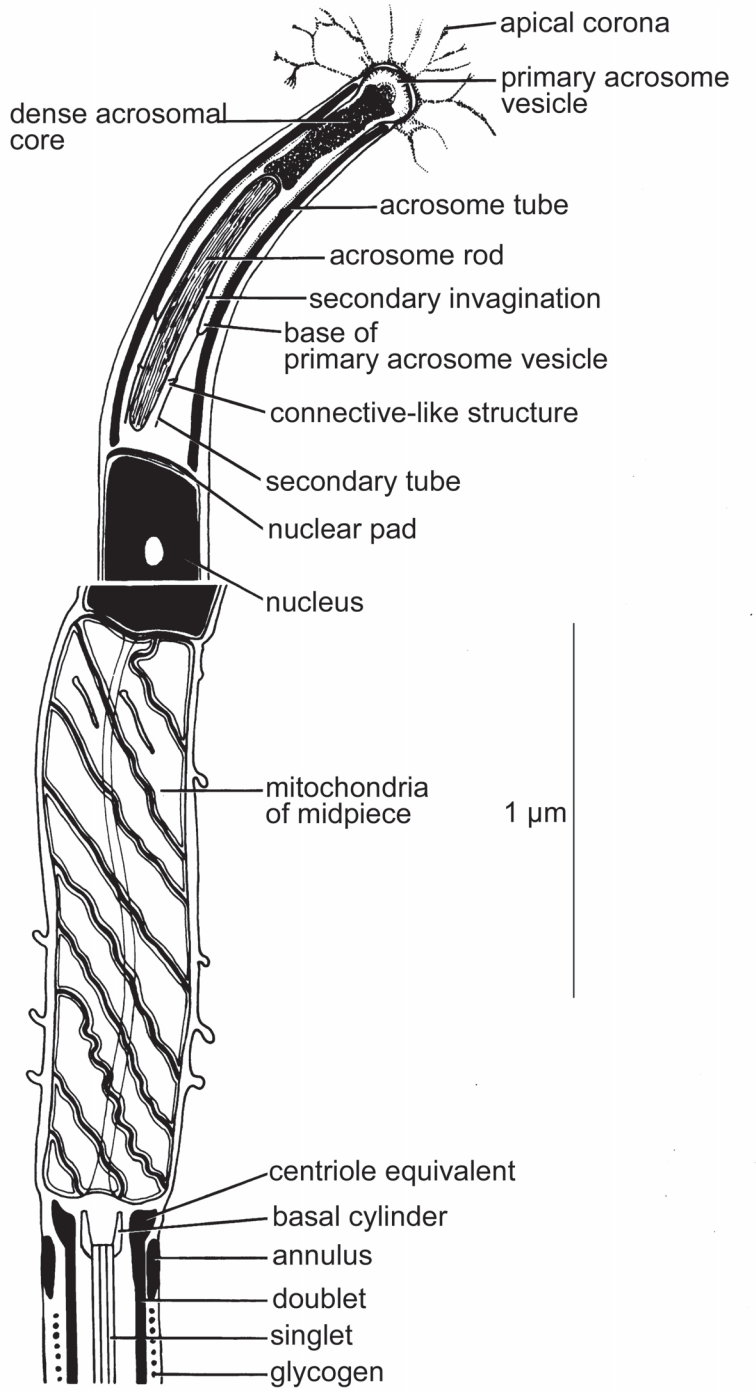


Fig. 8.48. *Bythonomus lemani* (Lumbriculidae). Longitudinal section of a spermatozoon by TEM. From Ferraguti, M. and Jamieson, B. G. M. 1987. Hydrobiologia 155: 123-134, Fig. 23.

sheath in *Rhynchelmis* and *Kinkaidiana,* but a sequence of prominent central sheath and tetragon fibers in the axoneme in *Bythonomus lemani*.

Haplotaxidae. The spermatozoa of the Tasmanian *Haplotaxis ornamentus* (Jamieson 1982) (Fig. 8.47B) and *Pelodrilus leruthi*, a subterranean species living in the Pyrenées, are substantially similar to each other (Ferraguti 1999). There is a long acrosome tube, and a comparatively small vesicle, only partly withdrawn into the tube. A thin acrosome rod with an apical enlargement (capitulum *sensu* Jamieson 1978a) is housed in the subacrosomal space, leaving a large basal chamber. A secondary tube is connected to the rod in *Haplotaxis*, but not so evidently in *Pelodrilus*. The tube ends with a limen in *Pelodrilus*, but is possibly closed in *Haplotaxis*. A flat nuclear pad separates the acrosome tube from the straight nucleus. Six mutually parallel mitochondria follow, and a flagellum with tetragon fibers. The two species examined are distant in space and phylogeny, thus the haplotaxid sperm models appear remarkably uniform.

Sparganophilidae. The acrosome tube of the spermatozoon of *Sparganophilus tamensis*, investigated by Jamieson *et al*. (1982) (Fig. 8.47C), is characterized by a long basal chamber ending proximally against a large limen and distally with the basal extremity of the vesicle. This vesicle is only partly withdrawn into the tube, projecting anteriorly as a bleb. The acrosome rod is contained within the subacrosomal space and partly projects outside the tube anteriorly. A short secondary tube surrounds the base of the rod. In *Sparganophilus*, as in the 'higher' families described below, the secondary tube has two clearly distinct parts: a cylinder surrounding the proximal extremity of the rod, and an oblique connection between the cylinder and the proximal extremity of the acrosome vesicle.

A nuclear pad with a central boss separates the acrosome from the straight nucleus. The midpiece is formed by eight parallel mitochondria, two of which are in line, one over the other. The flagellum has the typical tetragon fibers of the megadriles, as well as the usual glycogen granules.

Ocnerodrilidae. The spermatozoon of the peregrine ocnerodrilid species *Nematogenia panamensis* is 35 μm long and shows the conventional clitellate sequence of acrosome, nucleus, middle piece and tail. The acrosome is asymmetric, showing an acrosome rod crossing the vesicle to nearly touch the tube, and re-crossing the vesicle anteriorly. This condition is unique among investigated euclitellates, as is the structure of the acrosome tube, which seems to be decorated by longitudinal spiral furrows. The secondary tube and the limen are similar to those of other earthworms. The nucleus is straight as are the six midpiece mitochondria. The flagellum has one of the two usual oligochaete arrangements: a 9+2 axoneme with two central tetragon fibers, surrounded for most of its length by glycogen granules. The secondary tube and the limen are similar to those of earthworms (Bondi *et al*. 1993; Ferraguti *et al*. 1999). While the general features of *Nematogenia* spermatozoon are undoubtedly of crassiclitellate type, characters such as the shortness of the acrosome and the basal chamber were considered to indicate a plesiomorphic condition within the group.

Eudrilidae. Only a single species of the Eudrilidae has been examined for spematozoal ultrastructure, the circummundane *Eudrilus eugeniae* (Jamieson and Daddow 1992) (Fig. 8.49). Spermatogenesis in this species is illustrated for the first time in Fig. 8.43). This is an internally fertilizing species and investigation of species which extrude spermatozoa from spermathecae into the cocoon in the usual oligochaete mode is desirable. The

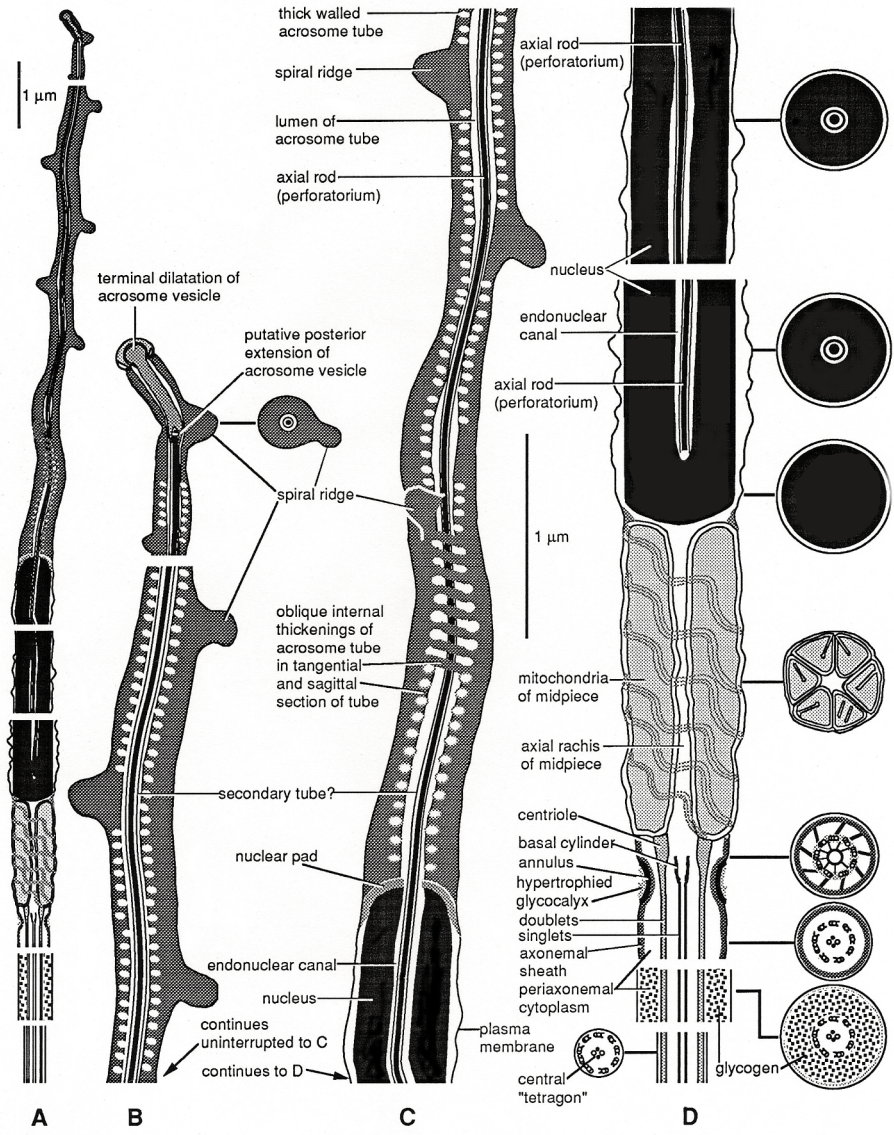


Fig. 8.49. *Eudrilus eugeniae* (Eudrilidae). Highly diagrammatic representation of the ultrastructure of the spermatozoon by TEM. From Jamieson, B. G. M. and Daddow, L. 1992. Journal of Submicroscopic Cytology and Pathology 24(3): 323-333, Fig. 2.

Eudrilus spermatozoon shows the following unique or unusual features relative to other oligochaetes: 1) The acrosome tube bears externally a spiral ridge or flange (also seen in some other oligochaetes, e.g. *Coralliodrilus*, Ferraguti and Erséus, 1999). The tube and its extension also bear helical ridges in leeches and some branchiobdellidans; it has a spiral tendency in lumbriculid and phreodrilid sperm (Jamieson 1981c; Ferraguti and Gelder 1991). The undulating nucleus and helical midpiece are unusual among earthworms. 2) The acrosome tube is greatly elongated, at 7.7 μm, though shorter than the maximum in other oligochaetes of 12.7 μm, reported for *Allolobophora chlorotica* by Jamieson *et al*. (1983). The tube is shorter in most leeches but in the rhynchobdellid leech *Theromyzon tessulatum* reaches the remarkable length of 55 μm, and 66 μm in the branchiobdellid *Cambarincola pamelae* (Gelder and Ferraguti 2001). 3) the acrosome tube is thickened around the subacrosomal space by oblique ribs. 4) Presence of an endonuclear canal containing the axial rod (an endonuclear canal is present in the tubificid *Rhizodrilus russus*, see Ferraguti *et al*. 1994, but does not contain a rod). 5) Presence of a subplasmalemmal sheath of dense material in the basal portion of the tail, reminiscent of that of the fish leech *Acanthobdella peledina*. 6) Presence of a wide band of cytoplasm beneath the plasma membrane of the anterior region of the axoneme. 7) Replacement of the two glycogen granules usually associated with each axonemal doublet in clitellates with radial rows of glycogen granules which occupy a wide band of cytoplasm peripheral to the axonemal doublets. The large amount of glycogen in a broad cytoplasmic zone resembles the condition in tubificine parasperm though it is there γ-glycogen (see 8.4.3).

As noted by Jamieson and Daddow (1992), it is not possible categorically to state which unique features of the sperm of *Eudrilus* are adaptations to the requirements of internal fertilization. Examination of sperm of externally (cocoon) fertilizing eudrilids is needed. However, all are apomorphies not seen in other oligochaete eusperm and coexist with highly apomorphic modifications of the reproductive system for internal fertilization in *Eudrilus*. The reproductive system rivals that of the similarly internally fertilizing leeches in its complexity. The apomorphies may therefore reasonably be considered to be adaptations for aspects of internal fertilization, presumably including specific requirements of altered sperm metabolism (increased glycogen storage), migration and storage of of sperm within the female system and peculiarities of sperm-egg interaction. The significance of elongation of the acrosome together with the extraordinary elongation of the axial rod, which varies between approximately 14 and 20 μm in length, are uncertain beyond its presumed relationship to internal fertilization. The length of the acrosome tube was shown in other oligochaetes to be highly correlated with that of the microvilli which constitute the zona pellucida of the egg (Jamieson *et al*. 1983). The egg of *Eudrilus* has yet to be studied.

Microchaetidae. Spermatogenesis in *Microchaetus pentheri* follows the familiar pattern known for other oligochaetes (Hodgson and Jamieson 1992).

As usual, spermatogenic stages develop around an anucleate cytophore from which they separate as mature spermatozoa. During spermiogenesis the nucleus elongates and becomes surmounted by a complex, elongate acrosome: the flagellar axoneme develops from the distal centriole. The centriole is positioned posterior to the midpiece. *Microchaetus* shows many plesiomorphic features in the structure of its acrosome, which are also seen in two other taxa of the Diplotesticulata, *Haplotaxis* (Haplotaxidae) and *Sparganophilus* (Spartganophilidae).

The spermatozoon has a long (3.8 μm) acrosome, with a tube only partly containing the acrosome vesicle, which projects anteriorly in a spheroidal bleb (terminal bulb in Hodgson and Jamieson 1992). The vesicle is deeply introflected at its basis, delimiting a large subacrosomal space extending up to the bulb. In the subacrosomal space there is a 1 μm long rod entirely contained in the tube. The rod is surrounded basally by a node-like sheath probably homologous to the secondary acrosome tube. A large basal chamber is delimited distally by the end of the acrosome vesicle and proximally by the limen terminating the nuclear end of the tube. A thin nuclear pad separates the acrosome from the rectilinear, 24 μm long, nucleus. Six parallel, radial mitochondria form the midpiece. A conventional megadrile flagellum follows, with tetragon fibers and glycogen granules (Hodgson and Jamieson 1992).

Hormogastridae. The Sardinian species *Hormogaster redii* has a typical megadrile (metagynophoran) spermatozoon (Fig. 8.47D). The acrosome is 2.7 μm long, with the vesicle completely withdrawn into the tube. An axial rod is housed in the basal invagination of the vesicle. A short secondary tube surrounds the base of the rod. There is a limen at the base of the tube, and a pad with central boss separates the acrosome from the nucleus. The straight nucleus is followed by a midpiece with six (or seven) straight mitochondria (Ferraguti and Jamieson 1984).

Lumbricidae. Comprehensive descriptions exist for spermatozoa of *Lumbricus terrestris* (Cameron and Fogal 1963; Anderson and Ellis 1968; Anderson *et al*. 1967, 1968; Anderson and Curgy 1969; Lanzavecchia and Lora Lamia Donin 1972; Shay 1972; Bergstrom and Henley 1973; Henley 1973) and of *Allolobophora* sp. (Troyer 1980; Troyer and Cameron 1980); see also an interpretation of their structure by Jamieson (1978a, 1981c) (Fig. 8.47E). The acrosome has been described and measured in several lumbricid species (Jamieson *et al*. 1983): *Eisenia fetida; Lumbricus castaneus*; *Allolobophora longa*; *L. rubellus*; *Allolobophora chlorotica* (Fig. 8.41A-E); *Dendrobaena octaedra*, *Eiseniella tetraedra* and *Aporrectodea caliginosa*. The observations point to a uniform sperm model for the family, with variations affecting the length of different portions. The acrosome has a length variable from 2.33 μm in *L. rubellus* to 12.7 μm in *A. chlorotica* (Jamieson *et al*. 1983), with the tube completely enclosing the vesicle, a 1 μm long rod in the basal invagination of the acrosome vesicle, and a short secondary tube around the base of the rod. The acrosome tube ends basally with a limen. There is a thin nuclear pad between the acrosome and the straight nucleus. The nucleus is 14.3 μm

long in *Allolobophora* sp. (Troyer 1980), and 9 μm in *Lumbricus terrestris* (Henley 1973). Six straight, 2 μm long, radial mitochondria follow, then a flagellum with tetragon fibers and glycogen granules. The tail plasma membrane shows two modifications: a flagellar necklace, i.e. a triple row of parallel particles at the base of the flagellum, and "long rows and aggregates of individual particles" longitudinally arranged (Bergstrom and Henley 1973) reminscent of the zipper lines of tubificids already mentioned.

Megascolecidae. The ultrastructure of the spermatozoa of six species of megascolecid was described by Jamieson (1978a); data discussed also in Jamieson (1981c) and reviewed by Ferraguti (1999): *Fletcherodrilus unicus* (Fig. 8.47F), *Cryptodrilus* sp., *Digaster longmani*, *Spenceriella* sp., *Amynthas* (=*Pheretima*) sp., *Amynthas corticis* (=*diffringens*) and that of *Amynthas rodericensis* was illustrated in a discussion of spermathecal function by Jamieson (1992) (Fig. 8.47G). There are variations in the size of the different organelles in the various species examined, but the general scheme is uniform. The acrosome is 1.7 (*Amynthas*) to 2.6 μm (*Digaster*) long, thus being much shorter than that of lumbricids. The acrosome vesicle is completely withdrawn and deeply invaginated at its base. The acrosome rod lies within the invagination, only its basal portion being external to it and surrounded by a distinctive secondary tube very close to it and obliquely connected to the posterior rim of the acrosome vesicle. The node-like form of this secondary tube is diagnostic of the investigated Megasolecidae. Posterior to this there is a short basal chamber. The acrosome tube terminates with an obvious medianly directed shelf-like exension, the limen, surmounting a thin nuclear pad and the domed extremity of the nucleus. The nucleus is straight and about 10 μm long. A midpiece follows, with six parallel mitochondria, of variable length (0.5-1.4 μm). The tail has tetragon fibers and glycogen granules. The low level of ATP in oligochaete (*Amynthas hawayanus*) sperm is discussed by Teisaire and Del (1989).

8.4.3 Double Spermatogenesis in Oligochaetes

The literature on oligochaete spermatogenesis contains many reports on the existence of "atypical" spermatozoa, reviewed by Fain-Maurel (1966) and Christensen (1980), uniformly interpreted as degenerating cells, without any genetic role in fertilization.

In was not until 1980 that two functional types of spermatozoa were found in *Limndodrilus hoffmeisterii*, a species belonging to the tubificid subfamily Tubificinae (Block and Goodnight 1980) and their function interpreted in another member of the same subfamily, *Tubifex tubifex*, as joining to form the spermatozeugmata (defined above) (Braidotti *et al*. 1980). They were the large rods (up to 2 mm long) already described in tubificids in the 19th century (Claparède 1861; Lankester 1871).

The spermatozeugmata. The first modern ultrastructural studies on spermatozeugmata were made on *Tubifex tubifex*, and showed that they contain two regions: an inner axial cylinder, and an outer cortex (Fig. 8.50I) (Braidotti and Ferraguti 1982; Ferraguti *et al*. 1988). In the axial cylinder the

fertilizing eusperm, resembling the conventional oligochaete spermatozoa are orientated in parallel. In the cortex a large number of paraspermatozoa is arranged with the nuclei facing the interior and the tails spirally coiled around and tightly connected by cell junctions (Fig. 8.50H). Only the extremities of the sperm tails are free (Fig. 8.50I) (Ferraguti *et al*. 1988). This organization proved to be valid, with minor variations, for other tubificine species examined (see below, and Ferraguti 1999).

The structure of *Tubifex tubifex* spermatozeugmata provides hints as to its functions: the spermatozeugmata hold together, by means of the junctional complex which connect parasperm tails, a large amount of euspermatozoa "ready for use"; the parasperm form the cortex of the spermatozeugmata and envelope the eusperm; the free ends of the parasperm tails are able to move, forming a metachronal wave; this probably transports fertilizing sperm towards the opening of the spermathecae at the moment of fertilization (Ferraguti *et al*. 1988). Other functions for parasperm cannot, however, be excluded: the parasperm may filter or process substances passing from the spermathecal lumen to the axial cylinder; the parasperm could also be a low-cost material to fill up the spermatheca of the partner, thus preventing further copulations (the 'eunuch effect' proposed for lepidopteran parasperm by Silberglied *et al*. 1984).

When the study of spermatozeugmata was extended to other tubificine species it was found that, while the presence of a cortex produced by parasperm and of an axial cylinder produced by eusperm was a common feature in all tubificines, the shape of parasperm nuclei and the presence of cell junctions varied in the different species. In *Tubifex tubifex* and in *Clitellio arenarius*, the parasperm nuclei change their shapes in the spermatozeugmata: in the first species they degenerate visibly even showing myelin figures, whereas in the latter the nuclei are coiled on themselves, leaving only the acrosomes outside the skein. In *Isochaetides arenarius* (Ferraguti *et al*. 2002b), in members of *Tubificoides* (Ferraguti *et al*. 1989, and unpublished), and in *Heterochaeta costata* (unpublished) the parasperm nuclei in the spermatozeugmata maintain their rectilinear shapes.

Cell junctions of two different types connect parasperm tails of *Tubifex tubifex* in the spermatozeugmata: septate junctions in the main tract, and scalariform junctions in a more distal portion (Ferraguti *et al*. 1988). In the second model of spermatozeugma studied, that of *Clitellio arenarius* (Ferraguti and Ruprecht 1992) the junctions were completely absent, whereas in species of *Tubificoides* only a limited number of septate junctions were present (Ferraguti *et al*. 1989, and unpublished). *Heterochaeta costata*, *Psammoryctides barbatus* (unpublished), and *Isochaetides arenarius* (Ferraguti *et al*. 2002b) showed a large number of septate junctions. As noted by Ferraguti *et al*. (2002b), it is possible that there is a connection between a freshwater habitat of a species and the production of large numbers of septate junctions.

Species of the sister subfamily to Tubificinae, the fully marine Limnodriloidinae, also produce spermatozeugmata (Marotta *et al*. 2003). In the genus *Limnodriloides* the five species examined have 'tubificine-type'

Fig. 8.50. A, B. *Tubifex tubifex* eusperm. **A**. Apical portion of the head. **B**. Basal portion of the nucleus, mitochondrial midpiece and proximal portion of the flagellum. Note the reduced size of the mitochondria and the 'conventional' aspect of the flagellar plasma membrane (× 20 000). **C**. Eusperm acrosome of *T. tubifex* fixed in the presence of tannic acid. The acrosome vesicle (paler) is almost completely withdrawn into the acrosome tube (× 55 000). **D**. Transition area between axial cylinder and cortex of a spermatozeugma of

Fig. 8.50 contd

spermatozeugmata, formed by eusperm surrounded by parasperm (those last are not connected by septate junctions, however), whereas members of *Smithsonidrilus* have spermatozeugmata of two types, each one formed by eu- or para-sperm only. Spermatozeugmata were also found in members of the other limnodriloidine genera examined, *Doliodrilus* and *Thalassodrilides*. Species of both genera produce eusperm only, but their spermatozeugmata differ significantly: a parsimony analysis indicates that these spermatozeugmata may even have arisen independently (Marotta *et al*. 2003). The same analysis suggests that, despite some morphological differences, the spermatozeugmata composed of both eusperm and parasperm may be homologous in the Tubificinae and *Limnodriloides* and that the simpler spermatozeugmata observed in *Smithsonidrilus* may be the result of an apomorphic secondary transformation of tubificine-like spermatozeugmata (Marotta *et al*. 2003).

The paraspermatozoa. The two types of sperm (eusperm and parsperm) differ in all their parts (Fig. 8.50). Table 8.3 lists the differences discovered to date in *Tubifex tubifex*. The other models studied in some detail in both tubificines and limnodriloidines showed the same type of differences, with species-specific features. In general it may be said that in paraspermatozoa:

1. Acrosomes are reduced in size and contents, or even absent (as in all Limnodriloidinae) (Fig. 8.50E,C)
2. Nuclei are much shorter (up to one tenth those of the eusperm in tubificines) and slender (Fig. 8.50A-B,G). Chromatin shows uncondensed areas in all tubificines
3. Mitochondria are always fewer in number then in euspermatozoa (only *T. tubifex* has two mitochondria in both sperm types). In the tubificines, however, the volume of the parasperm midpiece is about double that of eusperm (Fig. 8.50B,G).
4. Plasma membrane surrounding the flagellum is largely separated from the axoneme in all tubificines and most limnodriloidines (Fig. 8.50D,G,H).

Fig. 8.50 contd

Isochaetides arenarius. In this area, cross-sections of different regions of both eusperm and parasperm are visible (× 20 000). **E**. Parasperm acrosome of *T. tubifex*. The acrosome tube appears 'empty' and the vesicle is completely external to the tube (× 55 000). **F**. Ciliated funnel of *T. tubifex*: both parasperm and eusperm are visible among the cilia (× 3 000). **G**. An entire parasperm head of *T. tubifex*. Note the plasma membrane largely separated from the nucleus and from the axoneme, and the large mitochondria (× 20 000). **H**. Cross section of the cortex of an *I. arenarius* spermatozeugma. Prominent septate junctions connect parasperm tails (× 45 000). **I**. Spermatozeugma of *T. tubifex* broken and seen under scanning electron microscope. Part of the axial cylinder with eusperm is visible, as well as part of the cortex formed by parasperm. In the main portion of the cortex, parasperm tails are tightly packed, only their extremities are free, and form a metachronal wave (× 2 500). Abbreviations: a, acrosome complex; c, cilia; ce, ciliated epithelium; f, flagellum; m, mitochondria; n, nucleus. Arrowheads point to some eusperm sections; arrows point to some parasperm sections.

Table 8.3 Differences between the characters of euspermatozoa and paraspermatozoa in *Tubifex tubifex*.

Sperm components	*Eusperm*	*Parasperm*
Acrosome		
Acrosome tube	containing the acrosome vesicle	thinner: acrosome vesicle external
Acrosome vesicle	with dense contents	apparently empty
Acrosome rod	present	absent
Secondary tube	present	absent
Membrane particles on plasma membrane	regular array	absent
Nucleus		
Length	about 30 µm	about 3 µm
Shape	cylinder basally straight and apically twisted-column-shaped	comma-shaped, elliptical in cross-sections
DNA content	1C	approx. one eighth of that of the eusperm
Chromatin	fully condensed	with uncondensed areas
Shape in spermatozeugmata	maintained	lost
Cytoplasm	virtually absent	small amount present
Mitochondria		
Number	two, hemispherical	two, hemispherical
Volume	normal	double
Tail		
Basal body	extremely short; no microtubules visible	longer; doublets visible
Basal cylinder	present	irregular or absent
Axoneme	with 'tetragon fibers' in the central apparatus	with a conventional central apparatus
Glycogen	18 β-glycogen granules around the axoneme	large amount of γ-glycogen between axoneme and plasma membrane
Flagellar plasma membrane	close to the axoneme	largely separated from the axoneme
Particles arrangement on flagellar plasma membrane	interrupted zipper-lines	forming septate and scalariform junctions in spermatozeugmata

We do not know the biological meaning of the differences observed between the parasperm of the tubificine and limnodriloidine species, but, equally, this is not known for eusperm. It may be speculated that the reduction or absence of the acrosome and the reduced size of the nuclei and, at least as far as *Tubifex tubifex* is concerned, the reduction of their DNA content shown by Ferraguti *et al*. (1987) is related to their not being built for fertilization.

Genesis of the two sperm types in Tubificinae. In tubificids, as in all the oligochaetes studied (see 8.4.1), the cysts consist of a central cytoplasmic mass, the cytophore, to which the cells are connected through a narrow collar

(zonula collaris) (Fig. 8.51A). The cysts pass into the seminal vesicles where, in the eusperm line they undergo a series of nuclear divisions without cytoplasmic divisions (Fig. 8.52A), and finally undergo meiosis. Cysts at different developmental stages are mixed in the seminal vesicles thus the problem of distinguishing between cysts belonging to the two spermatogenic lines arises. At the spermatid stage the task is easy: the cysts of paraspermatids are much more numerous than those of euspermatids (see a discussion in Braidotti and Ferraguti 1982). Secondly, the parasperm cysts consist of several hundreds (1250 ± 900; n=114 in *Tubifex tubifex*, Ferraguti *et al*. 2002a) of small cells (Fig. 8.52G-H), whereas the eusperm cysts contain a smaller number (128 in *T. tubifex*: Fig. 8.52B-E) of larger spermatids (Ferraguti *et al*. 1983). Furthermore, pycnotic nuclei are always present in the central cytophore of the paraspermatid cysts (Fig. 8.51) whereas nuclei are absent from the eusperm cytophore (reviewed in Ferraguti 1999). Many ultrastructural details of spermatids and spermiogenesis also differ: in particular the parasperm nuclei have, from the early spermiogenetic stages, an irregular shape; chromatin condensation is also irregular; the microtubular manchette is incomplete (pointing to some kind of relationship between microtubular manchette, chromatin condensation and nuclear morphogenesis, see Ferraguti and Ruprecht 1992). The tail shows, from early spermiogenesis, wide separation of the plasma membrane from the axonemes.

The early spermatogenetic stages of both lines are easily recognized by counting the nuclei in each cyst (Fig. 8.52A). However, we could not distinguish between the two lines by their DNA content, whether by measuring single cells per cyst with a traditional method, Feulgen stain and densitometry (Ferraguti *et al*. 1987), or by measuring DNA content of the whole cysts under the confocal microscope (Boi *et al*. 2001). In other words, it was not possible to identify, in terms of DNA content, a line of spermatogenic cells containing a DNA amount being two or four times that of parasperm. Furthermore, DNA content of parasperm cysts (i.e. those having more than 128 cells), although extremely variable, is less or equal to that of the euspermatid cysts (i.e. those with 128 cells) (Boi *et al*. 2001; different figures were reported in Ferraguti 1999: for a discussion of the discrepancy see Boi *et al*. 2001). This rules out the possibility that parasperm are produced through an increased number of cell divisions. How is the commitment of the two sperm lines achieved? Two explanations were possible: either there is an early commitment of the two developmental lines, but this is not in terms of DNA content, or the spermatogenetic pathway is common in the two lines until the spermatocytes I stage (32 cell cysts), then some peculiar process occurs to produce paraspermatids.

Laboratory cohort cultures of *Tubifex tubifex* with a constant check of spermatogenesis have revealed that the production of parasperm begins before that of eusperm (Boi and Ferraguti 2001). However, since "... euspermatid and paraspermatid cysts and their precursors (i.e. meiotic cysts and fragmenting cysts) are present in the seminal vesicles at the same time,

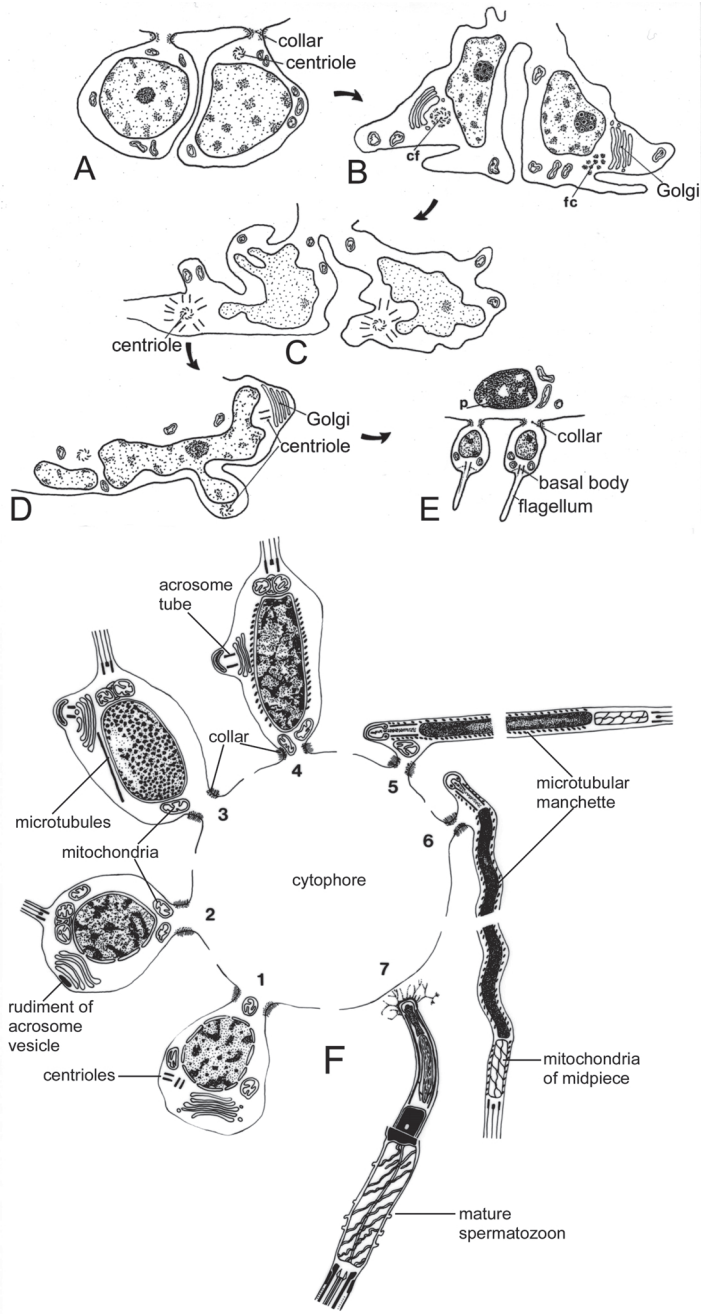


Fig. 8.51. A-E. The process of fragmentation during paraspermiogenesis in *T. tubifex* is here represented for a small portion of the cyst. For a description, see text and Boi *et al.* 2001 from which this figure is reproduced (with permission). **F**. Contrasted spermiogenesis in an oligochaete with only one sperm line, *Bythonomus lemani* (Lumbriculidae). Modified after Ferraguti, M. and Jamieson, B. G. M. 1987. Hydrobiologia 155: 123-134, Fig. 1.

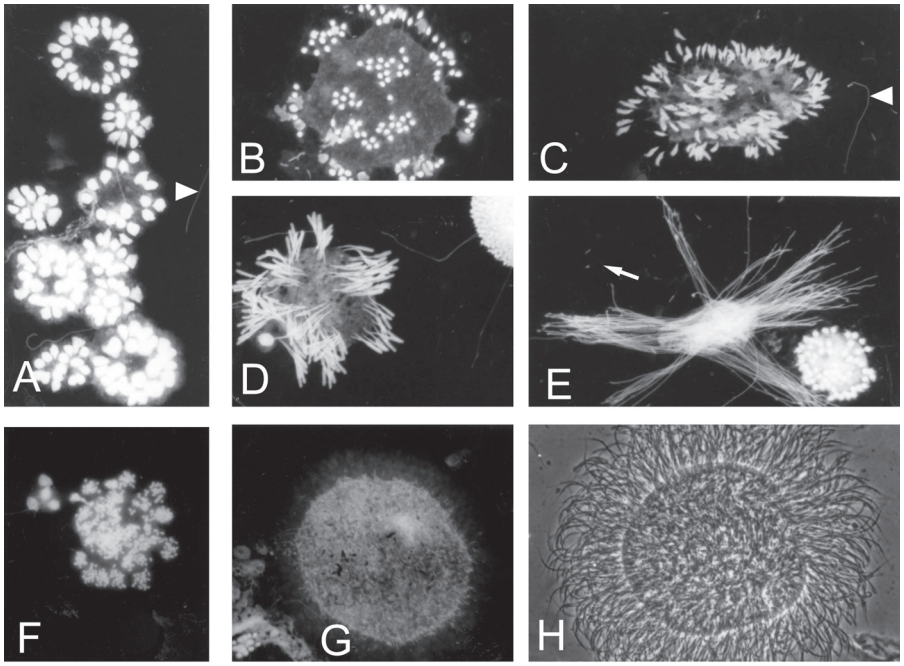


Fig. 8.52. Spermiogenesis and paraspermiogenesis in *Tubifex tubifex*. **A-G**. Optical micrographs (fluorescent microscope) of Feulgen-stained whole mounts of sperm cysts from the seminal vesicles. **A**. Nine premeiotic cysts (16 and 32 cells) (× 390). **B**. Euspermatid cyst at the beginning of spermiohistogenesis: the 128 nuclei are still roundish (× 390). **C-E**. progressive elongation of nuclei during euspermiogenesis. In E a (probably) 64 cells cyst is visible in the lower right corner (× 390). **F**. A (seemingly) 32 cells cyst undergoes the fragmentation process. At the upper left a four cells cyst (× 390). **G**. A paraspermatid cyst during the elongation process (× 280). **H**. A paraspermatid cyst at the same stage as the one in G as seen under phase contrast microscope shows an enormous number of flagella (× 280). Arrowheads point to two eusperm; arrow points to a parasperm.

the two sperm productions overlap. This leads us to exclude a sequential commitment due to hormone production during development, as is the case, for instance, of Lepidoptera (Friedländer 1997)" (Boi and Ferraguti 2001).

Independently from the presence of an early commitment, a peculiar process of cell division in *Tubifex tubifex* has been identified for which the term 'fragmentation' has been coined (Boi *et al*. 2001). The following account will be mainly based on these findings.

Fragmentation is a nuclear division which does not entail the formation of a spindle and a regular migration of equal portions of DNA into the daughter cells. The process of fragmentation is extremely complex and only partly understood (Fig. 8.51A-E). However, we were able to identify a population of cysts resembling the 32 cell cysts (euspermatocytes I), but characterized by the collapse of the collars connecting the single cells to the central cytophore (Fig. 8.52F) and by a process of *de novo* mass production of centrioles. The collapse of the collars is probably caused by a

depolymerization of the ring-forming actin (Boi *et al*. 2001) which in turn lets the nuclei 'slide' into the cytophore (Figs. 8.51B-D, 8.52F).

This phenomenon is accompanied by an impressive multiplication of the centrioles (Ferraguti *et al*. 2002a) due to the high number of spermatids produced by each paraspermatid cysts: each newly-formed centriole will, in fact, become the basal body of a parasperm. Curiously enough, multiplication of centrioles occurs through the model of *de novo* formation (deuterosomal mode), a model never before observed in an uniflagellated spermatozoon, but followed in the production of basal bodies in the multiciliated spermatozoa, like that of the termite *Mastotermes darwiniensis* (Baccetti and Dallai 1978) and the paraspermatozoa of certain gastropod molluscs (Healy and Jamieson 1981). It is, however, interesting to remember that in tubificine spermatozeugmata the cortex of parasperm will, in fact, behave as a multiciliated cell (Ferraguti *et al*. 1988).

The next stage of paraspermiogenesis is the formation of irregular chromatin lumps in each nucleus, which will become the paraspermatid nuclei (Fig. 8.51D-E). The last step of paraspermiogenesis is the migration of the newly-formed centrioles to the periphery of the cytophore where they will grow a flagellum (Figs. 8.51E, 8.52G-H). In the same area one of the 'lumps' of chromatin, now detached from the nuclei, migrates, accompanied by two mitochondria. Finally, actin re-polymerizes, the collars are re-formed, and the paraspermatid cyst assumes the typical final aspect with a large cytophore at the center, and hundreds of small paraspermatids at the periphery each with its own flagellum (Fig. 8.51E). The mechanism of irregular nuclear fragmentation produces a considerable variability of DNA content in the parasperm and explains the presence of degenerating nuclei in the common central cytoplasmic mass of the cytophore. We may suppose that the information for the production and working of the 'functional' parts of the parasperm (flagella, cell junctions, mitochondria) is already present in the common cytoplasm *before* fragmentation.

It is difficult, in our present state of knowledge, to speculate on a possible evolutionary origin of the dichotomous spermatogenesis in the tubificine-limnodriloidine assemblage. It seems pertinent to report that alterations of cell divisions have been described in the past during spermiogenesis in the oligochaete *Pheretima heterochaeta* (Cognetti de Martiis 1925) and that in *Tubifex tubifex* parthenogenesis occurs through a deep alteration of meiosis, the premeiotic doubling model (Christensen 1984; Baldo and Ferraguti 2005) as in oogenesis in some earthworms (see 8.5, below).

Among annelids there is only one other example of dichotomous spermiogenesis: that described in 13 *Protodrilus* species belonging to 22 different populations of the polychaete *Protodrilus* by von Nordheim (1987, 1989). Spermiogenesis has been followed with particular detail in *Protodrilus oculifer* (von Nordheim 1987). There were no evident differences between the two developmental lines at the stage of spermatogonia and spermatocytes, whereas euspermatids and paraspermatids were clearly recognizable.

It is tempting to establish a parallel between the tubificine and protodriline dichotomous spermatogenesis. In both cases, parasperm and eusperm jointly form sperm bundles which may be interpreted as a transport device for the fertilizing eusperm. In both cases, the parasperm of the different species resemble each other much more than do eusperm, thus suggesting some sort of 'arrest' of spermiogenesis. Finally, in both cases there seem to be no differences between the two developmental lines at spermatogonia and spermatocyte stages, whereas spermatids of the two types are easily distinguishable. However, this similarity may be ascribed to homoplasy as a close relationship of tubificines and protodrilines cannot readily be postulated.

8.5 MATING AND COITION (BARRIE G. M. JAMIESON)

Mating refers to the events surrounding insemination and in oligochaetes involves coition (copulation). The present account greatly augments the most recent review of coition in earthworms, that of Benham (1950).

Although hermaphrodite, oligochaetes are usually amphimictic, with copulation. Uniparental reproduction is, however, known and involves either self-fertilization, as in the *Enchytraeus buchholzi* and *E. bulbosus* (Dozsa 1995) or parthenogenesis, widespread in the megascolecid *Amynthas* (Gates 1972). Some populations, at least, of the cosmopolitan enchytraeids, *E. buchholzi*, and *E. bulbosus* have obligate uniparental reproduction. Presence of sperm in the spermathecae is attributed to entry from the cocoon as it passes over the spermathecal pores. In contrast, *E. coronatus* and *E. irregularis* reproduce only biparentally (Dozsa 1995). Parthenogenesis, as in earthworms results in diploid zygotes, without fertilization, owing to premeiotic doubling of the chromosome number in primary oocytes. Elegant investigations of this phenomenon were reported for the lumbricids *Eiseniella tetraedra*, *Allolobophora rosea, A. caliginosa* and *Octolasion lacteum* by Omodeo (1951, 1952, 1955).

Early limitation of knowledge of oligochaete anatomy to the Lumbricidae resulted in acceptance of the mode of copulation in lumbricids as the norm for oligochaetes. In lumbricids the male pores (usually on segment 15) are apposed in copulation to the clitellum of the partner and the exuded spermatozoa move in external seminal grooves to its spermathecae located anterior to the cliellum (Fig. 8.53). Seminal grooves are also utilized in some ocnerodriles and megascolecids. They are seen in the ocnerodrilid *Eukerria* which has two pairs of prostate pores connected by seminal grooves to the pair of male pores, the acanthodrilin arrangement (Figs. 8.9, 8.54A). They are also seen in the balantin reduction seen in the acanthodriline megascolecid *Balanteodrilus* (Fig. 8.54C) as in *Torresiella* Dyne (1997). In the vast majority of oligochaetes, however, the male pores are apposed directly to the spermathecal pores of the partner and are often located on permanent or transient protrusions, forming distinct porophores or 'penes', which are inserted into the spermathecal pores. This form of copulation is illustrated

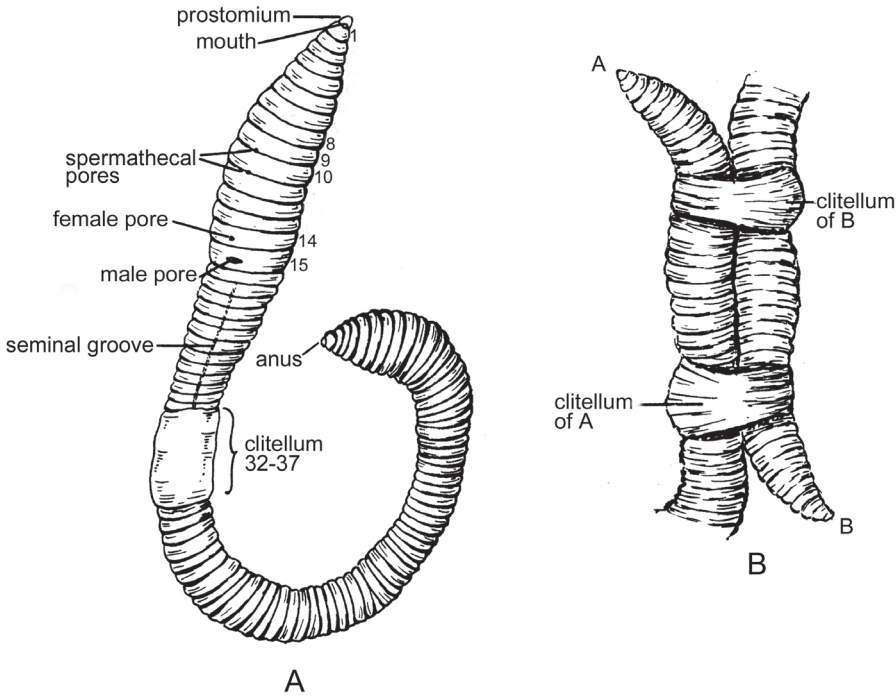


Fig. 8.53. *Lumbricus terrestris* (Lumbricidae). **A**. External features of worm turned slightly to one side to show genital pores, seminal groove and clitellum. **B**. Two worms in coition. The slime tube encloses the clitellum and apposed spermathecal pores. Relabelled after Jepson, M. 1951. Biological Drawings. Part II. John Murray, London, p. 31.

diagrammatically for the megascolecid (Megascolecinae) *Spenceriella* (Fig. 8.54, top) and *Metaphire* (Fig. 8.54D), which have the megascolecin arrangement with male and prostate pores united on segment 18, and is also exemplified by *Microscolex dubius* (Acanthodrilinae, Megascolecidae) (Fig. 8.54C) which has the microscolecin arrangement, with male and prostatic pores united on segment 17. Penetration may be aided by insertion of penial chaetae into the spermathecal orifices. This mode of insemination, with or without penial chaetae, is normal for the Megascolecidae. Coition has been described in detail for the megascolecid *Eutyphoeus waltoni* by Bahl (1927). This species is unusual for its family in copulating above ground. The most striking feature is the 'male cup' on segment 17 in the centre of which is a true penis from the tip of which protrudes a penial chaeta. Further forwards, on segment 7?, is a pair of spermathecal pores. In coition the two worms appose their ventral surfaces, with anterior ends pointing in opposite directions. Each male cup fits over the spermathecal papilla of the partner and the penis, and penial chaeta, is inserted into the duct of the spermatheca.

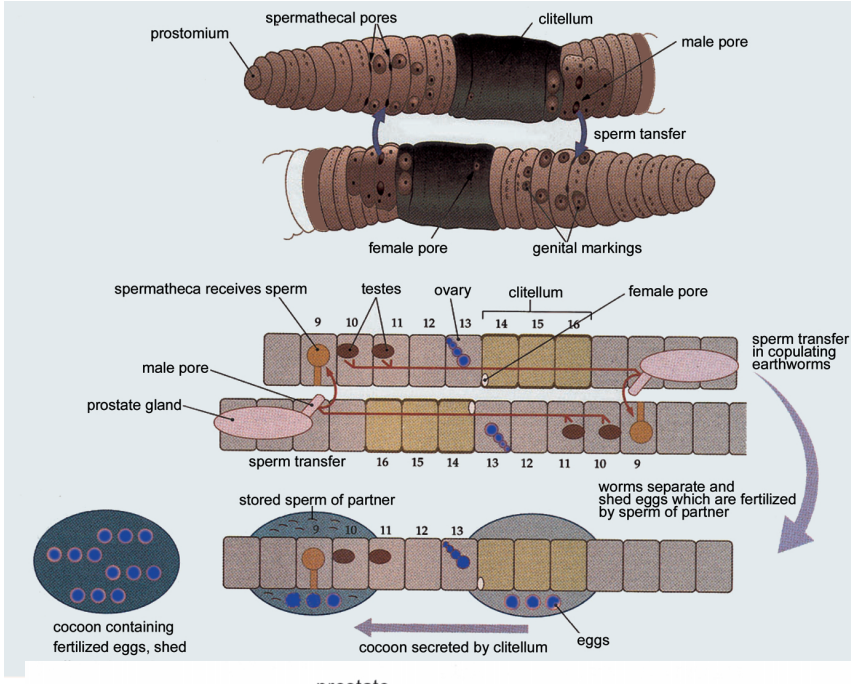
Benham (1950) recognized four external organs or structures employed in coition:

(1) **Genital markings**, including the tubercula pubertatis of lumbricids. These have been discussed in 8.2.4 above.
(2) '**Coupling chaetae'**, here termed genital chaetae and including penial and spermathecal chaetae; have been discussed in 8.2.3 above. *Lumbricus terrestris* uses 40 needle-like copulatory chaetae situated ventrally on segments 10, 26, and the clitellar segments, 31 to 38, to inject a substance into the mating partner from the chaetal glands. Compared to the normal (crawling) chaetae, these chaetae are longer and grooved. It has been proposed that the chaetal glands may produce an allohormone that manipulates the reproductive physiology of the mating partner (Koene *et al*. 2002). Some penial chaetae of megascolecids are illustrated in Fig. 8.55.
(3) **Claspers**, with which we may include the alae of *Glyphidrilus* and *Lutodrilus*. Claspers are best developed in the almid genus *Alma* for which their interspecific variation is illustrated in Fig. 8.11A,B. They are discussed in 8.2.4. Detail for *Alma tazelaari* is shown in Fig. 8.56.
(4) **A true penis**. Male porophores which act as what may be considered true penes are discussed for *Spenceriella* and *Eutyphoeus* in this section, above. The large penis like structure, accompanied by a seminal groove, of *Stuhlmannia variabilis* is illustrated in Fig. 8.10; it is presumed that this is inserted into the spermathecal aperture.
(5) **Seminal grooves** (added here) of various types. These include the semnal grooves of lumbricids, which run from the male pore, usually on segment 15 to the far posterior clitellum (Fig. 8.53) and that of the eudrilid *Stuhlmannia variabilis*, which runs from the male pore to the tip of the penis (Fig. 8.10). Other examples are the seminal grooves of *Eukerria* (Ocnerodrilinae) (Fig. 8.9) and of most Acanthodrilinae (Megascolecidae).

Precopulatory behaviour. Mating may be preceded by precopulatory behaviour.

Thus, mating of *Lumbricus terrestris* involves a pre-copulation behaviour sequence during which prospective partners visit each others burrows. Mate searching involves trail-following on the soil surface. This is followed by a series of, usually reciprocated, burrow visits. A burrow visit typically consisted of anterior segments insertion, for a period of 30 to 50 seconds, but also deeper burrow-penetrations, which sometimes lasted several minutes. Resident worms, when visited, either withdraw below ground completely or remain at the surface, with the first few anterior segments in view. Visiting worms normally retain their posterior segments in their own burrows. Partners often maintain close contact while moving back and forth between the burrow openings and the pre-copulation phase appears to be a specific courtship behaviour. Uninterrupted, the pre-copulation behaviour sequences lasted from 11 to 90 minutes. After a pre-copulation sequence, pairs adopt a static 's'-shaped copulation position of close ventral contact. Copulations lasts from 69 to 200 minutes (median 135 minutes). If other individuals touch the copulating pair, matings are shorter (Nuutinen and Butt 1997).

Colour Figure



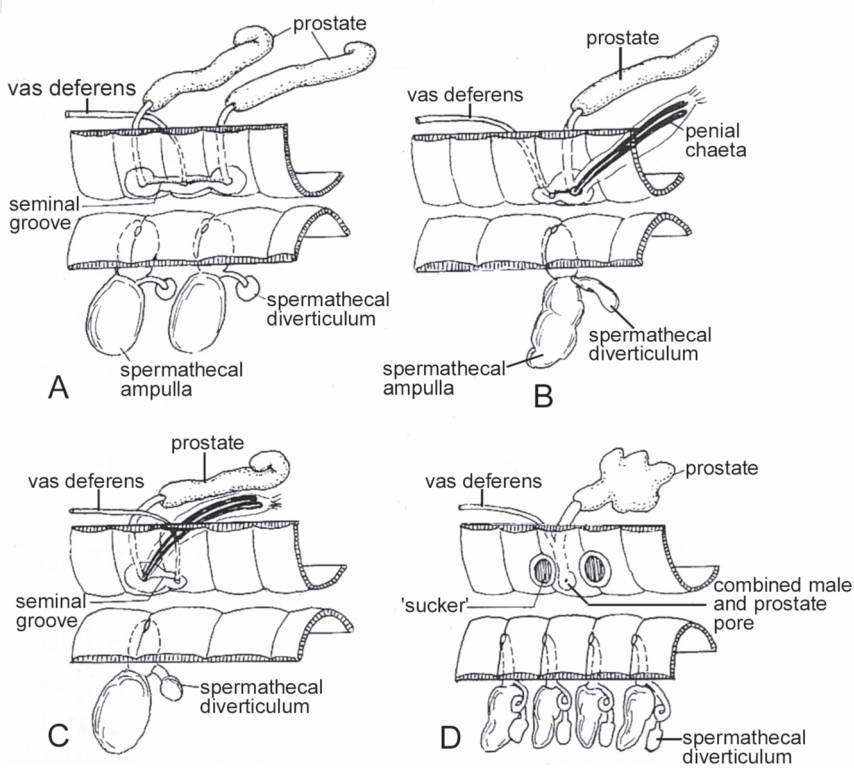
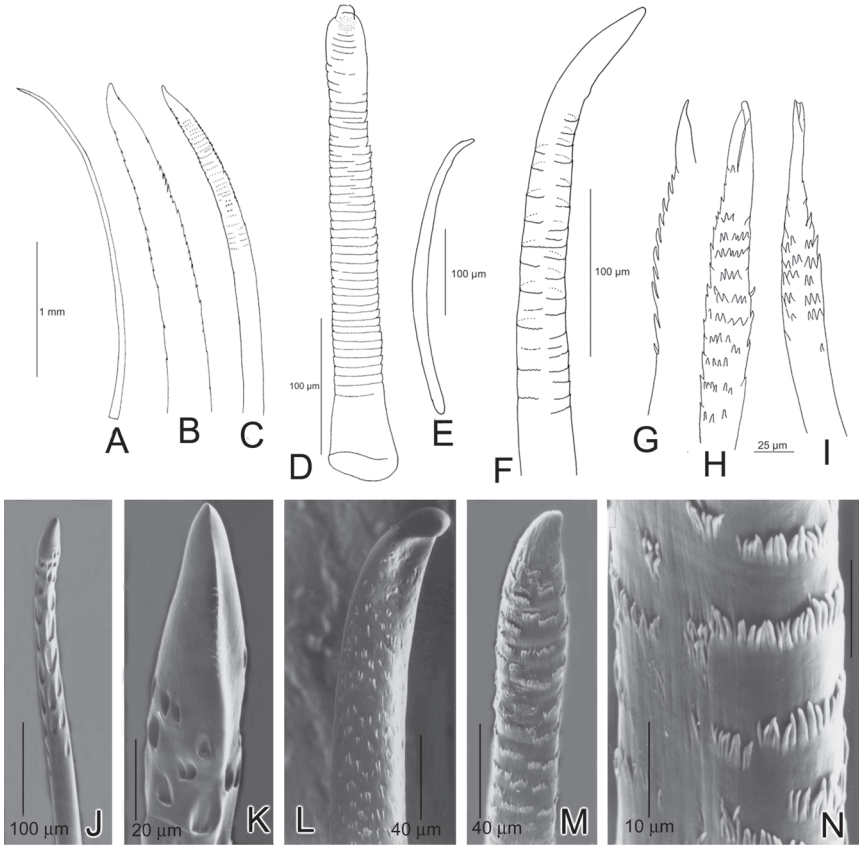


Fig. 8.54 contd



8.55. Genital chaetae. **A-I**. Australian Megascolecinae. **A-C**. *Heteroporodrilus mediterreus*. **D**. *Notoscolex camdenensis*. **E-F**. *Cryptodrilus polynephricus polynephricus*. **G-I**. *Digaster armifera*. From Jamieson, B. G. M. 2000. The Native Earthworms of Australia (Megascolecidae Megascolecinae). Science Publishers, Inc.: Enfield, New Hamphshire, Fig. 0.12, after various papers of Jamieson. **J-N**. New Caledonian *Acanthodrilus* (Megascoelcidae, Acanthodrilinae). **J**, **K**. *A. cavaticus*. Spermathecal setae. **L**. *A. cavaticus*. Penial chaeta. **M**, **N**. *A. chevalieri*. Penial chaeta. Unpublished, from the study of Jamieson, B. G. M. and Bennett, J. D. 1979. Bulletin du Muséum National d'Histoire Naturelle Zoologie Ser 1: 353-403.

Fig. 8.54 contd

Fig. 8.54. Top. Coition in *Spenceriella* (Megascolecidae). After Jamieson, B.G.M. 1994. Chapter 39. Annelids, Arthropods and Molluscs. Pp. 855-878. In B. Knox, P. Ladiges and B. Evans (eds), Biology, McGraw-Hill Book Company, Sydney, Fig. 39.8. Bottom. The copulatory apparatus of a mating couple of Ocnerodrilidae and Megascolecidae. **A**. The acanthodrilin condition in *Eukerria saltensis* (Ocnerodrilidae) **B**. The microscolecin reduction in *Microscolex* (Acanthodrilinae). **C**. The balantin reduction in *Balantodrilus* (Acanthodrilinae). **D**. The megascolecin reduction in *Metaphire saigonensis* (Megascolecidae). Note that only in the megascolecin reduction do the vasa deferentia open into the prostate ducts, whereas in other cases the male pores are independent of the prostate pores, to which they are connected by narrow seminal grooves. A-D slightly modified after Omodeo, P. 2000. Italian Journal of Zoology 67: 179-201, Fig. 9.

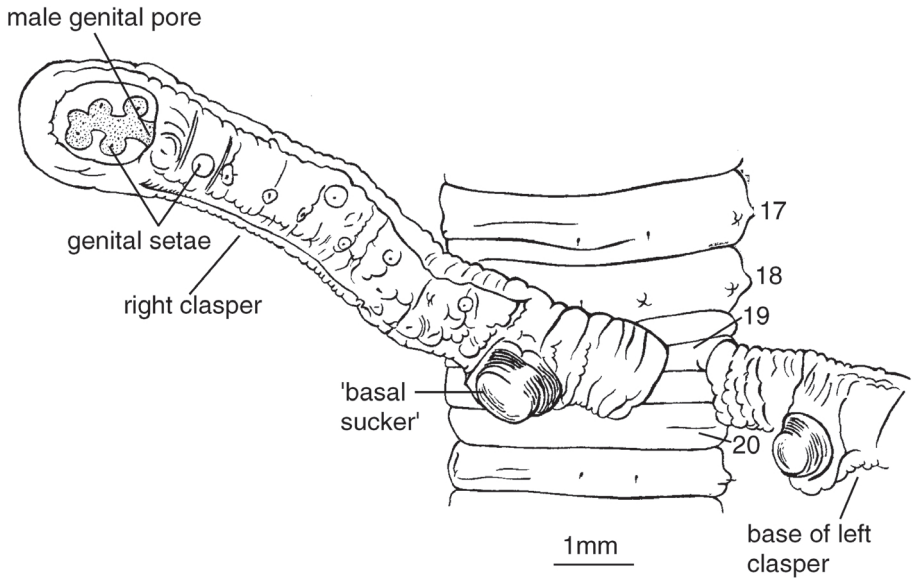


Fig. 8.56. *Alma tazelaari* (Almidae). Detail of claspers. After Jamieson, B. G. M. 1971. Glossoscolecidae. Pp. 41-72. In R. O. Brinkhurst and B. G. M. Jamieson (eds), Aquatic Oligochaeta of the World. Oliver and Boyd, Edinburgh, Toronto, Fig. 15.8G (*lapsus* for H).

In the Spanish endemic earthworm *Hormogaster elisae*, spermatogenesis ceases during summer months but spermatozoa are retained in the seminal funnels and spermathecae through the year, allowing copulation at any time, whenever conditions allow. *H. elisae* has two pairs of spermathecae variable in both shape and size; sperm storage is mainly in the second pair (Garvin *et al*. 1999).

Mated individuals of *Lumbricus terrestris* produced cocoons for up to 12 months after mating, while unmated individuals produced no cocoons. Hatchability of cocoons decreased to 11% in the sixth month after mating and zero thereafter. Median total production of viable cocoons is 5 per individual (range 0-21). There is no discernible relationship between cocoon production and length of copulation, individual longevity, or individual mass at mating. Both partners usually contribute to the production of viable cocoons, but within mating pairs there was a median difference of 4 cocoons. Median survival time after an experimental mating period was 9 and 11 months for mated and unmated earthworms, respectively (Butt and Nuutinen 1998).

8.6 FERTILIZATION, CLEAVAGE AND DEVELOPMENT

8.6.1 Sperm Entry, Polarity and Meiosis

For a molecular perspective on development, see Chapter **5**.

In *Tubifex*, sperm entry is restricted to the vegetal hemisphere, especially near the vegetal pole. A fertilization cone is formed (Shimizu 1982, and

literature therein). Two deformation movements then result in extrusion of the two successive polar bodies. The second deformation movement results in the formation of optically dense, yolk-free cytoplasm at the animal and vegetal poles, the so-called pole plasms mentioned below (see review by Jamieson 1981c; 1988a).

The meiotic apparatus (MA) of meiosis I is located away from the egg surface at the time of oviposition. The position of the animal pole is subsequently marked by attachment of one pole of the MA to the *Tubifex* egg cortex as an optically bright spot (Shimizu 1981, 1982). A centriole is found in the inner aster of the MA. Later a bulge develops at this site and is extruded as the first polar body. The second meiotic apparatus if formed at the site of extrusion and is tethered to the surface by the microfilamentous cortical layer. Eggs remain at metaphase II (for 90 minutes) and then enter anaphase, followed by extrusion of the second polar body. The deformation movements which occur during polar body formation are dependent on actin-containing microfilaments (Shimizu 1982).

8.6.2 Evidence for Polarity in the Primary Oocyte

A central problem in *Tubifex* development is the relationship between the pole plasm localization and cell determination. It is known that the pole plasms are segregated first to the D-cell, subsequently to the 2d- and 4d-cell (see 8.6.3, Fate maps, below), and finally to germ band cells (Shimizu 1982). During meiosis of the *Tubifex* primary oocyte, biosomatic elements (endoplasmic vesicles and mitochondria) and nutritive elements (lipid droplets and yolk granules) migrate within the cell by streaming movements (see review by Jamieson 1981c). Yolk granules may be concerned with organization as much as nutrition (Eckelbarger 1988). As a result of these segregational movements the components become arranged before the first cleavage division in a specific morphogenetic pattern of which the most conspicuous element is the concentration of mitochondria and endoplasmic vesicles at the animal and vegetal poles to form the two pole plasms. There is evidence that actin-like microfilaments in the cortex are responsible for accumulation of pole plasm (see review by Shimizu 1982).

8.6.3 Embryogenesis

The embryology of oligochaetes was reviewed and reinterpreted by Anderson (1971, 1973) and development in *Tubifex* was comprehensively studied and reviewed by Shimizu (1982). These studies form the basis of the present, albeit brief, review. Additional studies on the development of teloblasts in *Tubifex* (Goto *et al*. 1999a,b; Arai *et al*. 2000; Kitamura and Shimizu 2000a,b; Nakamoto *et al*. 2000; Shimizu *et al*. 2001) will also be summarized.

Fate maps. The fate maps of the blastulae of oligochaetes and polychaetes are basically similar (Anderson 1971, 1973) (Fig. 8.57) (see also chapter **5**). In both groups the 1st, 2nd and 3rd quartets of micromeres give ectoderm, with adult epidermis developing from 2d; 4d gives mesoderm; and macromeres 3 A, B and D give the midgut. In oligochaetes the process of

cleavage, in terms of cell lineages, which segregate the areas from one another and deformation movements, varies greatly according to the degree of yolkiness of the eggs which is least in the crassiclitellates (Anderson 1971, 1973).

Yolk content and cleavage. In the Enchytraeidae, Tubificidae *sensu stricto* and Lumbriculidae the egg is large, ranging in diameter from approximately 300 to 500 μm in *Enchytraeus albidus* and *Tubifex* to 1 mm in *Rhynchelmis* (references in Anderson 1973; Jamieson 1988a). The albumen which fills the cocoon and bathes the embryos appears to be of little significance as a source of food for development as this is provided by the internal yolk. The large (yolky) egg is deduced to be basic (plesiomorphic) in euclitellates. The size

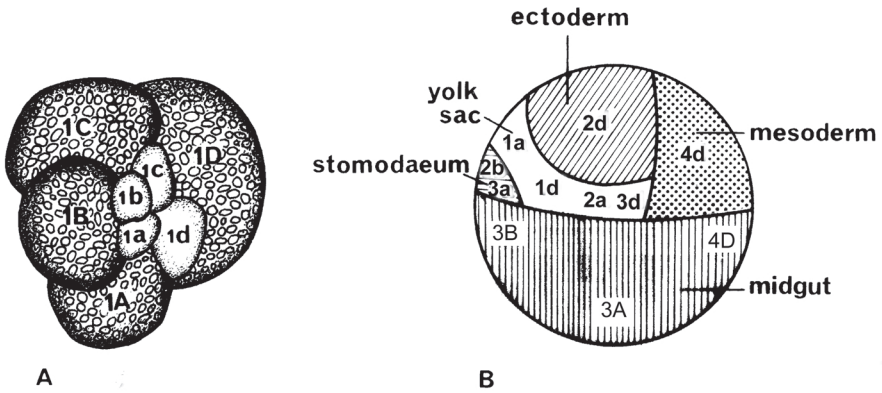


Fig. 8.57. Embryology of an oligochaete (*Tubifex*) embryo. **A**. 8-cell stage, anterodorsal view. **B**. Presumptive areas, lateral view. After Anderson, D.T. In R.O. Brinkhurst and B. G. M. Jamieson (eds), Aquatic Oligochaeta of the World. Oliver and Boyd, Edinburgh, Toronto, Figs. 2.1C, 2.2C.

and degree of yolkiness of the egg are imperfectly known for many oligochaete families but there is a reduction of yolk in the naids (now placed, as a subfamily, in the Tubificidae) and it is further reduced, presumably independently, in crassiclitellate families. In crassiclitellates, and to a lesser extent in naids, the eggs are smaller and the ambient albumen in the cocoon is exploited, in what is termed albumenotrophy, as a major source of nutrition during embryonic development.

The mesolecithal egg in microdriles gastrulates by epiboly and the late-forming archenteron is empty whereas the oligolecithal egg of megadriles (crassiclitellates) gastrulates by emboly and forms a large archenteron which engulfs the albumen contained in the cocoon (see Anderson 1971; Omodeo 2000).

Whether the egg be large or small, total, spiral cleavage, leading to a spherical blastula persists, as in polychaetes, but unlike the latter, the trochophore and, therefore, such regions as the presumptive prototroch are absent and there is no trace of larval organs or of metamorphosis.

Gastrulation is prolonged and is accompanied by formation of numerous segments from the posterior growth zone, leading to direct development of the adult organization.

Cleavage in the naidines *Stylaria* and *Chaetogaster* shows major modifications although they retain eggs of considerable diameter, 350 to 400 μm in *Stylaria*, and 400 to 500 μm in *Chaetogaster*. The blastula is attained through fewer cell divisions than in *Tubifex* and consequently has a large 3d cell instead of a large 4d cell (Anderson 1971, 1973).

Earthworms (crassiclitellates) have small eggs (in lumbricids 70 μm in *Dendrobaena subrubicunda* and 100 to 120 μm in *Eisenia fetida*; references in Jamieson (1988a) exhibiting a more marked reduction in yolk than that seen in naidids. Their cleavage is correspondingly modified relative to that of *Tubifex*. The D quadrant is emphasized to an even greater degree than in naids but resemblance of the blastula to that of oligochaetes with yolky eggs is retained and the usual 4d cell is present posteroventrally (Anderson 1971, 1973).

Cleavage is least modified in the large yolky eggs of tubificids and lumbriculids and we will take development in *Tubifex* as described by Shimizu (1982) as an example.

Developmental stages in *Tubifex* are illustrated in Fig. 8.58. The *Tubifex* egg is fertilized at metaphase of the first meiosis, that is, as a primary oocyte. After extrusion of polar bodies, pole plasms comprising endoplasmic reticulum and mitochondria accumulate around the animal and vegetal poles. The developmental stages of polar body formation are characterized by a dynamic shape change called deformation movement. The first cleavage produces the smaller AB- and larger CD-cell. The second cleavage gives rise to four cells: A, B, C and D; the D-cell is larger than the other three cells. Thereafter, these four cells divide in a spiral cleavage pattern, producing micromeres and yolky macromeres. The pole plasms are segregated into 2d- and 4d-cells resulting from divisions of the D-cell. The descendant cell ($2d^{111}$) of 2d-cell and 4d-cell exclusively participate in teloblastogenesis. The stages of teloblastogenesis as seen by by SEM are illustrated in Fig. 8.59. Four ectoblasts and a mesoblast are located on either side of the embryo, and bud off small cells forming germ bands. Gastrulation movement consists of two events: 1) ventral shift and ensuing coalescence of germ bands, and 2) epibolic expansion of a micromere-derived epithelial sheet over the endodermal cells (Shimizu 1982). In naidines gastrulation is wholly eliminated; the presumptive midgut, M cells and the ectoteloblast cells attain their definitive positions directly as a result of cleavage and proceed directly into organogenetic activity. In earthworms, in contrast, gastrulation by invagination (emboly) occurs as a secondary development relative to gastrulation by overgrowth of the large presumptive midgut by definitive ectoderm which occurs in yolky clitellate embryos (Anderson 1971, 1973). Organogenesis begins during the gastrula stage. The ectodermal germ bands are responsible for the ventral nerve cord and circular muscle layer. The mesodermal organs are exclusively derived from the stem cells produced by

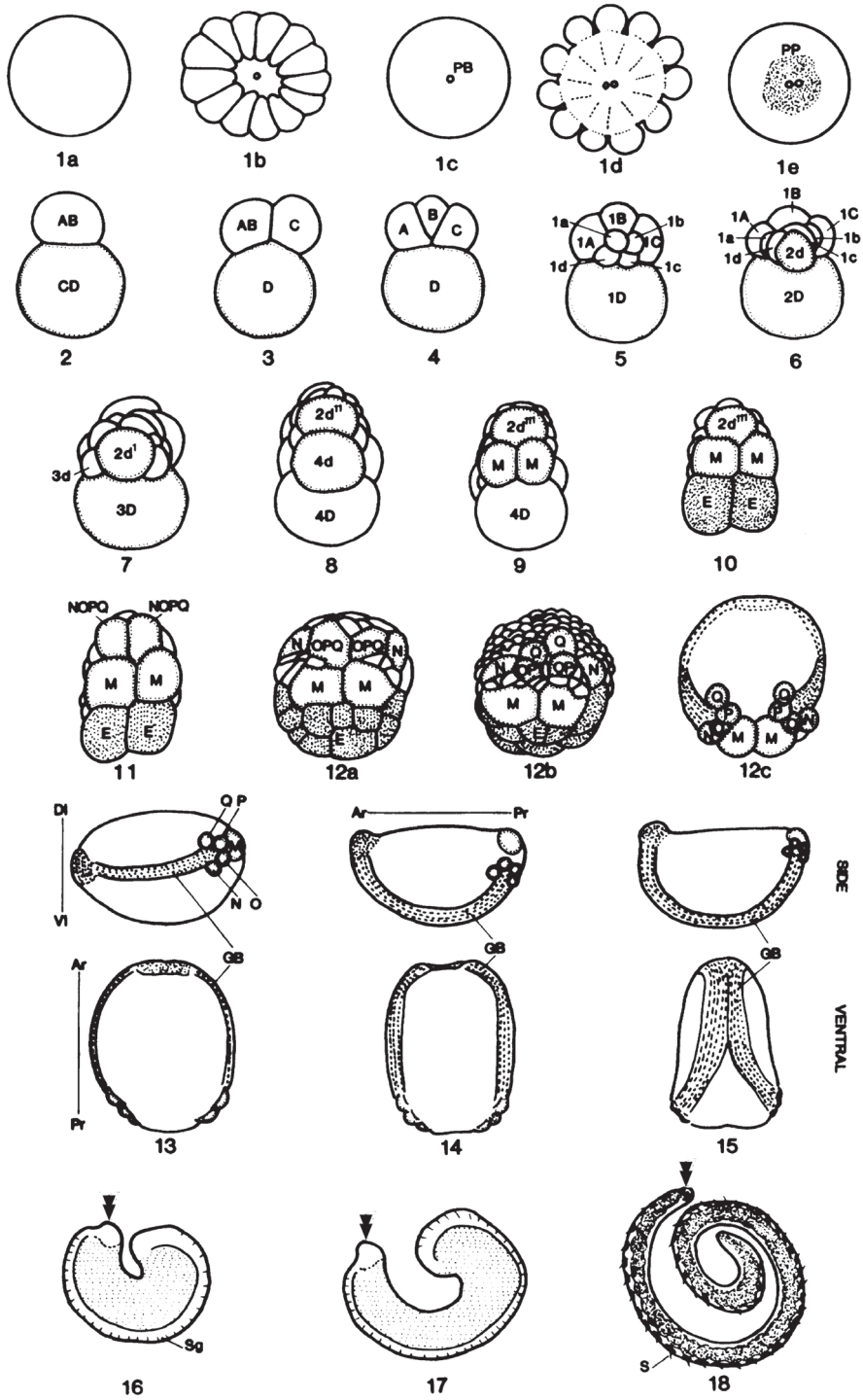


Fig. 8.58 contd

the mesoblasts. Gastrulation is followed by elongation of the embryo. It finally changes its shape to become vermiform. The embryonic period lasts for two weeks at 18°C, and is divided into 19 stages (Shimizu 1982).

Significance of pole plasm. It appears that the acquisition of both pole plasms accounts for the totipotency of the D-cell quadrant and for formation of organs, as opposed merely to endoderm and ectodermal epithelium produced by isolated A, B and C cells. The pole plasm is devoid of yolk granules. Its major components are mitochondria and its different metabolism may be responsible for the asynchronous *Tubifex* cleavage which is led by the D-quadrant. Differences in cleavage patterns of D cells compared with other cells may be governed by the presence of pole plasm as this determines the position of the MA. The somatoblasts of the early embryo thus differ from each other in the different proportions of primary constituents of the egg cytoplasm (mitochondria and ER) and in the distinct patterns of spatial distribution of these (see review by Shimizu 1982).

Origin of ectoderm and mesoderm (Teloblasts and their fate). The following account of the fate of teloblasts in *Tubifex* is based chiefly on that of Shimizu *et al*. (2001) and the findings of Arai *et al*. (2000); Goto *et al*. (1999a,b); Kitamura and Shimizu (2000a,b) and Nakamoto *et al*. (2000) which they review. The relevant events of development, as summarized by Shimizu *et al*. (2001) are illustrated in Fig 8.60A-H.

As in other clitellates, embryonic development in *Tubifex* is characterized by the generation of five bilateral pairs of teloblasts (designated M, N, O, P and Q), which serve as embryonic stem cells to produce germ bands on either side of the embryo (Goto *et al*. 1999a; Shimizu *et al*. 2001) (Fig. 8.60C,D). Each teloblast divides repeatedly to produce primary blast cells which are arranged in a coherent longitudinal column or bandlet (Fig. 8.60D). Four of the five bandlets on each side of the embryo join together to form an ectodermal germ band, while the remaining bandlet becomes a mesodermal germ band underlying that of the ectoderm (Fig. 8.60H). A large part of the tissues comprising body segments has been assigned to the progenies of the teloblasts. Goto *et al*. (1999a) followed the fate of the progenies of each teloblast using horseradish peroxidase tracers. M teloblasts give rise to nearly all of the mesodermal tissues, which included circular and longitudinal muscles, coelomic walls, nephridia (in segments VII and VIII) and primordial

Fig. 8.58 contd

Fig. 8.58. Diagrammatic illustration of developmental stages in *Tubifex*. Stages 1a-12c, animal pole view; stages 13-15, side and ventral view; stages 16-18, side view. N, O, P, and Q denote ectoblasts; NOPQ, OPQ, and OP denote precursor cells of ectoblasts. Mesoblasts (M) are located posteriorly behind ectoblasts (stage 12). Anteroposterior (Ar-Pr) and dorsoventral (DI-VI) axes are indicated for stages 13-15. Double arrowheads (stages 16-18) point to the anterior end of the embryo. E, endodermal cell; GB, ectodermal germ band; PB, Polar body; PP, pole plasm; S, chaeta; Sg, segment. After Shimizu, T. 1982. Development in the freshwater oligochaete *Tubifex*. Pp. 283-316. In F. W. Harrison, and R. R. Cowden (eds), Developmental Biology of Freshwater Invertebrates. Alan R. Liss, Inc., New York, Fig. 3.

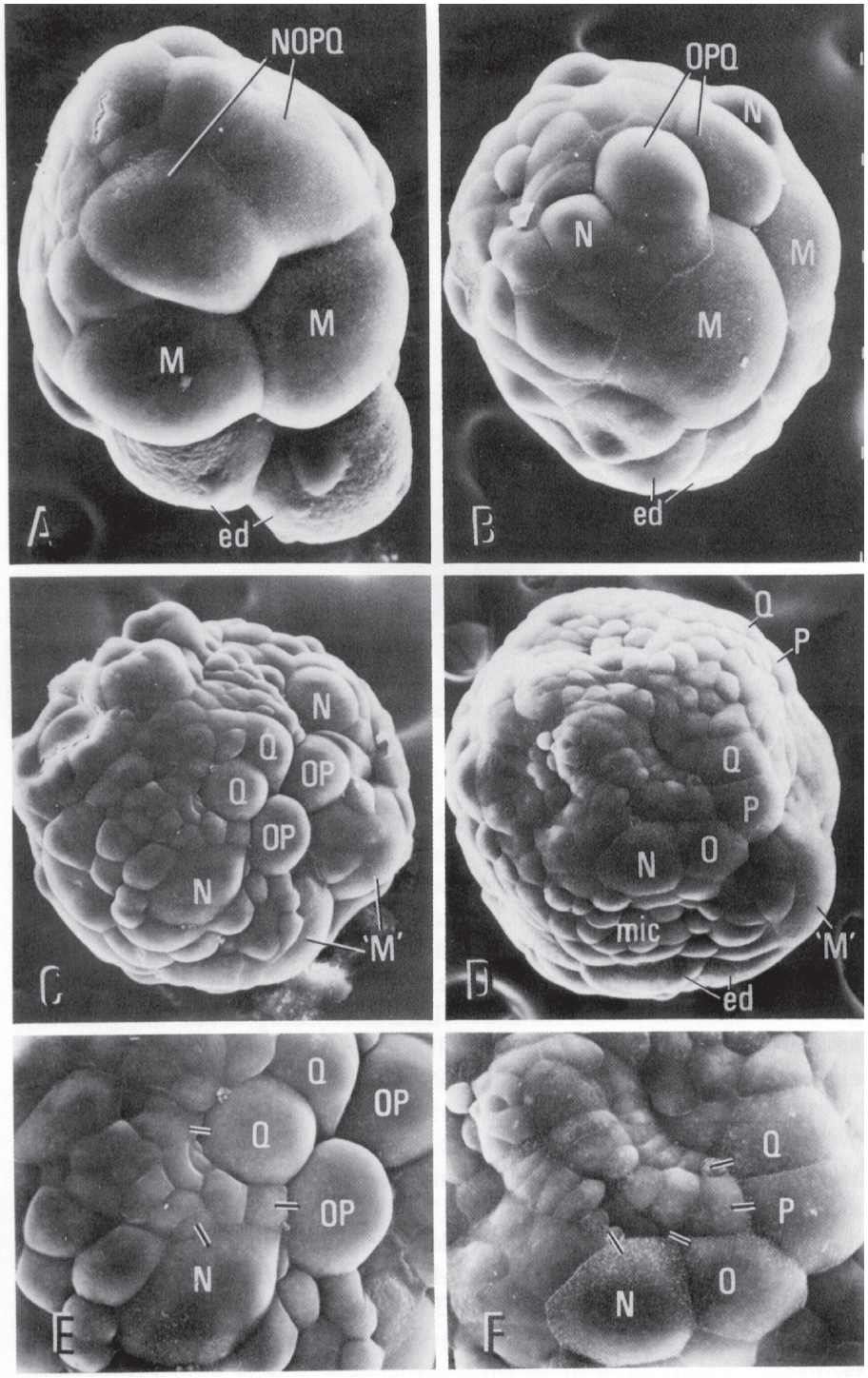


Fig. 8.59 contd

germ cells (in segments X and XI). Although few in number, M teloblasts also contributed cells to the ventral ganglion. Similarly, each of the ectoteloblasts, N, O, P and Q, made a topographically characteristic contribution to the ectodermal tissues such as the nervous system (i.e. ganglionic cells and peripheral neurones) and epidermis, all of which exhibited a segmentally repeated distribution pattern (Goto *et al*. 1999a).

In tubificids and enchytraeids, products of the proliferation of primordial germ cells (PGCs) spread forward through the mass of yolky midgut cells during gastrulation; in each genital segment they proliferate to form the testes and ovaries projecting into the coelom and covered by somatic peritoneum. However, it is reported that in *Eisenia* the primordial germ cells which, as in other earthworms, first become recognizable in the walls of the genital segments, cannot be traced to the products of division of the pair of cells cut off as the first products of the mesoteloblasts as these products regress late in gastrulation (see Anderson 1971, 1973). As stated by Shimizu (1982) it has yet to be firmly established that PGCs undergo migration.

Ectodermal bands and segmentation. Segmentation of the ectoderm in *Tubifex* is a process of separation of 50-µm-wide blocks of cells from the initially continuous ectodermal germ band (GB), a cell sheet consisting of four bandlets of blast cells derived from ectoteloblasts (N, O, P and Q). The initially linear array of blast cells in each ectodermal bandlet gradually changes its shape in a lineage-specific manner. These morphogenetic changes result in the formation of distinct cell clumps, which are separated from the bandlet to serve as segmental elements (SEs). SEs in the N and Q lineages each consist of clones of two consecutive primary blast cells. In contrast, in the O and P lineages, individual blast cell clones are distributed across SE boundaries; each SE is, therefore, a mixture of a part of the preceding anterior clone and a part of the next posterior clone (Shimizu *et al*. 2001).

The P and Q teloblasts uniquely give rise to additional ectodermal tissues, namely ventral and dorsal chaetal sacs, respectively. Furthermore, O teloblasts make a contribution to the nephridiopores in segments VII and VIII as well. Ectoteloblasts and mesoteloblasts are the main source of ectodermal and mesodermal segmental tissues, respectively, but all of the teloblasts produce more types of tissue than has previously been thought.

Fig. 8.59 contd

Fig. 8.59. A-D. CSFM Micrographs illustrating the sequence of teloblastogenesis. **A**. Stage 11. **B**. Stage 12a. **C**. Stage 12b. **D**. Stage 12c. Ectoblasts are designated N, O, P, and Q; NOPQ, and OP denote Precursor cells of ectoblasts. M, mesoblast. Mesoblasts in **C** and **D** are covered with epithelium and indicated by 'M.' ed, endoderm; inic, cells derived from micromeres. × 120. **E**,**F**. Higher magnification of **C** and **D**, showing details of initiation of ectodermal gerrn band formation. Bars indicate relations between ectoblasts and their offspring. × 210. After Shimizu, T. 1982. Development in the freshwater oligochaete *Tubifex*. Pp. 283-316. In F. W. Harrison, and R. R. Cowden (eds), Developmental Biology of Freshwater Invertebrates. Alan R. Liss, Inc., New York, Fig. 10.

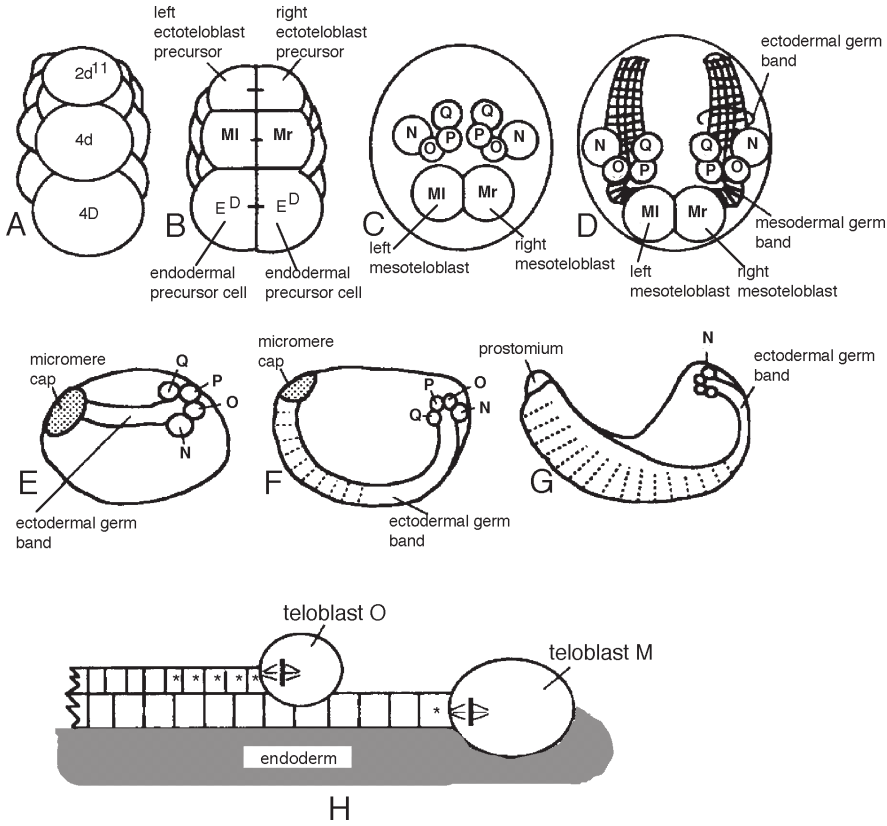


Fig. 8.60. Summary of *Tubifex* development. **A** and **B**. Posterior view with dorsal to the top. **C** and **D**. Dorsal view with anterior to the top. **E**-**G**. Side view with anterior to the left and dorsal to the top. **A**. A 22-cell stage embryo. Cells $2d^{11}$, 4d and 4D all come to lie in the future midline. **B**. 4d divides bilaterally into left and right mesoteloblasts (MI and Mr); $2d^{111}$ derived from $2d^{11}$ divides into a bilateral pair of ectoteloblast precursors (NOPQl and NOPQr), and 4D divides into a pair of endodermal precursor cells E^D. **C**. An embryo at about 30 h after the bilateral division of 4d. Only teloblasts are depicted. NOPQ on each side of the embryo has produced ectoteloblasts N, O, P and Q. **D**. A two-day-old embryo following the bilateral division of 4d. Only teloblasts and associated structures are depicted. At this stage, a short ectodermal germband (EGB) extending from the ectoteloblasts N, O, P and Q is seen on either side of the embryo. A mesodermal germ band (MGB) extending from the M teloblast is located under the ectodermal germ band. **E**-**G**. Morphogenesis of the ectodermal germ band. Embryos are shown in **E** 2.5, **F** 4, and **G** 6 days after the 4d cell division. **E**. The germ band (EGB) is associated, at its anterior end, with an anteriorly located cluster of micromeres (called a micromere cap; MC), and it is initially located at the dorsal side of the embryo. **F**. The germ bands (EGB) on both sides of the embryo elongate and gradually curve round toward the ventral midline and finally coalesce with each other along the ventral midline. **G**. The coalescence is soon followed by dorsalward expansion of the edge of the germ band, Pr, prostomium. **H**. Longitudinal section showing the relative positions of the endoderm (end) and bandlets extending from teloblasts M and O. Anterior is to the left and posterior is to the right. The bandlet (germ band) derived from the M teloblast is overlain by the O-bandlet and is underlain by the endoderm. Asterisks indicate the presence of a single primary blast cell in each block of the bandlet. The remaining blocks individually represent a cell cluster, which is derived from a single primary blast cell. After Shimizu, T. *et al.* 2001. Hydrobiologia 463(123): 123-131, Fig. 1.

Without the underlying mesoderm, separated SEs fail to space themselves at regular intervals along the anteroposterior axis (Nakamoto *et al*. 2000; Shimizu *et al*. 2001). Nakamoto *et al*. (2000) suggest that ectodermal segmentation in *Tubifex* consists of two stages: first, autonomous morphogenesis of each bandlet leading to generation of segmental elements and, secondly, the ensuing mesoderm-dependent alignment of separated segmental elements (Nakamoto *et al*. 2000; Shimizu *et al*. 2001). Some of the epidermis of the *Tubifex* embryo is reported to derive from the temporary yolk sac (Anderson 1971, 1973).

Mesodermal segmentation. Using lineage tracers, in *Tubifex* Goto *et al*. (1999b) showed that segmental organization arises sequentially in the anterior-to-posterior direction along the longitudinal axis of the mesodermal germ band, a coherent column of primary blast cells that are produced from the mesodermal teloblast. Shortly after its origin, each primary blast cell undergoes a spatiotemporally stereotyped sequence of cell divisions to generate three classes of cells (in terms of cell size), which together give rise to a distinct cell cluster, a mesodermal compartment (Fig. 8.61). Each cluster is composed of descendants of a single primary blast cell; there is no intermingling of cells between adjacent clusters. Relatively small-sized cells in each cluster become localized at its periphery to form coelomic walls including an intersegmental septum thus establishing individuality of segments.

Ablation experiments show that these features of mesodermal segmentation are not affected by the absence of the overlying ectodermal germ band, that each primary blast cell serves as a founder cell of each mesodermal

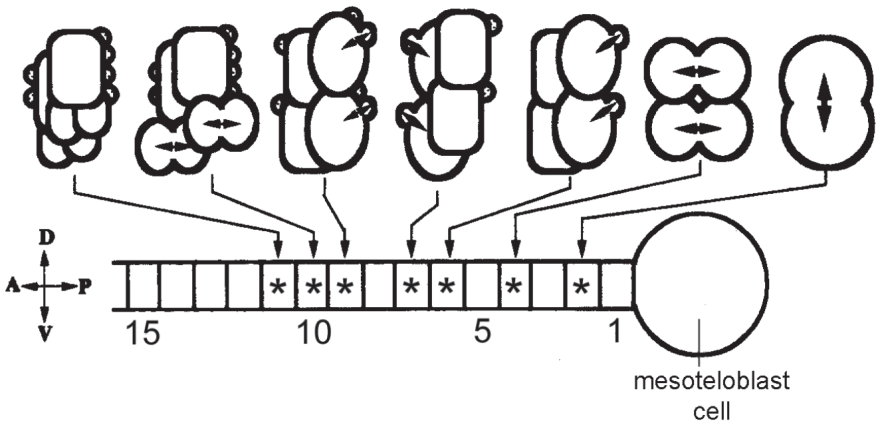


Fig. 8.61. Schematic summary of pattern and sequence of divisions in mesodermal blast cells. Inequality and direction of divisions are reflected by position and orientation of mitotic spindles in dividing cells. The mesodermal germ band (GB) extending from the mesoteloblast (M) cell is illustrated in the lower part of the figure; each block in the GB represents a cell cluster. Arrows indicate the approximate position, along the GB, where each division occurs. A—anterior; D—dorsal; P—posterior; V—ventral. After Shimizu, T. *et al*. 2001. Hydrobiologia 463(123): 123-131, Fig. 4, adapted from Goto A. *et al*. 1999. International Journal of Developmental Biology. July 43(4): 317-327, Fig. 4.

segment and that the boundary between segments is determined autonomously. In contrast with development of the ectoderm (see below), the metameric body plan of *Tubifex* thus arises from an initially simple organization (i.e., a linear series) of a segmental founder cell for each segment (Goto *et al*. 1999b; Shimizu *et al*. 2001). Using alkaline phosphatase activity as a biochemical marker for segments VII and VIII it appears that segmental identities in primary M-blast cells are determined according to the genealogical position in the M lineage and that the M teloblast possesses a developmental program through which the sequence of blast cell identities is determined (Shimizu *et al*. 2001).

8.6.4 Organogenesis

Somite formation. On each side of the embryo a somite derives from a single mesodermal stem cell produced by the mesoblast (Shimizu 1982). The mode of segmentation of the mesoderm, and the overlying ectoderm has been outlined above.

Coelomic walls. The coelomic walls of the somite are differentiated into lateral somatopleure (somatic mesoderm), median splanchnopleure (splanchnic mesoderm) and the transverse epithelium covering the septa. The longitudinal muscle of the body wall differentiates from the somatic mesoderm. The splanchnic mesoderm is differentiated into the gut musculature and the overlying splanchnic peritoneum which on the midgut (and elsewhere) usually forms the cholagogen tissue (Shimizu *et al*. 2001).

Prostomium. Shimizu *et al*. (2001) state, for *Tubifex*, that the prostomium and the cerebral ganglia (brain) originate from cells that are not the progeny of teloblasts. The prostomium, at least, derives from a 'micromere cap' (Fig. 8.60E-G). This contrasts with the view (Anderson 1971, 1973) that the prostomium originates from the anterior ends of the ectoblast bands and their underlying mesodermal bands and that the cerebral ganglia develop from the ventral neuroblast components at the extreme anterior end of each ectoblast band.

Peristomium. The oligochaete peristomium appears to be a single segment formed by fusion of the first, bilateral pair of segmental somites, and overlying segmental ectoderm, immediately behind the prostomial rudiment. The neuroblast components of the segmental ectoderm giving rise within it to a single pair of ventral ganglia (Anderson 1971, 1973).

Stomodeum. In yolky (lecithotrophic) euclitellate embryos, as part of the development of the gut, the stomodeum eventually grows back through the first segments of the segmenting embryo, develops a lumen and differentiates as the lining epithelium of the pharynx. Continuity between the buccal and pharyngeal lumen is established. Albumenotrophic embryos, in contrast, develop a precociously functional embryonic pharynx lined by cilia. In earthworms this is developed during gastrulation while that of naidines arises as an independent albumenotrophic invagination. It is later transformed into or (earthworms) replaced by the definitive pharynx. In *Tubifex*, the large mass of yolky cells, or in *Rhynchelmis* a syncytium, filling

the interior of the embryo develops more or less directly into a midgut epithelium by developing a central split. In naidines a provisional midgut sac becomes connected with the provisional pharynx and presumably acts temporarily in feeding on the ambient albumen. The walls of this sac later merge and become syncytial before resorption of the central part of the syncytium and differentiation of the definitive midgut. In earthworms the midgut sac formed as a result of invaginate gastrulation is already connected with the provisional pharynx and takes over the albumenotrophic role played in cleavage by albumenotrophic cells. The proctodeum forms, like the pygidial cells which surround it, from cells of the temporary yolk sac ectoderm. The formation of the proctodeum in naidines, in which the provisional ectoderm does not contribute to the definitive ectoderm, requires elucidation (Anderson 1973).

Blood vessels. The ventral blood vessel (VBV) develops in *Tubifex* by separation of the apposed walls of the ventral mesentery and the segmental commissural vessels by separation of the apposed walls of the intersegmental septa; thus the blood vascular system occupies the site of the former blastocoel. In *Criodrilus* and *Eisenia*, however, the VBV is first apparent between the ventral mesentery and the floor of the midgut. In *Tubifex* the dorsal vessel develops precociously, as a paired vessel between the upper edges of the somites and the lateral surfaces of the yolky midgut. Later, as the edges of the somites come together in the dorsal midline, the two half-vessels combine into a single, dorsal longitudinal vessel in the resulting mesentery (Anderson 1971, 1973). In contrast, Shimizu (1982) states that all blood vessels develop between the endoderm and splanchnopleure of the somites.

Nephridia. That nephridia are ectodermal derivatives, as is often stated, has been controversial. Origin from a single nephridioblast is generally accepted (Anderson 1971, 1973). Anderson noted that the septal location of the nephridioblast had been taken as evidence that the cell has a mesodermal origin but he considered the possibility of an earlier derivation from ectoderm. Goto *et al*. (1999a) demonstrated that embryonic nephridia of *Tubifex* are mesodermal, being descended from M teloblasts. These provisional protonephridia develop anteriorly in *Tubifex*, *Rhynchelmis*, and earthworms but were considered to be absent in naidines (Anderson 1973). However, Bunke (2003) has convincingly demonstrated origin of metanephridia of the naidine *Dero digitata* from three nephroblast cells in the frontal epithelium of a septum, suggesting its mesodermal origin. One cell produces the rudiment of the canal; another the ciliated mantle cell, and the third produces a flame of cilia that beats into the canal lumen, the so-called flame cell. In *Tubifex hattai* each nephridium-like structure was traced back, by histochemical staining, to a single cell (detected by alkaline phosphatase staining) that emerges in the mesodermal territory in the ventrolateral region of each of segments 7 and 8 at the late gastrula stage (Kitamura and Shimizu 2000b).

Gonoducts. Where development of the gonoducts has been investigated in clitellate embryos, they have been identified as coelomoducts. In

oligochaetes each gonoduct arises as a thickening of the coelomic epithelium opposite a gonad. The thickening develops as a funnel while the base of the thickening grows out as a duct. A small ectodermal invagination establishes the opening to the exterior. A detailed study of the development of the gonoducts and prostates in Tubificidae by Gustavsson and Erséus (1997, 1999) has been discussed in 8.2.11.2.

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