

II

Selected Groups of Annelida

Non-leech Clitellata

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with contributions by Marco Ferraguti^{2*}

8.1 PHYLOGENY AND SYSTEMATICS

8.1.1 Definition of Clitellates

The Clitellata Michaelsen 1919 are annelids which include the oligochaetes (earthworms and their allies), branchiobdellids (ectoparasites of freshwater crayfish) and leeches. They are defined by the possession of a modification of the epidermis, the clitellum, which is located at least partly behind the female pores and which secretes a cocoon in which the eggs are deposited. They were renamed Euclitellata (a term not employed in this volume though widely used) by Jamieson because a clitellum also occurs in questid polychaetes though there anterior to the female pores (Jamieson 1983c). *Questa* has since been placed, from 18S rRNA, close to the eunicid polychaetes (Erséus and Källersjö 2003) but showed firm relationship to the Orbiniidae (with 95% jackknife support) in an unpublished analysis of 215 annelid taxa, using 18S, 16S, 28S, COI and H3, by Siddall *et al.* (pers. comm.).

In view of the synonymy of the terms Clitellata and Oligochaeta *sensu lato*, containing oligochaetes *sensu stricto*, acanthobdellids, branchiobdellidans and true leeches (Siddall *et al.* 2001; Erséus *et al.* 2002) it is appropriate to adopt the term Clitellata for the combined assemblage and to dispense with the term Oligochaeta as an official name, a procedure also adopted by Erséus and Källersjö (2003) (but see Chapter 9). Nevertheless, the term 'oligochaete' will here be used as a common noun for Oligochaeta *sensu stricto*, to avoid the inconvenience of referring to them as non-branchiobdellidan, non-acanthobdellid, non-leech Clitellata. Alternatively they may be referred to as 'oligochaetous clitellates', though excluding the similarly characterized *Acanthobdella*.

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*Section 8.4

8.1.2 Molecular Evidence for Higher Clitellate Relationships

Molecular studies have confirmed monophyly of the clitellates, using 18S rRNA (Moon *et al.* 1996; Struck *et al.* 2002; Erséus and Källersjö 2003); or 18S rRNA (Winnepenninckx *et al.* 1998); 18S and mtCOI (Martin 2000; Siddall *et al.* 2001); cytochrome oxidase I (Siddall and Bureson 1998; Nylander *et al.* 1999) or elongation factor lalpha (Kojima 1998; see also a review by McHugh 2000). An apparent exception to clitellate monophyly in a parsimony analysis of 18S rRNA was considered questionable (Martin 2001, see below).

With regard to the position of the clitellates within the Annelida, molecular analysis has indicated that clitellates form a clade within the Polychaeta and that polychaetes are a paraphyletic or polyphyletic group (Kojima 1998; McHugh 2000; Martin 2001; Rota *et al.* 2001; Siddall *et al.* 2001; Erséus and Källersjö 2003).

Paraphyly of the Oligochaeta, with leeches and/or branchiobdellidans lying within the oligochaete clade, has long been suspected on morphological grounds (Michaelsen 1928; Brinkhurst and Nemeč 1986; Jamieson *et al.* 1987; Jamieson 1988b; Brinkhurst 1989; Brinkhurst and Gelder 1989; Purschke *et al.* 1993). Derivation of leeches from a lumbriculid-like ancestor had been suggested on morphological grounds by Michaelsen (1928-1932), Brinkhurst and Nemeč (1986) and Jamieson *et al.* (1987). These relationships are being increasingly confirmed from molecular analyses. Thus, using 18S rDNA and mitochondrial cytochrome c oxidase subunit I, parsimony analysis by Siddall *et al.* (2001) of the combined data-set gave complete support for monophyly of leeches (Hirudinea), branchiobdellidans (Branchiobdellida), and acanthobdellidans (Acanthobdellida), as already shown in Siddall and Bureson (1998). Monophyly of the oligochaete order Lumbriculida with these three groups was also strongly supported. These results provided support for the hypotheses that leeches and branchiobdellidans are sister groups, that acanthobdellidans are sister to them, as suggested by Livanow (1931), and that together with the family Lumbriculidae they all constitute a clade within Oligochaeta. Synonymy of the Clitellata and the more commonly used Oligochaeta was therefore proposed. It was considered that leeches branchiobdellidans, and acanthobdellidans should be regarded as orders equal to their closest relatives, the order Lumbriculida. However, leaving aside the issue of recognizing ordinal ranks, these clitellate entities are not here considered to be equivalent in rank.

Martin (2001) had also investigated phylogenetic relationships of clitellates using 18S rRNA, aligning on the basis of secondary structure and applying maximum parsimony (MP) and maximum likelihood (ML). In contrast to ML, parsimony did not recover the monophyly of clitellates. However, a close scrutiny of the data suggested a spurious attraction between some polychaetes and clitellates. Acanthobdellida and Euhirudinea were reconciled in their traditional Hirudinea clade and were included in the

Oligochaeta with the Branchiobdellida 'via the Lumbriculidae' as a possible link between the two assemblages. The exact position of Hirudinea and Branchiobdellida within oligochaetes and of Clitellata within the Polychaeta remained unresolved. Polytomies within annelids were considered to indicate an extremely ancient radiation of polychaetes and emergence of clitellates. It has since emerged that the polychaete sequences taken from another source which led to the non-monophyly of Clitellata by Martin (2001) contained sequencing errors; these have been replaced in genbank with correct sequences (Siddall, pers. comm.).

Jamieson *et al.* (2002) found support, from 28S rDNA, for the following clades, among others: (1) Enchytraeidae; (2) Naididae + Tubificidae; (3) Lumbriculidae; (4) Lumbriculidae + (Hirudinida + Branchiobdellida); (5) Crassicitellata; and (6), within the Crassicitellata, Ocnero-drilidae + (Acanthodrilinae + Megascolecinae) (Fig. 8.1). The leech-branchiobdellid relationship has been controversial owing to the concern that molecular artifacts (under the rubric of long branch attraction) undermine confidence in the result. This study revealed significant base composition variation in the 28S ($p < 0.001$ PAUP* test), in particular the leeches and the branchiobdellids being distinct from other clitellates. Bootstrap support for the leech-branchiobdellid grouping was fairly high in maximum parsimony (MP) (76%) but not sustained in maximum likelihood (ML) (16%), and the grouping was conspicuously absent using LogDet, a method that is supposed to compensate for nonstationarity (Lockhart *et al.* 1994). Furthermore, parametric data-sets made to trees that did not contain this clade, when analysed with MP, grouped them at high bootstrap frequency ($> 70\%$); it was therefore suspected that MP support was artifactually high. Conversely, it was considered that low ML support might be due to long branch repulsion effects (see Siddall 1998). Thus, although the branchiobdellid-leech clade was supported it was not without reservation.

Erséus and Källersjö (2003) have given a tree based on 18S analysis using Clustal and PAUP (Fig. 8.2) which confirms a sister group relationship of Hirudinida and Branchiobdellida with *Acanthobdella* as their plesiomorph sister group; the Lumbriculidae formed the plesiomorph sister group of the *Acanthobdella* through Hirudinida assemblage.

Hugall *et al.* (unpublished), in a study using a different computational method (for details see Enchytraeidae, below) for the three genes 18S, 28S and COI, corroborated this clade but in a sequence ((Hirudinea) ((Branchiobdellida) (Acanthobdella, Lumbriculidae))) (Fig. 8.5).

8.1.3 Phylogeny and Classification of Oligochaetes

Sensu Stricto

Oligochaetes *sensu stricto* are marine, freshwater and terrestrial. Unlike leeches, only one species is known to have parasitic forms, in snails. The circummundane naidine *Chaetogaster limnaei*, found throughout the world in the mantle cavity of snails where it is known to reduce numbers of trematode cercariae (see Fernandez *et al.* 1991), has a truly parasitic subspecies,

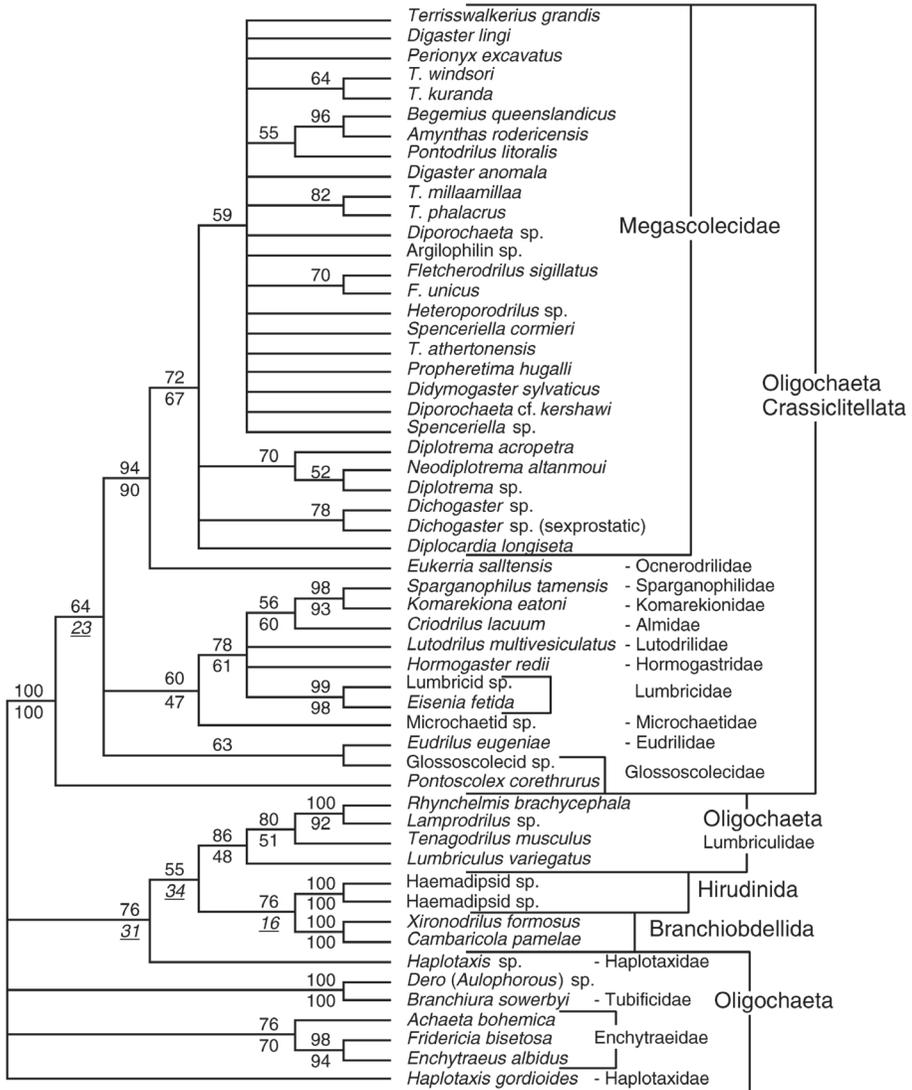


Fig. 8.1. 28S rDNA maximum parsimony majority rule consensus of 1000 bootstrap resamples, five random sequence additions each with tree bisection reconnection swapping. Basal polytomy rooted with *Haplotaxis gordioides* (Hartmann, 1821). Maximum parsimony bootstraps above node, maximum likelihood bootstrap values below (200 resamplings from reduced 36 taxa set using only nine Megascolecidae). Those underlined were not recovered in the majority rule. Family and some higher level groups indicated. From Jamieson *et al.* 2002. *Zoosystema* 24(4): 707-734, Fig. 2. © Publications Scientifiques du Muséum national d'Histoire naturelle, Paris.

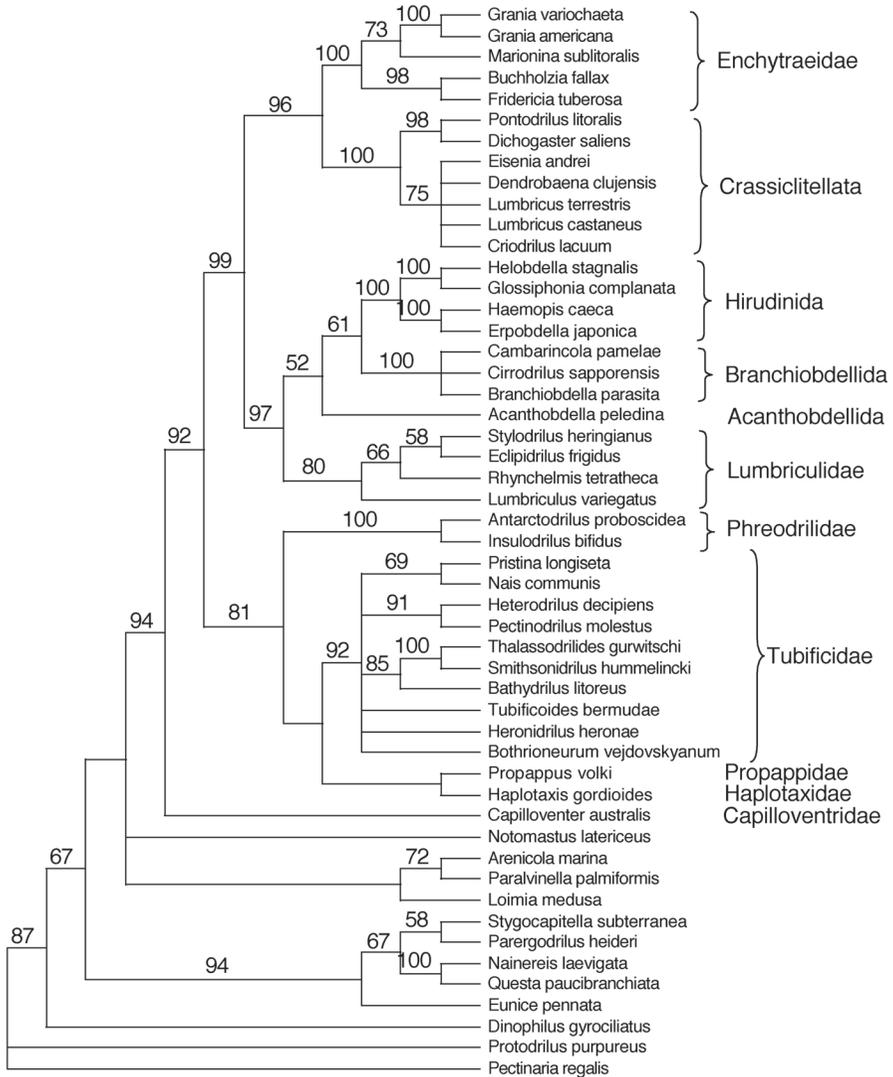


Fig. 8.2. 18S rDNA phylogeny of basal groups of Clitellata (Annelida). From Erséus, C. and Källersjö, M. 2003. *Zoologica Scripta*: 33(2): 187-196, Fig. 4.

C. l. vaghini (Gruffydd 1965). An Indian naidid, *Haemonais*, inhabits the mantle cavity of *Vivipara bengalensis*. The pheodrilid *Astacopsidrilus* is commensal in the branchial chamber of Australian crayfish (pers. obs.) and Rota (pers. comm.) reports that *Lumbricillus catanensis* is abundant in the branchial chambers of freshwater crabs in Sicily; and that *Enchytraeus carcinophilus* lives in the same habitat in *Gecarcinus lagostoma* from Trinidad Island, Brazil. The enchytraeid *Fredericia parasitica* ingests mucus and, apparently, coelomocytes from the surface of *Allolobophora robusta*, and the

highly modified, sucker-bearing *Aspidodrilus kelsalli* on an African earthworm probably takes similar food although the gut also contains 'mud' and vegetable matter (see Jamieson 2000). *Haereodrilus reichelti* is a *Diporochaeta*-like species which lives on the surface of another megascolecine, the giant *Digaster grandis* in South East Queensland (Dyne, in Jamieson 2000)

With the exception of some earthworm-like genera, aquatic oligochaetes are usually small and are loosely termed microdriles. However, some species of the 'microdrile' assemblage are as large as earthworms, namely giant enchytraeids and some Moniligastridae. Microdriles are characterized by a plesiomorphic type of clitellum in which, like the epidermis from which it is derived, there is only a single layer of cells. Its simple structure and limited ability to secrete nutrients into the cocoon correlate with the production of small numbers of large, yolky eggs. However, naidines do show some degree of albumenotrophy (see 8.6.6. Embryogenesis. Yolk content and cleavage). Prostate glands usually secreting into the male ducts, or the glandular sperm funnels in enchytraeids, presumably add secretions to the seminal fluid and contribute to the small amount of albumen in the cocoon.

A major evolutionary innovation in earthworms (loosely termed megadriles), in contrast, has been the development of a clitellum consisting of several layers of cells with the ability to secrete large proteinaceous reserves into the cocoon (Jamieson 1971c). Correlated with this, the eggs possess little yolk, are therefore small and are produced in large numbers (Jamieson 1992). In morphocladistic analysis, all families with multilayered clitella were found to form a single clade, named the Crassicitellata (Jamieson 1988b). Thus acquisition of a multilayered clitellum was deduced to be a monophyletic event. However, Omodeo (2000), in an informative review of evolution and biogeography of megadriles, implied that a multilayered clitellum has arisen more than once when he derived the Eudrilidae (with multilayered clitellum) from the Alluroididae (with clitellum consisting of a single layer of cells) independently of other earthworms. Jamieson (1974) had also suggested a relationship between the Alluroididae and Ocnerodrilidae but neither of these familial relationships is supported morphocladistically (Jamieson 1988b).

The higher classification of the Oligochaeta *sensu stricto* adopted here (Table 8.2) is based on the morphocladistic parsimony analysis by Jamieson (1988b), but is modified to take into account molecular (rDNA and RNA) findings (Figs. 8.1, 8.2, 8.5-8.7). The morphocladistic analysis did not include branchiobdellidans and leeches. It produced a single most parsimonious tree resulting in the classification shown in Fig. 8.3 and 8.4A, B. No great significance was attached to Linnean and other rank names above the familial level but they at least served to show the relative levels in the phylogenetic hierarchy, which it is cumbersome to represent by any numerical system. Ranks are omitted from the revised classification in Table 8.2. Detailed definitions of oligochaete families are given by Jamieson (1978b; 1980) and features of their reproductive anatomy are discussed in Section 8.2 below and are partly shown in Fig. 8.4.

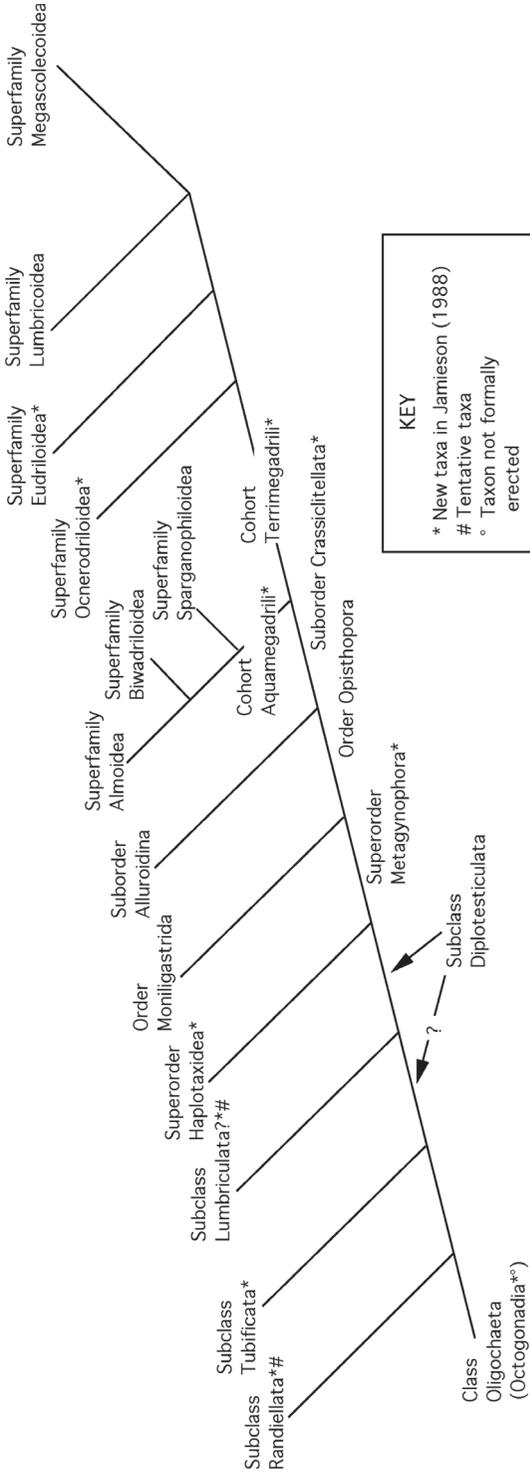


Fig. 8.3. Cladogram to show sister-group relationships of superfamilies and high taxa recognized by Jamieson (1988) in a morphocladistic analysis. The Randiellidae (not computed) were tentatively assigned to a new subclass regarded as the most plesiomorphic descendant of a presumed octogonadal ancestor. From Jamieson, B.G.M. 1988. Cladistics 4(4): 367-402, Fig. 4.

Colour
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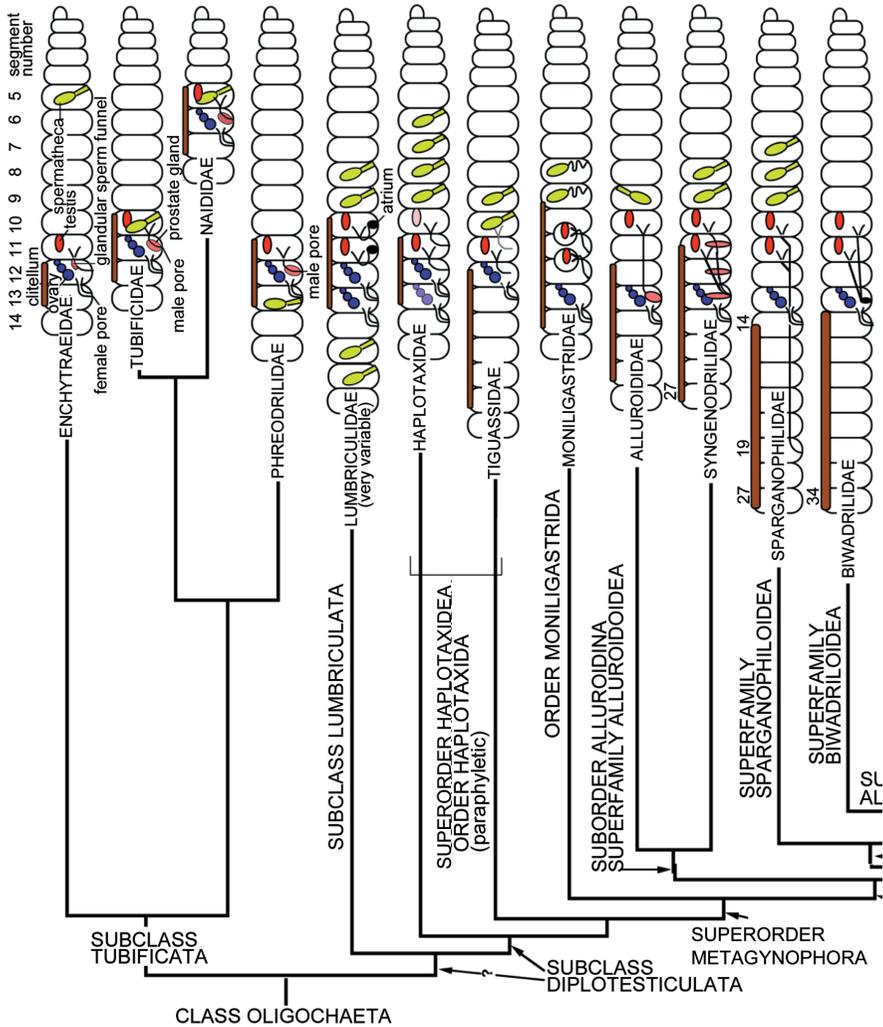


Fig. 8.4. A and B. Cladogram derived from a single most parsimonious tree, represented only to families, showing the taxonomy adopted in a morphocladistic analysis. Naididae were not included in the original

Although the Jamieson (1988b) classification is more complex than that advanced, without recourse to parsimony cladistics by Brinkhurst and Jamieson (1971), it confirmed the order Lumbriculida, family Lumbriculidae and the order Moniligastrida (a single family, Moniligastridae). It restricted the Haplotaxida (previously the Haplotaxidae and putative opisthopore descendants of a common ancestor shared with these) to the Haplotaxidae and Tiguassidae and recognized the order Opisthopora (male pores more than one segment behind the segment

support. One of these, named the Aquamegadrili, contained the aquatic megadrile families Sparganophilidae, Biwadrilidae, Almidae (with *Criodrilus*), and Lutodrilidae, with, questionably, the Kynotidae. The other, named the Terrimegadrili, contained the predominantly terrestrial families Ocnodrilidae, Eudrilidae, Microchaetidae, Hormogastridae, Glossoscolecidae, Lumbricidae and Megascolecidae (Jamieson 1988b). We will see in the discussion of molecular analyses that recognition of Aquamegadrili and Terrimegadrili is not supported, though at present only a single family (Komarekionidae) transgresses the division.

The morphocladistic analysis (Jamieson 1988b) attributed a basal position to the Eudrilidae and especially the Ocnodrilidae relative to the other terrimegadrile families, whereas these two families are usually considered close to the Megascolecidae. We will see that molecular data do not support this distant position for ocnodrilids but reunite them with the Megascolecidae. The single eudrilid included in the molecular analysis (Fig. 8.1) associates with the Glossoscolecidae and is not confirmed as a sister taxon of the Ocnodrilidae + Megascolecidae but this finding requires confirmation from larger samples.

In the morphocladistic analysis (Jamieson 1988b), the Haplotaxidae and *Tiguassu*, confirming suspected paraphyly of the Haplotaxidae, were successively basal to the Metagynophora, the combined clade being the Diplostesticulata which questionably contained the Lumbricidae (no longer placed there), the latter forming the subclass Lumbriculata. The microdrile families Tubificidae (and Naididae) and the Phreodrilidae, and the Enchytraeidae formed a monophyletic clade, termed the Tubificata, the sister-taxon of the Lumbriculid-Diplostesticulata clade. As discussed below, this basal position of the Enchytraeidae is particularly challenged by some molecular analyses (Siddall *et al.* 2001; Erséus and Källersjö 2003; Hugall *et al.*, unpublished).

Impact of molecular studies. In the analysis of leech relationships by Siddall *et al.* (2001), which used polychaete outgroups, a sufficient representation of oligochaete families was included to allow assessment of hitherto uncertain interrelationships of major groups of families. Thus, although Siddall *et al.* (2001) did not refer to it, the suggestion of Stephenson (1930) that Tubificidae and related families were derived groups was contraindicated. Enchytraeidae were divorced from the Tubificidae + Naididae and had an unexpected association with the two oligochaete families represented. Their analysis pointed to the existence of the following main groups of oligochaetes [my enumeration]: 1) an aquatic 'microdrile' clade consisting of Tubificidae (including Naididae) and Phreodrilidae and 2) a clade consisting of 2a) the Enchytraeidae (aquatic and terrestrial and sometimes of 'megadrile' proportions) with earthworms (e.g. Lumbricidae and Megascolecidae) and 2b) a well defined clade consisting of Lumbriculidae + Acanthobdellidae + Branchiobdellidae + leeches. It should be noted, however, that linkage of the latter clade (2b) with the enchytraeid + earthworm clade was given no numerical support

in their consensus tree. The 18S analysis of Erséus and Källersjö (2003) (Fig. 8.2) with its more detailed representation of the Oligochaeta *sensu stricto*, confirmed the major groupings of Siddall *et al.* (2001).

It should be noted, however, that in the study of Erséus *et al.* (2002), the Enchytraeidae and Lumbricidae, arbitrarily chosen as the outgroup, failed to form a monophylum in relation to the ingroup consisting of Tubificidae, Naididae, Phreodrilidae and Lumbriculidae.

Many families remain to be analyzed for molecular sequences. It is particularly desirable that the Moniligastridae, putatively the most basal group of the Metagynophora and the plesiomorph sister-group of the Opisthopora, and the Alluroididae, which are putatively the most basal of the Opisthopora and form the plesiomorph sister-group of the Crassicitellata (Jamieson 1988b), should be included.

8.1.4 Phylogeny of the Oligochaete Families

The following section will be structured according to the morphocladistic analysis (Jamieson 1988b) but departures from the latter will be noted, some additional families included and some groupings and names recognized in the analysis will be questioned or replaced in light of new evidence chiefly from molecular analyses. Features of the reproductive systems of the various families will be included in Section 8.2, below. After this discussion in the context of the morphocladistic analysis, a revised, simplified classification of the oligochaetous clitellates will be given.

Capilloventridae. This family was not included in the morphocladistic analysis (Jamieson 1988b) though it was provisionally placed in the Tubificata. Despite the complex and probably apomorphic condition of the midpiece of the spermatozoon, Ferraguti *et al.* (1996) considered spermatozoal ultrastructure to indicate that capilloventrids were basal oligochaetes. The 18S analysis of Erséus and Källersjö (2003) (Fig. 8.2) confirms capilloventrids as the most basal known oligochaetes and the plesiomorph sister group of the Proppapidae + Haplotaxidae + Phreodrilidae + Tubificidae clade. The basal position of *Capilloventer* is further confirmed in the 215 taxon analysis (Siddall, pers. com.).

Randiellata. Whether the little known Randiellidae deserve a position, as the subclass Randiellata, basal to all other oligochaetes requires further investigation. Evidence for recognition of the subclass Randiellata is discussed in the taxonomic review of reproductive systems below (8.2.11.1).

Tubificata. Order Tubificida. The Tubificida was the sole taxon of the Tubificata and the latter name can therefore be regarded as redundant. These were defined as having one pair of testes followed by one pair of ovaries; male ducts plesiomporous (penetrating one intersegment). They included the Tubificidae, Enchytraeidae, Phreodrilidae, Dorydrilidae, and Opisthocystidae. The Capilloventridae, though not included in the character matrix, were tentatively included but are here removed (see above). It was considered possible that the imperfectly known Randiellidae (see above) should be included. Inclusion of the Enchytraeidae is now questionable.

Propappidae. These were not included in the morphocladistic analysis but appear to be referable to the Tubificida. Separation (Coates 1986) of the Propappidae from the Enchytraeidae in which they were formerly grouped appears to be supported by the 18S analysis of Erséus and Källersjö (2003) (Fig. 8.2) in which *Propappus* forms a clade with the Haplotaxidae, Phreodrilidae and Tubificidae, far separated from the Enchytraeidae.

Enchytraeidae. The position of the Enchytraeidae particularly needs reinvestigation. From general morphology (Jamieson 1988b) and sperm ultrastructure (Jamieson 1981c, 1983a; Ferraguti and Erséus 1999) the Enchytraeidae have been considered to be a more basal group than the Tubificidae and other oligochaetes and to have no close affinity with crassicitellates. Siddall *et al.* (2001) demonstrated a grouping (2a) of Enchytraeidae and earthworms (crassicitellates) on the basis of 18S rDNA and mitochondrial cytochrome oxidase subunit I. However, the analysis, and the enchytraeid + (lumbricid+*Pontodrilus*) clade, may not be robust as one of the lumbricids (*Eisenia fetida*) forms the sister taxon of the megascolecid *Pontodrilus*, the remaining lumbricid (*Lumbricus terrestris*) being the sister taxon of that clade. Lumbricids show greater molecular homogeneity in our studies (Jamieson *et al.* 2002) and *Pontodrilus* is an undoubted, if somewhat aberrant, member of the Megascolecinae from analysis of the three genes 28S + 12S + 16S.

In support of the above proposition of Siddall *et al.* (2001), Erséus and Källersjö (2003) (Fig. 8.2) show enchytraeids as the plesiomorph sister group of a small sample of crassicitellate species in the Megascolecidae, Lumbricidae and Almididae (*Criodrilus*). Insofar as crassicitellates must have arisen from a 'microdrile' group, origin of crassicitellates from an ancestor shared with the Enchytraeidae deserves consideration. Enchytraeids resemble at least some crassicitellates in having terrestrial members, simple chaetae (though the crassicitellate *Pontoscolex* has some bifid somatic chaetae), in including some species of 'megadrile' proportions, and in location of the spermathecal pores well anterior to the testes, a location which seems basic for crassicitellates (with haplotaxids). Other 'microdrile' families have been eliminated in molecular studies from sharing direct ancestry with crassicitellates.

The sister group relationship of enchytraeids and crassicitellates cannot yet be considered certainly established, however. Thus, Hugall *et al.* (unpublished) (Fig. 8.5; Table 8.1), used combined nuclear 18S, 28S and mitochondrial COI genes to assess oligochaete, and particularly enchytraeid, relationships. Computation used the MrBayes Markov Chain Monte Carlo (MCMC) likelihood program developed in the Manhattan project. MCMC provides an efficient means of obtaining an estimate of relative likelihood (or relative probability of occurrence) of models across a very large number of competing possibilities while giving the relative support for each possibility. A consensus tree was obtained for all three genes, excluding (Fig. 8.5A), or including (Fig. 8.5B), the third position in mtDNA CoI. Taxa included in the analysis are listed in Table 8.1. Most of the operational taxonomic units were

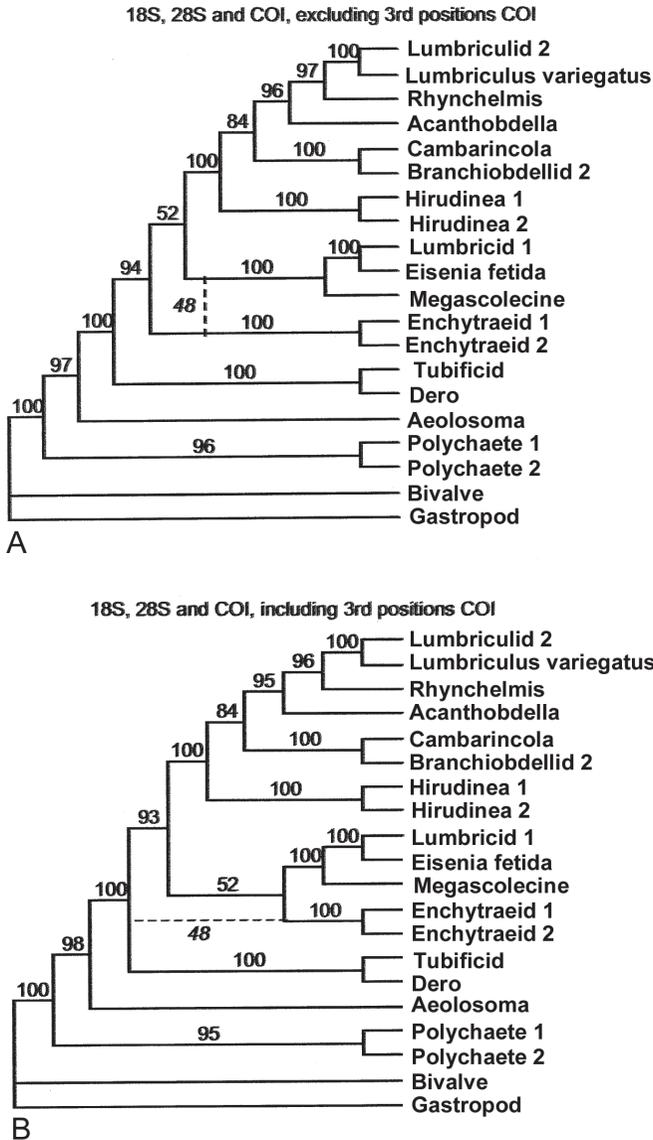


Fig. 8.5. Analyses of phylogenetic relationships of annelids using combined nuclear 18S, 28S and mitochondrial COI genes.

Genes used 18S, 28S (nuclear genes) and COI (mtDNA). Not all taxa have all genes (see Table 8.1). Computation uses the MrBayes Markov Chain Monte Carlo likelihood program. Consensus trees for all three genes but excluding the third position in mtDNA Col. The dashed lines indicate the result of constraining enchytraeids as the sister group of crassiditellates. **A.** Excluding third bases in mtDNA COI. **B.** Including third bases. Taxa are as listed in Table 8.1. Note that most of the sequences are combined from more than one species. For instance, the megascolecine combines different genes for *Pontodrilus litoralis*, *Perionychella cf kershawi*, and *Pheretima* sp. In contrast *Lumbriculus variegatus* denotes a single species for all three genes. (From Hugall *et al.*, unpublished.)

Table 8.1 Species sampled, accession numbers and base sites for molecular phylogenetic analysis. The Operational Taxonomic Units listed in the first column are those included in the cladograms in Fig. 8.5 (From Hugall *et al.*, unpublished).

18S			
<i>OTU</i>	<i>18S</i>	<i>Accession</i>	<i>Sites</i>
Lumbricidae			
Lumbricid 1	<i>Lumbricus rubellus</i>	Z83753	1711
<i>Eisenia fetida</i>	<i>Eisenia fetida</i>	X79872	1713
Megascolecidae			
Megascolecinae	<i>Pontodrilus bermudensis</i>	-	0
Enchytraeidae			
Enchytraeid 1	<i>Enchytraeus</i> sp., Kim	Z83750	1716
Enchytraeid 2	<i>Enchytraeus</i> sp., Aguinaldo	U95948	1713
Lumbriculidae			
<i>Lumbriculus variegatus</i>	<i>Lumbriculus variegatus</i>	AF209457	1709
Lumbriculid 2	<i>Trichodrilus diversisetosus</i>	AJ252318	480
<i>Rhynchelmis</i>	<i>Rhynchelmis shamanensis</i>	AJ308512	1705
Tubificidae			
Tubificid	<i>Tubifex</i> sp.	U67145	1712
Naidinae			
<i>Dero</i>	<i>Dero digitata</i>	AF021879	1708
Branchiobdellida			
<i>Cambarincola</i>	<i>Cambarincola holti</i>	AF115975	1712
Branchiobdellid 2	<i>Xironogiton victoriensis</i>	AF115977	1711
Hirudinea			
Hirudinea 1	<i>Erpobdella punctata</i>	AF116002	1713
Hirudinea 2	<i>Hemiclepsis marginata</i>	AF115981	1714
Acanthobdellida			
<i>Acanthobdella</i>	<i>Acanthobdella peledina</i>	AF099953	1711
Aphanoneura			
<i>Aeolosoma</i>	<i>Aeolosoma</i> sp.	Z83748	1706
Mollusca			
Bivalve	<i>Glycymeris</i> sp.	X91978	1711
Gastropod	<i>Helix aspersa</i>	X91976	1708
Polychaeta			
Polychaete 1	<i>Nereis limbata</i>	U36270	1701
Polychaete 2	<i>Lanice conchilega</i>	X79873	1710
28S			
Lumbricidae			
Lumbricid 1	Lumbricid sp.	AY048498	670
<i>Eisenia fetida</i>	<i>Eisenia fetida</i>	AY048508	670
Megascolecidae			
Megascolecinae	<i>Perionychella kershawi</i>	AY048484	670
Enchytraeidae			
Enchytraeid 1	<i>Achaeta bohemica</i>	AF406595	664
Enchytraeid 2	<i>Fridericia bisetosa</i>	AF406596	659

Table 8.1 contd

Lumbriculidae

<i>Lumbriculus variegatus</i>	<i>Lumbriculus variegatus</i>	AF406594	651
Lumbriculid 2	<i>Lamprodrilus</i> sp.	AF406592	668
<i>Rhynchelmis</i>	<i>Rhynchelmis brachycephala</i>	AF406593	668

Tubificidae

Tubificid	<i>Branchiura sowerbyi</i>	Jamieson <i>et al.</i> 2003	659
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Naidinae

<i>Dero</i>	<i>Dero aulophorus</i>	AF406598	648
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Branchiobdellida

<i>Cambarincola</i>	<i>Cambarincola pamelae</i>	AF406601	657
Branchiobdellid 2	<i>Xironodrilus formosus</i>	AF406600	663

Hirudinea

Hirudinea 1	MGLeech	Jamieson <i>et al.</i> 2003	667
Hirudinea 2	FWLeech	Jamieson <i>et al.</i> 2003	649

Acanthobdellida

<i>Acanthobdella</i>	<i>Acanthobdella peledina</i>	-	0
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Aphanoneura

<i>Aeolosoma</i>	<i>Aeolosoma</i> sp.	-	0
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Mollusca

Bivalve	<i>Mytilus edulis</i>	Z29550	666
Gastropod	<i>Chloritis anxantheri</i>	Hugall <i>et al.</i> , unpublished	658

Polychaeta

Polychaete 1	<i>Phyllodoce</i>	Hugall <i>et al.</i> , unpublished	669
Polychaete 2	<i>Amphitritides harpa</i>	AF185180	265

COI

Lumbricid 1	<i>Lumbricus terrestris</i>	U24570	651
<i>Eisenia fetida</i>	<i>Eisenia fetida</i>	-	0
Megascolecinae	<i>Pontodrilus bermudiensis</i>	AF003256	650
Enchytraeid 1	<i>Achaeta bohemica</i>	-	0
Enchytraeid 2	<i>Fridericia tuberosa</i>	AF064047	573
<i>Lumbriculus variegatus</i>	<i>Lumbriculus variegatus</i>	other	632
Lumbriculid 2	<i>Agriodrilus vermivorus</i>	AJ252319	573
<i>Rhynchelmis</i>	<i>Rhynchelmis alyonae</i>	AJ252320	573
Tubificid	<i>Tubifex tubifex</i>	U74076	651
<i>Dero</i>	<i>Dero digitata</i>	AF054195	573
<i>Cambarincola</i>	<i>Cambarincola holti</i>	AF116012	651
Branchiobdellid 2	<i>Xironogiton victoriensis</i>	AF116014	651
Hirudinea 1	<i>Hemiclepsis marginata</i>	AF003259	651
Hirudinea 2	<i>Erpobdella punctata</i>	AF003275	651
<i>Acanthobdella</i>	<i>Acanthobdella peledina</i>	AF003264	651
<i>Aeolosoma</i>	<i>Aeolosoma litorale</i>	AF054188	573
Bivalve	<i>Mytilus edulis</i>	M83762	622
Gastropod	<i>Albinaria coerulea</i>	NC_001761	648
Polychaete 1	<i>Neanthes japonica</i>	D38032	504
Polychaete 2	<i>Paralvinella palmiformis</i>	U74070	651

represented by sequences combined from more than one species where these unequivocally belonged to the same, named OTU. For instance, the megascolecine OTU combined different genes for *Pontodrilus litoralis*, *Perionychella* cf *kershawii*, and *Pheretima* sp. In contrast some OTUs denote a single species (*Lumbriculus variegatus*, *Acanthobdella peledina*, *Eisenia fetida*) or a single genus (*Rhynchelmis*, *Cambarincola*, *Dero*, *Aeolosoma*) for all three genes. A composite bivalve (*Glycymeris*, *Mytilus*), gastropod (*Helix*, *Chloritis*) and two composite polychaetes constituted the outgroup. With regard to the position of the Enchytraeidae, represented by two composite OTUs, the enchytraeid clade was the plesiomorph sister-group (at 94%) of a large crassicitellate + leech (Hirudinea + branchiobdellid + acanthobdellid + lumbriculid) clade when third position COI was excluded. The molecular analysis of Erséus *et al.* (2002) also failed to group enchytraeids exclusively with earthworms (represented by Lumbricidae), as is also the case in Fig. 3 (not shown) of Erséus (2003). In the Hugall *et al.* analysis, if enchytraeids were constrained as the sister-group of the crassicitellates, support was only 48% (Fig. 8.5A). When third position COI was included, however, the enchytraeid clade was the plesiomorph sister-group of the Crassicitellata represented by lumbricids and megascolecids (Fig. 8.5B) as suggested by Siddall *et al.* (2001) and Erséus and Källersjö (2003). However, in this case the support was at only 52% whereas if enchytraeids were constrained as the sister-group of the larger clade (including leeches and allies), support was a close 48%. In both analyses the tubificid-naid clade was the most basal in the Oligochaeta (Hugall *et al.*, unpublished) but capilloventrids were not included in the analysis.

Tubificidae and Naididae. The combined data set of Siddall *et al.* (2001) did not resolve relationships within the tubificid + naidid clade.

Phylogenetic analyses by Christensen and Theisen (1998) based upon the D3 domain of the nuclear 28S rRNA gene and part of the mitochondrial COI gene indicated that the family Naididae is a subordinate group within the family Tubificidae and is most closely related to the subfamily Rhyacodrilinae. Based upon relative genetic distances they hypothesized that the origin of Naididae occurred relatively early in the evolution of Tubificidae, though a fairly late event compared to the basic radiation among Annelida. Erséus and Källersjö (2003) and Erséus *et al.* (2000, 2002), using cladistic analysis of sequences of the 18S rDNA gene, also showed that the Naididae must be subsumed in a monophyletic Tubificidae, and are related to some "rhyacodriline" groups within the Tubificidae but further stated that "naidids" may not even constitute a monophyletic group. The family name Naididae was formally suppressed by Erséus *et al.* (2002) as a junior synonym of the Tubificidae (Application to the ICZN by Erséus and colleagues for recognition of this decision is pending as the name Naididae in fact has chronological precedence). The subfamily Naidinae, within the Tubificidae, was recognized by Erséus and Gustavsson (2002) and Erséus (2003). A tubificid+naid clade was confirmed from 28S in Jamieson *et al.*

(2003) (Fig. 8.1) and in the combined 18S, 28S, and COI analysis of Hugall *et al.* (unpublished) (Fig. 8.5).

The analyses of Erséus *et al.* (2000, 2002) corroborated the idea that the Tubificinae are monophyletic but indicated that the Rhyacodrilinae and the Phallodrilinae are not. *Bathydrilus* did not appear to be closely related to other “phallodriline” genera. Limnodriloidinae appeared monophyletic in the first study but were unresolved in the second.

Of tubificid genera tested for monophyly, several were fully supported by the data, namely *Ainudrilus*, *Bathydrilus*, *Limnodrilus*, *Smithsonidrilus*, *Thalassodrilides*. Strong (but not 100%) jackknife support was obtained for *Heterodrilus*, *Tectidrilus* and *Heronidrilus*. The gutless genera *Inanidrilus* and *Olavius* were found not to be sister-taxa but collectively formed a reasonably well supported group (85%). Only one genus (*Tubifex*) was refuted, and two (*Tubificoides* and *Limnodriloides*) were unresolved from other taxa (Erséus *et al.* 2002).

Tubifex tubifex siblings. Molecular analyses have also proved of value in determining species limits. Thus, an analysis, using mitochondrial 16S ribosomal DNA, of the relationship within the genus *Tubifex* identified six genetically distinct lineages of *T. tubifex* from North America and Europe that were separated by genetic distances comparable to those found for “well-defined” species of *Limnodrilus*. Therefore, the existence of several morphologically indistinguishable, thus cryptic, species of *Tubifex* in North America and Europe was suggested (Beauchamp *et al.* 2002).

Narapidae. This South American family (not included in the morphocladistic analysis) has only one known species, in freshwater (Righi and Varela 1983) (see also 8.2.11.2). *Narapa* resembles the enchytraeid *Achaeta* in (always?) lacking chaetae.

Phreodrilidae. The 18S gene of the phreodrilid *Insulodrilus bifidus* provided the first molecular evidence that phreodrilids are closely related to tubificids (Erséus *et al.* 2002), corroborating previous conclusions based on morphology (e.g. Jamieson 1988b). Phreodrilids, albeit represented only by a single species, formed the sister to a clade consisting of all included Tubificidae and Naididae. The combined Phreodrilidae + Tubificidae (including Naididae) formed the sister to the Lumbriculidae. Leeches and branchiobdellids were not included in the analyses of Erséus *et al.* (2002). However, in the later analysis, which included these (Erséus and Källersjö 2003), phreodrilids were the sister-taxon to a clade containing *Propappus*, *Haplotaxis*, and a monophyletic tubificid assemblage (Fig. 8.2), though support was inconsistent and may have depended on the questionable coding of gaps as fifth states.

Parvidrilidae. This family is known only from *Parvidrilus strayeri*, described by Erséus (1999) from a stream-bed in northern Alabama, U.S.A., and *Parvidrilus spelaeus* from subterranean waterbodies of southern Europe (Martinez Ansemil *et al.* 2002). *Parvidrilus* has the capilloventrid character of hair chaetae in ventral and dorsal bundles (a symplesiomorphy?). Its affinities have not been investigated in morphocladistic or molecular

analyses. The Parvadrilidae have been considered to be most closely related to two, largely Southern Hemisphere, aquatic clitellate families, Capilloventridae and Phreodrilidae, and to have possible relationship to the Narapididae (Erséus 1999). Erséus (2003) suggests that they may be the Northern Hemisphere sister group of the Phreodrilidae. Martinez *et al.* (2002) endorse the phreodrilid relationship. They consider familial ranking of parvadrilids to be sustained on the basis of three autapomorphies: the location of the chaetae in a markedly posterior position within the segments; the lateral development of the clitellum, with large clitellar cells in relation to the body diameter; and the singular glandular pouches present in the mid-dorsal line of the mesosomal segments.

Opistocystidae. This little known family contains *Dorydrilus* with a few species, in the Americas and Africa (see Stacey and Coates 1996), and *Lycodrilus*, with five species in Lake Baikal. *Lycodrilus* was considered to merit a new family, Lycodrilidae, by Chekanovskaya 1962 (see Chekanovskaya 1981) who considered the Opistocystidae to be very closely related to the Naididae.

Dorydrilidae. This monogeneric family, with three species, occurs in western Europe. They and the Opistocystidae have not been included in morphocladistic or molecular analyses. The reproductive systems are discussed in 8.2.

Lumbriculata. In the morphocladistic analysis (Jamieson 1988b), the Lumbriculata, erected (as a subclass) in that work, contained only the order Lumbriculida, family Lumbriculidae (acanthobdellids, branchiobdellids, and leeches were not included in the analysis). The name Lumbriculata is now available for the lumbriculid + branchiobdellid + acanthobdellid + hirudinid (leech) assemblage that has been confirmed from molecular investigations (Siddall *et al.* 2001; Jamieson *et al.* 2002; Erséus and Källersjö 2003; Hugall *et al.*, unpublished; see discussion above).

Lumbriculidae. The definition of the Lumbriculidae in Jamieson (1988b) included the following characters: Sperm with weak connectives. Acrosome short. Some male ducts recurved from the funnels to end in the same segment as their testes, i.e. quasiprosoporous. Often multigonadal. Nephridia intersegmentally connected. Myofilaments square in cross section. Some of these characters have been investigated in only a small number of species. For further details of the reproductive system see 8.2.

Diplotesticulata. The name Diplotesticulata was coined by Yamaguchi (1953) for all non-microdrile oligochaetes. As a grouping it corresponded with the Opisthopora *sensu lato* of Michaelsen (1928-1932) (see Michaelsen 1928) which included the non-opisthoporous family Haplotaxidae in which typically each pair of male pores is in fact plesioporous (i.e., in the segment immediately following the corresponding pair of testes) in addition to the 'megadriles' which are truly opisthoporous in having the male pores more than one segment behind the most posterior testes. The Opisthopora was retained (Jamieson 1988b) as a more restricted group, excluding haplotaxids, as in this account. The Diplotesticulata, if the Lumbriculidae are excluded,

also corresponds with the order Haplotaxida (*sensu* Jamieson 1978b, 1980). The Diplotesticulata formed a clade in the morphocladistic analysis (Jamieson 1988b) (Figs. 8.3, 8.4A,B) with or without inclusion of the Lumbriculidae. The diplotesticulate condition, connoting two pairs of testes, was recognized as a symplesiomorphy but its use was satisfactorily descriptive in view of the cohesion and monophyly of the group. The group had a single unifying apomorphy: presence of well-developed connectives from the secondary tube to the axial rod of the spermatozoon. However, the Diplotesticulata, although not specifically tested, does not appear to be supported in the molecular analysis of Erséus and Källersjö (2003) (Fig. 8.2) in which the Haplotaxidae grouped with the Propappidae.

Haplotaxidea. The superorder Haplotaxidea is redundant as it contained only the Order Haplotaxida *sensu stricto*, the latter being paraphyletic for the Haplotaxidae and the monotypic Tiguassuidae. There is a trend to loss of the posterior ovaries, a loss which is constant in *Tiguassu*. This contrasts with loss of the anterior ovaries in the Metagynophora (Jamieson 1988b).

Haplotaxidae. From a combination of classical and computer-aided methods Brinkhurst (1988) divided the Haplotaxidae into several genera: *Haplotaxis*, *Alphadrilus*, *Delaya*, *Hologynus*, *Heterochaetella*, *Pelodrilus* and *Villiersia*. *Haplotaxis* was retained for the *gordioides*-like species with specialized pharynx and chaetae associated with a predaceous habit. *Adenodrilus* was placed in *Haplotaxis* in Brinkhurst and Jamieson (1971). *Tiguassu (reginae)* was elevated to the family Tiguassidae *Metataxis (eliae) sensu* Omodeo was placed *incertae sedis* in the order Lumbricina (Brinkhurst 1988).

Haplotaxis has been regarded by many authors as a representative of a putative octogonadal ancestor of all megadriles (references in Erséus 2003), a view supported in the morphocladistic analysis. However, if the single *Haplotaxis* species included in the 18S analysis of Erséus and Källersjö (2003) was indeed the type-species, *H. gordioides*, *Haplotaxis* would appear to be a member of the tubificid clade in which it is closely allied to *Propappus* (Fig. 8.2). However, support for this relationship was weak as two of the four trees were unresolved and the other two differed from each other. Only when gaps were treated as new states was there greater than 50% jackknife support for the tubificid relationship, but this placed *Haplotaxis* in a large clade.

In the 28S rDNA analysis (Jamieson *et al.* 2002) the position of *Haplotaxis gordioides* was unresolved apart from the demonstration that it lies outside the Crassiclitellata. Paraphyly of the genus *Haplotaxis*, suggested by Gates (1972) and several later workers, was endorsed as the two *Haplotaxis* species did not group together, one forming the plesiomorph sister group of the lumbriculid through leech clade (the Lumbriculata *sensu lato*) (Fig. 8.1).

Metagynophora. The Metagynophora, then a new coinage, formed a superorder within the Diplotesticulata (Jamieson 1988b). Loss of the anterior ovaries with retention or possibly acquisition of ovaries in segment 13, so that a segment lacking gonads intervenes between the last or only testes and

the ovaries, diagnoses all megadrile oligochaetes (moniligastrids through the Megascolecidae). This condition is also present in some supposed haplotaxids. This unifying condition, the sole universal synapomorphy of its possessors, has warranted the term *Metagynophora* (Jamieson 1988b) for this assemblage. Only three species in the entire assemblage are known to have two pairs of ovaries, in segments 12 and 13. These are the almid *Glyphidrilus kukenthali*, the glossoscolecoid *Enantiodrilus borellii*, and the megascolecoid *Diplocardia sandersi* (see Jamieson 1978b, 1980). Where seen in megadriles, this rare, quadriovarian condition presumably represents a reversal to, rather than retention of, the presumed plesiomorphic condition but has an interesting implication of retention of the underlying genome, or of an epigenetic switch, for production of ovaries in both segments. The metagynophoran clade has not been tested by molecular means.

Moniligastrida. The plesiomorphic sister-group of the Opisthopora contains only the family Moniligastridae. For nomenclatural purposes these were placed (Jamieson 1988b) in an order Moniligastrida (an arbitrary ranking) coordinate with the order Opisthopora. Moniligastrids are indigenous in southeast and eastern Asia. They are terrestrial and, despite a common belief that they are amphibious, no moniligastrids are known to be aquatic or limicolous (Gates 1972). They have yet to be subjected to molecular analysis. Reproductive characters are discussed in 8.2.

Opisthopora. Nested immediately within the *Metagynophora* is the alluroidid through megascolecoid assemblage (Jamieson 1988b). The nominal apomorphy of the Opisthopora is movement of the male pores from the plesiomorphic location one segment behind the last or only testes (the plesioporous condition) to a more posterior location, commencing with segment 13 or its homeotic equivalent. The name Opisthopora was coined by Michaelsen (Nachtrag 1932 in Michaelsen 1928) for this condition though for reasons which are not clear he included the plesioporous family Haplotaxidae (= Phreoryctidae), which are excluded here and in Jamieson (1988b). In one known haplotaxid, *Haplotaxis violaceus*, the anterior vasa deferentia traverse more than one segment, discharging near the posterior pair of vasa in segment 12 but the latter ducts remain plesioporous. Remarkably, the validity of the Opisthopora has not been tested by molecular phylogenetics, as its most plesiomorphic family, the Alluroididae, has not been sequenced. However, the largest opisthopore group, the Crassicitellata, is strongly confirmed as monophyletic (Fig. 8.1, 8.2) (Jamieson *et al.* 2002; Erséus and Källersjö 2003). These, at least, exclude *Haplotaxis*.

Alluroidina. Alluroidoidea. These have opisthoporous male pores in segment 13. The Alluroidoidea of Brinkhurst and Jamieson (1971) computed in the morphocladistic analysis (Jamieson 1988b) as the plesiomorphic sister-taxon of the Crassicitellata. It contains the Syngenodrilidae Smith and Green 1919, and the Alluroididae Michaelsen 1900, neither of which has been included in molecular analyses. Their reproductive systems are discussed in 8.2.

Alluroididae. The Alluroididae have been reviewed by Brinkhurst (1964), Jamieson (1968b, 1971a) and Omodeo (1996). They occur in inland waters of equatorial Africa and South America but have also been found (Jamieson 1968b) in moist soil near streams.

Syngenorilidae. No specimens of the East African family Syngenorilidae have been found since definition of the family for *Syngenorilus lamuensis* by Smith and Green (1919). The habitat of *Syngenorilus* is unknown.

Crassiclitellata. The remainder of the Oligochaeta are unified by a striking synapomorphy, a multilayered clitellum. This group was termed (Jamieson 1988b) the Crassiclitellata. The Crassiclitellata is confirmed as a monophylum in the molecular analyses (Jamieson 2000; Jamieson *et al.* 2002; Hugall *et al.*, unpublished), a result which is not contradicted by 18S analysis (Siddall *et al.* 2001; Erséus and Källersjö 2003). Thus, at least for the taxa represented, the multilayered clitellum arose only once. It is noteworthy that in the morphocladistic analysis Crassiclitellata remained a monophyletic group even when the character pertaining to the clitellum was omitted from the analysis (Jamieson 1988b). The maximum parsimony majority rule consensus tree for 549bp of 28S only (Fig. 8.1) gives 100% bootstrap support for the Crassiclitellata versus outgroup taxa. Maximum likelihood analysis differs little from a maximum parsimony bootstrap tree.

Aquamegadrili and Terrimegadrili. Some molecular analysis was made (Jamieson 2000; Jamieson *et al.* 2002) of division of the Crassiclitellata on morphocladistic evidence into two groups, the Aquamegadrili and Terrimegadrili (Jamieson 1988b). Aquamegadrili have an aquatic or semi-aquatic mode of life, and include the families Sparganophilidae (Holarctic), Biwadrilidae (Japan), Almidae (mostly warm tropics but including *Criodrilus*, in the Mediterranean region etc.) and Lutodrilidae (Southern Neartic). It is not unlikely that the aquamegadrile families, irrespective of mono- or polyphyly of the group, have always had an aquatic or amphibious existence. The remainder of the Crassiclitellata were predominantly terrestrial, hence the term Terrimegadrili. These included the superfamilies Lumbricoidea, Eudriloidea and Megascolecidea.

The 28S analyses (Jamieson *et al.* 2002) are inconsistent with partition of crassiclitellates into Aquamegadrili and Terrimegadrili and with monophyly of the Aquamegadrili but this is entirely due to the inclusion of *Komarekiona* as sister-taxon of *Sparganophilus*. This pair was previously supported in a phenetic analysis, though in a phylogenetic analysis *Komarekiona* had the terrimegadrile *Microchaetus* as its nearest neighbor (Jamieson 1978b); it was also supported in a cladistic phylogeny by Omodeo (1998). Qiu and Bouché (1998) consider the Komarekionidae to be the junior synonym of the terricole family Ailoscolecidae, a relationship mooted by Jamieson (1978b). The representatives (*Sparganophilus*, *Criodrilus* and *Lutodrilus*) of the original aquamegadrile taxa, with *Komarekiona*, lie within a paraphyletic Terrimegadrili (Figs. 8.1 and 8.5). The superfamilies originally

constituting the Aquamegadriili may, nevertheless, be retained, though where monofamilial they are possibly redundant. Michaelsen (1932) divided what are here termed the Crassicitellata into two groups: the Lumbricina and Megascolecina. The 28S rDNA analysis (Jamieson *et al.* 2002) (Fig. 8.1) indicates a monophyletic core for each of the groups Megascolecina (Megascolecidae and Ocnerodrilidae) and Lumbricina (Sparganophilidae, Komarekionidae, Almidae (*Criodrilus lacuum*), Lutodrilidae, Hormogastridae, Lumbricidae, and Microchaetidae) but both groups are rendered paraphyletic by the successive basal taxa Glossoscolecid sp. + *Eudrilus* (Eudrilidae) and *Pontoscolex* (Glossoscolecidae).

Sparganophiloidea. This contained only the Sparganophilidae in the morphocladistic analysis (Jamieson 1978b, 1988b).

Sparganophilidae. This is a Nearctic freshwater family, with representation, possibly by introduction, in the United Kingdom and France. The type-species, *Sparganophilus tamesis* Benham (Fig. 8.40) was shown to be the senior synonym of the American *S. eiseni* Smith by Jamieson (1971c). The Komarekionidae and Ailoscolecidae were separated from the Sparganophilidae, in the Terrimegardili, in the morphocladistic analyses (Jamieson 1978b, 1988b) but the 28S rDNA analysis suggests a very close relationship of *Sparganophilus* and *Komarekiona*. Evidence for inclusion of the Ailoscolecidae with these is given in the discussion of reproductive systems in 8.2.11.4.

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. *Komarekiona* seems little advanced beyond a sparganophilid level of organization. It is therefore noteworthy that *Komaerkiona* formed a clade with *Sparganophilus*, with very high bootstrap support, in molecular analyses (Jamieson *et al.* 2002) (Figs. 8.1, 8.6). *Ailoscolex* has yet to be included in molecular analyses.

Ailoscolecidae. The single genus, *Ailoscolex*, occurs terrestrially in southwestern France. *Ailoscolex* appears to have close affinities with the family Komarekionidae (see 8.2), which was subsumed in it by Sims (1980, 1982), and with the Sparganophilidae.

Biwadriloidea. Biwadrilidae. *Criodrilus bathybates* Stepenson (synonym *C. miyashitai* Nagase and Nomura) was made the type of the monotypic *Biwadrilus*, in the subfamily Biwadrilinae, by Jamieson (1971c). As the family Biwadrilidae it was placed in a superfamily Biwadriloidea by Jamieson (1988b) which formed the plesiomorph sister-taxon of the Almidae + Lutodrilidae. *Biwadrilus* has yet to be subjected to molecular analysis that might clarify its relationships. Omodeo (2000) suggests that *Biwadrilus* (removed from *Criodrilus* by Jamieson 1971c) may belong to his resurrected Criodrilidae

Superfamily Almoidea. As constituted in the morphocladistic analysis these contained the Almidae, Lutodrilidae and tentatively the Kynotidae.

Almidae (including *Criodrilus*). The Almidae Duboscq was resurrected as the Alminae (containing *Alma*, *Callidrilus*, *Glyphidrilus*, *Drilocrius*,

Glyphidrilocrius and *Criodrilus*) within a broad Glossoscolecidae by Jamieson (1971c) who argued against placement of its species in the Microchaetidae by Gates (1972). An innovation was the inclusion of *Criodrilus*, in a tribe Criodriliini, within the Alminae. The Alminae was subsequently restored to familial rank (Jamieson 1988b). The family is aquatic, falling within the Aquamegadrili and occurs in Tropical Africa and the Nile Valley; Central and South America; India; Oriental Region. *Criodrilus* occurs in Europe and the Middle East. In the 28S rDNA analysis the sole representative, *Criodrilus lacuum* (Criodrilineae) was the sister-taxon of *Sparganophilus* + *Komarekiona* (Fig. 8.1). There is a need for inclusion of members of the Alminae, particularly *Alma*, in molecular analyses. Omodeo (2000) has restricted the Almidae to *Alma* (the other almine genera were placed by him in a family Glyphidrilidae) but it is here considered that the restricted Almidae is insufficiently distinct in comparison with the differences between other families. Furthermore, *Drilocrius alfari* (Fig. 8.38C) which resembles *Alma* in having claspers, though with male pores basal to, not apical on these, constitutes a convincing link with the Almidae *sensu lato*.

Pop *et al.* (2002) gave a maximum parsimony tree for 15 lumbricid species and *Criodrilus lacuum* based on 16S rDNA and for 15 species based on CO1 analysis, both cladograms being rooted with a mollusc species. Agreement between the two trees was low. *Criodrilus* necessarily associated with lumbricids as only these accompanied it in the analyses. They nevertheless considered that "separation [of *Criodrilus lacuum*] from the Lumbricidae to the family Criodrillidae or Glossoscolecidae (Oligochaeta) is supported by these genetic characters". Inclusion of *Alma* in an analysis will be necessary to test the present author's contention that the affinities of *C. lacuum* lie with the Almidae.

Lutodrilidae. The family Lutodrilidae is known from a single species of 'swamp worm' in Louisiana (McMahan 1976, 1979). Morphocladistic analysis suggests that the Lutodrilidae are closely related to the Almidae (Jamieson 1988b). In the 28S rDNA analysis they lie in the same clade as the almid *Criodrilus* (in the absence of *Alma* from the study) but do not show an unequivocal close relationship with this (Fig. 8.1).

Kynotidae. The Kynotidae is a monogeneric, terrestrial family with about 12 species, restricted to Madagascar. The phylogenetic position of the Kynotidae is uncertain. In morphocladistic analysis it was questionably the sister-group of the Almoidea (Jamieson 1988b) (Fig. 8.4A,B). It had been considered the sister-group of the Lumbricoidea in an intuitive phylogeny (Jamieson 1971b) and maintained this position in a previous Hennigan study (Jamieson 1978b).

Glossoscolecidae. The Glossoscolecidae forms the dominant group of earthworms in tropical South America north of the Juramento-Salado River (to the south of which acanthodrilids predominate) and much of the Caribbean. They occur in a wide range of terrestrial habitats although most species are forest dwellers. *Pontoscolex corethrurus* is circummundane in warmer regions of the world. One of the largest species (*Rhinodrilus fafner*)

has been reported to reach over 2 m in length and 2.5 cm in diameter (Sims 1982).

The tribe Glossoscolecini *sensu* (Jamieson 1971c) is usually, as here, given familial rank (Righi, in many papers; Gates 1972; Sims 1980, 1982; Jamieson 1988b; Jamieson *et al.* 2002). The family appeared paraphyletic in molecular analyses (Jamieson 2000; Jamieson *et al.* 2002) (Fig. 8.1) and included *Eudrilus* but this relationship requires further testing. Samuel James (pers. com.) has independently noted the similarity of *Eudrilus* to glossoscolecids in morphological studies.

Eudrilidae. Eudrilids are restricted to Africa (Ethiopian Region) as natives. One species, *Eudrilus eugeniae*, has become widely distributed around the warmer parts of the world and is cultured as the 'African Night Crawler'. The male pores of eudrilids lie in segment 17, as is also typical of Ocnodrilidae, with which they have been considered to be closely related. Molecular studies do not support this relationship but suggest glossoscolecoid affinities (Jamieson 2000; Jamieson *et al.* 2002). The Eudrilidae has formerly been associated with the Megascolecidae and Ocnodrilidae in a superfamily Megascolecoida (Jamieson 1978b) or in a separate subfamily Eudriloidea as the sister-group of the Lumbricoidea + Megascolecoida *sensu stricto*, in the morphocladistic analysis (Jamieson 1988b) (Figs. 8.3, 8.4 A,B). In the molecular analyses (Jamieson 2000; Jamieson *et al.* 2002) (Figs. 8.1, 8.6), *Eudrilus* always has a glossoscolecoid sp. (a lumbricoid *sensu* Jamieson 1978b) as its sister-taxon, with moderate bootstrap support. The other glossoscolecoid exemplar, *Pontoscolex*, may or may not link with these. There is no molecular support in these analyses for regarding eudrilids as the unique sister-group of the Ocnodrilidae + Megascolecidae assemblage. The molecular placement of the Eudrilidae with the Glossoscolecidae may be correct but it requires further investigation as there are morphological similarities between this family and the Ocnodrilidae with which both a cladistic and phenetic analysis linked them in a previous study (Jamieson 1978b); furthermore, ocnodrilid and eudrilid interchaetal ratios are closely, and uniquely, similar (Jamieson unpublished). Omodeo (2000) recognized the Eudriloidea (as did Jamieson 1988b); he derived eudrilids independently (from alluroidids), thus also noting their distinctness, but that origin goes contrary to the present confirmation of the monophyletic nature of the Crassicitellata.

Lumbricoidea. The Lumbricoidea as defined by Jamieson (1978b, 1988b) included the Lumbricidae (Holarctic), Glossoscolecidae (Neotropical), Microchaetidae (Ethiopian, south of the Kalahari), Hormogastridae (Western Palaearctic, Tyrrhenian), Komarekionidae (Nearctic), and Ailoscolecidae (Palaearctic), of which the latter two families are here placed in the Sparganophiloidea. Relationships of some of these families and the validity of the grouping Lumbricoidea were investigated from molecular sequences by Jamieson (2000) and Jamieson *et al.* (2002) (Figs. 8.1, 8.6).

The Lumbricoidea is incompatible with the 28S data (Fig. 8.1) or combined gene data (Fig. 8.6) but principally because the glossoscolecoid sp.

never groups with the remainder of the lumbricoids in the best trees but groups equivocally with *Eudrilus* which is here tentatively placed in the resurrected superfamily Glossoscolecoidae. However, testing the unity of the majority of the remaining Lumbricoidea will necessarily require more sampling (Jamieson *et al.* 2002). In these molecular analyses the Lumbricoidea is a paraphyletic clade, though with considerable monophyly (*Sparganophilus*, *Komarekiona*, *Criodrilus*, *Lutodrilus*, *Hormogaster*, lumbricids, and the microchaetid forming a clade). This paraphyletic lumbricoid assemblage also contains the Ocnodrilidae and Megascolecoidae in addition to the Eudrilidae.

In the revised classification (Table 8.2) the Lumbricoidea *sensu* Jamieson (1988b) is provisionally revived for the non-megascolecoid families, with the exception that the Eudriiloidea is tentatively subsumed in the Glossoscolecoidae, as indicated by the molecular analyses. In Table 8.2, lumbricoid families are listed in the order that they occur in the DNA analyses (Figs. 8.1, 8.3).

Microchaetidae. Microchaetidae are restricted to South Africa and include the largest known earthworms, *Microchaetus microchaetus*, with a maximum reported length of 7 m and diameter of 7.5 cm. Molecular analysis (Jamieson 2000; Jamieson *et al.* 2002) (Fig. 8.1) indicate that microchaetids lie in a different clade from the Glossoscolecidae in which they were formerly placed as a tribe (Jamieson 1971c). With the Hormogastridae they occupy a sparganophiloid + almoid + lumbricoid clade (Fig. 8.1).

Lumbricidae. Lumbricids are native in the Holarctic, chiefly in Europe. From the 28S analysis they share a clade with the Sparganophilidae, Komarekionidae, *Criodrilus lacuum* (an atypical exemplar for the Almidae), Lutodrilidae and Hormogastridae (Fig. 8.1).

Hormogastridae. Hormogastrids are known from two subfamilies that occur mainly around the western Mediterranean. Molecular analyses (Jamieson 2000; Jamieson *et al.* 2002) (Fig. 8.1) indicate that hormogastrids lie in a different clade from the Glossoscolecidae in which they were formerly placed as a subfamily (Jamieson 1971c). They show somewhat unresolved relationships with the Sparganophilidae, Komarekionidae, Almidae (*Criodrilus*), Lutodrilidae and Lumbricidae on molecular grounds (Fig. 8.1). Hormogastrids were considered the sister taxon of the Lumbricidae by Rota and Omodeo (1992) and Omodeo (2000).

Tumakidae. In the Colombian family Tumakidae, monotypic for *Tumak hammeni* Righi 1995, the calciferous glands closely resemble those of some Glossoscolecidae but the gizzard instead of being preclitellar, is postclitellar, in segment 25. As noted by Omodeo (2000), in accordance with the 'rule' of Bouché (1972) the gizzard thus lies outside the clitellar zone. The calciferous gland, at 20/21, lies within the posterior limit of the clitellum. *Tumak* differs from the Glossoscolecidae in the posterior and reverse position of its calciferous glands and gizzard, intraparietal male ducts and presence of dorsal pores. Despite these differences, it seems likely that *Tumak* originated

Table 8.2. A revised, simplified classification of the Clitellata

Clitellata Michaelsen 1919

- ☒ Eoclitellata¹ - Capilloventridae Harman and Loden 1984^{DNA}
- ☒ Neoclitellata² - all other Clitellata
- ✧ **Unnamed clade, here unresolved**
 - ⊙ **Randiellata?** Jamieson 1988b
 - Randiellidae Erséus and Strehlow 1986
 - ⊙ **Tubificata** Jamieson 1988b
 - Tubificidae Vejdovsky 1884^{DNA}
 - (including Naidinae Ehrenberg 1831^{DNA})
 - Narapidae? Righi 1983
 - Opistocystidae?
 - Dorydrilidae? Cook 1971
 - Parvidrilidae Erséus 1999
 - Phreodrilidae Beddard 1891^{DNA}
 - Propappidae Coates 1986^{DNA}
 - Haplotaxidae? Michaelsen 1900^{DNA}
 - Tiguassuidae? Brinkhurst 1988
- ✧ **Unnamed clade**
 - **Lumbriculata** Jamieson 1988b
 - ☐ Lumbriculidae Vejdovsky 1884^{DNA}
 - ☐ **Unnamed clade**
 - “ **Branchiobdellida** Odier 1823^{DNA3}
 - “ **Hirudinea** Lamarck 1818^{DNA}
 - Acanthobdellida** Livanow 1905^{DNA}
 - Hirudinida sensu Siddall (Euhirudinea** Lukin 1956)
 - **Unnamed clade**
 - ◆ Enchytraeidae Vejdovsky 1879^{DNA4}
 - ◆ **Metagnophora** Jamieson 1988b
 - ⌘ Moniligastridae Claus 1880
 - ⌘ **Opisthophora** Michaelsen 1932
 - ☆ **Alluroidina** Jamieson 1978b
 - Alluroididae Michaelsen 1900
 - Syngnodrilidae Smith and Green 1919
 - ☆ **Crassiclitellata** Jamieson 1988^{DNA}
 - * **Paraphyetic, requiring resolution**
 - Biwadriloidea** Jamieson 1978b
 - Biwadrilidae Jamieson 1971
 - Glossoscolecoida** Emend.
 - Glossoscolecidae Michaelsen 1900^{DNA}
 - Eudrilidae Claus 1880^{DNA?}
 - Tumakidae Righi 1995
 - Sparganophiloidea** Jamieson 1978b⁵
 - Ailoscolecidae Bouché 1969
 - Komarekionidae Gates 1974^{DNA}
 - Sparganophilidae Michaelsen 1918^{DNA}
 - Lumbricoidea** Gates 1976. Emend.
 - Microchaetidae Michaelsen 1900^{DNA}
 - Lumbricidae Claus 1876^{DNA}

Table 8.2 cont'd

	Kynotidae? Jamieson 1971
	Hormogastridae Michaelsen 1900 ^{DNA}
	Almoidea Jamieson 1978b ⁶
	Lutodrilidae McMahan 1978 ^{DNA}
	Almidae Duboscq 1902 (including <i>Criodrilus</i> ^{DNA})
*	Megascolecoidea Jamieson 1978b
	Ocnerodrilidae Beddard 1891 ^{DNA}
	Megascolecidae Rosa 1891 ^{DNA}
	Acanthodrilinae Vajdovsky 1884 ^{DNA}
	Megascolecinae Rosa 1891 ^{DNA}

¹Eoclitellata, new taxon, is defined as Clitellata with capillary setae in the dorsal and ventral bundles. Atria, prostates and penes are absent

²Neoclitellata, new taxon, is defined as Clitellata lacking capillary setae in the ventral bundles or if these are present (Parvidrilidae) they are markedly anterior in their segments, a peculiar lateral clitellum is present and glandular pouches are present in the mesosomal segments.

³Whether the Acanthobdellida or the Branchiobdellida is nearest to Hirudinida is equivocal from molecular evidence. Neither shows the anterior extension of the acrosome tube diagnostic of Hirudinida. The single mitochondrion is here considered a clear synapomorphy of Acanthobdellida and Hirudinida, contrasting with the plesiomorphic multiple mitochondria of the sperm of branchiobdellids and oligochaetes *sensu stricto*. However, the retention of some setae in in *Acanthobdella* but not in branchiobdellids is plesiomorphic.

⁴The phylogenetic position of the Enchytraeidae is particularly controversial (see text).

⁵The close grouping of the *Sparganophilus* and *Komarekiona* in the molecular analyses prompts extension of the subfamily to include the Komarekionidae and, because of its morphological resemblance to the latter, the Ailoscolecidae.

⁶Only *Lutodrilus* and *Criodrilus* were included in the molecular analysis. They lay within the 'lumbricoid' clade and were paraphyletic relative to each other. It is nevertheless envisaged that the Lutodrilidae and Almidae are closely related. The position of *Criodrilus*, which grouped with *Alma* in the morphocladistic analysis (Jamieson 1988b) remains controversial.

within the Glossoscolecidae or, at most, is its sister-taxon and separate familial rank is questionable.

Ocnerodriloidea and Megascolecoidea. The families Megascolecidae, Ocnerodrilidae and Eudrilidae were tentatively included in the superfamily Megascolecoidea in Jamieson (1978b, 1980). However, in the morphocladistic analysis (Jamieson 1988b) (Fig. 8.6A,B), the Eudrilidae (superfamily Eudriloidea) and especially the Ocnerodrilidae (superfamily Ocnerodriloidea) occupied a basal position relative to the other terrimegadrile families. Molecular data (Jamieson 2000; Jamieson *et al.* 2002) do not support this distant position for ocnerodrilids but reunite them with the Megascolecidae (Figs. 8.1, 8.6). As we have seen, the single eudrilid included in the molecular analysis associates with the Glossoscolecidae and is not confirmed as a sister taxon of the Ocnerodrilidae + Megascolecidae.

Megascolecidae. The largest, most speciose, earthworm family is the Megascolecidae for which a Pangean origin has been suggested (Jamieson 1981a). They are native in the Nearctic, Ethiopian, Oriental, Australian, eastern Palaearctic (China, Japan, Korea) and southern Neotropical Regions,

Colour Figure

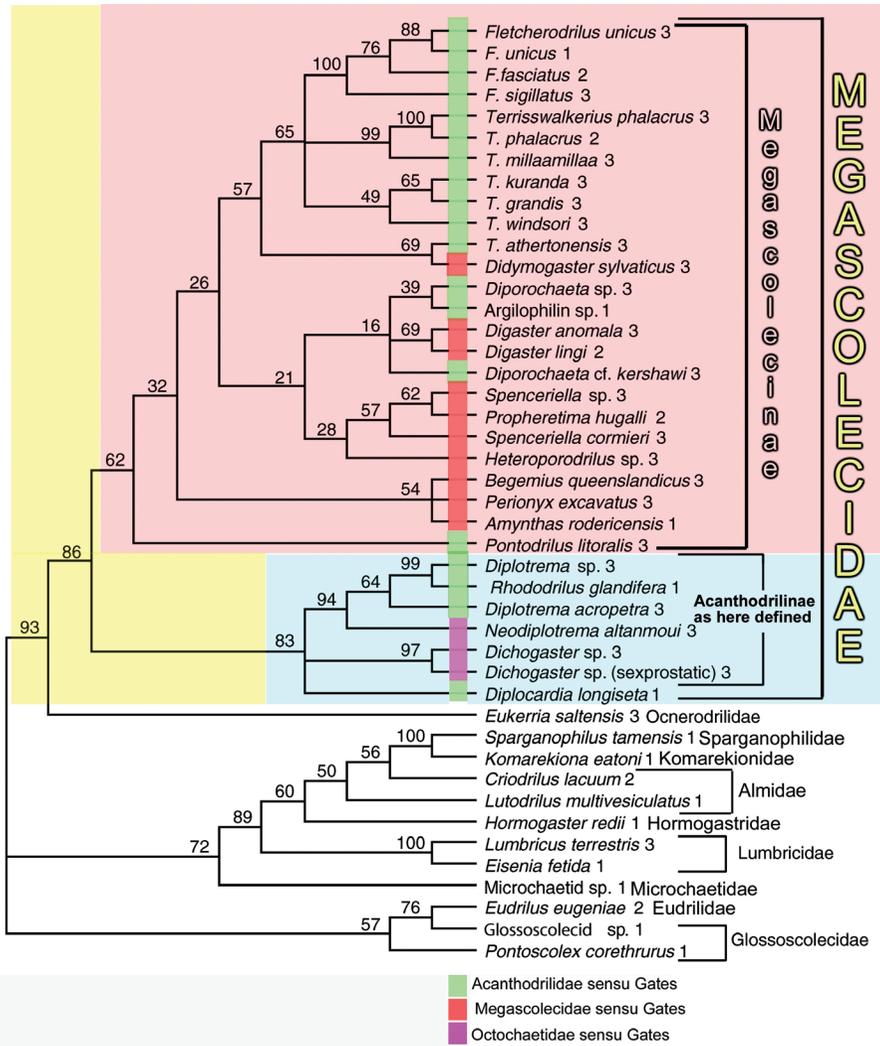


Fig. 8.6. Strict consensus of six maximum parsimony trees using the combined data. Taxa with one, two or three genes indicated. Heuristic search, 1371 sites unweighted 50 random additions tree bisection reconnection with steepest decent, with bootstrap consensus values from 500 resamplings (heuristic search, unweighted, five random additions, tree bisection reconnection with steepest descent). 1, 28S (12S only for *Rhododrilus glandifera* Jamieson 1995); 2, 28S + 12S; 3, 28S + 12S + 16S. The narrow colored column indicates the attribution of species which would conform with the classification of Gates (1959, 1972); note the extreme polyphyly of the Acanthodrilidae and Megascolecidae *sensu* Gates. The Octochaetidae *sensu* Gates represented (with two or three pairs of prostates) are poly- or paraphyletic and lie within the Acanthodrilinae *sensu* Jamieson. Other 'octochaetids' differing in having megascolecine male pores (not included in this analysis, such as *Celeriella*) would not be expected to group within the Acanthodrilinae. Redrawn after Jamieson *et al.* 2002 *Zoosystema* 24(4): 707-734, Fig. 3. © Publications Scientifiques du Muséum national d'Histoire naturelle, Paris.

with Central America. In S. America North of the Juramento-Salado River the large family Glossoscolecidae largely replaces the Megascolecidae. In the Ethiopian Region, particularly in Tropical West and East Africa, the family Eudrilidae greatly exceeds megascolecids in numbers of genera. Currently recognized subfamilies of the Megascolecidae are the Acanthodrilinae and Megascolecinae, with or (as supported here) without the Ocnerodrilinae. Megascolecidae *sensu stricto* differ from the Ocnerodrilidae in that calciferous glands, if present, are not in, or are not restricted to, segments 9 and 10; their reproductive systems are discussed in Section 8.2. Indigenous acanthodriles are predominant in the earthworm faunas of the southern and eastern portions of North America, Mexico, Guatemala, southern S. America, S. Africa, New Zealand, New Caledonia, and parts of Australia (especially the Northern Territory and Queensland). The native range of the Ocnerodrilidae includes the warmer parts of N. and S. America, the Dominican Republic, Africa, India and Burma (Jamieson 1981a).

Several, sometimes widely divergent classifications of megascolecoid earthworms have been proposed since publication of Stephenson's monograph (1930) of the Oligochaeta (see Michaelsen 1928-1932; Pickford 1937; Omodeo 1958; Gates 1959; Lee 1959; Sims 1966, 1967; Jamieson 1971d, 1971e, 1971f, 1978b, 1988b). Particular attention has been paid to the system of Gates (1959), supported by Sims (1966, 1967), and the alternative classification of Jamieson (1971d, e, f) as both of these systems are widely used. Detailed discussion of the alternative classifications may be found in the 1971 papers.

Analysis of 28s rDNA (Fig. 8.1), complemented by 12s and 16s mitochondrial rDNA (Fig. 8.6), of megascolecoid oligochaete sequences, has been performed using maximum parsimony, maximum likelihood, bootstrap and neighbor joining procedures, with one (Lumbricidae) to nine non-megascolecoid families as the outgroup (Jamieson 2000; Jamieson *et al.* 2002). The widely used classification by Gates (1959) of the non-ocnerodriline Megascolecidae into three groups (Acanthodrilidae, with tubular prostates and holonephridia; Octochaetidae, with tubular prostates and meronephridia; and Megascolecidae, with racemose prostates and holo- or mero-nephry) was not supported by the molecular data. Thus the Octochaetidae and Acanthodrilidae, so defined, were not endorsed as *Neodiploptrema* (Octochaetidae *sensu* Gates) paired unequivocally with *Diploptrema* (a true acanthodriline), and *Dichogaster* (Octochaetidae *sensu* Gates) grouped with these. Diagnosis of Megascolecidae by racemose prostates is invalid, as *Amyntas*, *Begemius*, *Didymogaster*, *Digaster*, *Heteroporodrilus*, *Prophetitima*, *Spenceriella*, and *Perionyx* (all with racemose prostates and Megascolecidae *sensu* Gates) did not collectively form an exclusive monophylum. The Ocnerodrilidae, represented by *Eukerria*, was sister-group of the Acanthodrilinae + Megascolecinae, and joint monophyly of the three taxa was supported (Figs. 8.1, 8.6, 8.7). If the Ocnerodrilidae are regarded as a family, the Acanthodrilinae and Megascolecinae are subfamilial in the Megascolecidae.

Although Gates' (1959) classification was not supported, the alternative classification of Jamieson (1971d, e, f) also required modification in the light of the molecular study. Thus subdivision of the Megascolecinae into the tribes Perionychini, Dichogastrini, and Megascolecini (long suspected of being grades rather than clades) did not have molecular support (Figs. 8.1, 8.6, 8.7). The Perionychini proved to be a para- and polyphyletic assemblage. The

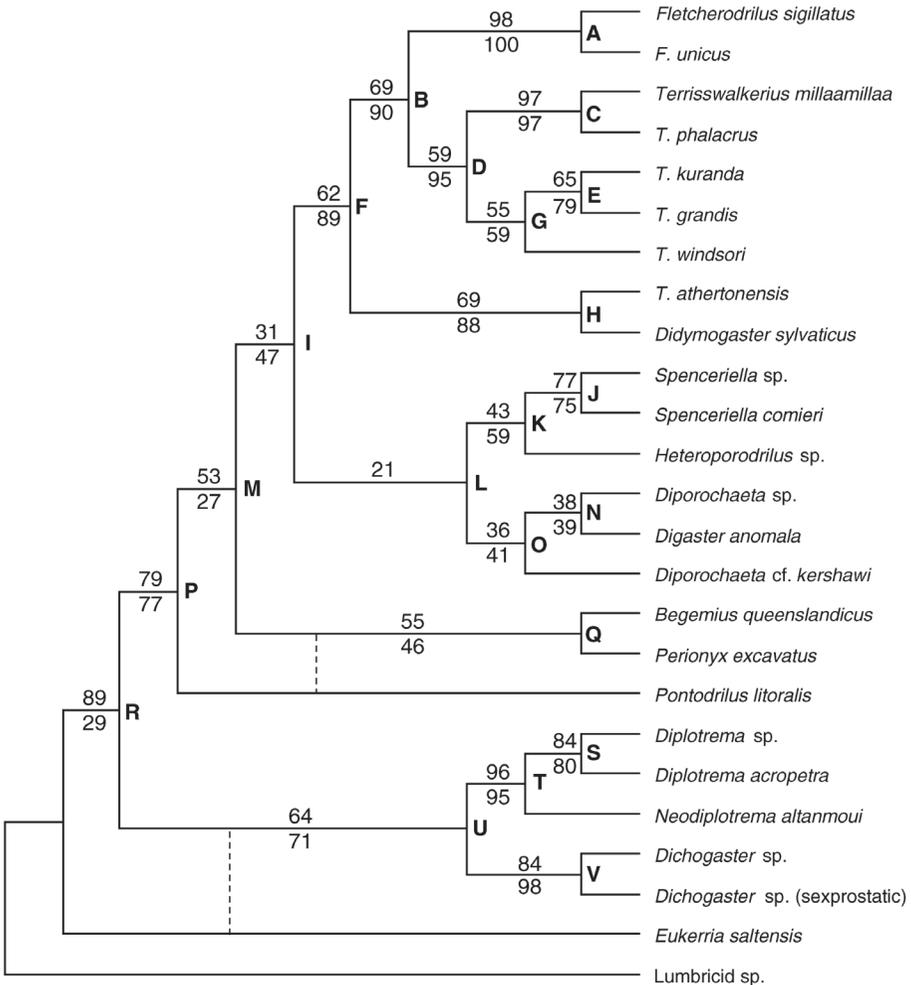


Fig. 8.7. Maximum Likelihood tree from the sum of the 28S rDNA and mtDNA partitions optimized for GTR-G model separately (SUM model best tree). Selected from 14586 near parsimonious reverse constraints trees. The Maximum Parsimony bootstrap tree is the same. Dashed lines indicate the two topology differences found in the combined data model Maximum Likelihood tree (COMBO model). Bold letters are clade labels for Table 4 in Jamieson *et al.* 2002. Maximum Parsimony and Maximum Likelihood bootstrap values above and below branches respectively. Partition support shown in Table 4. From Jamieson, B. G. M. *et al.* 2002. *Zoosystema* 24(4): 707-734, Fig. 4. © Publications Scientifiques du Muséum national d'Histoire naturelle, Paris.

Dichogastrini were disbanded as *Dichogaster* associated with the Acanthodrilinae. Maximum likelihood testing confirmed non-monophyly of the Dichogastrini. This confirmed the observation from an earlier cladistic study (Jamieson 1978b) that "the diphyletic origin of the Dichogastrini emerges clearly." It is uncertain that the residue of the Dichogastrini, represented by *Digaster* and *Didymogaster*, with male pores on segment 18, is monophyletic. Jamieson (2000) referred to them as Megascolecinae with the notoscolecine arrangement of nephridia (the *Notoscolex*-group). The Megascolecini (defined by meronephridia differing from those of notoscolecins) were not found to be monophyletic. Recognition of further higher groupings and confirmation of genera awaits a more detailed survey.

8.1.5 Spermatozoal Phylogeny

Jamieson (1983a) compared spermatozoal ultrastructure of a range of oligochaetes with a holomorphological phylogeny of the group. Plesiomorph characters for the oligochaete spermatozoon were proposed. The chief trends from these plesiomorphies were deduced to have been elongation of the acrosome and its tube; withdrawal of the acrosome vesicle and the axial rod into the acrosome tube and development of a knob-like tip on the rod (capitulum); development of connectives from the secondary tube to the axial rod (though some possibility was noted that the reverse, absence of connectives, is plesiomorph); detorting and shortening of the midpiece (or possibly, again, the reverse) with an increase in numbers of mitochondria from the plesiomorph four to eight or a reduction to two (though capilloventrids suggest that the multiple mitochondria might be plesiomorphic); modification of the base of the tube to form a limen of variable form; and, in one line (lumbricids) flattening of the tip of the nucleus and correspondingly of the limen. Sperm ultrastructure, examined for 9 oligochaete families, corresponded well with taxonomic and phylogenetic groupings. However, convergent similarity of the phreodrilid sperm to that of the Lumbricina suggested a corresponding alteration of fertilization biology in the phreodrilids. The results indicated that the Haplotaxidae were the plesiomorph sister-group of the opisthopores, though they did not unequivocally contraindicate acceptance of a *Haplotaxis*-like form as a stem form of the Haplotaxida (opisthopores and Haplotaxidae) and Tubificida (Jamieson 1983a).

In a parsimony analysis of spermatozoal ultrastructure, Jamieson *et al.* (1987) drew the following conclusions as to relationships within oligochaetes. The Haplotaxida (*Haplotaxis* through Megascolecidae) were found to form a discrete monophyletic group with inclusion, however, of the reproductively exceptional tubificidan *Phreodrilus*. *Bythonomus*, representing the Lumbriculidae, the phylogenetic position of which had been so controversial, formed the plesiomorphic sister group of the Haplotaxida, a position which was supported from other evidence. The Tubificida, represented by two tubificids (*Rhizodrilus* and *Limnodriloides*) and by the enchytraeid *Lumbricillus*, appeared plesiomorphic relative to the

lumbriculid + haplotaxid assemblage and to lie at the base of the tree, but all three appeared mutually paraphyletic. Monophyly of the Tubificida could not, however, be considered conclusively refuted from the small sample used. *Lumbricillus* (Enchytraeidae) appeared to have the most plesiomorphic sperm in the investigated oligochaetes. The Megascolecidae (*Amyntas* and *Fletcherodrilus*) formed the highest affinity and most apomorphic group. The implications of relative apomorphy of the Lumbriculida (*Bythonomus*) were considered profound. It was stated that 'Branchiobdellids and leeches are generally regarded as sharing a common ancestry with lumbriculids and would, because of the revised position of the latter, cladistically constitute part of the Oligochaeta *sensu lato*' (Jamieson *et al.* 1987).

A major example of the utility of spermatozoal ultrastructure for testing phylogenetic relationships, in this case at the species level, is that for *Enchytraeus* by Westheide *et al.* (1991). Species of *Enchytraeus* are morphologically rather similar; they are difficult to distinguish or even inseparable on conventional light microscopic methods. In individuals from 19 populations of different origin and in some cases unknown species identity the ultrastructure of mature spermatozoa was investigated. Their morphometric and qualitative data were used as morphologic taxonomic characters. With the exception of a genetically polymorphic field population of *E. albidus*, the material investigated came from genetically monomorphic laboratory inbred strains. The structure of the thread-like spermatozoa generally conformed with the sperm type known for the family. Numerical characters used were 1) length of the acrosome, 2) length of the corkscrew-like nucleus, 3) number of helical spires of the nuclear flange, 4) periodicity of the flange spires proximally and distally, 5) angle of the flange to the longitudinal axis of the nucleus, 6) length of the midpiece, 7) number of the mitochondrial helical spires, and 8) length of the flagellum. Besides the shape of the nucleus, the complex structure of the acrosome provided further specific qualitative differences such as, length ratio of primary acrosome vesicle to the entire acrosome, extension of a subdistal electron-dense material, and absence or presence of specific structures underneath the primary acrosome vesicle. Eight populations showed highly significant differences from each other and from the remaining populations on the basis of morphometric data alone. In addition, these populations—recognizable as different species on conventional methods—could also be discriminated by qualitative differences of their spermatozoa. The spermatozoa of 10 other populations appeared to be highly similar on the length of their nuclei. Number of nuclear spires, length of acrosome, flange spire periodicity and especially structural details of the acrosome, however, separated these 10 populations into two significantly different groups, one with six species and the other with four. The group of four, which had also been recognized in parallel, independently conducted non-morphological investigations, was considered to belong to one species. Certain morphological data divided the group of six into two groups of three, as was also supported by non-morphological methods and crossbreeding experiments—both groups being distinct species. Statistical

comparisons generally showed no or only slight variability of morphometric data for sperm of one individual. The spermatozoal variability between individuals of genetically monomorphic laboratory cultures was higher but in no case did it prevent species identification. The greatest intraspecific variation was observed in an *E. albidus* field population: spermatozoa of individuals differing in the pattern of single enzymes deviated significantly in their morphometric data, and on the basis of these data alone they were inseparable from spermatozoa of certain other species. However, qualitative spermatozoal characters were not affected and species could easily be discriminated on the basis of acrosomal structure (Westheide *et al.* 1991).

The influence of patterns of spermatozoal ultrastructure on hypotheses of phylogenetic relationships within the Tubificidae was examined by Erséus and Ferraguti (1995) for species representing 15 different genera. A parsimony analysis of a combination of spermatozoal and conventional morphological characters supported that the Phallodrilinae, Limnodriloidinae and Tubificinae are monophyletic taxa, and that the Rhyacodrilinae as currently defined is a paraphyletic group (Erséus and Ferraguti 1995).

The sperm ultrastructure of three Naididae (*Paranais frici*, *P. litoralis* and *Stylaria lacustris*) and two Lumbriculidae (*Rhynchelmis brachycephala* and *R. alyonae*) was described to supply spermatological evidence towards a better understanding of the phylogeny of the two families. Naidid spermatozoa were found to be similar to one another, particularly with regard to the acrosome (with the acrosome tube bent to one side), and the shape and number of mitochondria. The various morphological characters did not militate against the previously proposed inclusion of naidids within the Tubificidae. The two lumbriculid species examined showed a characteristic conical indentation at the apex of the nucleus, a feature formerly described only in Branchiobdellida, recognized as having possible affinities with Lumbriculidae (Ferraguti *et al.* 1999).

Presence of an acrosome tube appears to be distinctive of clitellates (Jamieson 1981c). A structure resembling an acrosome tube was recognized in vestimentiferan pogonophorans by Jamieson (1987b); however, this has since been found to differ from that of the oligochaete sperm in having continuity with the nucleus although it does not show nuclear (DAPI) staining (Ferraguti and Bright, pers. comm.). Features of oligochaete sperm shared with onychophorans (see Jamieson 1986) are: a cylindrical midpiece interpolated between the basal body and the nucleus; presence of subacrosomal material possibly homologous with an acrosome tube; location of the sole, distal, centriole (basal body) behind the midpiece; and absence of a proximal centriole at maturity. However, an onychophoran-clitellate sister-group relationship is not widely supported.

8.1.6 Revised Classification of the Clitellata

The following revised classification of the Clitellata, with particular reference to oligochaetous clitellates is based chiefly on the morphocladistic

classifications (Jamieson 1978b,1988b) and the classification of Brinkhurst and Jamieson (1971), modified according to molecular analyses discussed above. Sister-groups are indicated by similar symbols.

8.2 ANATOMY AND EVOLUTION OF THE OLIGOCHAETE REPRODUCTIVE SYSTEM

Before considering the anatomy of the reproductive system of individual families, some discussion of shared features, such as the gonoducts, clitellum and male pores will be given.

8.2.1 Gonoducts

Oligochaete gonoducts are coelomoducts but otherwise the genital ducts and associated glands in oligochaetes offer no clear homologies with those of polychaetes (see Westheide 1988). Retention of separate coelomoducts and metanephridia, universal for oligochaetes, has been reported only in capitellid and some nereidid polychaetes. Oligochaete features of few gonadal segments (basically apparently two testicular followed by two ovarian segments), and presence of spermathecae and a clitellum are seen in questid polychaetes. From a consideration of spermatozoal ultrastructure resemblances between questids and oligochaetes were considered to be homoplastic convergences (Jamieson 1983b) and this is confirmed from analysis of 18S sequences (Erséus *et al.* 2002; see also Siddall *et al.* pers. comm. 8.1.1).

Oligochaetes are hermaphroditic but some reports of protandry exist. A tubificid, *Mitinokuidrilus excavatus*, has been described in which the sexes are separate, though whether this condition represents consecutive hermaphroditism is uncertain; 'male' worms have testes, seminal vesicles, male ducts and small spermathecae; 'female' individuals possess clitellum, ovaries, ovisac, female ducts and fully developed spermathecae. No mature worms with intermediate sexual condition were observed (Takashima and Mawatari Shunsuke 1998).

8.2.2 The Clitellum

The structure of the oligochaete clitellum was investigated by light microscopy by several workers from 1890 to 1980; for reviews, particularly of its ultrastructure and function, see Fernandez and Benito (1987); Jamieson (1981c, 1988a,b, 1992) and Welsch *et al.* (1984). The structure of the clitellum in several crassicitellate families is illustrated by light microscopy in Brinkhurst and Jamieson (1971).

The clitellum is a specialization of the epidermis for secreting the cocoon in which the eggs are deposited and into which spermatozoa, received into spermathecae from the partner, are extruded to bring about fertilization. Some eudrilids are exceptional in fertilizing the eggs internally before they are shed into the cocoon, entry of sperm from the spermatheca into the ovarian system being demonstrated (Jamieson 1958, 1967; Sims 1967). The clitellum is one

cell thick in all microdriles and in the Haplotaxidae, Moniligastridae and Allurodidae but in all other oligochaetes, comprising the Crassiclitellata, as the name suggests, the clitellum consists of more than one layer of cells. The single-layered condition of the moniligastrid clitellum is confirmed under that family below (Fig. 8.30).

The crassiclitellate condition is interpreted as a modification allowing increased secretion of nutrient materials into the cocoon, a change correlated with a great reduction in both yolk content and size of the eggs and increasing dependence of the embryo for its nutrition on the fluid contained in the cocoon (albumenotrophy) (Jamieson 2000). In megascolecids, where there are usually one or more spermathecal diverticula, the albumen received into the cocoon from the clitellum is presumably augmented from the contents of the spermathecal atrium, the allosperm usually entering from the diverticulum.

Our knowledge of the products and function of the clitellar secretions has recently been considerably augmented. In the clitella of *Eisenia fetida*, the amount of total proteins decreases but the level of soluble proteins increases during puberty. In the cocoons, an increase of total proteins and soluble proteins correlates with the development of the embryos. Among the soluble proteins of the mature clitellum, three major proteins (A, B and C) have been separated by electrophoresis: A, glycolipoprotein (molecular weight = 450 kDa), B, glycoprotein (MW = 350 kDa) and C, also glycoprotein (MW = 150 kDa). Only A was present in the cocoons at all stages of development. Although this protein is not provided by the oocyte, it has the same characteristics as a typical vitellogenin. After experimental denaturation of the soluble proteins, four polypeptides were obtained. Two of them, a and c, are involved in the making of this 'vitellogenin' (Rouabah Sadaoui and Marcel 1995).

With regard to the glucidic and lipidic components of the clitellar epithelium and the cocoon albumen in *Eisenia fetida* it has been shown that in the clitellum, the sugar concentration increases greatly (about 5-fold) during differentiation. Several monosaccharides increase in quantity during puberty. In addition, glucose is prominent in immature clitella, whereas mannose is the main sugar in mature clitella. About 50% of the dry weight of recently deposited cocoons is carbohydrate. The sugar concentration decreases in the albumen as hatching approached. The amount of total lipids in the clitellum increases during maturation. The levels of neutral lipids, however, varies very little. Phospholipids begin at low levels at the beginning of differentiation, and increased dramatically thereafter. The amount of fatty acids in the clitellar epithelium reached a maximum during the submature stage. Large amounts of fatty acids are stored in the cocoon. Palmitic, stearic, oleic and vaccenic acids preponderate in both the clitellum and cocoon (Rouabah Sadaoui and Marcel 1995b).

The reproductive innovation of crassiclitellates allows juveniles to hatch when they have attained a size and energy resources suitable for the terrestrial environment. The size of worms newly hatched from the cocoon is

said to be proportional to the number of clitellar segments involved in secreting the cocoon but also to the amount of yolk (Omodeo and Magaldi 1951, cited in Omodeo 1998).

In some species of lumbricids at least, the cocoons are deposited in cocoon chambers composed of faeces from the maternal earthworm; the principal importance of the chambers lies in protection against loss of water but it is suggested that the chambers also supply the cocoons with nutritive fluid and that they are a means of incubation care (Ramisch and Graff 1985).

The oligochaete clitellum commonly occupies only a small number of segments in megadriles and as few as two segments in microdriles. The greatest development of the clitellum, in terms of length, occurs in the Almoidea and particularly in the Almidae. In this family, in the genus *Alma*, the number of clitellar segments varies from 20 to 69. *Alma*, like other almidoids, is also unusual in the posterior location of the clitellum. This is correlated with the post-testicular position of the spermathecae as in oligochaetes the cocoon, after secretion by the clitellum effectively moves forwards to receive sperm from the spermathecae. In *Alma* the anterior border of the clitellum varies from segment 35 to 247 and the posterior border from segment 49 to 295. In *Criodrilus* the clitellum begins from segments 14 to 16 and extends for 30 to 34 segments. In the Microchaetidae, the clitellum is also long, beginning on segment 11 to 14 and occupying as many as 44 segments though sometimes a more modest six segments; and in *Hormogaster* it begins on segment 13 or 14 and extends to segment 24 or as far as 32. The oligochaete clitellum is always behind, or includes, the female pore(s), as the cocoon has also to receive the ova in its forward movement.

In the Glossoscolecidae the male pores are intraclitellar, as in *Pontoscolex corethrurus*, or (*Opisthodrilus*) postclitellar, they are also intraclitellar, near the anterior border of the clitellum, in the Microchaetidae, Hormogastridae and Almidae, including *Criodrilus*, though anteclitellar in some almidids. In *Biwadrilus bathybates*, the male pores are immediately anteclitellar, relative to a clitellum of approximately 20 segments. The shorter clitellum is invariably well anterior to the male pores in the Lumbricidae.

The clitellum is anterior to the male pores for most or all of its length in the Ocnerodrilidae, Eudrilidae and Megascolecidae in which it occupies a small number of segments (the minimum being three segments, in pheretimoids and some *Spenceriella* species). The ocnerodrilid *Nematogenia lacuum* is exceptional for this assemblage in the length of the clitellum, occupying approximately 13 segments, commencing on segment 14, with the result that the male pores (on segment 17) are well forward of its posterior end.

Some details of clitellar histology are given by Jamieson (1971b), exemplified for *Sparganophilus* and *Alma* in Fig. 8B,D,E (see also those genera in 8.2.11). A detailed review of the ultrastructure and function of components of the clitellum is given by Jamieson (1992). No ultrastructural investigation of the megascolecid clitellum has been published. The histology and histochemistry of the clitellum of the pheretimoid *Amyntas hawayanus*

(=*Pheretima hawayana*) has been investigated by light microscopy (Lufty 1965). The following components of the clitellum were observed: cuticle, supporting cells, sensory cells, mucus secreting cells, large granule cells, fine granule cells and, around the female pore, cells of the "plug."

In taxa with elongate clitella the cocoons are correspondingly long and in almines they are many times longer than wide. The longest recorded in the Alminae are those of *Alma multisetosa* where the clitellum may occupy 57 segments and in which the cocoons attain a length of 130-155 mm and may contain 32 embryos. In *A. nilotica*, with a clitellum of 40 to 60 segments, the cocoons (Fig. 8.8F) reach a length of 110 mm and each contains 8-22 young. They average 42 mm (by 12 mm) in *Glyphidrilus annandalei* in which each contains, on average, seven ova. They reach 70 mm in *Criodrilus lacuum*, in which they release from 2-8 young. Their elongate spindle-shape in the Alminae contrasts with the more nearly ovoid form of cocoons of other families and appears to be characteristic (diagnostic?) of the subfamily as perhaps is the relatively large number of young released. The cocoons of *Sparganophilus tamesis* (= *S. eiseni*), with a clitellum of 14 segments (Fig. 8.8C), are intermediate in length, almost ovoid, though with attenuated extremities, and release from 1-4 young (References in Jamieson 1971b). The cocoons of large earthworms, such as the megascolecid *Megascolides australis* are several centimeters long and ovoidal.

8.2.3 Chaetae Associated with Reproduction

Modified chaetae with a function in reproduction are termed genital chaetae. Those at the male and/or prostatic pores are distinguished as penial chaetae as there is evidence that during copulation they are protruded into the spermathecal orifices of the partner. Other genital chaetae may occur in association with glandular modifications of the epidermis. Those associated with spermathecae are termed copulatory chaetae by some authors but are here termed, less ambiguously, spermathecal chaetae. Penial and spermathecal chaetae are known in some tubificids. Penial chaetae occur in the alluroidid *Brinkhurstia* and in the biwadrilid *Biwadrilus bathybates* and are widespread in crassicitellates (see below).

Cuadrado and Martínéz-Ansemil (2001) have provided an investigation and discussion of external structures used in sperm transfer in tubificids, from which the following account is drawn. Judging from the location of genital pores, direct sperm transfer with simple apposition of male and spermathecal pores, or with penetration of differentiated structures such as penes or pseudopenes, appears to be the mechanism of sperm transfer (insemination) used by most tubificids. In some it is aided by penial setae.

Observations on the genital region of *Potamotheix* and *Psammoryctides* suggest that the embrace of the partners, with formation of a ventral protrusion between spermathecal pores and a retraction of the male pores area, could allow contact between male and spermathecal pores, even in species where the latter are clearly lateral. When male and spermathecal pores are aligned, an embrace is not essential to keep them in contact.

The ciliated (?) and glandular porophore of *Protuberodrilus tourenqui* could allow the approach of male and spermathecal pores and the adherence of the two worms during sperm transfer. In *Peristodrilus montanus*, it is likely that the cilia close to the penial chaetal muscles facilitate location of the correct anchorage site. The combined action of the penial chaetal muscles and the strong dorso-ventral muscles could allow the penial chaetae to enter into the anchorage bridge pockets to firmly hold the couple while the pseudopenes enter the spermathecal pores. The small epidermal papilla present in the midventral line of the spermathecal segment of four investigated species of *Krenedrilus*, combined with their penial chaetae, probably represent a similar anchorage system. The 'X-shaped mid-ventral slit' of *Bathydriulus rohdei* described by Jamieson (1977a) may facilitate the mutual holding of copulating worms (Erséus 1981). A great number of species belonging to several genera from all the tubificid subfamilies have similar penial chaetae that, based on form, location, and orientation, may also have an anchorage function.

In *Rhyacodrilus falciformis* the large and deeply grooved falciform penial chaetae, assisted by several bundles of cilia, may enter into the spermathecal pores, clasping the couple and assisting in sperm transfer.

Five of the seven species (*Peristodrilus montanus*, *Protuberodrilus tourenqui*, *Rhyacodrilus falciformis*, *Psammoryctides barbatus*, *Potamothrix bavaricus*, *P. hammondiensis* and *P. heuscheri*) studied by Cuadrado and Martínéz-Ansemil (2001) had spermathecal chaetae. *Protuberodrilus tourenqui* also has spermathecal-type chaetae in two non-spermathecal segments (9 and 12). All these chaetae are gutter-shaped, as are most tubificid spermathecal chaetae. Several reasons indicate that they act as piercing chaetae whose principal role is perhaps stimulation, rather than attachment of the concopulants or sperm transfer: (a) they are generally long enough to penetrate deeply into the coelomic cavity; (b) some species from several genera have spermathecal chaetae together with typical anchorage penial chaetae (e.g. *Krenedrilus*, *Rhizodrilus*, *Limnodriloides*); (c) in many species, the spermathecal chaetae are not located near the spermathecal pores, and there are even some species with supplementary sets of spermathecal-type chaetae in non-spermathecal segments (e.g. *Protuberodrilus* and *Krenedrilus*). If a mechanical stimulation in fact takes place, the secretions of the large glands associated with spermathecal chaetae, present in many tubificids, could serve to firmly attach the partners while spermathecal chaetae protract and retract alternatively to stimulate the worms. The possibility of chemical stimulation by inoculation of secretions into the blood stream (Feldkamp 1924) deserves further investigation. Either chemical or mechanical stimulation could help to contract the atrium or to contract and expand the coelomic cavity, producing embrace and release, and aspiration, movements aiding sperm transfer.

There are other structures and mechanisms, in other tubificids, that probably represent further means of holding partners during sperm transfer: the common median chamber (bursa) into which male pores open in some

species (e.g. some *Rhizodrilus*, some *Monopylephorus*); the development of a cuticular penial sheath of various lengths and forms (e.g. *Limnodrilus*, *Aktedrilus*) and the development of special penial chaetae (e.g. *Adelodrilus*, *Inanidrilus*). The long and horn-shaped penis sheath of several *Aktedrilus* species could be interpreted as a means of embracing [holding] the partner and bringing the sperm into the dorsally opening spermatheca (*Aktedrilus* is devoid of penial chaetae). Erséus (1979) and Erséus and Baker (1982) interpreted the giant penial chaetae of *Adelodrilus* and *Inanidrilus* as structures used to transfer sperm into the spermathecae of the mate; the small penial chaetae would be used for attachment (Cuadrado and Martínéz-Ansemil 2001).

Space permits mention of only a few examples of genital chaetae in megadrile families. They do not appear to occur in the Moniligastridae and Ocnodrilidae. They occur in all 'aquamegadrile' families, excepting the Sparganophilidae. In terrestrial earthworms (former Terrimegadrili) they are seen in the Kynotidae, Hormogastridae, Microchaetidae, Lumbricidae, Glossoscolecidae, Eudrilidae and Megascolecidae (Fig. 8.55). In *Pontoscolex corethrurus* one or both chaetae of the ventral couples in some of segments 14-22 are modified as genital chaetae with longitudinal rows of gouges (Gates 1972; Jamieson, pers. obs.). In the Eudrilidae the penial chaetae assume a great variety of bizarre form (Jamieson 1967) including so-called rolled chaetae (Stephenson 1930). In the acanthodriline Megascolecidae, penial chaetae are often present associated with the prostate glands. In the Australian acanthodriline *Diploptrema* and its apomorphic relative *Neodiploptrema*, and the New Caledonian, *Acanthodrilus* (Fig. 8.55K-N) which appears to be the sister genus of *Diploptrema*, the penial chaetae may be longer than the thickness of the body and usually have distinctive sculpturing. The spermathecal setae of *Acanthodrilus* and *Diploptrema* are sharply pointed and have longitudinal arrays of approximately alternating gouges or notches (Fig. 8.55J,K).

Penial chaetae are present in a considerable proportion of the genera of the Megascolecinae, in which they vary interspecifically though they may retain generic or higher characteristics (Fig. 8.55).

8.2.4 Male Pores and Accessory Genital Markings

The male pores are the external openings of the vasa deferentia. There is a single pair, even where there are two pairs of testes and vasa deferentia, except in the marine microdrile *Randiella*, most Haplotaxidae, those Moniligastridae with two pairs of testes, and the Indian megascolecid *Hoplochaetella*, all of which have two pairs of male pores. Only in the Randiellidae (Erséus and Strehlow 1986) and typical Lumbriculidae are they prosoporous, lying in the same segment as the corresponding testes. By definition, they lie in the segment behind the single pair or last pair of testes in plesioporous oligochaetes: all of the remaining microdriles, which have a single pair of testes; most Haplotaxidae, which typically have two pairs of testes; and the Moniligastridae, which have one or two pairs of testes.

The segmental positions of the male pores that are typical of the various families are indicated in Fig. 8.4A,B. Location of the pores relative to the clitellum in crassicitellates is briefly discussed in Section 8.2.2 on the clitellum.

In the Megascolecidae, the position of the male pores and their relationship to the openings of the prostates is of great importance taxonomically from the specific to the subfamilial level (Fig. 8.8A). At the specific level, the position of pores relative to chaetal rows is much used. At higher levels the numbers of prostate pores and their locations relative to the male pores are important. The various arrangements in the Megascolecidae are discussed under that family in 8.2.11. In the acanthodrilin condition grooves which is usually regarded as conduits for sperm are present, joining the two pairs of prostate pores to the intermediate male pores (Fig. 8.8A).

The genital field illustrated for the African ocnodrilid *Pygmaeodrilus nabugaboensis*, suggests utilization of a seminal groove or tract (Jamieson 1957). In the eudrilid genus *Stuhlmannia variabilis* (Fig. 8.10) it appears that spermatozoa are conveyed via a seminal groove to the summit of the penis, with further incorporation of the two in *S. asymmetrica*. The most remarkable copulatory mechanism in oligochaetes is perhaps that seen in the genus *Alma* (Figs. 8.11, 8.56) in which a pair of long claspers, each of which bears a male pore distally, grips the post-testicular, and often far posterior, spermathecal region of the partner.

In the Megascolecidae, more than any other oligochaete group, so-called accessory genital markings are well developed, as in *Spenceriella penolaensis* (Fig. 8.12A) and *Heteropodrilus thomsoni* (Fig. 8.12B). These markings usually have the form of paired and/or single pit-shaped or protuberant, segmental and/or intersegmental glandular modifications of the body wall, which may be circular, elliptical or ridge-like or have other forms. They rarely form elongate structures over two or more segments somewhat resembling the tubercula pubertatis of lumbricids. Their function is imperfectly understood but it is likely that they have a role in species recognition and that they may have a "key in the lock" function in copulation, not by mutual insertion, but by precise apposition to structures on the partner. Their prevalence in every other Australian genus makes their absence in the Wet Tropics genus *Terrisswalkerius* all the more remarkable. Presumably they were lost in an ancestor of the 17 or so currently known species of this genus although a plesiomorphic absence cannot be discounted (they are also absent in the Indian genus *Celeriella* which appears related). The distribution of accessory genital markings has great taxonomic value for species identification.

8.2.5 Female Pores

Much of the variation in the location of the female pores in the Oligochaeta is indicated in Fig. 8.4A,B. They are always located in the segment behind the corresponding ovaries. Predominantly, there is only one pair of female pores as there is only one pair of ovaries in most microdriles and in all but

Arrangement of male and prostatic pores in the Megascolecidae

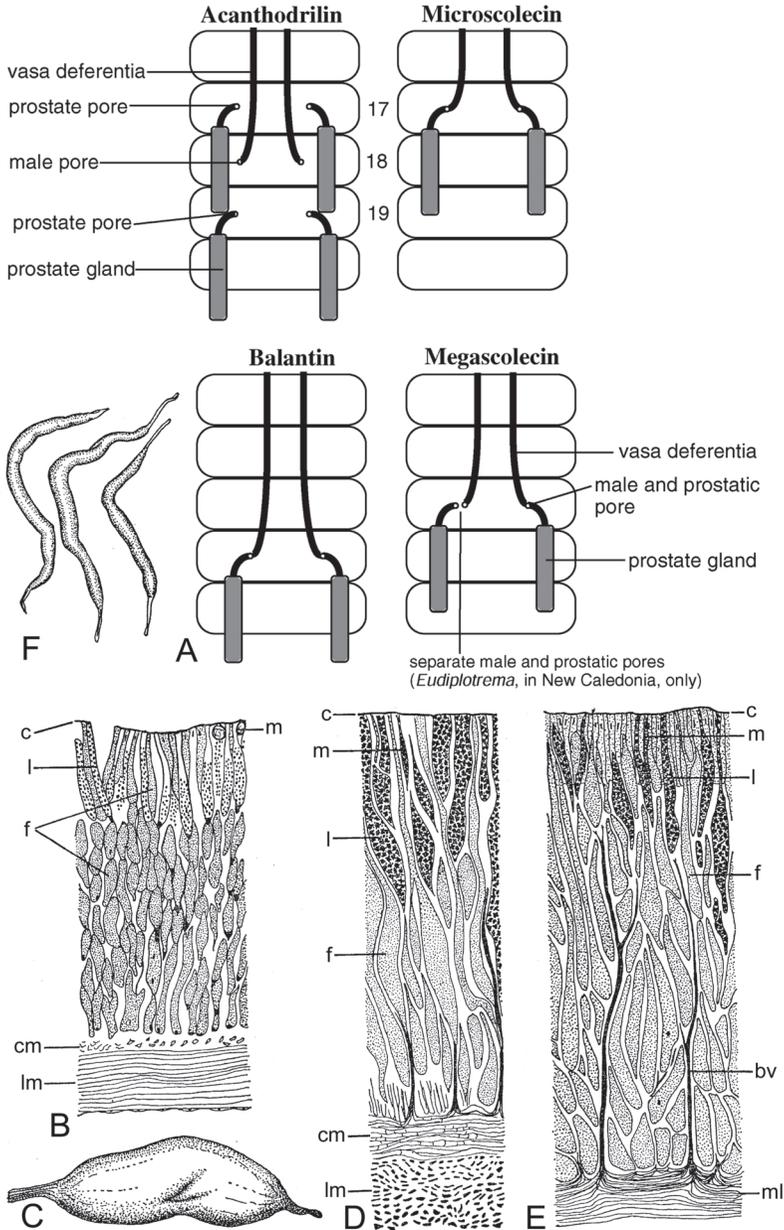


Fig. 8.8. A. Arrangement of male and prostatic pores in the Megascolecidae. Original. B. Optical section of clitellum of *Sparganophilus tamesis* (Sparganophilidae). C. Cocoon of same. D, E. Optical section of clitellum of D. *Alma emini* and E. *A. nilotica* (Almidae). F. cocoons of *A. nilotica*. After Jamieson, B. G. M. 1971b. Anatomy: 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. 1.6, from various sources.

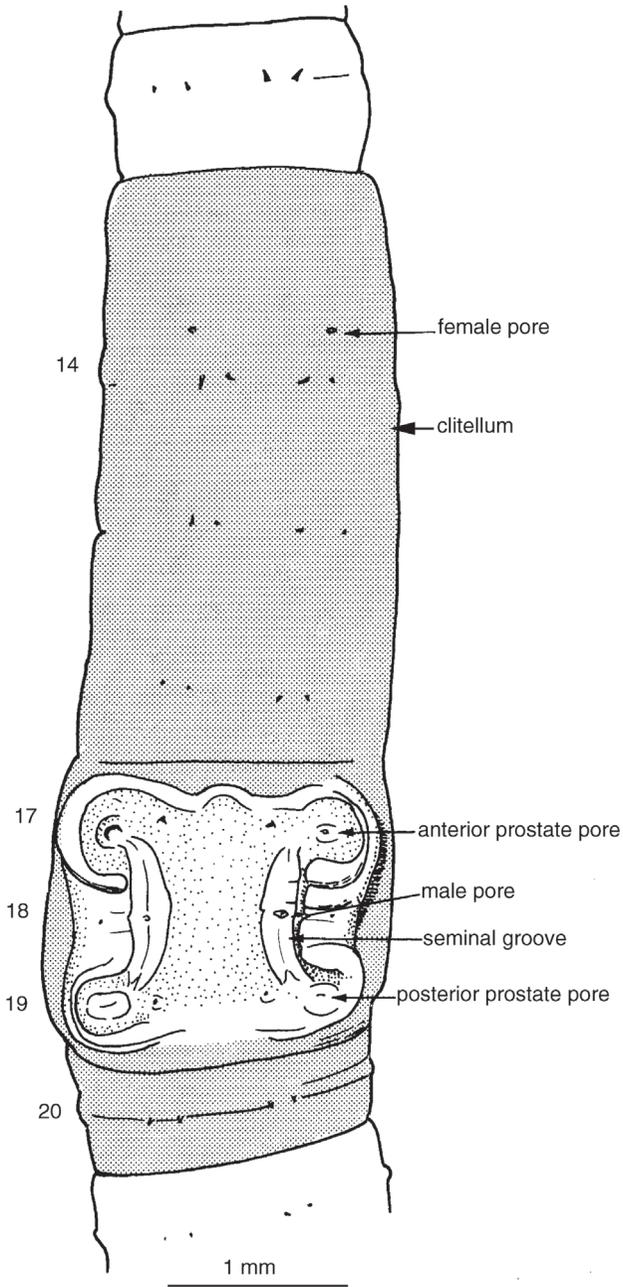


Fig. 8.9. *Eukerria borellii* (Ocneroдрilidae). Genital field, showing seminal grooves connecting prostate and male pores. After Jamieson, B. G. M. 1970. Bulletin of the British Museum (Natural History) Zoology 20: 131-172, Fig. 7F.

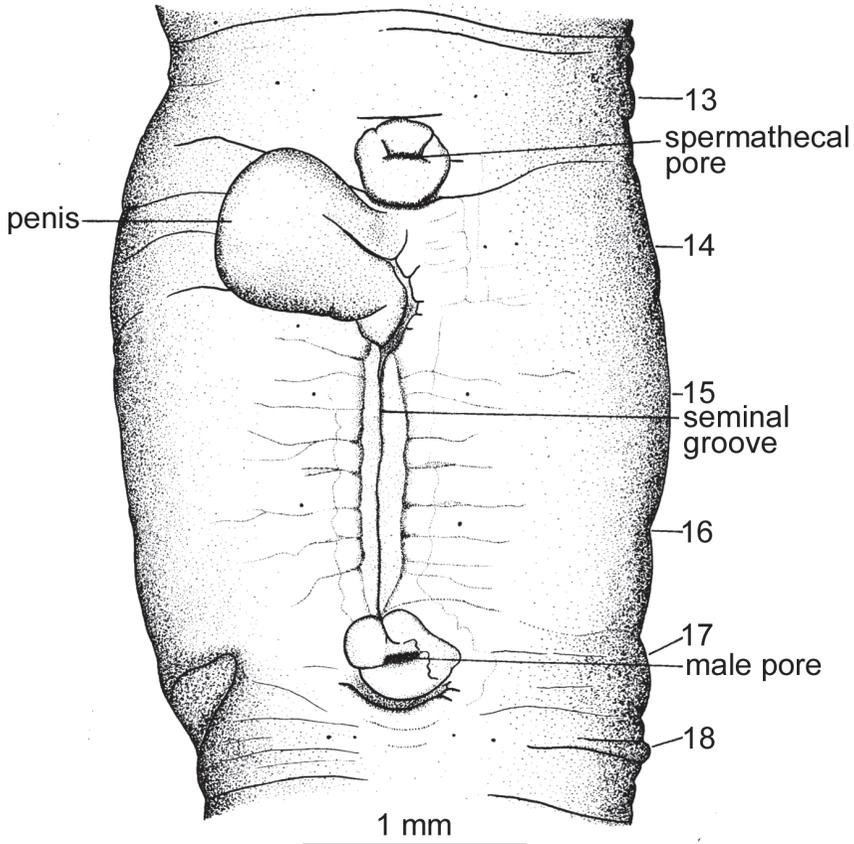


Fig. 8.10. *Stuhlmannia variabilis* (Eudrilidae). Genital region, showing penis and seminal groove. After Jamieson, B. G. M. 1967. *Journal of Zoology* (London) 152 79-126, Fig. 12.

three species of the Metagynophora. There are one or two pairs in the Lumbriculidae. Female pores are usually inconspicuous. In the circummundane *Eudrilus eugeniae*, unusual for the Eudrilidae in having a pair of combined spermathecal and female pores, these are conspicuous and act as vulvae in internal fertilization. In most eudrilids the female pores are separate from the spermathecal pores. The location of the female pores relative to the chaetal lines has some value in taxonomy. Fusion as a single pore is almost invariable in Megascolecinae of the *Pheretima* group and is seen also in *Propheretima* and in some species of other genera.

8.2.6 Spermathecal Pores

Spermathecal pores are generally inconspicuous, with the notable exception of the Eudrilidae in which they form large, often unpaired orifices (Fig. 8.10). In microdriles they are far pretesticular in enchytraeids but lie in the testis segment in tubificids, including naids, whereas in phreodrilids they occupy

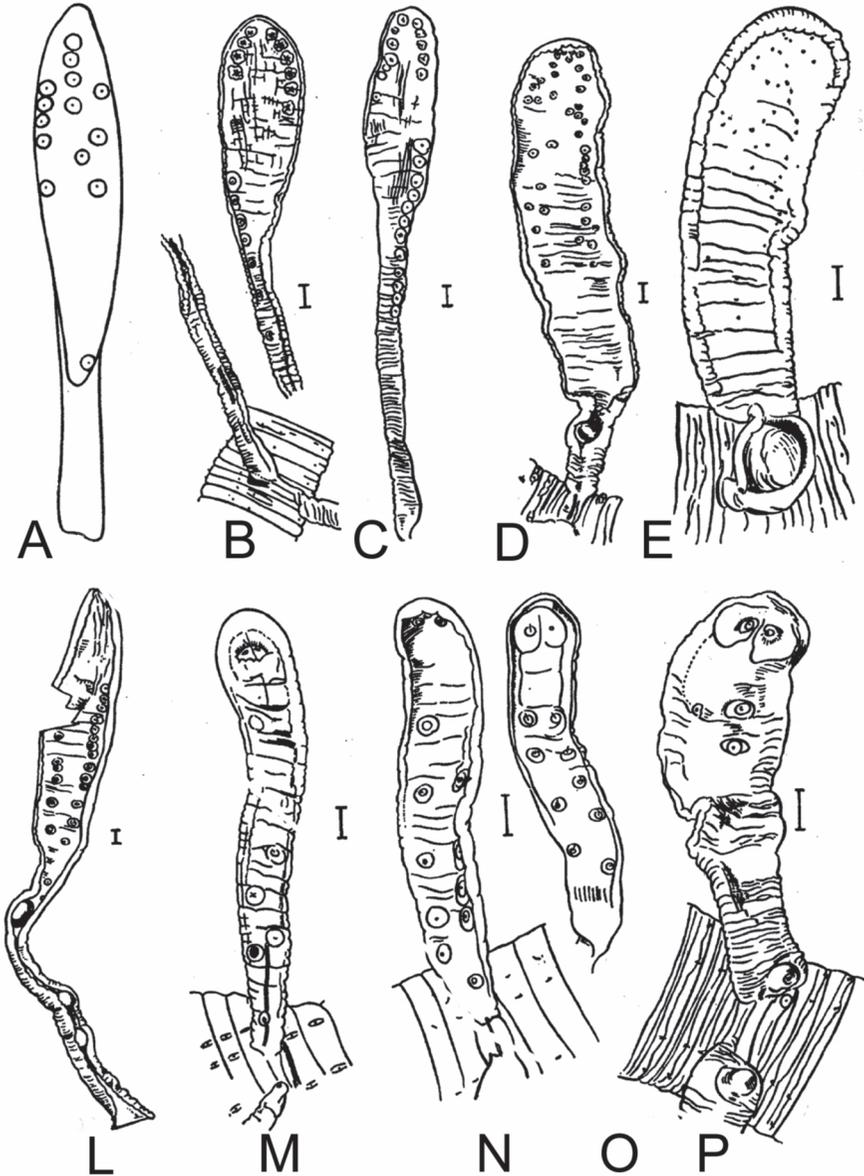
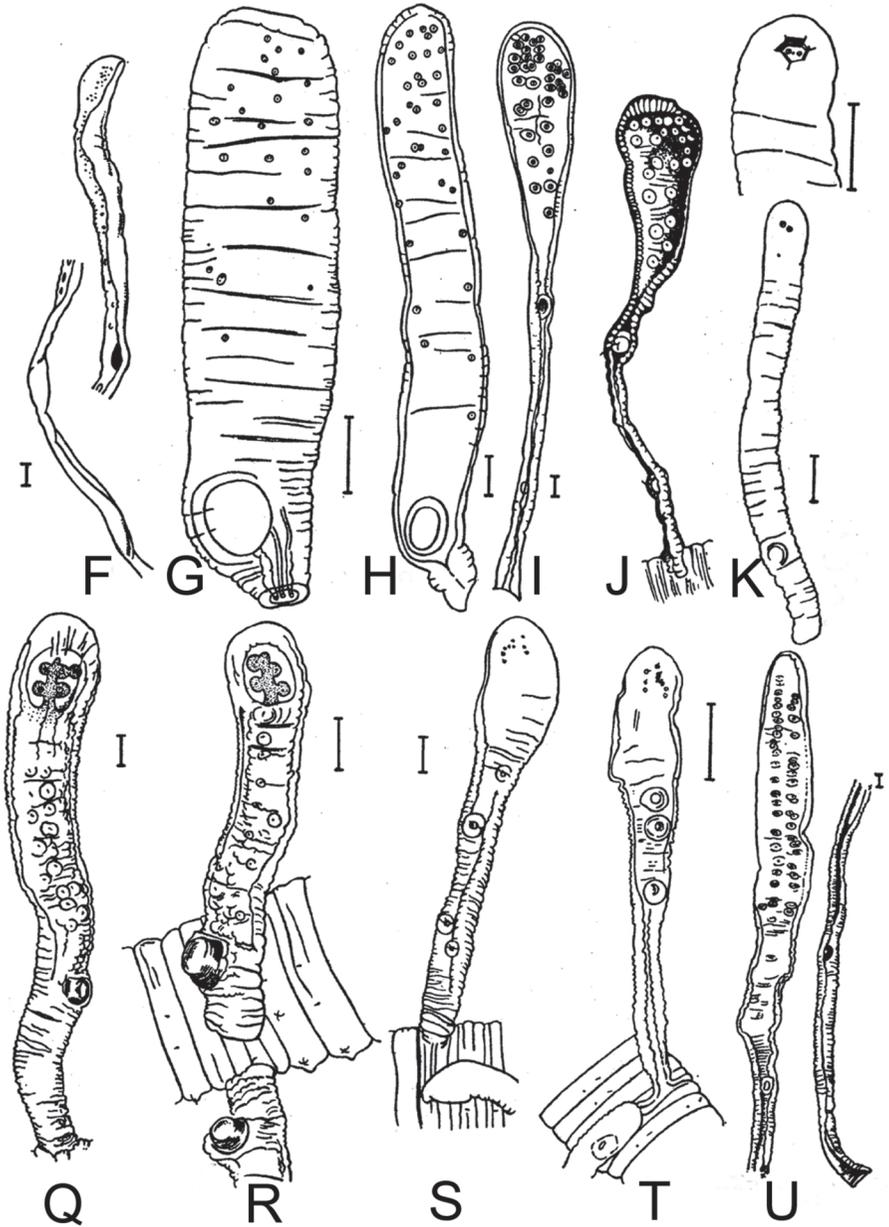


Fig. 8.11. Variation in claspers of *Alma* (Almidae). **A.** *Alma basongonis*. **B.** *A. emini* (Ituri). **C.** *A. emini* (= *aloyssiabaudiae*). **D.** *A. eubranchiata eubranchiata*. **E.** *A. eubranchiata catarrhactae*. **F.** *A. multisetosa*. **G.** *A. nilotica* (Egypt). **H.** *A. nilotica* (Sudan). **I, J.** *A. pooliana*. **K.** *A. stuhlmanni*. **L.** *A. kamerunensis*. **M.** *A. millsoni millsoni*. **N, O.** *A. millsoni millsoni* (= *schultzei*). **P.** *A. millsoni zebangui*. **Q, R.** *A. tazelaari*.



S. *A. togoensis* (Type, Togo). T. *A. togoensis* (Ghana). U. *A. ubangiana*. Scale bar 1 mm. With some corrected labelling, 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. 15.7, 15.8.

Colour
Figure

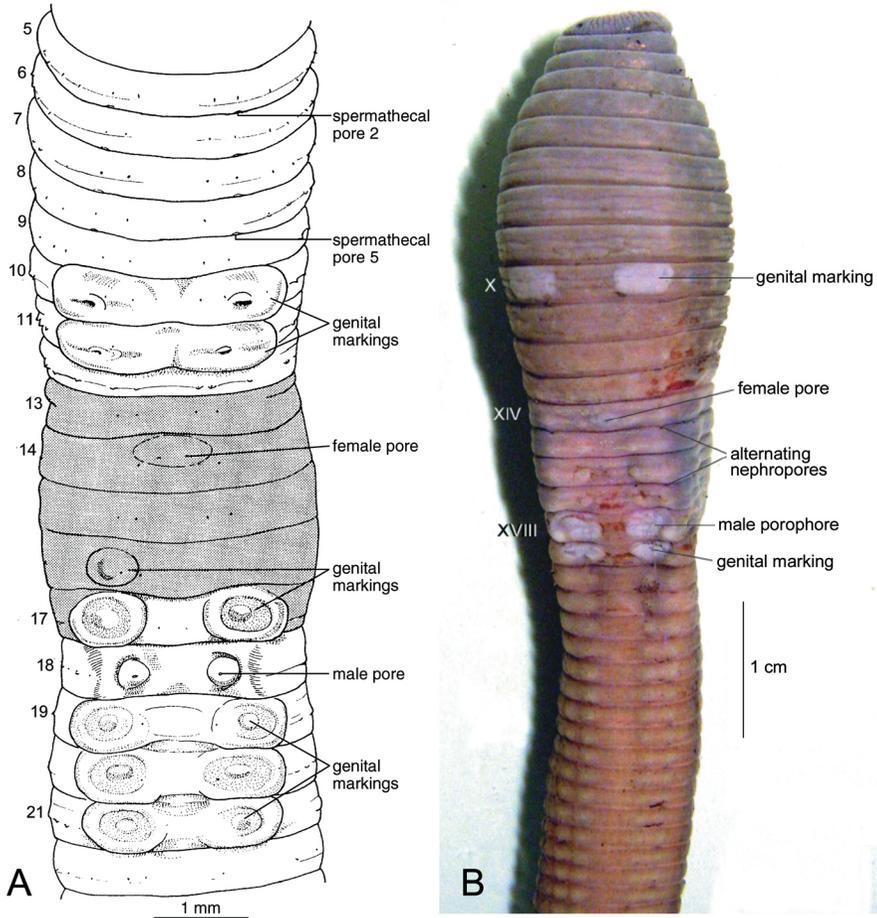


Fig. 8.12. Example of genital markings in megascolecine earthworms (Megascolecidae). **A.** *Spenceriella penolaensis*. After Jamieson, B. G. M. 1974. Earthworms (Oligochaeta: Megascolecidae) from South Australia. Transactions of the Royal Society of South Australia 98: 79-112, Fig. 9B. **B.** *Heteroprodriilus thomsoni* Blakemore; specimen from Coolumb, Queensland. From Jamieson, unpublished.

the segment of the female pore; in these three groups there is only one pair, with rare exceptions in the Tubificidae. In lumbriculids, spermathecal pores are variable in number, anterior or posterior to the testes (Pinder and Brinkhurst 1994). Haplotaxids have one to four pairs of spermathecal pores, anterior to the testis segments. In alluroidids the pores are pretesticular in one to three of segments 6 to 9, at or near their anterior margins, lateral to dorsal, paired or single. They are also pretesticular in moniligastrids, sparganophilids, *Biwadriilus*, ocnodrilids, glossoscolecids (rarely extending into the testis-segments) and megascolecids. In hormogastrids and lutodrilids they are paired or multiple, testicular and posttesticular. In the Almididae, the pores are post-testicular, rarely continued into and in front the

testis-segments, sometimes translocated into the hindbody, and usually multiple in an intersegment; in *Alma* they occupy some or all of the eight chaetal lines, or are more numerous, in seven to 37 intersegments (or exceptionally 138 intersegments) from 18/19 to 253/254, thus well behind the testis-segments. In the Kynotidae and Microchaetidae they are post-testicular, though in microchaetids they may also occupy the last testicular segment, and are paired or multiple. In the Lumbricidae spermathecal pores are usually paired, with two to eight pairs in furrows 5/6 to 19/20. They are absent from *Criodrilus* and *Biwadrilus* which have external spermatophores (ectospermatophores) and are probably biparental but are also absent from the two circummundane species *Ocnerodrilus occidentalis* (Ocnerodrilidae) and acanthodrilid *Microscolex dubius* (Megascolecidae, Acanthodrilinae) which lack spermatophores and are deduced to be parthenogenetic (Gates 1972; Jamieson 1974a).

The segmental location of spermathecal pores and their position relative to chaetal lines is much used for specific identification in megascolecids. For instance, in Australian megascolecids they are usually intersegmental though in *Didymogaster* they are segmental in location; there are maximally five pairs, or, in *Fletcherodrilus*, five midventral pores or are multiple in intersegments 2/3-8/9 in *Geoffdyneia rubens* (Fig. 8.16) (see also Spermathecae, 8.2.8).

8.2.7 Prostate and other Glands and Bursae Associated with the Male Ducts

Various types of so-called prostate glands occur in microdriles. In lower Metagynophora, capsular, smooth-surfaced prostate glands occur in the Moniligastridae and receive the male ducts (Fig. 8.14). These and other accessory sex glands have been reviewed by Adiyodi (1988).

The most plesiomorphic of the Opisthopora, the alluroidids, have tubular or bulbous atria which also receive the male ducts or discharge with the latter but separately from them into a terminal chamber; they have an internal epithelium surrounded by a muscular sheath outside which secretory ("prostate") cells are usually present (see Jamieson 1971a; Omodeo and Coates 2000) (Fig. 8.32).

In the crassicitellate megadriles, dilatations or chambers at the ectal end of the vasa deferentia and protruding into the coelom occur in *Kynotus*, *Criodrilus*, commonly in *Glossoscolex* and some other glossoscolecids and in *Biwadrilus*. The large gland in *Criodrilus* is probably responsible for secreting the ectospermatophore of this species and is remarkably similar to the "prostate" of the lumbricid *Bimastos palustris*. The "copulation glands" of *Biwadrilus* are glandular prostate-like organs associated with the genital chaetae and are similar in internal structure to the genital chaeta glands of *Microchaetus*. In the Almidae, only *Callidrilus ugandaensis* has compact glands which may be considered true prostate glands, though probably not homologous with the prostates of other megadriles. The posterior prostate-like glands of *Sparganophilus tamesis* and all of these glands in *S. smithi*,

resemble those of *Callidrilus* in having glandular cells grouped around an epithelial layer which lines an intraglandular duct. *Sparganophilus* differs, however, in the unbranched condition of the duct and, in *S. tamesis* (= *S. eiseni*) by differentiation of a terminal, externally recognizable duct (see details in Jamieson 1971a).

True prostates, that is glands with a distinct ectal duct and associated with the male terminalia, are typical of the Ocnerodrilidae and Megascolecidae. They have been termed metaprostates (Jamieson 1978b) to distinguish them from the euprostate, which is a dilated ectal loop of the male duct, seen in the Eudrilidae.

Three chief types of prostates are recognized in the Megascolecidae (Fig. 8.13) and are here exemplified by Australian taxa. These are tubular, tubuloracemose or racemose. Tubular prostates are slender and elongate and have a single central lumen without side branches. They have their most typical development in the Ocnerodrilidae, and in the Megascolecidae are characteristic of the Acanthodrilinae, though in *Diplorema scheltingai* they are tubuloracemose or even racemose and in *Exxus* they are racemose, and are well exemplified in some Megascolecinae, e.g. *Megascolides australis*. They are also seen, *inter alia*, in *Cryptodrilus tenuis*, *Diporochaeta frenchi* and *Plutellus manifestus* (Fig. 8.13A-C) but the latter two species exemplify a tendency in Australian species for some thickening of the gland.

In tubuloracemose prostate glands, there is again a central lumen, but thickening of the gland, and often mammilation of the surface, is such as to suggest that groups of cells discharge their secretions by ductules into the central lumen; an example, albeit of relatively simple form, is shown for *Graliophilus macedonensis* in Fig. 8.13D. The various species of *Terrisswalkerius* exemplify transitions from tubular to tubuloracemose prostates.

In the racemose prostate, the external form is such as to suggest that the duct branches immediately within the gland. This is clearly the case where the gland is bipartite, as in *Digaster armifera* (Fig. 8.13I). An extreme case of the racemose form is seen in *Trinephrus fastigatus* (Fig. 8.13E), in which the duct forms several branches external to the gland. A unipartite racemose gland is exemplified by *Notoscolex camdenensis* (Fig. 8.13H), a deeply incised form by *Heteroporodrilus shephardi* (Figs. 8.13G), and a unipartite racemose gland, which may be an extreme development of the tubuloracemose type, in *Anisochaeta mudgeanus*.

The structure of the prostate glands of the peregrine megascolecine *Lampito mauritii* has been investigated by Gupta *et al.* (1999). Two types of secretory cells are present. Type 1 cells with a broad basal region and a long apical region contain electron-dense oval secretory granules with an increased density at the core region. Numerous electron-lucent granules with fine filamentous and electron-dense amorphous materials also occur at the basal region of these cells. Type 2 cells contain electron-lucent mucous-like secretory granules. This cell type contains exceptionally large Golgi complexes with 20-23 stacked cisternae. Both cell types open into a common

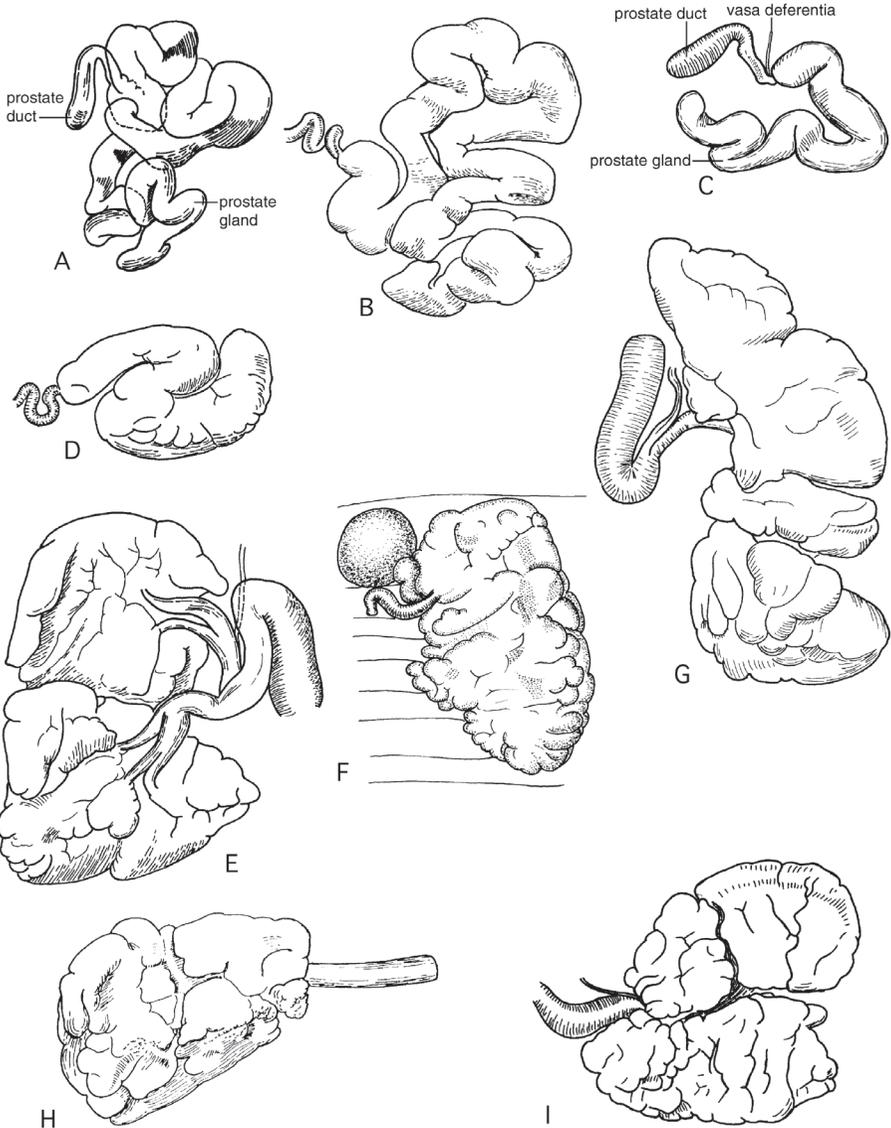
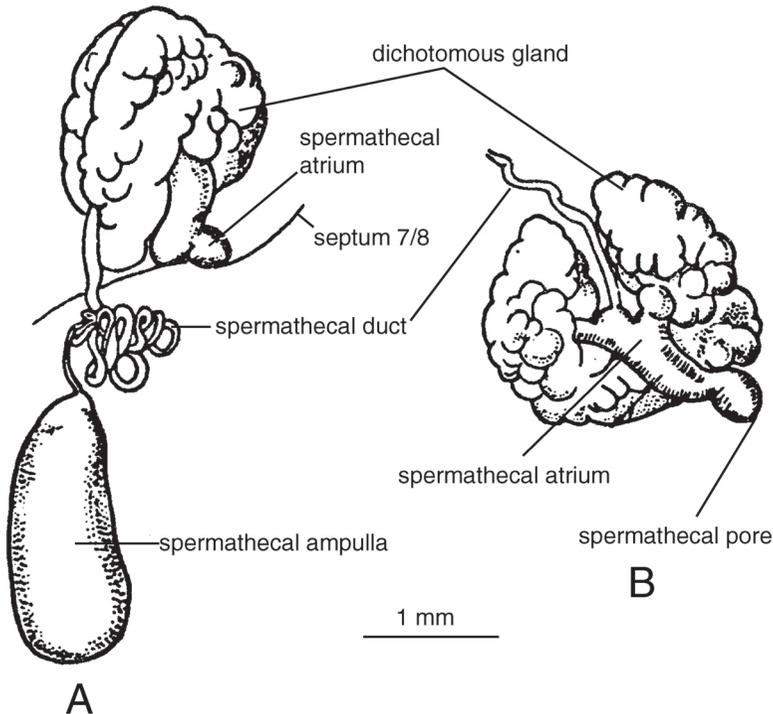


Fig. 8.13. Prostate glands. Some variation in Australian Megascolecinae (Megascolecidae). **A.** Tubular (*Cryptodrilus tenuis*). **B.** Tubular (*Diporochoeta (Vesiculodrilus) frenchi*). **C.** Tubular (*Plutellus manifestus*). **D.** Tubuloracemose (*Graliophilus macedonensis*). **E.** Racemose, bipartite with external branching of the duct (*Trinephrus fastigatus*). **F.** Racemose, unipartite, extending through several segments (*Anisochaeta mudgeanus*). **G.** Racemose, deeply incised (*Heteroporodrilus shephardi*). **H.** Racemose, unipartite, in a single segment (*Notoscolex camdenensis*). **I.** Racemose bipartite, with internal branching of duct (*Digaster armifera*). From Jamieson, B. G. M. 2000. CD ROM. The Native Earthworms of Australia (Megascolecidae Megascolecinae). Science Publishers, Inc., Enfield, New Hampshire, Fig. 0.39.

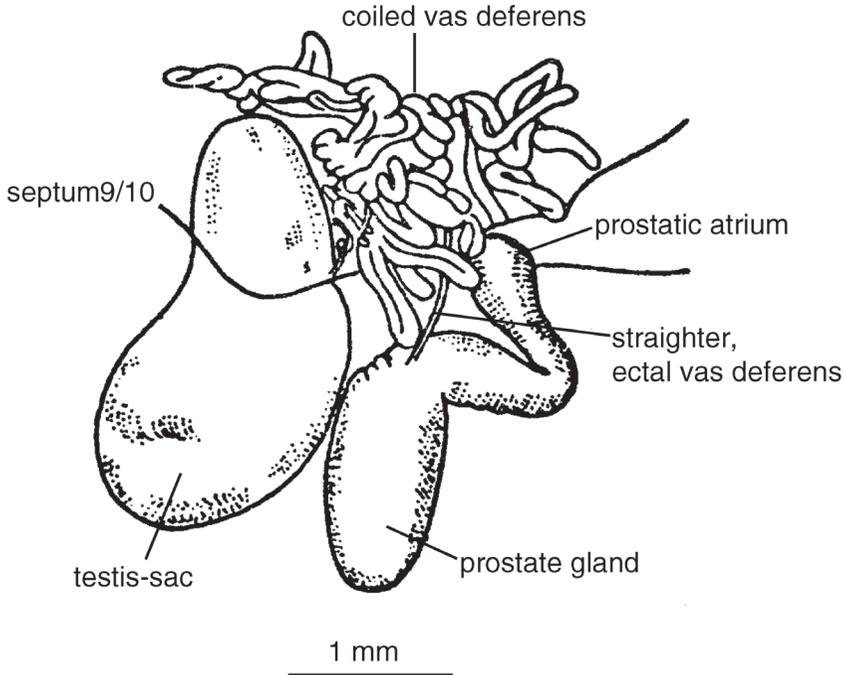
lumen and numerous microtubules are visible at the apical end. Junctional complexes such as desmosomes and septate junctions are present in this glandular tissue.

8.2.8 Spermathecae

Spermathecae are epidermis-lined sacs which receive spermatozoa from the partner during copulation and store them for later expulsion into the cocoon and fertilization of the eggs. Each spermatheca has a sac-like ampulla and a duct. The duct may be so short that the ampulla is virtually sessile on the body wall, but is usually some significant fraction of the length of the ampulla or longer. The spermathecae of the Moniligastridae are almost unique in the Oligochaeta in the great length of their ducts, a development paralleled by the great length of the vasa deferentia which Bourne (1894) found to measure 9.5 inches in only a single segment. In the advanced genera *Drawida* and *Moniligaster*, there is a large atrium at the ectal end of the spermathecal duct and in *Moniligaster* (Fig. 8.14), this has developed a peculiar "dichotomous gland". The similarity of the spermathecal atrium to the prostates at the ends of the male ducts (Fig. 8.15) suggests that a common morphogenetic controlling mechanism may be involved in the production of



8.14. *Moniligaster troyi*. Spermathecal apparatus. After Jamieson, B. G. M. 1977. Evolutionary Theory 2: 95-114, Fig. 5C.



8.15. *Moniligaster troyi*. Male genital apparatus. After Jamieson, B. G. M. 1977. *Evolutionary Theory* 2: 95-114, Fig. 5E.

the spermatheca and prostate (Jamieson 1977b). These appendages of the spermathecal ducts do not, however, store sperm; their tubular constituents are here shown to consist of a tall glandular epithelium (Fig. 8.31C,D,E).

In most Megascolecidae and a few species of some other families there are one or more diverticula connected with the spermathecal duct or sometimes with the lower part of the ampulla. In all diverticulate megascolecid spermathecae, except, as far as is known, *Megascolides australis* (Van Praagh 1995) and *Microscolex macquariensis* (Jamieson, unpublished), the spermatozoa are stored in the diverticulum. Megascolecid spermathecae are pretesticular. There is usually one pair, occasionally a single midventral spermatheca, per thecal segment but some pheretimoids have several spermathecae per segment. The latter, polythecal condition is known in only one megascolecine Australian species, *Geofdyneia rubens* (Fig. 8.16). In the Ocnero-drilidae spermathecae rarely (*Pygmaeodrilus*, some *Gordiodrilus*) have diverticula; in some ocnero-drile species the diverticula differ from those of megascolecids in not containing spermatozoa, which are stored in the ampulla, as in *Pygmaeodrilus montiskenyae* (Fig. 8.17), but in at least *P. nabugaboensis* (see Jamieson 1957), the diverticula and not the spermathecal ampullae are inseminated. In the Glossoscolecidae, usually lacking diverticula, each of the two spermathecae of *Glossoscolex schutti* has a pair of diverticula of the duct which receive sperm (Michaelsen 1918).

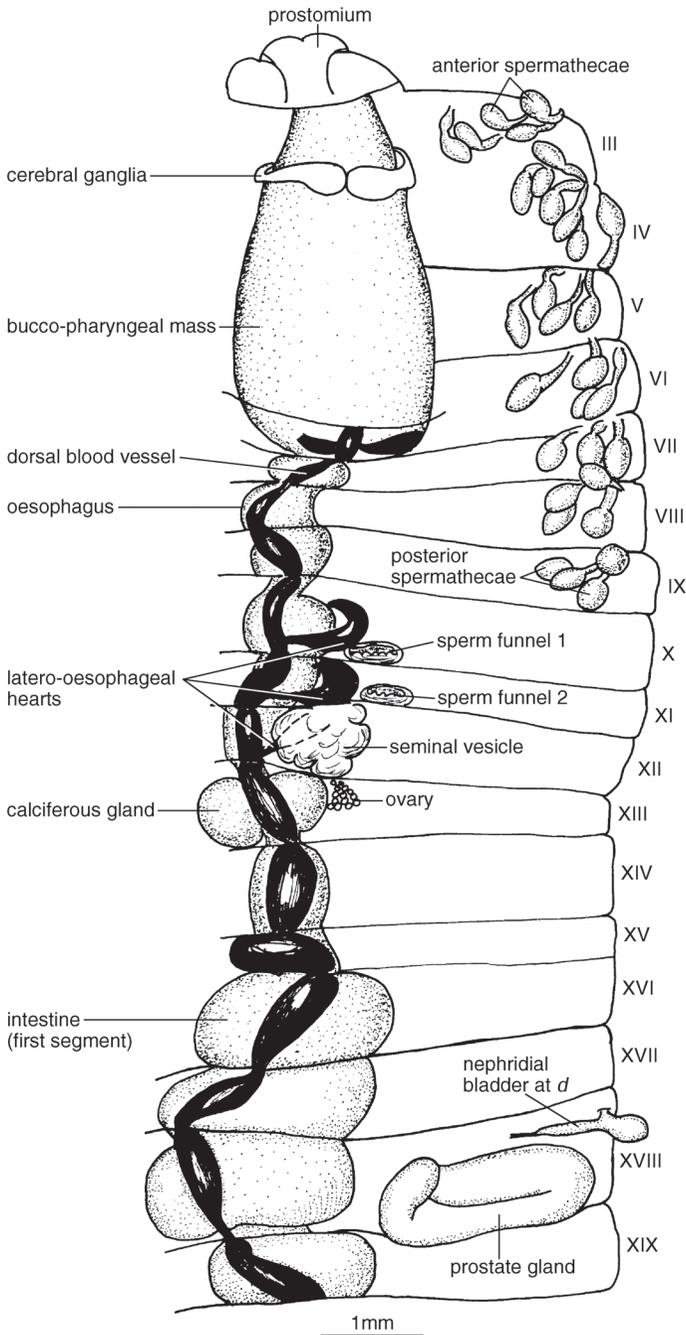
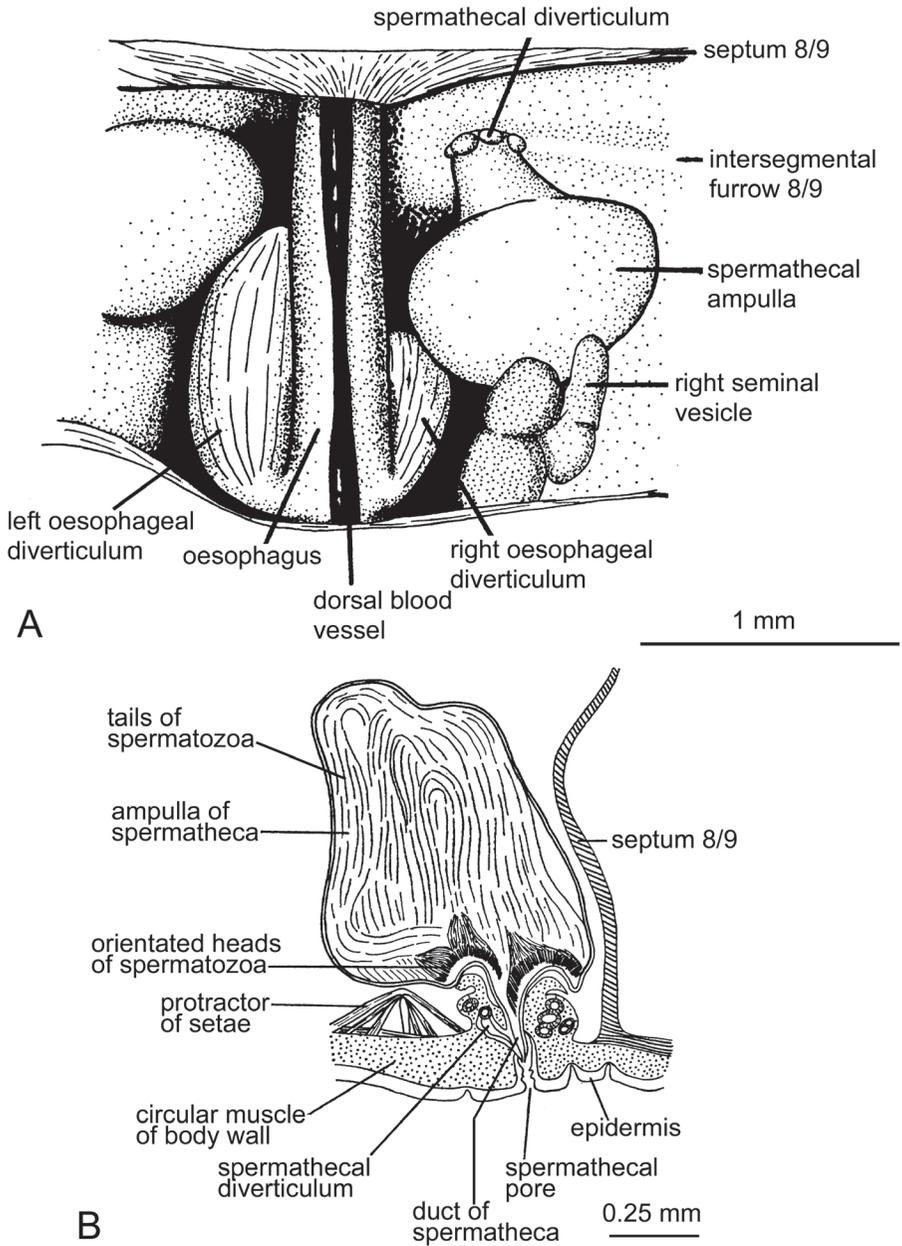


Fig. 8.16. *Geofdyneia rubens* (Megascolecidae). An example, rare in megascolecids, of multiple spermathecae in a segment. From Jamieson, B. G. M. (2000). CD ROM. The Native Earthworms of Australia (Megascolecidae Megascolecinae). Science Publishers, Inc., Enfield, New Hampshire, Fig. 14.2.



8.17. *Pygmaeodrilus montikenya* (Ocnerodrilidae). **A.** Dorsal dissection to show the right spermatheca. **B.** Vertical section of a spermatheca, showing spermatozoa in ampulla. After Jamieson, B. G. M. 1965. *Annals and Magazine of Natural History* 8: 95-107, Figs. 4 and 5.

In the Enchytraeidae, e.g. *Lumbricillus rutilus*, the ental end of each spermatheca (often in the form of an ampulla) of which there is a pair in segment 5, opens into the lateral aspect of the alimentary canal (Stephenson 1930). Westheide (1999: 199) has stated that, in *Marionina preclitellochaeta*, the ampullae are intestinal pockets and not part of the epidermal invaginations. In the same paper, Westheide (1999: 210) draws out of the study of the spermathecae-gut connections in three enchytraeid species an interesting speculation on the origin of the whole taxon Oligochaeta: "In principle, all sperm are wasted that do not fertilize an egg, and in general these are the great majority. In a hermaphrodite species with direct transfer of sperm, there is no loss of male gametes. In this case, each individual is capable of reproducing and all the sperm that are not transferred and remain in the body can be resorbed—that is, recycled—and contribute to the energy budget for female reproduction just like all allosperm that are transferred but fail to fertilize eggs. Special structures, such as the genitointestinal connections... may serve to introduce allosperm into the recycling process in a particularly simple and rapid manner. Finally, this idea leads to the assumption that the evolution of hermaphroditism should be favored in small species.A conclusive phylogenetic inference that could be drawn from this reasoning for a taxon composed exclusively of hermaphrodites, as the Clitellata are, is that its stem species was small-bodied (about several millimetres) and reproduced by direct sperm transfer".

In the Megascolecidae, the number, shape, and length relative to the ampulla of the spermathecal diverticula are important taxonomically and are used in specific identification or even at the generic level. Thus the genus *Simsia* in south eastern Australia typically has distinctive rosette-like multiloculate diverticula. Although the form of the spermatheca may allow specific distinction within a genus, the same or a similar form may, nevertheless, occur in other genera. Some major variations in the form of spermathecae exemplified by Australian megascolecids are illustrated in Fig. 8.18.

Spermathecae are absent from *Criodrilus lacuum*, *Criodrilus bathybates*, *Ocnerodrilus occidentalis* and *Microscolex dubius* (see 8.2.6, above).

In the megascolecids *Amyntas hawayanus*, *A. morrissi* and *Metaphire californica*, most of the sperm are found within the diverticulum as is usual for Megascolecidae. The 'bladder' (ampulla) is the secretory portion and releases a mucous secretion, identified as acid carboxylated and/or neutral glycosaminoglycans linked to neutral proteins. The spermathecal content is thought to play a role in nourishing spermatozoa since the male gamete itself has little intrinsic energy. (Omodeo 2000 suggests that some of the ampullary secretion also enters the cocoon). Cells of the ampulla show a vertical cytoplasmic zonation, and the epithelial surface displays slender, widely spaced, ramified microvillousities. The apical zone is filled with dense vesicles. The central zone with mitochondria, Golgi complexes and rough endoplasmic reticulum is highly developed. The basal zone is made up by vertical compartments consisting of basal plasma membrane folds which

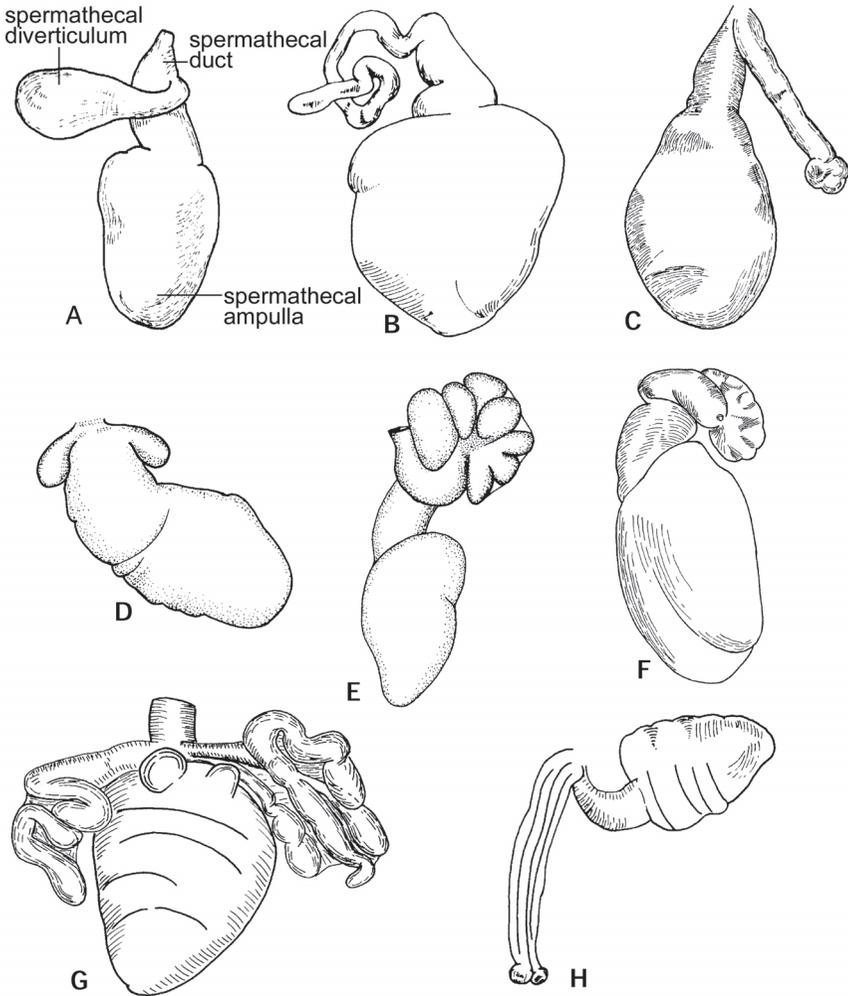


Fig. 8.18. Contrasted types of spermathecae in the Megascolecinae (Megascolecidae). **A.** Unidiverticulate and uniloculate (*Arthurdrilus burniensis*). **B.** The same, elongate (*Terrisswalkerius nashi*). **C.** Unidiverticulate with terminal loculi (*Gemascolex similis*). **D.** Bidiverticulate, simple (*Anisochaeta enormis*). **E.** Multiloculate (*Digaster amifera*). **F.** The same (*Anisochaeta (=Oreoscolex) saccarius*). **G.** Bidiverticulate and multifurcate (*Heteropordrilus tryoni*). **H.** With two lumina and apically multiloculate (*Terrisswalkerius oculatus*). Jamieson, B. G. M. 2000. CD ROM. The Native Earthworms of Australia (Megascolecidae Megascolecinae). In Science Publishers, Inc., Enfield, New Hampshire, Fig. 0.41.

contain glycogen. Diverticulum cells are of lesser height with the apical surface presenting cytoplasmic projections where sperm cells are stored and vesicles having a dense secretion different from that of ampullary cells (Teisaire and Roldan 1995).

In the megascolecid *Amyntas rodericensis* evidence has been tentatively advanced for holocrine secretion by cells of the epithelium of the

spermathecal diverticula of materials putatively nutritive for spermatozoa contained in the diverticula and it has further been tentatively suggested that the sperm acrosomes contribute to lysis of the epithelium (Jamieson 1992).

8.2.9 Spermatophores

Spermatophores in oligochaetes have been defined by Jamieson (1978c) and discussed in detail by Ferraguti (1999). In oligochaetes, spermatophores have been found attached to the body wall or within the spermathecae; they are termed ectospermatophores and endospermatophores respectively. It may be difficult to determine whether the endospermatophores are produced by the inseminating concopulant or by the spermathecal wall. Spermatophores secreted by the spermatheca, reported in some Eudrilidae and Megascoelidae are termed thecacysts (Jamieson 1978c).

As reviewed by Ferraguti (1999), (ecto)spermatophores have been reported in no less than 27 lumbricid species by Bouché (1975) as structures, presumably deposited by the partner, attached to the exterior of the body; they are less than one mm long, with a flat base and a small mass of sperm wrapped in a chitinous sheath. The sheath is produced by the atrial gland (Perel' 1978). There are two types of spermatophores: club-shaped ones, and flat ones, inserted into the integument. The presence of spermatophores in *Spermophorodrilus albanianus* has led Bouché (1975) to suppose that the cocoon, during its anterior migration, would collect the spermatophores and fertilization should occur within the cocoons, as usual in oligochaetes: thus hypodermic impregnation is not suspected. Earlier Jamieson (1971c) had similarly suggested that sperm from the ectospermatophores (received from the partner) of *Criodrilus* were shed into the cocoon. Ferraguti (1999) considered it questionable that this mechanism could be attributed to all the lumbricid species in which spermatophores have been found (Ljungström 1968) as the segments with spermatophores vary from the clitellum to the pre-clitellar region (Perel' 1978). These locations do not here appear to constitute an impediment to deposition of ectospermatophores or their spermatozoa into the cocoon as it effectively moves forward along the body. It is noteworthy in this regard that they are never postclitellar.

Where spermatophore-bearing species are known to be parthenogenetic, the spermatophores have been regarded as 'residual organs'. Perel' (1978) argues that the exchange of spermatophores adhering to the partner's body wall could have been the original way of insemination in Lumbricidae, preceding the 'invention' of spermathecae. However, it is evident that spermathecae were developed very early in oligochaete evolution (Ferraguti 1999) and Westheide (1999) has argued for direct transfer as the primitive mechanism.

Ectospermatophores consisting of an ovoid chamber with an opening at one end and a stalk at the opposite end occur in the tubificids *Bothrioneurum* and *Bacescuella* attached to the body wall in the clitellar region (Mann 1984). Both species lack a spermatheca, thus a possible function of the spermatophores is evident.

In the almoid *Criodrilus lacuum* ectospermatophores are found in a number variable from two to six attached to the ventral surface, before and behind the genital pores, in some of segments 13-19 (Jamieson 1971c) (Fig. 8.19). (The clitellum lies in segments 14-47.) They have the appearance of small horns, a few millimeters long, with a chitinous wall, containing a large

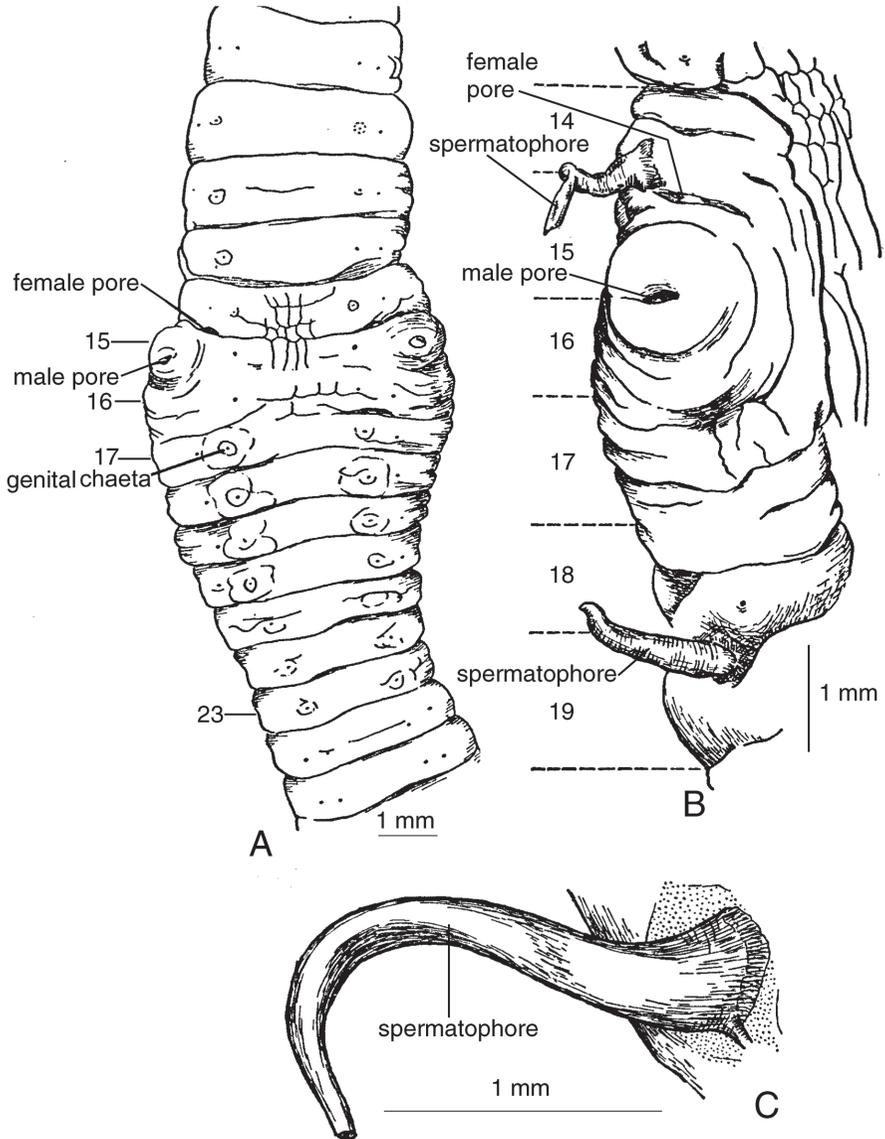


Fig. 8.19. *Criodrilus lacuum* (Criodrilinae, Almidae). **A.** Genital region. **B.** Genital region, showing attached ectospermatophores. **C.** An ectospermatophore attached to the body wall. After Jamieson, B. G. M. 1971. Glossoscolecidae. Pp. 41-72. In R. O. Brinkhurst and B. G. M. Jamieson (eds), *Aquatic Oligochaeta of the World*. Oliver and Boyd, Edinburgh, Toronto, Fig. 15.11F,G,H.

amount of spermatozoa arranged in very regular layers (Ferraguti and Jamieson, unpublished in Ferraguti 1999). (Ecto)spermatophores have also been reported for the lutodrilid *Lutodrilus vesiculatus* (McMahan 1979) (Cuadrado and Martínéz-Ansemil 2001) and for the bathydrilid *Bathydrilus* (= *Criodrilus*) *bathybates* by Nagase and Nomura (1937).

In other clitellates, ectospermatophores have been found in some branchiobdellids and they are widely distributed among hirudineans, being present in all Rhynchobdellida and in Erpobdelliformes among the Arhynchobdellida (Ferraguti 1999; see also chapter 9 of this volume).

Jamieson (1967) was not able to confirm the presence of a spermatophore within the spermatheca of the eudrilid *Stuhlmannia variabilis* which was illustrated by Beddard (1901). That author also reported spermatophores, within the spermatheca, in the same family, in *Polytoreutus*. Whether they were endospermatophores or thecacysts is uncertain. Internal spermatophores have also been reported for the acanthodriline megascolecid *Dichogaster austeni* (see Stephenson 1930).

8.2.10 Spermatozeugmata

Spermatozeugmata may be defined as sperm aggregates implanted in the spermatheca by the concopulant, characterized by a repetitive order of the spermatozoa and the presence of some sort of cementing agent, but lacking a proper capsule (Ferraguti *et al.* 1989; Ferraguti 1999). Spermatozeugmata are said to be formed in the spermathecal ducts during copulation (Dixon 1915). However, the cementing substance has also been said to originate from the atrial epithelium (Jaana 1982). Spermatozeugmata are known from tubificids of the subfamilies Tubificinae, Limnodriloidinae, Phallodrilinae and Rhyacodrilinae. The shape and organization varies within some of the subfamilies, such as Limnodriloidinae but is diagnostic in others, such as Tubificinae (references in Ferraguti 1999).

All investigated tubificines, with the exception of *Aulodrilus* and related forms, have spermatozeugmata with the shape of rods 1-2 mm long, with a diameter of less than 100 μm . The spermatozeugmata are formed by eusperm and parasperm (for terminology see Healy and Jamieson 1981) as they are in some Limnodriloidinae (see sperm dimorphism, 8.4.3, below). Sperm dimorphism is not involved in the Phallodrilinae where there is a single model of spermatozeugma described for *Bathydrilus formosus*. In *Bathydrilus* the single spermatozeugma present in the spermathecae is a skein composed of euspermatozoa with the acrosomes in the central portion, and nuclei and tails wound around this (references in Ferraguti 1999).

In the Rhyacodrilinae, spermatozeugmata which consist of only one type of spermatozoon have been reported in *Rhyacodrilus arthingtonae* by Jamieson (1978c) and *Rhizodrilus russus* by Ferraguti *et al.* (1994). In both there are also many free spermatozoa. In *R. russus*, the spermatozeugmata are formed by a sort of cap surrounding the anterior portion of a sperm bundle, the sperm tails being free. The cap is formed by an electron-dense sheath 0.7 μm thick, terminating with a characteristic rim. The spermatozeugmata are apparently

identical to those described by Martin and Giani (1995) for another rhyacodriline, *Epirodilus michaelsoni* (see Ferraguti 1999).

8.2.11 Taxonomic Survey of Reproductive Systems

Overview. Notable distinctions of the Clitellata from most polychaetes are the restriction of the gonads to a few segments and their hermaphroditism. The plesiomorphic arrangement for oligochaetes is very tentatively considered to be two testicular followed by two ovarian segments (Brinkhurst and Jamieson 1971). In the Lumbriculata the numbers of gonads are very variable (particularly when branchiobdellids and leeches are included). In the Tubificata and Diplostesticulata there are one or two pairs of testes, excepting the lutodrilid crassicitellate *Lutodrilus multivesiculatus* (see McMahan 1976) which, by interpolation, has 10 testicular segments. Two pairs of testes (the holandric condition), in segments 10 and 11, is the predominant condition in the megadriles (Metagynophora), but reduction to a single pair in segment 10 (the proandric condition) occurs *inter alia* in most Alluroididae (*Kathrynella guyanae* Omodeo 1996 has testes in segment 11, and male pores in 14, a displacement of one segment behind the usual alluroidid positions), some glossoscolecids, some *Microchaetus* species and, allowing for segmental deletions, in the moniligastrids *Hastirogaster* and *Eupolygaster*. The alternative, metandric condition, testes in segment 11 only, is widespread in the Metagynophora, including the moniligastrids *Moniligaster* and *Drawida*, allowing for segmental deletions, and characterizes some megascolecid genera (e.g. *Begemius* and *Trichaeta*). Development of spermatogonia or spermatocytes through spermatids to spermatozoa usually occurs in pouches of the septa which delimit the testis segments. These pouches, the seminal vesicles, are maximally, in earthworms, four pairs, on the anterior and posterior septa of the testis segments. Sometimes there is a pair of "pseudovesicles" of unknown function, or sometimes containing spermogenetic stages, at septum 13/14. The number and form of the seminal vesicles has some taxonomic value.

Leaving aside derived members of the Lumbriculidae, the maximum number of functional ovarian segments in oligochaetes is two, seen only in some haplotaxids and three exceptional species of crassicitellates among some 28 oligochaete families, the remainder having a single ovarian segment (Jamieson 1992). The arrangement of reproductive organs is summarized in Fig. 8.4A,B.

8.2.11.1 Subclass Randiellata

Randiellidae. The Randiellidae is a small marine family the reproductive system of which is imperfectly known (Erséus and Strehlow 1986; Erséus 1997). The number and position of the gonads in this family are unclear, but *Randiella caribaea* appears to have at least one testis in segment 10 and one ovary in segment 12 (Erséus 1997) whereas two pairs of testes followed by questionably one or two (paired?) ovaries was previously reported (Erséus and Strehlow 1986). The possibly euprosoporous condition of the male ducts

(male pores in the same segment as their testes), if confirmed, was considered by Jamieson (1988b) to merit placement in a separate basal subclass, the Randiellata. Distribution of the gonads, over more than two segments, certainly appears to merit separation from the Tubificata. Spermathecae are known to be dorsal or lateral.

8.2.11.2 Subclass Tubificata

Order Tubificida. Suborder Tubificina. Tubificidae. In the Tubificidae and the Naididae, which were convincingly subsumed as a subfamily in the former by Erséus *et al.* (2002) there is typically one pair of testes followed in the next segment by one pair of ovaries (the monotesticulate condition). The male pores are in the segment behind the testis segment (the plesioporous condition). The testis segment is usually 10 (Tubificidae *sensu stricto*) or is more anterior in 'naids'. The male duct in Tubificidae *sensu lato* comprises a funnel, a vas deferens, an atrium and, frequently, a copulatory structure (male bursa). There may also be a prostate gland (diffuse or compact) in association with the duct. A tubificid reproductive system is exemplified in Fig. 8.20 by that of *Macquaridrilus bennettiae*, a species which is, however, unusual for the Tubificidae in possessing a spermathecal diverticulum (Jamieson 1968a) and by *Rhyacodrilus arthingtonae* (Fig. 8.21).

The development of the male and oviducal system has recently been clarified by Gustavsson and Erséus (1997, 1999). The funnel and vas deferens in *Clitellio arenarius*, *Tubificoides benedii*, *Heterochaeta costata*, *Rhyacodrilus coccineus*, *Monopylephorus rubroniveus* (Rhyacodrilinae) and the 'naid' *Stylaria lacustris* originate from peritoneal (mesodermal) cells in the posterior septum

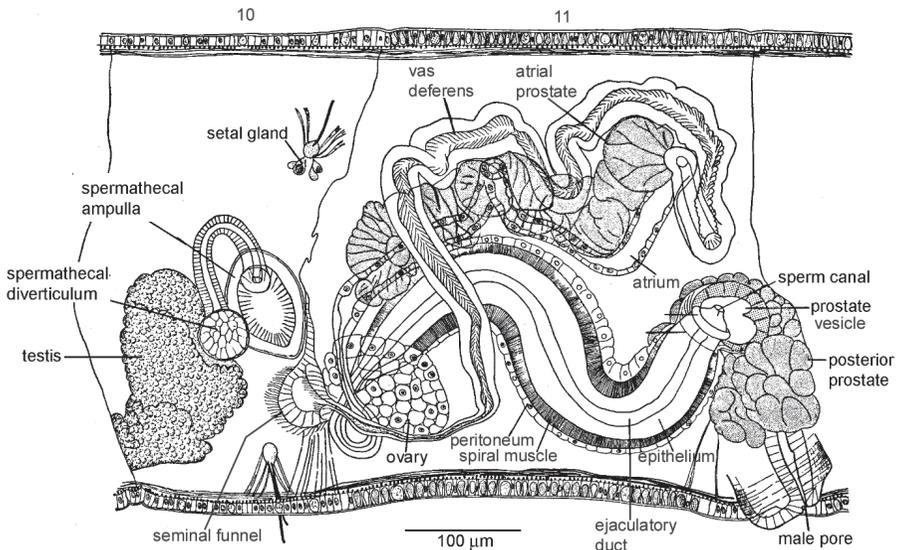


Fig. 8.20. *Macquaridrilus bennettiae* (Tubificidae). Exemplifying the genital organs of a tubificid. This species is unusual in having a spermathecal diverticulum. After Jamieson, B. G. M. 1968a. University of Queensland papers. Department of Zoology 3: 55-69, Fig. 5.

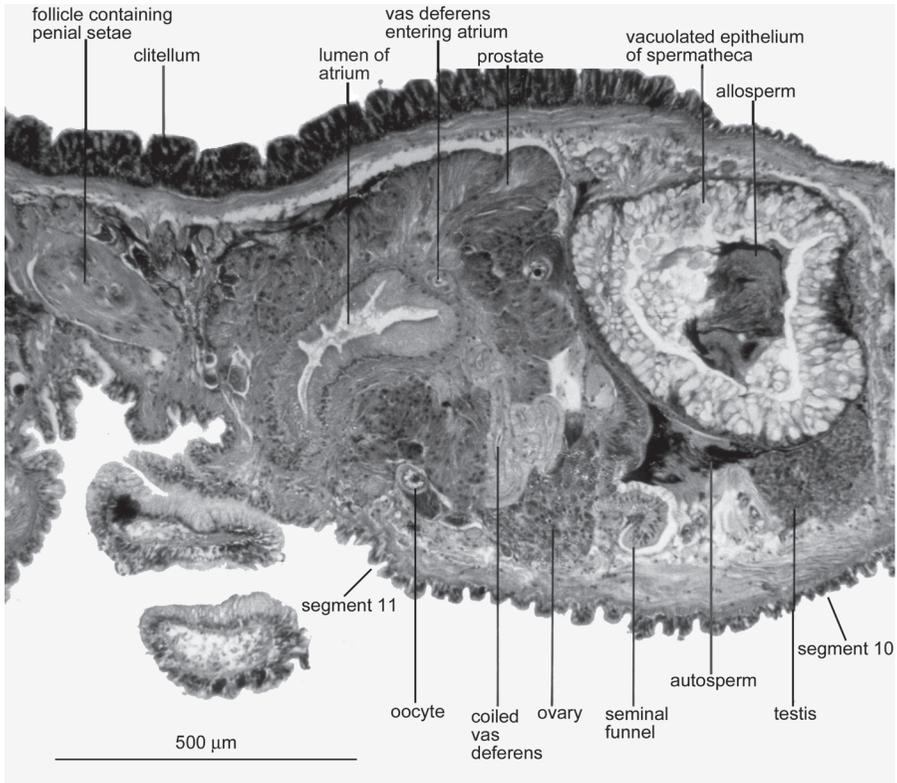


Fig. 8.21. *Rhyacodrilus arthingtonae* (Tubificidae). Photomicrograph of genital organs. After Jamieson, B. G. M. 1978. Proceedings of the Royal Society of Queensland 89: 39-43, Plate 5.

in the testis segment. The atrium develops from an epidermal (ectodermal) invagination but a typical atrium is not formed in *M. rubroniveus* in which the entire duct is of mesodermal origin. In the latter species, a shallow epidermal invagination occurs, into which both male ducts open; it bears resemblance to a copulatory structure, which usually forms from a secondary invagination, rather than to a proper atrium. The prostate glands have different origins in different taxa; in *S. lacustris*, *R. coccineus* and *M. rubroniveus* they differentiate from the peritoneal (mesodermal) cells surrounding the atrium but in *H. costata* and *T. benedii* they develop by a multiplication of the atrial (ectodermal) cells; *C. arenarius* lacks a prostate gland. The extent of the diffuse prostates in rhyacodrilines varies: in *R. coccineus* the cells cover the atrium, whereas in *M. rubroniveus* they cover only a part of the vas deferens.

In all of these species, the spermatheca is confirmed as being of ectodermal origin, developing as an invagination of the epidermis, and the female ducts develop from peritoneal (mesodermal) cells in the posterior septum of the ovarian segment. However, in *M. rubroniveus* the first rudiment of the oviduct disappears and a proper duct never develops. The position of

the oviduct differs; in *S. lacustris* the female duct is lateral and the pore is in the ovarian segment, while in the tubificids studied the duct is ventral with the pore in the segment behind the one containing the ovary (Gustavsson and Erséus 1997, 1999).

Naidinae are notable in having asexual, in addition to sexual, reproduction. They reproduce asexually by paratomic fission, including regeneration of a fixed number of anterior segments in secondary individuals. This provides a great potential for population growth (Erséus 2003).

Phreodrilidae. Phreodrilids have a pair of testes in segment 11 and a pair of ovaries and male pores in 12. Spermathecal pores are ventral or dorsal on 13, often with vestibulae; spermathecal ampullae in 13 or usually more posterior. The female funnels are on septum 12/13 or entering into spermathecal vestibulae. Atria are present, usually with a very narrow lumen, without prostate glands. Pendant penes or eversible pseudopenes are usually present. Asexual reproduction is rare (Pinder and Brinkhurst 1994). Internal fertilization is suspected in some species.

Opistocystidae. Opistocystids are typical plesiopores but the gonads are unusually posterior in *Opistocysta*. In *O. funiculus* (in South America and Africa) testes are in segment 21, ovaries in 22, sperm funnels on 21/22, male pores on 22, female pores apparently in 22/23, and spermathecal pores anterior on 23. Seminal vesicles extend from 21/22 into 22 and ovisacs from 22/23 to 26. *O. corderoi* has a similar configuration of genital organs but they are further forward, the testes being in segment 14 or 15, with the clitellum at least from 15-16.

In *Lycodrilus*, tentatively placed in the Opistocystidae, the genitalia have the tubificid arrangement: testes in segment 10, ovaries in 11, male pores in 11, female pores in 11/12. Spermathecae are in segment 8 or 10 and spermatophores are present. There are small atria with stalked prostates (Cook 1971b).

Dorydrilidae. Dorydrilids are plesioporous, with one pair of testes and funnels in segment 9 and male pores in 10. Spermathecal pores are in segment 10 or 11, or both, spermatheca being atrial or post atrial or both. The ovaries are in segment 10. A pair of vasa deferentia joins a pair of large, muscular atria in 10 (Cook 1968, 1971a). Cook has espoused derivation of *Dorydrilus* from a lumbriculid ancestral form but if affinities lie with the Tubificidae this origin is unlikely in view of the wide phylogenetic separation of lumbriculids and tubificids indicated from molecular studies.

Enchytraeidae. The Enchytraeidae, Propappidae and Capilloventridae have what may be the most plesiomorphic condition of the male ducts known in oligochaetes, the usual absence of a male atrium. If this is a symplesiomorphy it could not be used to unite these families. However, some enchytraeids have a well developed male atrium (Fig. 8.22) as in most other oligochaete families.

The Enchytraeidae is one of the largest families of oligochaetes and is abundant terrestrially as well as in freshwater and marine habitats.

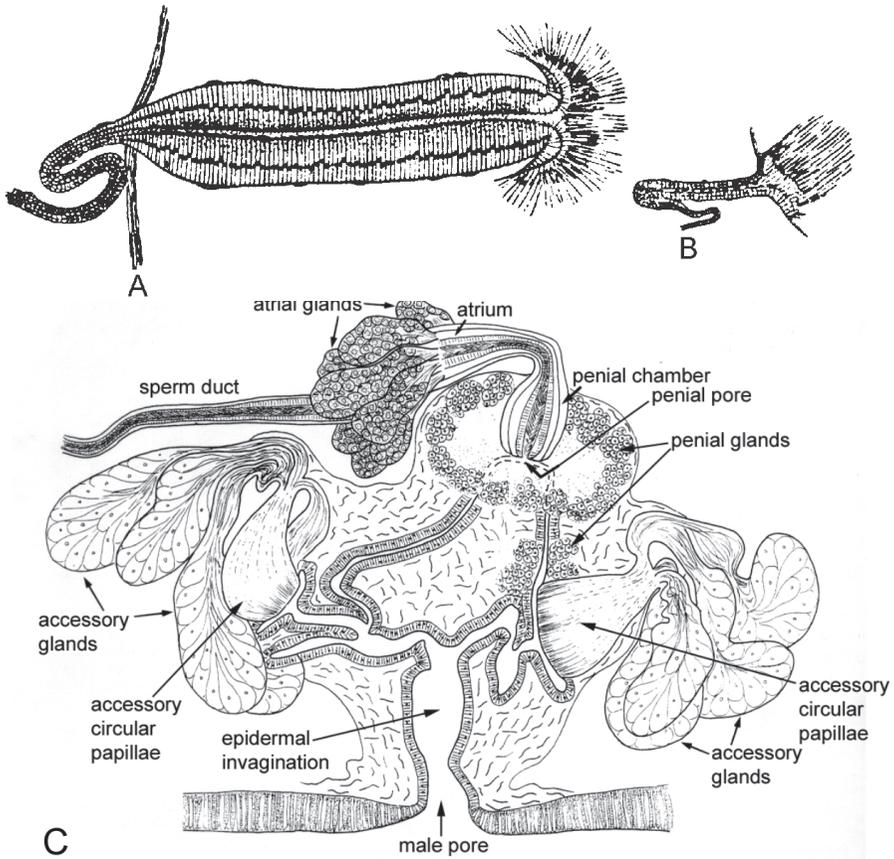


Fig. 8.22. **A.** *Enchytraeus albidus* (Enchytraeidae). Seminal funnel. Note prostate-like preseptal expansion. **B.** *Propappus glandulosus* (Propappidae). Seminal funnel. From Michaelsen, W. 1928. *Oligochaeta*. Pp. 1-118. In W. Kükenthal and T. Krumbach (eds). *Handbuch der Zoologie*. 2. Walter de Gruyter and Co, Berlin, Fig. 45. **C.** *Mesenchytraeus antaeus*. Ectal male apparatus showing atrium, accessory glands, penial glands and a penial chamber or bulb. The apparatus is highly complex, contrary to what is usually envisaged for enchytraeids. After Rota, E. and Brinkhurst, R. O. 2000. *Journal of Zoology London* 252 (1): 27-40, Fig. 6.

Enchytraeids have a pair of testes in segment 11 followed by a pair of ovaries, in 12, in which segment the male pores are situated. Spermathecae are paired in segment 5, with pores in 4/5 (Fig. 8.4A). They have a long swollen glandular preseptal duct immediately posterior to each seminal funnel (Fig. 8.22A) and this may be regarded as an alternative strategy to having prostate glands for providing seminal secretions. Other male glands, and penial bulbs, are usually absent. However, the absence or weak development of atria and associated glands is possibly size dependent (see *Mesenchytraeus antaeus*, below). Many details of the reproductive anatomy of *Fridericia* are given in the valuable monograph of Schmelz (2003).

Although most enchytraeids are microdriles, some are as large as earthworms. *Mesenchytraeus antaeus* an unpigmented giant enchytraeid from Vancouver Island, reaches, in fixed material, 61×2.9 mm (with the diameter maximal in the midbody). Its spermathecal pores open laterally in the middle of segment 5. The male apparatus has unusually long sperm funnels (extending over 10 segments) and two groups of large accessory glands opening through two circular papillae at the base of each penial bulb, independently of the penial pores (Rota and Brinkhurst 2000). The ectal male apparatus of this species (Fig. 8.22C), which includes an atrium, accessory glands, penial glands and a penial bulb, is highly complex, contrary to what is usually envisaged for enchytraeids. This perhaps suggests that simplicity of the reproductive system in small species is a secondary condition.

Most enchytraeids have no sperm sacs (seminal vesicles) or ovisacs, apparently by loss. These sacs are, however, present in *Mesenchytraeus*, *Grania*, *Randidrilus* and a few species in other genera (Omodeo 1998).

Propappidae. The Propappidae were removed from the Enchytraeidae because they have characters which are not seen in the latter family: sigmoid, nodulate, bifid chaetae; large epidermal glands posterior to each chaetal bundle; spermathecal pores at septum 3/4; and glandular parts of the vasa deferentia located posterior to 11/12 (Fig. 8.22B) unlike the preseptal glandular enlargement in enchytraeids. Another significant, unique character state is location of the single pair of ovaries in segment 13, except in *Propappus arhyncotus*, rather than in the enchytraeid location in the male pore segment, 12 (Coates 1986). The presence of a one-segment hiatus (requiring confirmation) between testes and ovaries is here considered homoplastic with the Metagynophora.

Capilloventridae. Capilloventrids have one pair of testes in segment 11 and a pair of ovaries and male pores in 12. Atria, prostates and penes are absent. Female pores are in 13 (to be confirmed in some species). Spermathecal pores are lateral at 6/7, with the ampullae in segment 7 (Pinder and Brinkhurst 1994). With their simple organization, including dorsal and ventral bundles of hair chaetae (shared with Parividrilidae), as in some polychaetes, the capilloventrids appear to be the most plesiomorphic oligochaete (and clitellate) family and this position has been confirmed in 18S rDNA parsimony analysis (Erséus and Källersjö 2003). Their plesiomorphy possibly militates against regarding the octogonadal condition as primitive for oligochaetes.

Parividrilidae. In ovigerous specimens of the type-species *Paravidrilus strayeri* (Fig. 8.23), a clitellum is developed as a pair of lateral rows of a few, large, swollen epidermal cells in segments (9-) 10-12 (-13, -14). It is unclear whether gonads are paired or unpaired; testes (testis?) are in segment 11, ovaries (ovary?) in 12. The other reproductive organs include a U-shaped muscular 'genital body', in segment 12 and a V-shaped 'copulatory organ', consisting of two conjoined penes (or pseudopenes) in a penis sac or sheath, in segments 12-13; the exact nature and function of these structures are unknown (Erséus 1999). Martinez-Ansemil *et al.* (2002), for the second known