Handbook of Zoology
Gastrotricha, Cycloneuralia and Gnathifera

Volume 2:
Nematoda

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Founded by Willy Kükenthal
Editor-in-chief Andreas Schmidt-Rhaesa

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DE GRUYTER

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Introduction

There are more than a few people who think that all nematodes more or less look the same and are morphologically uniform in shape, as expressed by the common notion that a nematode is "a tube within a tube". How narrow minded this is! Nematodes are extremely diverse and abundant; they have occupied almost every possible habitat and are of extreme economic and medical importance. About 27,000 species are currently described, but it is more than likely that this reflects only a fraction of the real diversity.

Despite this vast diversity, nematodes are often reduced to a single species, *Caenorhabditis elegans*, which has become a pet and model animal for developmental biology and genetics. It is extremely well known in many cellular, developmental and genetic details. *C. elegans* was proposed as a model organism by Sydney Brenner in 1965. Earlier, in the 19th century, another nematode, *Parascaris equorum*, was an important model organism.

The concentration on one model species provides excellent and deep insights into particular aspects of this species. Every single cell of the body of *C. elegans* is known, along with the developmental origin of all those cells during cleavage from the egg. In achieving this goal, it was fortunate that *C. elegans* has a constant cell number (eutely) and a strictly determinate cleavage. It is generally assumed and reflected in textbooks that these features are common features of all nematodes, but this is not the case, and the nematode ancestor probably did not have a strictly determinate cleavage or eutely (e.g., Voronov & Panchin 1998, Voronov et al. 1998, Schierenberg 2000). This example shows how important it is to proceed on two paths: study model species in detail but always keep the broad diversity of a taxon in mind.

Fortunately, there is large interest in nematodes, and the initial concentration on *C. elegans* as *the* model nematode is now broadening to include more species. Because nematodes are so diverse in habitat and life style, the community of nematode students has split into several subgroups. Some are working with free-living forms, others with plant parasites or with animal parasites. This volume tries to bring the diverse information on nematodes together. Unfortunately, it was not possible to find authors for every nematode taxon, and therefore a few taxa could not be included. Nevertheless, the authors have contributed an impressive collection of chapters covering many aspects of nematode biology and diversity.

One topic that could not be treated in the desired detail is a review of nematode phylogeny and systematics. This aspect is replaced by a very short introduction, but excellent literature is available on this topic.

In some cases, a different terminology is applied for similar structures. The most prominent example is whether the postembryonic, non-adult stages of nematodes should be named juveniles or larvae. The decision of authors to use their terminology was respected here, which leads in some cases to a not unified terminology among chapters.

As volume editor, I want to thank all my colleagues who contributed to this volume. Sadly, we lost one colleague, Odile Bain, during the process of producing this volume. She died in 2012. We will keep her in memory.

Andreas Schmidt-Rhaesa

Literature

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Dedication

Dr. Odile Bain passed away on October 16, 2012, shortly after finalizing the manuscript for the chapter on the Spirurida. As her co-authors, we would like to dedicate this chapter to her for having been such an inspiring colleague, mentor and friend. Having been the driving force from the start, Odile approached this work as she did her research – with utter professionalism and competence, great dedication and infectious enthusiasm. While she would usually cite filarial worms, their biology and host relationships as her field of expertise, it soon became apparent that she had accumulated a wealth of knowledge on virtually all the major groups within the Spirurida and had first-hand experience in the taxonomy, morphology and development of many taxa included in this chapter. A biologist by training, Odile had been employed in the field of parasitology since she joined the Muséum National d'Histoire Naturelle, Paris, France in 1964. The outstanding quality of her research made her a recipient of the Bronze Medal of the Centre National de la Recherche Scientifique (CNRS), France. In 1984, she was awarded the Prix Foulon of the French Academy of Sciences, and she was one of a select few who were granted the title of Director of Research, Exceptional Class by the CNRS. As the world authority on filariae and filarioses, she was a member of the steering committee on filarioses of the World Health Organization until 1995. It is a testimony to her human qualities, however, that despite her extraordinary accomplishments as a scientist, evidenced by over 350 publications in scientific journals and contributions to a number of books, many of her colleagues will remember her first and foremost for the exceptional person she was. A free-thinker with a versatile mind and a great sense of humor, generously reaching out to others and freely sharing her vast expertise.

Kerstin Junker and Yasen Mutafchiev

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Wilfrida Decraemer, August Coomans and James Baldwin

1 Morphology of Nematoda

Nematode morphology forms the basis for understanding nematode development, function, behavior, evolution and relationships. Nematodes are apparently simple organisms, but their similar appearance is deceiving as these animals possess amazing plasticity to adapt to a wide range of conditions and habitats. In the last decade, morphological studies have developed toward a more integrated approach, combining classical and modern tools and techniques, such as electron, confocal and 4D microscopy (Bumbarger et al. 2007, Ragsdale & Baldwin 2010), with information from molecular analyses and gene studies (Bert et al. 2008).

Nematodes are generally described as small, nonsegmented animals with typical thread-like bodies (*nema* = thread in Greek). They are mostly translucent, allowing observation of their internal anatomy by light microscopy without dissection or sectioning. Nematodes possess an apparently simple and relatively conserved basic body plan that consists of an external cylinder (the body wall) and an internal cylinder (the digestive system), which are separated by a pseudocoelomic body cavity filled with fluid under pressure and containing a number of cells and other organs, such as the reproductive tract.

1.1 General and external morphology

1.1.1 Size

Free-living and plant-parasitic nematodes are usually smaller than 1 mm. The smallest free-living marine nematode species so far recorded was a female of *Halalomus minutus* (Desmoscolecida), 82 µm long, that was described from a sandy beach in Togo, whereas the longest species is *Cylicolaimus magnus* (Leptosomatidae, Enoplida), 21–34 mm long, first recorded from the English Channel. The shortest plant-parasitic nematodes are less than 300 µm long (*Neopsilenchus minor*, Tylenchidae), and the longest may grow up to 12 mm (*Paralongidorus epimikis*, Longidoridae). Animal parasites can be considerably longer, showing some correspondence with the body size of their host. An extreme example is the human parasite *Dracunculus medinensis* (Spirurida), the Guinea worm, which is 1.2 m long, and the largest species described is *Placentonema gigantissima* (Spirurida), a parasite of the placenta of the sperm whale, with females up to 8.4 m and male specimens more than 6 m long. Large intraspecific variation has been observed in body length in several species, with the larger specimens being up to 3 times longer than the smaller specimens (Geraert 2006). Food availability and feeding time may influence body size. Nematode growth is not limited to juvenile stages and molting processes, but also occurs during the adult stage. In large nematodes, such as animal parasites, a large body size is achieved primarily by adult growth, which can be achieved by continued cell proliferation but is often due to massive somatic polyploidy by endoreduplication (Flemming et al. 2000).

1.1.2 Shape and dimorphism (Fig. 1.1 A–G)

About 99% of all known nematodes have long and narrow cylindrical body shapes, round in cross section (roundworms) and tapered toward both ends, usually more so posteriorly toward the tail region. More rarely, the body is also strongly tapered anteriorly, as in *Rhynchonema* spp. (Monhysterida), making it more difficult to differentiate the anterior end from the posterior end at low magnification. Bodies can also be short and robust, as in the plant-parasite *Criconemella* (Tylenchina) or the free-living marine *Desmoscolex* (Desmoscolecida) species. Upon fixation, the body can vary from straight to more or less ventrally curved (C-shaped or J-shaped), up to spirally wound. Aberrant body shapes appear in different types. For example, in obligate plant parasites, such as cyst (*Heterodera*) and root-knot nematodes (*Meloidogyne*), female bodies are extremely swollen but also in some animal parasites (e.g., *Tetrameres*, a parasite of chickens). In the free-living marine families Epsilonematidae and Draconematidae, the bodies are ε- or S-shaped, respectively, with swollen pharyngeal and mid-body regions. In *Desmoscolex*, the body has a strongly annulated appearance, distantly resembling oligochaetes, due to the presence of extra cuticular bands of foreign particles embedded in secretory products. An even stranger appearance is observed in the female of *Sphaerularia bombi*, a parasite of bumblebees. In development toward the adult stage, the reproductive system is extruded and swells approximately 300 times, forming an uterium with

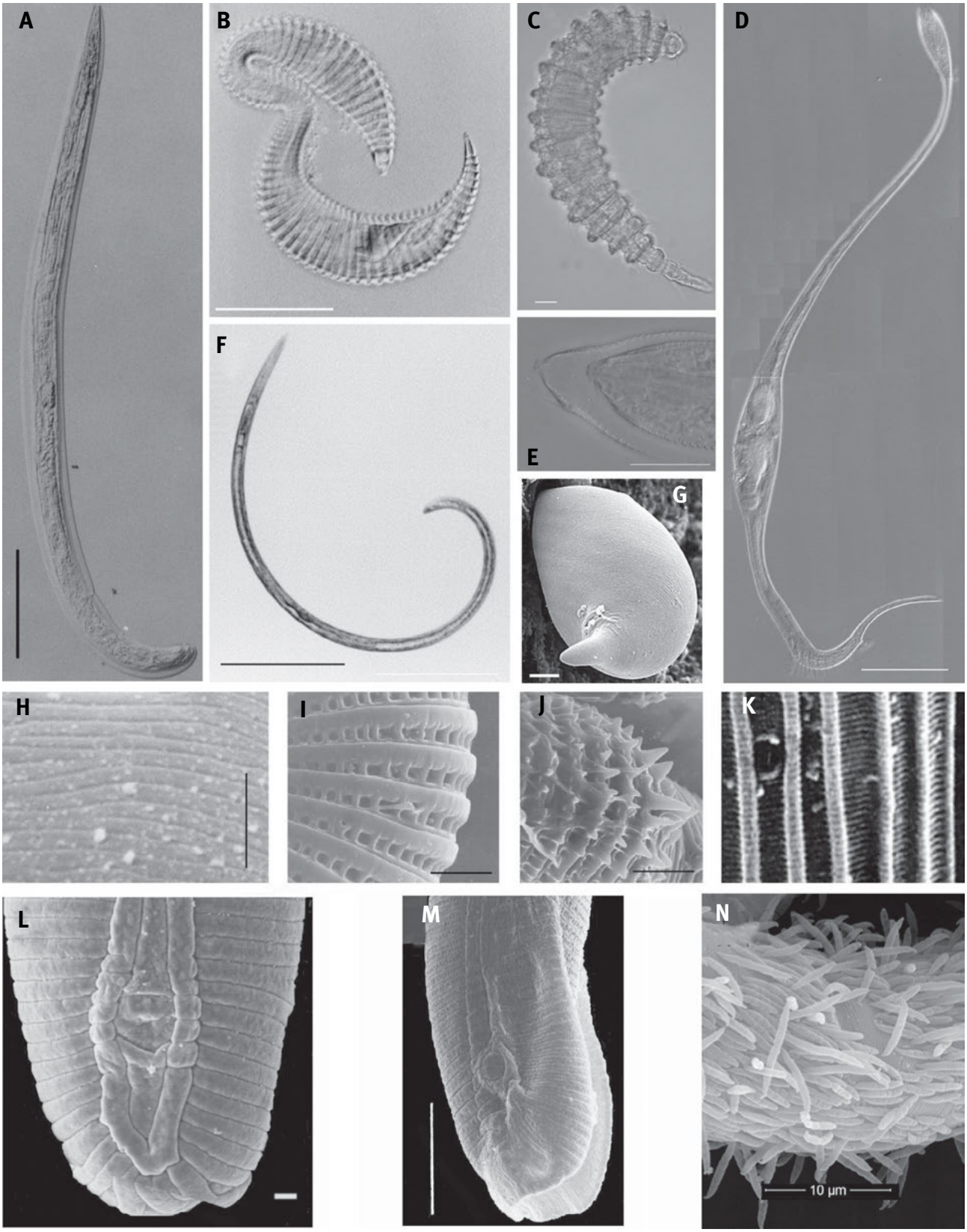

Fig. 1.1: Diversity of body shapes and cuticle ornamentation. A, J-shaped body of a *Trichodorus* male; B, ε-shaped body of *Epsilonema*; C, Short fusiform body of *Desmoscolex*; D, S-shaped body of *Tenuidraconema*; E, Head-region molting *Tenuidraconema*; F, Open C-shaped body of *Xiphinema*. SEM micrographs. G, Swollen posterior body region of *Heterodera glycines* female; H, Body cuticle with transverse striae; I, Annulated body cuticle with vacuoles; J, Annulated body cuticle with spines; K, Body cuticle with longitudinal ridges; L, Aerolated lateral field, arrow; M, Posterior body region showing caudal alae in *Scutellonema*; N, Ectosymbiontic bacteria in a Stilbonematid. Scale bars: A, B, D, F 100 µm; C, H, I, J, K, M, N 10 µm; E 20 µm; L 1 µm.

the small female body (2 mm) remaining attached to it. Aberrant body shapes often indicate loss of locomotion, as in the swollen female of cyst and root-knot nematodes, or an aberrant locomotion pattern, as in Draconematidae and Epsilonematidae, which move on their ventral side with the additional help of specialized setae (see 1.1.4., 1.2.1 & 1.3) instead of by undulating movements in a dorsoventral plane. *Desmoscolex* and Criconematid species show an aberrant earthworm-like progress.

Sexual dimorphism can be found not only in body shape (root-knot nematode males are slender vermiform, whereas females are swollen) but also in body size. An extreme case of the latter is found in *Trichosomoides crassicauda*, where the male is only 2 mm long and resides within the vagina and uterus of the 5 times longer female.

1.1.3 Orientation and *body symmetries* (Fig. 1.2 A–B)

Externally, the nematode body can hardly be subdivided into body regions apart from an often tapered tail region or an exceptionally swollen pharyngeal region (Draconematidae). The mouth is usually located terminally at the anterior end. The ventral side can be recognized by the position of the secretory-excretory (SE) pore and the vulva and anus in females or the cloacal opening in males. The nematode body, more or less circular in cross section, has two fundamental symmetries: a bilaterally and a triradially symmetrical body plan. Coomans (1978) proposed a precise terminology for both types of symmetries that permits the correct description of any structure.

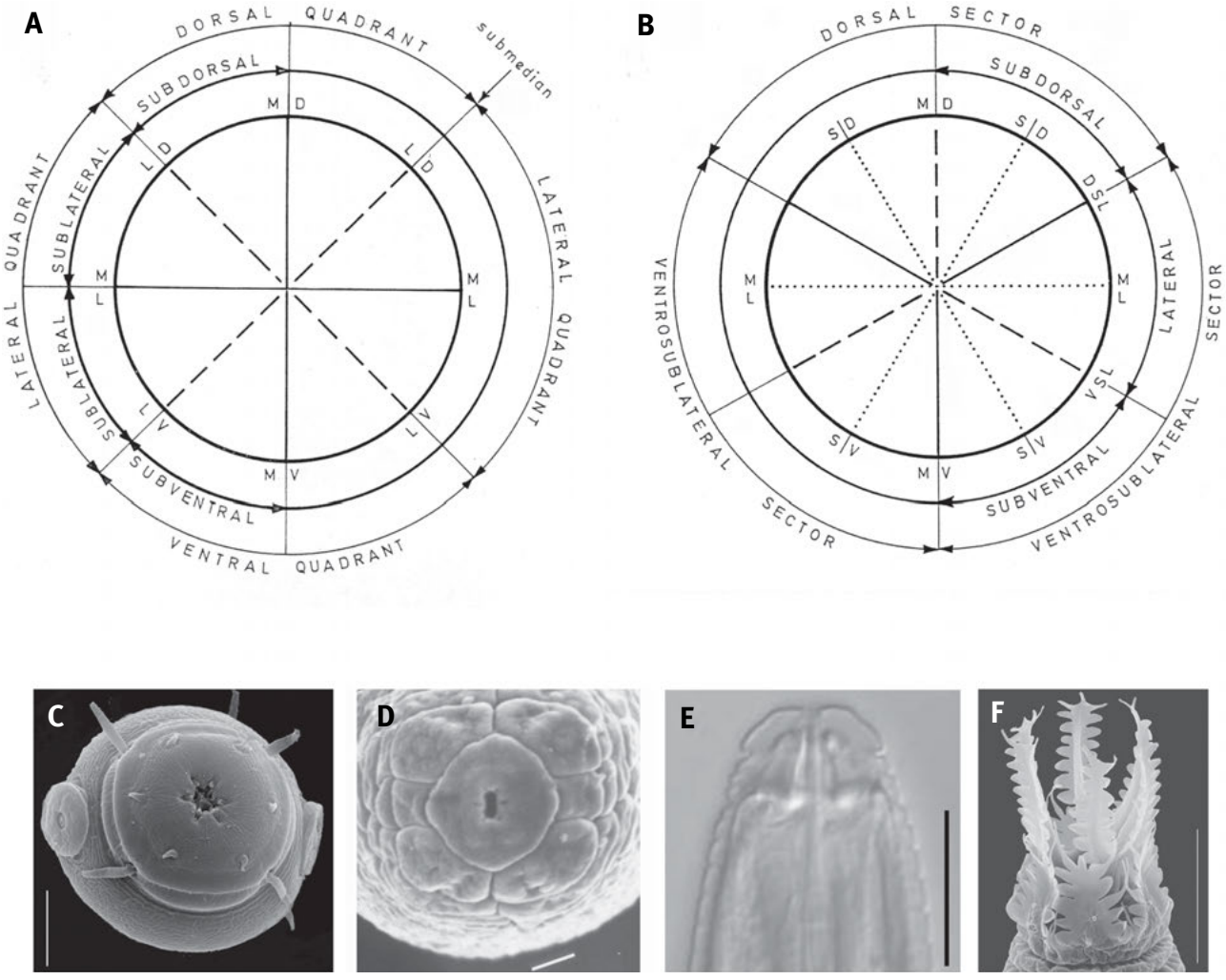

Fig. 1.2: Nematode symmetries. A, Scheme bilateral symmetry (after Coomans 1979); B, Triradial symmetry (after Coomans 1979). SEM microphotographs. C, Lip region in *Desmodora* with fused lips; D, En face view of *Scutellonema* with oral disc and lip areas; E, Head region in *Meloidogyne*, cephalic framework, male; F, *Acrobeles* sp. showing elaborate probolae. Scale bars: C 5 µm; D 1 µm; E, F 10 µm.

The bilaterally symmetrical body plan applies to all structures, with the exception of the pharynx (esophagus). This body plan has four quadrants, two lateral, a dorsal and a ventral, bordered by laterodorsal and lateroventral radii. The quadrants can be divided into equal subregions, respectively, by mediolateral, mediodorsal and medioventral radii resulting in a total of four sublateral, two subdorsal and two subventral regions.

The triradially symmetrical body plan only applies to the pharynx and lip region. It has three equal parts, one dorsal and two ventrosublaterals, delimited by two dorsosublateral and one medioventral radii. The dorsal sector here is larger than in the bilaterally symmetrical body plan; it can be divided into two subdorsal sectors, also larger than the division of the dorsal quadrant of the bilateral symmetry. The laterosubventral sectors can be divided into a lateral (smaller than in the bilateral symmetry) and a subventral sector (larger than in the bilateral symmetry).

At the anterior end or lip region, the bilateral symmetry of the body fuses with the triradial symmetry of the pharynx, resulting in radial (often hexaradial) symmetry (De Coninck 1942, 1965). The radial symmetry at the anterior end is supposed to have originated in relation to a semi-sessile life in which the nematode is attached to a substrate by means of secretions from the caudal glands and occupies a vertical position, with the anterior end completely surrounded by the medium. This behavior is considered to be a plesiomorphic trait and can be frequently observed in aquatic nematodes. The symmetry was, however, retained in forms that lost the semi-sessile life because, in most of them, the anterior end has a smaller diameter than the remainder of the body, and in this way it is completely surrounded by the medium.

1.1.4 Body ornamentation (Fig. 1.1 H–N)

The body cuticle may appear smooth, although it often shows transverse markings such as striae or annuli and/or longitudinal markings such as striae or ridges. In transverse striation, the incisures are fine and superficial, whereas annuli are delimited by deeper incisures that involve more than one cuticle zone (Decraemer et al. 2003) and that are farther apart. When both transverse striae/annuli and longitudinal striae are present all over the body, the cuticle has a tessellate appearance. Longitudinal ridges may be present all over the body, with or without an internal support, in animal parasites. In a significant number of nematodes, the cuticular pattern is different at the lateral body sides, in which case one speaks of “lateral fields”. In Tylenchomorpha, the lateral fields may rise above the body contour and form longitudinal ridges separated by deep incisures. The number of ridges or the number of longitudinal incisures of a lateral field is of taxonomic importance but should be counted at mid-body as the number decreases toward the extremities. The lateral longitudinal ridges may be interrupted by the transverse striae (areolated lateral field), show anastomoses (fusion) or it may disappear in obese females. Alae are thickened wings of cuticle that are found mainly laterally or sublaterally on the body. According to the body region in which they occur, one distinguishes cervical alae, lateral alae and caudal alae. In some taxa, the caudal alae in the tail region of males are well developed and form a copulatory bursa. More elaborate types of cuticular ornamentation may be observed, such as spines, setae, papillae, tubercles, warts, bands, plates or rugae. Cuticular differentiations may also occur at or around the vulva, the anus and in the caudal area of males as well as at the tail tip. Some nematode taxa have a body cuticle covered by foreign material, as in *Desmoscolex*. The outer appearance of the nematode may also be influenced by the adherence of other organisms, such as bacteria. For example, in the genera of the subfamily Stilbonematinae, adhering Cyanobacteria give these marine nematodes a “hairy” appearance.

Surface markings such as annulation may facilitate locomotion, whereby the rings are retrorse (Epsilonematidae, Criconematidae). Lateral ridges also allow some degree of widening of the body. Spines and ridges in Spirurida aid in locomotion or function as mechanisms for scraping the host mucosa or as means of attachment to intestinal villi. In *Glochinema bathyperuvensis* (Epsilonematidae), dense spiny cuticular ornamentation provides balance in the uppermost sandy mud layers in deep-sea habitats.

1.1.5 Lip and head region (Fig. 1.2 C–F, Fig. 1.8)

The lip region can be continuous with the body contour or be offset from the rest of the body, either by a depression (shallow indentation) or a constriction (sharply offset); furthermore, the lip region may be expanded with respect to the adjoining body. The oral opening is usually terminal, rarely displaced toward ventral or

dorsal side. The basic pattern of the lip region consists of six separate lips radially arranged around the terminal oral opening as two subdorsal, two subventral and two lateral lips (see 1.5.1.2.). The lips can fuse into pairs of two, resulting in three lips (one dorsal and two ventrosublateral, as in the cephalobid *Chiloplacus* and in *Ascaris*) or fuse into two sets of three, resulting in two lateral lips (e.g., *Pseudonchus*). In some forms, the lips are unequal in size (e.g., *Pseudacrobeles pulcher*, with reduced lateral lips). The lips may be separated from one another or partially (e.g., *Mononchus*) to completely fused (*Trichodorus*). In Tylenchomorpha, the anterior end shows an amalgamated, usually hexagonal lip region with lip-like differentiations described as lip sectors, and the area around the "oral" opening may be differentiated into an oral and/or labial disc. In this group, the real oral opening may be displaced internally and connected by a prestoma to the prestomatal opening. In Tylenchomorpha, the cephalic region is internally supported by a poorly to heavily sclerotized cuticular cephalic framework that may be variously developed. The lip region carries a concentration of anterior sense organs with a primitive arrangement in two circlets of six labial sensilla each (papillae or setae), followed (either on the lips or postlabial) by four cephalic sensilla (papillae or setae). Laterally and often posterior to the lips are chemoreceptor organs or amphids. In some taxa, the labial area may bear membranous appendages (*Enoploides,* Enoplidae) or cuticular projections or probolae (Cephalobidae). Probolae may be simple, deeply bifurcated to very complex and form a major character in genus and species identification. In addition, cuticular outgrows or interlabia can also be found between the lips (*Travassosinema thyropygi*). In some species, lateral cuticular outgrows develop over the primary lips and replace them; they are referred to as false lips or pseudolabia. In some marine species, the lips can be retracted within a strongly sclerotized head region or "helmet" (*Epsilonema*).

1.1.6 Tail region (Fig. 1.3 A–I)

According to species, the tail varies in shape from long filiform to short and broadly rounded. The tail shape may be similar throughout all developmental stages or differ between juvenile stages or between juveniles and adults or show sexual dimorphism. The male tail in several genera may be enveloped by a bursa. The tail plays an important role in locomotion; a long tail for example, appears advantageous in aquatic habitats and is considered to be plesiomorphic. The tail may also help in anchoring the body or as a tool during eclosion (*Trichodorus*) of the juvenile from the eggshell.

1.2 Integument or body wall

The body wall of nematodes is composed of an external non-cellular cuticle, lined inside by the epidermis (hypodermis) or main progenitor of the cuticle, and the somatic musculature composed of longitudinal muscles only.

1.2.1 Body cuticle (Fig. 1.4 A–D)

The body cuticle is a flexible and resilient exoskeleton that plays a key role in the development, growth and survival of all nematodes. It is a complex conserved system that together with the epidermis protects the animal from harsh environmental conditions. The body cuticle is semi-permeable and plays a role in the secretion-excretion or uptake of substances, providing an internal physiological balance. As nematodes do not have a circular body musculature, the cuticle serves as antagonist for the contraction of the longitudinal somatic muscles during undulatory locomotion and the inner high turgor pressure system of the body cavity (pseudocoel). The strong structure of the cuticle prevents radial deformation of the body and possible obstruction to locomotion. The exoskeleton is composed of a complex extracellular matrix (ECM) synthesized at the end of embryogenesis and prior to hatching, and subsequently it is formed by the epidermis at the end of each of the four juvenile stages prior to molting. The structure of the body cuticle varies from a simple and thin (some plant-parasitic nematodes) to a highly complex, multilayered structure, as in some free-living marine species. It covers not only the entire surface of the animal but invaginates at openings and lines the stoma, rectum and cloaca in males, the vulva in females, the S-E duct and some sensory organs (e.g., amphidial canal, phasmid duct, somatic papillae and setae). The cuticle may appear smooth although it often shows different types of ornamentation (see previously). It exhibits great diversity externally as well as internally at the ultrastructural level among taxa but also intraspecifically among different developmental stages, between sexes or at different sites on the body. This variability is

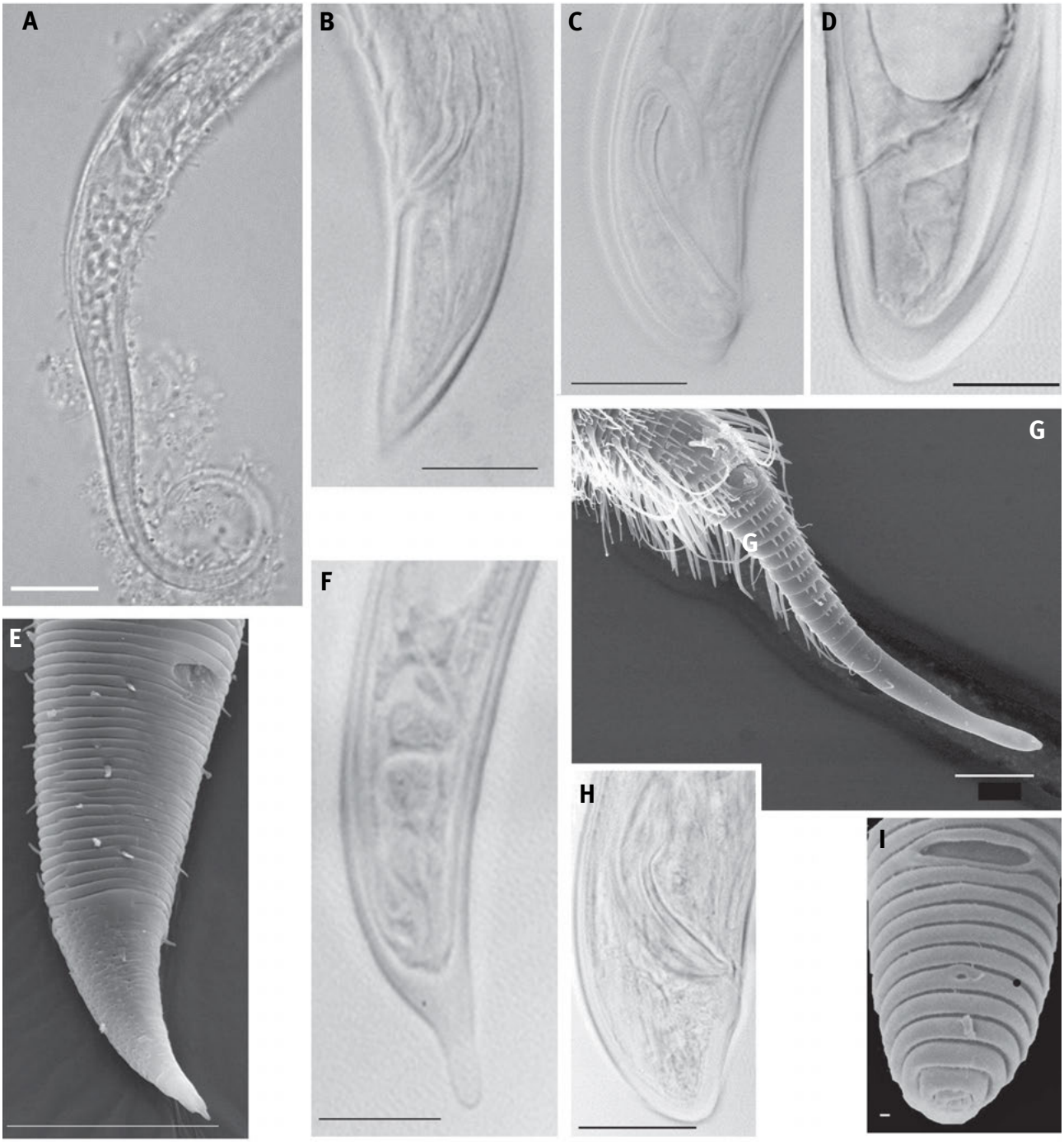

Fig. 1.3: Tail shapes. A, Filiform tail in *Dichromadora*; B, Dorsally convex conoid tail in *Paralongidorus*; C, Short rounded tail, *Trichodorus* male; D, Short rounded tail in a *Longidorus* female; E, SEM microphotograph of a conoid tail in *Croconema*; F, Juvenile tail with digit in *Longidorus*; G, SEM microphotograph of fine conical tail in *Glochinema*; H, Short, dorsally convex, ventrally slightly indented tail in a *Mesodorylaimus* male; I, Short, stout conical tail in *Hemicriconemoides*. Scale bars: A 10 µm; B–D, F–H 20 µm; E 40 µm; I 1 µm.

especially pronounced in parasitic species where it shows an adaptation to different life cycles such as a free-living stage and a parasitic stage (root-knot nematodes) or different types of parasitic stages (a plant-parasitic and an insect-parasitic stage as in *Fergusobia*). More rarely, the body cuticle may be completely absent as in females of *Fergusobia* sp. when living as parasites of *Fergusonina* flies; in the parasitic life stage the digestive system has degenerated and direct food uptake occurs through the microvilli of the epidermis (Giblin-Davis et al. 2001).

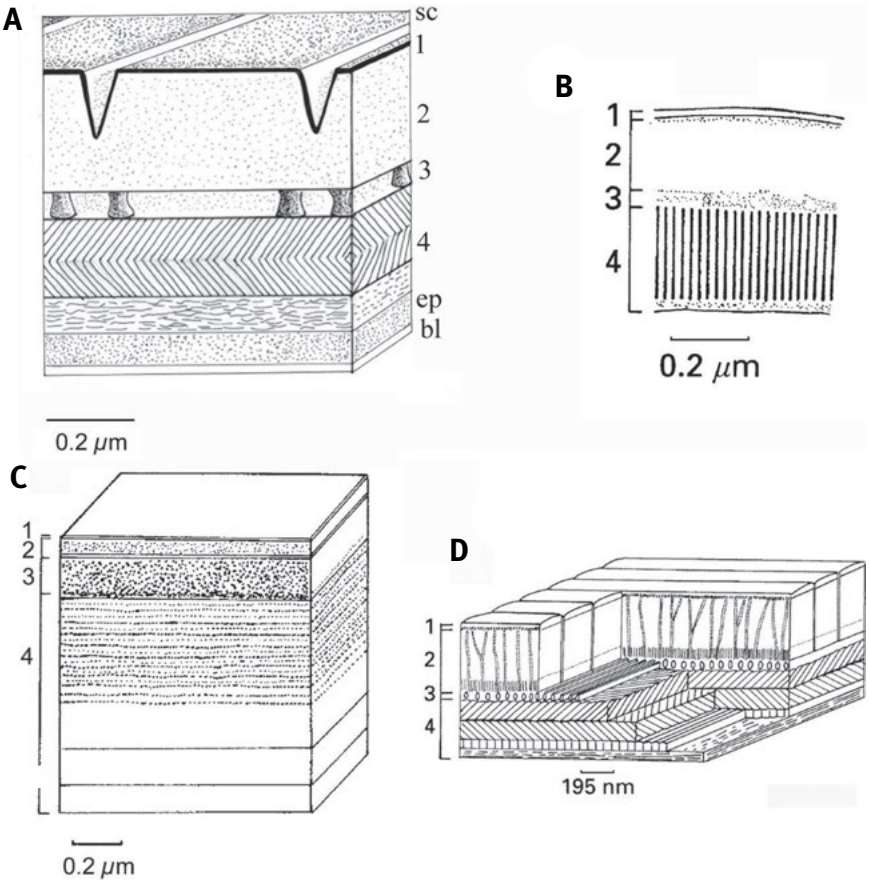

Fig. 1.4: Diagrammatic representation of the ultrastructure of the body cuticle (transverse section in front view). A, *Caenorahbditis elegans*, adult; B, *Pratylenchus penetrans*; C, *Trichodorus cylindricus*; D, *Xiphidorus balcarceanus* (B–D after Decraemer et al. 2003).

The major biochemical component of the acellular body cuticle present in all zones, except epicuticle and surface coat, consists of highly cross-linked collagen-like structural proteins with a characteristic glycine-X-Y tripeptide repeat flanked by conserved cysteine. Collagen biosynthesis is a multistep process involving synthesis of procollagen in the endoplasmic reticulum and including modifications catalyzed by specific enzymes (Page & Johnstone 2007; Stepek et al. 2010). Next to collagens, the cuticle contains cuticlins (proteins that are insensitive to collagenase), soluble proteins such as glycoproteins and lipids (Blaxter & Robertson 1998); cuticlins are restricted to the cortical zone and play a role in the formation of the cuticular alae by the seam cells (see epidermis). Depending on the taxon, 40 to over 180 cuticle collagen genes have been found, but mutants of the majority of these genes have not resulted in detectable phenotype differences (Johnstone 1994; Jones et al. 2011). In *C. elegans*, only a restricted number (21) of collagen mutants (e.g., *dpy*-2, 3, 7, 8 or 10) result in morphological defects such as short fat worms (dumPY) without cuticle annuli (Fig. 3 B in www.wormbook.org). Some collagen genes are only active in some developmental stages, and their number appears reduced in parasites such as root-knot species (Abad et al. 2008). The cuticle structure and its collagen components are conserved throughout the phylum Nematoda. For example, interspecific conservation of *dpy*-31 enzymes of the bone morphogenetic protein class (astacin metalloprotease), associated with cuticle defects when mutated, was detected in free-living *C. elegans* as well as in the animal parasites *Haemonchus contortus* and *Brugia malayi*. Collagens and cuticlins are synthesized and secreted by the epidermis.

The ultrastructure of the body cuticle shows a conserved basic pattern consisting of four main parts: (1) the epicuticle at the external surface, then (underneath) (2) the cortical zone (= exocuticle), (3) the median (= mesocuticle) and (4) the basal zone (= endocuticle) (Fig. 1.4). The boundaries between the different parts may be difficult to discern. In addition, the epicuticle surface is covered by a surface coat or glycocalyx (Bird & Bird 1991, Decraemer et al. 2003). Maggenti (1979) observed that zone(s) can be missing and that some ultrastructural features, such as radial striae (cortical zone) or struts (median zone), could serve to analyze deep relationships within the Nematoda. However, according to Decraemer et al. (2003), several of these ultrastructural features appear as products of convergent evolution.

The epicuticle is always present and trilaminar, composed of non-collagenous proteins as cuticlins (Fujimoto & Kanaya 1973) and lipids. It appears to act as a hydrophobic barrier to diffusion. In cyst nematodes, quinones and polyphenols in the epicuticle are the result of phenol oxidase activities in the tanning of the female cuticle and the formation of a resistant cyst wall. In *C. elegans*, epicuticle cuticlins play stage-specific roles in the formation of the cuticular alae, and one of the genes encoding for these proteins probably contributes to the thickening of the cuticle in the dauer stage (Cassada & Russell 1975). The epicuticle is the first layer to be laid down during molting.

A glycoprotein-rich, negatively charged surface coat or glycocalyx overlies the epicuticle. It is a highly dynamic layer synthesized from the epidermis, the S-E system and gland cells (pharyngeal glands, caudal glands, amphid, phasmid cells and somatic setae) (Sharon et al. 2002). In natural environments, it may facilitate locomotion by lubrication (Bird et al. 1988) and protects the nematode from microbial predators, parasites and pathogens. For example, the attachment of endospores of the bacteria *Pasteuria* (a biocontrol agent of plant-parasitic nematodes) to the nematode cuticle is host specific and reveals genetically inherited surface coat variation suggesting that the surface coat is part of the immune system (Davies et al. 2008). In *C. elegans*, genes have been identified that alter the surface coat in such a way as to affect bacterial pathogenesis, e.g., affect the adherence of a bacterium biofilm (Darby et al. 2007). In the root-knot nematode *Meloidogyne incognita*, an increase in lipophilicity of the surface coat was triggered by in vitro root exudates and allowed the animal to adapt to survive plant defense processes (Davies & Curtis 2011). The surface coat of animal parasites (*Trichinella spiralis*) and plant-parasitic nematodes (*Meloidogyne incognita*) shares immune-dominant epitopes secreted inside their hosts. In a group of marine sediment inhabiting nematodes, the Stilbonematinae, the body cuticle is covered by ectosymbiotic sulfur-oxidizing bacteria enveloped in a mucus layer produced by a conspicuous system of glandular sensory organs beneath the cuticle. Bulgheresi et al. (2006) discovered that the stilbonematid *Laxus oneistus* binds its symbionts by secreting specific antibodies (Ca^{2+}-dependent mannose-specific lectins named Mermaid) onto the posterior bacterium-associated region of the cuticle. Surprisingly, the carbohydrate recognition domain of this mannose-binding protein was similar, both structurally and functionally, to a human dendritic cell-specific immune receptor, a discovery that could lead to a method to block interactions with pathogens such as *Mycobacterium tuberculosis* in humans.

The cortical zone underneath the epicuticle may be more or less uniform in structure (e.g., amorphous) or exhibit radial striae (in longitudinal (LS) and transverse (TS) sections of the body), or it may show a differentiation into two layers, e.g., an outer layer without differentiation and an inner layer with radial striae. A radially striate cortical zone (visible at high magnification as fine osmiophillic rods separated by electron-light material showing a specific periodicity only slightly varying interspecifically) is largely known from free-living aquatic (mainly marine) taxa and from free-living developmental stages of some mermithid insect parasites and provides the cuticle with the strength to prevent radial expansion due to dissipation of hydrostatic pressure upon contraction of the longitudinal body muscles during locomotion. Cortical radial striae extend all around the body without interruption laterally (*Enoplus*) (Yushin & Malakhov 1989).

The median zone is greatly variable and may be absent. It consists of a homogeneous matrix that may contain fluid and globular bodies (*Hirschmanniella)*, column-like structures or struts (*Enoplus, Sabatieria*) or fiber material (*Xiphidorus*). Differences may be found in the literature in the morphological delimitation of the median and basal zones between free-living aquatic and the parasitic taxa. At present, the terminology of Bird (1984) has been generally accepted, and the spiral fiber layers are considered part of the basal zone. The median zone can be strongly reduced (e.g., in free-living marine *Monoposthia*).

The basal zone is usually structurally more complex than the outermost zones and may contain radial striations, sublayers of spiral fibers or other fibers and laminae. The basal zone may be very thin (*Desmolaimus*) to very thick (*Mermis*). Spiral fiber layers form the outermost part of the basal zone in many nematodes and are considered typical for large animal parasitic forms (e.g., *Nippostrongylus brasiliensis*) where they are described as giant fibers. However, spiral fiber layers have also been observed in smaller animals (≤1 µm length, e.g., in J2, J3, J4 and in adults of *C. elegans*) (Decraemer et al. 2003). Similar to radial striae, three spiral fiber layers forming two helices spiraling around the body in opposite directions (e.g., *Ascaris*) provide resistance to the longitudinal body muscle contraction by the arrangement of the fibres at an angle above 54°44′ with the longitudinal body axes, an angle at which there

is a maximum volume at the lowest pressure. Normal body movement causes a 10%–15% range of alteration in body length, with indication of a 75° to 73° range for the spiral angle (in *Ascaris suum*: 75°30) (Harris & Crofton 1957). The existence of such a high-pressure system explains why approximately 95% of all nematodes known have a cylindrical body shape and why nematodes have a strong cuticle (O'Grady 1983). Many species lack spiral fiber layers and instead possess radial striae similar in structure to those described for the cortical zone (Popham & Webster 1978) though not considered homologous, e.g., basal radial striae are interrupted at the level of the lateral fields, whereas cortical radial striae are continuous around the body (Decraemer et al. 2003). The presence of basal radial striae is, apart from one lineage in the Enoplea (Trichocephalida), related to the secernentean taxa in the Chromadorea and is considered primitive as it is more widely distributed in juveniles. The presence of basal radial striae appears related to different functions. Priess & Hirsh (1986) found that basal radial striae appeared to be a fundamental requirement for maintaining body shape after elongation of the embryo in *C. elegans*; mutant embryos sqt-3 (e2117), which retracted after normal elongation to become a short, fat nematode, lacked a striated basal cuticle layer. Basal radial striae appear to induce some physical constraints e.g., to growth, which may explain their absence under certain conditions. For example, in obese endoparasitic females of Heteroderinae the thick basal zone retains only patches of radial striae and in animals with lateral alae allowing small changes in the diameter of the nematode, basal radial striae are replaced at the lateral fields by fiber layers (different from the spiral fibers). Basal radial striae also appear to be involved in locomotion as they disappear in J2 of *Meloidogyne* shortly after the juvenile becomes a sedentary endoparasite. The presence of basal radial striae also appears related to protection from environmental harsh conditions such that they are present e.g., in the thickened cuticle of the J3 dauer of *C. elegans* but not in the active J3.

An exceptional extracuticular sheath has been described for all parasitic stages (J2, J3, J4 and females) of the plant-parasite *Hemicycliophora arenaria*. It is composed of a trilaminate outer layer and four inner ones in the female (two in the male) covering a cuticle. Both, the "sheath" and normal cuticle are formed when the nematode molts and therefore should be regarded as two parts of the same cuticle (Johnson et al. 1970). The latter coverage is different from the cuticular sheath that encloses, for example, the infective J3 of trichostrongylid nematodes, where it is formed by the non-ecdysed cuticle of the J2. The non-feeding, ensheathed, somewhat resistant obligate stage in their life-cycle enables the free-living J3 to survive stressful environmental conditions. Exsheathment of the infective trichostrongyle juveniles marks the transition from a free-living to a parasitic existence and usually requires a stimulus from a host to bring about exsheathment and to continue development.

The body cuticle is semi-permeable and metabolically active. Direct evidence that H^+ and organic anions are extruded across the cuticle/epidermis complex in nematodes was obtained in *A. suum* studies (Thompson & Geary 2002), but the molecular mechanisms that underlie organic acid excretion across the cuticle in nematodes have not been defined. At the same time, the cuticle forms a barrier that protects the nematodes against biotic and abiotic factors from its environment. In some nematodes, pore-like canals have been observed through the cuticle (Mounport et al. 1997).

1.2.2 Molting and formation of J1 cuticle

Most nematodes molt four times during their development. During molting, the cuticle covering the body, body openings and outlets is shed and reconstructed. However, between molts there is also the continuous production and export of surface components. Molting occurs in a series of steps but shows quite some variation among taxa. In general, four main steps can be distinguished: (1) a lethargus period during which all nematode activities become arrested and the cuticle becomes loose from the tip of the head, in the buccal cavity and around the tail; immobility of the nematode is presumable due to separation of the basal zone of the cuticle from the surface of the epidermis by disconnection of the hemidesmosomes; (2) the apolysis during which the cuticle separates from the epidermis showing change in its ultrastructure (numerous mitochondria, ribosomes, endoplasmatic reticulum); (3) secretion of components of the new cuticle at the level of the epidermis visible at first as fibrous material between the old cuticle and the epidermis and (4) ecdysis or rupture and shedding of the old cuticle. A characteristic of new cuticle formation is the occurrence of epidermal folds known as *plicae* over which the new cuticle becomes highly convoluted (Yushin et al. 2002). The new folded cuticle enables the nematode to increase in length after ecdysis (Bird & Bird 1991).

In the infective J3 of trichostrongylids, the separated cuticle of the J2 is not shed but retained as a

protective sheath, and exsheathment occurs upon infection. In cases where the first (*Meloidogyne javanica*) or first and second molt (*Ascaris lumbricoides, Toxacara canis*) occur within the egg, the inner zones of the old cuticle are broken down and probably resorbed, leaving just a thin sheath of the cortical zone (Bird & Bird 1991). Different molting patterns may relate to the nematode's environment. For instance, in a confined space where nutrition is limited (e.g., in the egg) it would be an advantage to be able to resorb the old cuticle and recycle its protein, or a sedentary nematode might have difficulty escaping from its cuticle if it did not resorb it. On the other hand, shedding the old cuticle could be an advantage for a parasitic nematode (e.g., *Nippostrongylus*, which lives among the villi of the host's intestine) because slow resorption could leave it open to dislodgement by its host's peristaltic movements. A similar argument could apply for free-living nematodes, which would need to begin feeding and move as soon as possible to escape, e.g., predation. The type of molting can also be related to the existence of a high-pressure system and the necessity to replace a cuticle that has become too thin and too weak to prevent radial expansion of the body. Further, at each molt, the cuticle is reconstructed. It may show stage-specific differences and play an important role in parasitic nematodes in the resistance of the invading nematodes to non-specific and specific immune attack.

The molting process and cuticle synthesis still largely remain to be resolved. Frand et al. (2005) studied endocrine and enzymatic regulators of molting in *C. elegans* through a genome-wide RNA-interference (RNAi). They found that inactivation of 159 genes interfered with molting; the majority of the genes idientified probably act at all four molts because their inactivation prevents molting from several juvenile stages. There is some evidence that orphan nuclear hormone receptors are involved in that they are liganded by cholesterol and steroid-derived hormones (ecdysone hormones) (Kuervers et al. 2003, Jones et al. 2011). Modern classifications group nematodes together with insects within the Ecdysozoa or molting animals (Aguinaldo et al. 1997). Although nematodes and insects have a different cuticle composition, the regulatory neurosecretory control systems show common features.

1.2.3 Epidermis (hypodermis)

The epidermis consists of a single outer cellular or multinucleate syncytial layer (depending on the taxa or developmental stage) that connects the body cuticle through desmosome-like structures to the somatic muscles. A major function of the epidermis is the secretion of the body cuticle. The epidermis also plays a major role in the development of the basic body shape, as do the body muscles. During embryogenesis, the cytoskeleton organization of epidermal cells consisting of actin microtubules and microfilaments shape the dorsal and ventral cells, inducing elongation of the embryonic tadpole stage into a vermiform shape (Pries & Hirsh 1986, Costa et al. 1997). The epidermis also interacts with other tissues and internal organs through different types of specialized epithelial cells. These include seam cells (responsible for formation of alae), interfacial epithelial cells, such as the socket and sheath cells of sensory organs, and epithelial cells associated with the outlet of the S-E system, such as the S-E pore cell and S-E duct cell. Further examples of specialized epithelial cells are the marginal cells of the pharynx (at the tip of the lumen rays) and rectal, vulval and cloacal epithelia (see 1.6.4, 1.6.7). Some of these specialized epithelial cells do not produce cuticle (Chisholm & Hardin 2005). The epidermis is metabolically very active and forms, together with the body cuticle, a complex system involved in the processes of excretion, secretion, osmoregulation and transport of nutrients. The epidermis probably functions as the true limiting membrane responsible for maintaining an internal equilibrium (Thompson & Geary 2002).

The epidermis does not form an equally thick layer around the body. It bulges out internally into the pseudocoel, forming four main longitudinal chords (one dorsal, one ventral and two lateral); hereby, the somatic longitudinal muscles are consigned into four fields. Apart from the four well-developed epidermal chords, up to four smaller secondary chords may be present, especially in the anterior body region (Chitwood & Chitwood 1950). The lateral chords extend nearly over the entire length of the body except for the tips of the head and tail; their position is often demarcated externally by lateral differentiations of the body cuticle, such as lateral alae that are present, e.g., in J1, dauer J3 and the adult of *C. elegans*. Most details at the cellular level are known for *C. elegans* where the epidermis of the hermaphrodite consists of 12 cylindrical syncytia linked by desmosomes: six in the anterior body region, followed posteriorly by a seventh cylindrical syncytium (hyp7) covering most of the body and four other epidermal cells in the tail (Altun & Hall 2009).

A cellular epidermis is considered as primitive and is found in free-living taxa and parasitic species such as

Trichinella and *Xiphinema*, but also in juveniles (e.g., J3) of large animal parasites that possess a syncytial epidermis in adults (e.g., *A. lumbricoides*). The outline of epidermal cells in free-living nematodes has been visualized using silver impregnation techniques (Malakhov 1994). The cell pattern shows a regular mosaic with cells arranged in 5–12 longitudinal rows, except at the extremes and around the vulva in female. Cell membranes (plasmalemmas) separating the cells may be only present in the chords, especially the lateral chords. Epidermal nuclei are located in the four chords; the dorsal chord as a rule has only nuclei in its anterior part (pharyngeal region), and the nuclei of the lateral chords may be similar or dissimilar in shape, arranged in three rows with the middle row possessing fewer but large nuclei.

During embryogenesis, the epidermis is derived from the founder cells AB and C (see chapter on embryology). In *C. elegans*, the major epidermal precursors are located on the dorsal surface of the early embryo.

The epidermis has several types of specialized cells. Seam cells, for example, are responsible for the formation of the lateral alae in rhabditids (e.g., *C. elegans*); they are arranged in two mid-lateral longitudinal lines, linked to the epidermis. At hatching, two rows of 10 seam cells, each embedded in the hyp7 syncytium, can be observed (Sulston et al. 1983). During postembryonic development, each seam cell will further divide into a posterior seam cell that will elongate, whereas the anterior daughter cell will become detached and fuse to the epidermal syncytium of hyp7. These lateral cell fusions are essential for the growth of the hyp7 syncytium, generation of neurons, molting and elongation of the juvenile (Podbilewicz 2006). A mid-J4 *C. elegans* has 30 seam cells and 98 syncytial epidermal nuclei. The maintenance of seam-cell fates in juvenile stages possessing them requires the GATA transcription factor *elt*-1, a key regulator of neural function (Smith et al. 2005). Loss of *elt-1* function results in a hypermotility phenotype, whereas over expression results in a phenotype of reduced motility or paralysis.

Arcade cells are specialized epithelial cells present around the gymnostom (part of the buccal cavity posterior to the cheilostom). They are interfacial cells connecting the body cuticle and epidermal syncytia of the lip region with the anterior most part of the pharynx. Arcade cells are present in many free-living and parasitic taxa and appear to be arranged in an anterior and a posterior group of cells (syncytia), e.g., *C. elegans* has three anterior arcade cells and a posterior group of six cells. In tylenchs, both arcade syncytia line the stylet shaft, supporting the hypothesis that the stylet shaft and cone (main parts of the stomatostylet) in tylenchid plant-parasites are homologous with the gymnostom in the bacteriovorous cephalobids, a sister group of the tylenchs. Ultrastructural studies are very important because they allow detailed three-dimensional reconstructions and provide for example tests of homology and the evolution of feeding structures for plant parasitism (Ragsdale et al. 2008). In Tylenchomorpha, the epidermis is also responsible for the formation of the cephalic framework.

Throughout the Nematoda, there is considerable variation in the structure and configuration of the epidermis during development, e.g., due to specialization to parasitism. In a number of insect parasites such as *Bradynema* sp. and *Fergusobia* sp., the cuticle and feeding apparatus are degenerated, and the epidermis becomes convoluted into microvilli for uptake of nutrients in the animal-parasitic stage (Riding 1970, Giblin-Davis et al. 2001). The epidermis of some free-living nematodes (e.g., *Geomonhystera disjuncta* and *Diplolaimella dievengatensis*) contains fluid-filled vacuoles that are thought to form a compartmented hydrostatic skeleton. This condition might be considered as a primitive condition relative to the more derived state that allows for the antagonistic locomotion system (Van de Velde & Coomans 1989a).

The epidermis shows several types of specializations, with different functions such as epidermal glands, caudal glands and ventral gland(s)/renette cell of the S-E system.

1.2.4 Epidermal glands (Fig. 1.7 F–G)

The presence or absence of epidermal glands was an important character in the two group classification system of Chitwood (1958) in nematode systematics: (1) the former Adenophorea[1] (derived from Greek words meaning "bearing glands") usually have epidermal glands with outlets through pores or specialized setae, caudal glands and a single-cell S-E, usually with a non-cuticularized terminal duct, and phasmids (sensory organs) are absent and (2) the Secernentea (derived from the Latin *secernentem*, a secretory organ) is a name referring to the cuticle-lined duct(s) of a

1 In newer classifications that consider molecular data, Class Secernentea is considered to be of the order Rhabditina within Chromadorea, whereas Adenophorea, primarily included within the Enoplia, is paraphyletic (De Ley & Blaxter 2002, 2004). The old names are here referenced to provide historical continuity.

tubular S-E system; they have no epidermal nor caudal glands, but do possess phasmids. Epidermal glands are unicellular (Fig. 1.7 F). Ultrastructure research of the free-living aquatic nematode *Chromadorina germanica* showed that these gland cells are often associated with a somatic receptor of a bipolar nerve cell (Lippens 1974); glandular secretion is released to the surface of the cuticle via a common duct of the gland and nerve cell that leads to a pore. These epidermal glands may be involved in the secretion of the cuticle surface coat. Epidermal glands may occur throughout the body length, mainly located in or at the lateral and ventral chords; gland cells may be separated from each other (e.g., *Dorylaimellus*) or arranged in bacillary bands (*Capillaria, Trichuris*).

Bacillary bands are modified epidermal gland cells opening through a complex cuticular pore. The cell membrane of the gland cell beneath the pore is highly convoluted, forming a lamellar apparatus; four to six dendritic processes are inserted in the gland cell (Wright & Chan 1973). Bacillary bands occur in some of the larger trichurid nematodes (Enoplea) that are parasitic in mammals. These structures may be restricted to a particular body region (lateral pharynx region in *Trichurus*) or distributed along the length of the body in ventral and lateral regions (e.g., in the genus *Capillaria*). Similarities in structure between bacillary cells and cuprophilic cells present in the intestine of insects and secreting proteins essential in osmoregulation supported the presumption that bacillary cells are also involved in osmoregulation. This hypothesis was abandoned when *Trichuris* specimens incubated in bromophenol blue, a dye that changes from blue to yellow in cuprophilic cells due to acid secretion, did not show a change in color and thus did not support osmoregulation. A second hypothesis that bacillary cells may have an absorption function was tested using a fluorescent technique. The pores of bacillary cells were strongly fluorescent, showing that macromolecules enter the pores and thus prove their absorption function. According to Tilney et al. (2005), some of the bacillary cells could also have a chemosensory function. Less specialized gland cells are thought to produce a lubricating secretion that facilitates movement.

The best-known epidermal glands are (1) the ventral gland(s) of the S-E system, usually a single cell, present in most nematodes and also indicated as a renette (cell) and (2) the caudal glands.

In general, caudal glands consist of three (sometimes two or five) unicellular glands, each composed of a globular body and a narrow duct that opens to the exterior, either subterminally or terminally through a valve or spinneret. The three cell bodies usually occupy the space dorsally from the rectum (pre-anal) and/or the proximal part of the tail. In some nematodes (enoplids), the caudal gland cell bodies can migrate very far anteriorly to the tail region. The three cells are usually arranged one after the other (= in tandem); occasionally one lies in front of the two others at the same level. The three ducts usually open into a common ampulla that leads to a terminal (or rarely subterminal) spinneret. The latter is composed of a small sclerotized funnel that can be closed by a conical plug. A minute muscle running from the plug to the dorsal body wall operates the plug; upon muscle contraction, the plug is withdrawn from the funnel, and a sticky secretion escapes from the spinneret. Once outside the body, this secretion quickly hardens, enabling the nematode to attach itself temporarily to a substrate. In a few marine nematodes (e.g., *Sphaerolaimus gracilis, Theristus caudasaliens*), two additional "release" glands have been discovered in the tail region (Turpenniemi & Hyvärinen 1996). Their secretion would dissolve the cement produced by the "adhesive" glands, allowing the nematode to free itself from the substrate. The presence of such a duo-gland system may be far more general than so far recorded.

1.3 Musculature (Fig. 1.5 A–F)

Somatic muscles comprise the internal layer of the nematode body wall, next to the thin interchordal epidermis. They provide the physical means of locomotion, generally sinusoidal, that is characteristic of the phylum (Baldwin & Perry 2004). The somatic muscle layer is interrupted by chords protruding inward from the epidermis, generally in the lateral, dorsal and ventral positions. Consequently, the somatic muscle layer is organized into four fields (Fig. 1.5 A), with each field enclosed in a basal lamina tube that lies between the chords. Within each field, the somatic muscles are organized as a single layer of intersecting rhomboid-shaped cells, and they are anchored to the adjacent interchordal epidermis by transepidermal hemidesmosome attachments and associated fibrils (Baldwin & Hirschmann 1975, Moerman & Fire 1997, Ding et al. 2004, Zhang & Labouesse 2010). Muscles are further secured to one another and to adjacent epidermal chords by attachment plaques (Hall & Altun 2008).

The basic unit of the contractile portion of the muscle cell is the sarcomere, with each sarcomere composed of a pattern of thick (myosin-containing) and thin (actin-containing) contractile filaments oriented

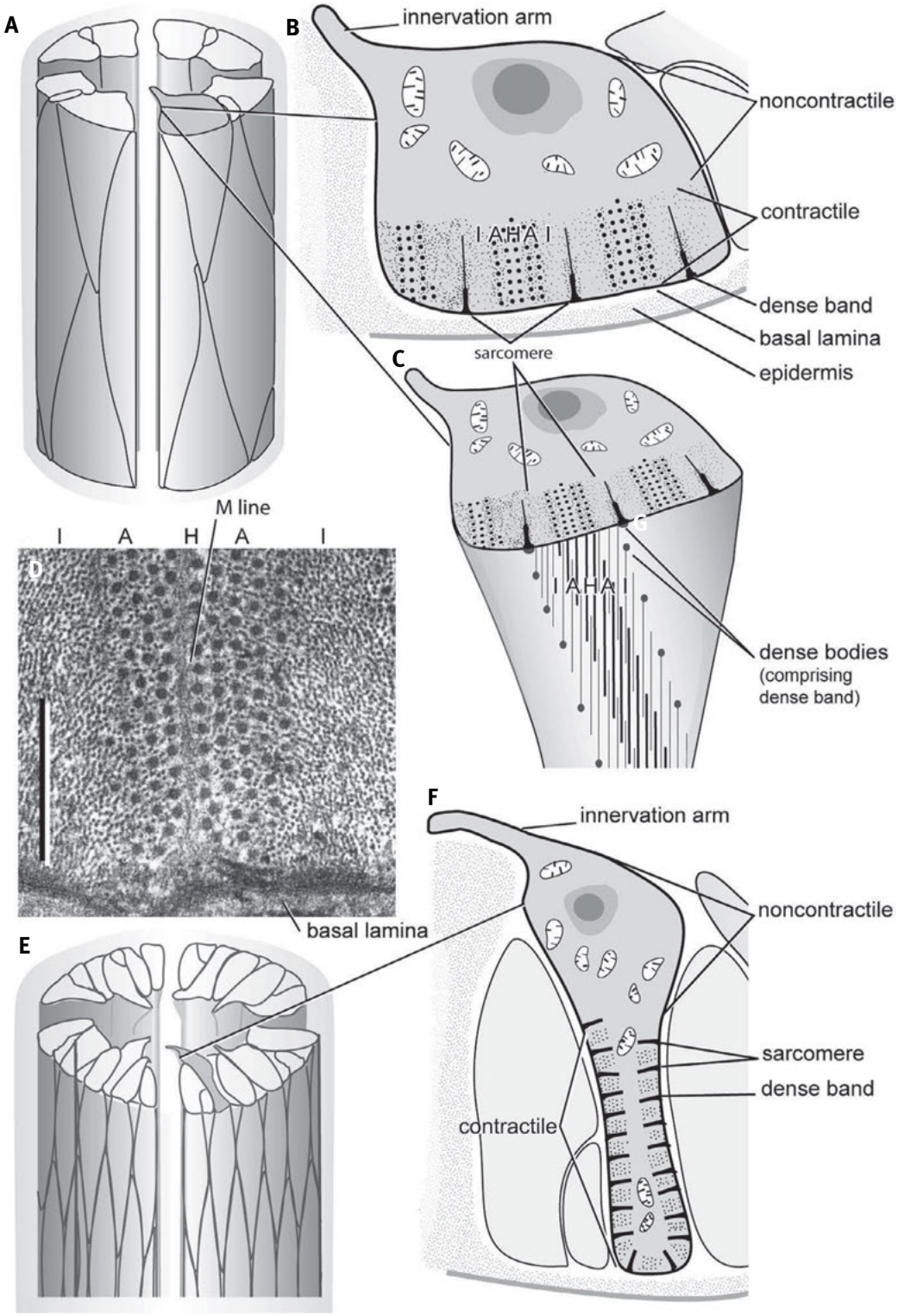

Fig. 1.5: The nematode muscle system. Each part is a schematic diagram unless otherwise indicated. A, Thick cross section of a nematode showing only somatic musculature; the four quadrants of musculature are each comprised of spindle-shaped platymyarian muscle cells. B. Transverse section of platymyarian muscle cell; C, Partial lateral view from B showing the oblique arrangement of dense (bodies) band and I, A, H bands defined by their composition respectively as thin (I), thin with thick (A), and thick (H) filaments; D, Transverse TEM section of platymyarian muscle cell including I, A, H bands and M-line; the M-line is defined by its position in the H band and its cytoskeletal connection with the cell membranel; E, Thick cross section of a nematode showing only somatic musculature; the four quadrants of musculature are each comprised of spindle-shaped coelomyarian muscle cells. F, Transverse section of coelomyarian muscle cell. A, B, E, F redrawn from Baldwin and Perry (2004).

parallel to the longitudinal axis of the nematode cell (Fig. 1.5 B–D, F). The central region of thick filaments is bisected on either side by thin filaments, and there is a transition region on either side with thin and thick filaments arranged in a highly specific lattice. The result is a pattern defining an individual sarcomere of thin filaments (I-band), followed by thick plus thin filaments (A-band), a region of exclusively thick filaments (H-band) and additional A- and I-bands. Generally but not always in nematodes, the limits of a sarcomere are defined by a band of dense bodies or plates (analogous to the Z-line of vertebrate muscle) that function to anchor and align thin filaments. In addition, a membranous M-line runs through the center of the H band and participates, with the D-band, in anchoring filaments to the cell membrane and underlying epidermis (Hall & Altun 2008). However, during the early stages of molting, the proteins identified with these attachment structures decrease and thus contribute to the molting process with respect to detachment of the muscles from the underlying basal lamina, epidermis and cuticle (Zaidel-Bar et al. 2010). The genetic and molecular mechanisms regulating myofilament structure in sarcomeres and systems of attachment of sarcomeres are a focus of ongoing research in *C. elegans* (Meissener et al. 2009, Qadota & Benian 2010).

Within each muscle cell, it is the repetition of sarcomeres and the bands that compose them that give a striated appearance (Fig. 1.5 B–D). In *C. elegans*, an individual sarcomere is only about 1 µm wide, and indeed the size of the sarcomere may be functionally constrained. The number and organization of sarcomeres within a given muscle cell are highly variable depending on the size and locomotion requirements of diverse species.

Several features of somatic muscles of nematodes appear to be unusual among invertebrates and together contribute to defining the uniqueness of phylum Nematoda. For example, the actin- and myosin-containing filaments of sarcomere bands are offset in the longitudinal access of the cell at an angle of 5°–7°, resulting in distinctive "oblique striation" (Fig. 1.5 C). This oblique striation, although unusual, is not exclusive to nematodes (Hope 1960) and is considered to have functional significance in the smooth bending of the nematode body (Burr & Gans 1998).

A further distinction in nematodes is the organization into contractile, noncontractile and innervation-arm regions (Fig. 1.5 B, F). That is, each muscle cell, in addition to the contractile region composed of sarcomeres, includes a noncontractile body or "belly" that is primarily comprised of cytoplasm, a nucleus with a small spherical nucleolus, abundant mitochondria, ribosomes, glycogen and other small organelles (Fig. 1.5 B, C, F). Often the noncontractile body does not extend the entire length of the rhomboidal muscle cell so that the narrow termini might be entirely comprised of the contractile region. From the noncontractile body, the nematode muscle cell also has one or more muscle arms that extend to nerve processes with which they primarily synapse in the ventral or dorsal nerve cord or the nerve ring. The distal portion of the muscle arms form gap junctions between muscle cells, and these may have a role in "electronic coupling" and regulating synchronous contractions among muscle cells (Hall & Hedgecock 1991). Although the innervation arm in nematodes is unusual, apparently a similar arrangement occurs in some other invertebrates, including some gastrotrichs (Teuchert 1977).

Although unusual features of somatic muscles contribute to defining nematodes, there are also a number of aspects of somatic muscles that vary among nematode species and even among the juvenile and adult stages of the same species. Most prominent among these differences is the organization, within the overall rhomboidal shape, of contractile and noncontractile regions with variations described as platymyarian (Fig. 1.5 A–C), coelomyarian (Fig. 1.5 E, F) and circomyarian.

The platymyarian muscle cell is relatively simple, with the contractile region composed of a single row of sarcomeres that closely follows the contour of the interchordal epidermis; internal to this region is the noncontractile body from which arms extend (Fig. 1.5 A–C). With sarcomeres typically about 1 µm wide, a muscle cell, for example, in *C. elegans*, may grow to be as wide as ten sarcomeres (Hall & Altun 2008). Platymyarian muscle cells occur widely throughout the phylum, and particularly so in nematodes less than 1 mm long; however, the configuration limits the numbers of sarcomeres and thus seems to be constrained by overall nematode size.

In the coelomyarian muscle cell, the contractile region is also a single row of sarcomeres. In this case, however, the row is folded along those sides of the cell that are adjacent to other muscle cells or epidermal chords, therefore, the row of sarcomeres appears "U-shaped" in transverse section (Fig. 1.5 F). The coelomyarian row of sarcomeres primarily extends perpendicularly, with only a few sarcomeres parallel, to the interchordal epidermis. In coelomyarian muscle, there is a prominent noncontractile body that balloons into the pseudocoelom. The region is continuous with additional noncontractile material sandwiched between the arms of

the "U" or the fold of the row of sarcomeres. Muscle cell arms extend from the noncontractile body. Compared to the platymyarian muscle, the coelomyarian muscle accommodates a greater number of sarcomeres per unit of perimeter of the nematode circumference (Fig. 1.5 A, B). In this way, it provides sufficient contractile power to accommodate the needs of larger nematodes. For example, *Ascaris* sp. adults, being about 20–50 cm long and 5–6 mm wide, have somatic musculature of approximately 50,000 coelomyarian cells. However, the much smaller J2 of the same nematode has only 83 platymyarian cells (Stretton 1976). Coelomyarian muscle cells are also present in smaller nematodes such as *Anoplostoma rectospiculum* (Malakhov, 1994). Hirumi et al. (1971) have shown an intermediate category (shallow coelomyarian) between platymyarian and coelomyarian muscle cells with the intermediates mostly representative of free-living and parasitic species that are a few mm long.

Circomyarian is reported as an additional type of muscle cell in which a row of sarcomeres completely encircles a noncontractile core that includes the nucleus (Hope 1960, Maggenti 1981), but this type of cell is not well documented by transmission electron microscopy (TEM). Notable because the noncontractile belly of coelomyarian muscles may be confined to the midregion of the rhomboidal cell, the tapering ends, when viewed in transverse section, may be composed of a circle of sarcomeres enclosing a noncontractile region, but this is not truly circomyarian (Hope 1960). Circomyarian arrangement can occur in specialized muscles, such as pro- and retractor muscles.

1.4 Nervous system

1.4.1 History and overview

The nematode nervous system, being relatively tractable for study, has been a topic of investigation for nearly 200 years (e.g., Otto 1816). Chitwood & Chitwood's (1950) review highlighted the microscopic skills of pioneers in elucidating the basic patterns of nervous systems of large parasites, including ascarids (Bütschli 1874, Rohde 1885, Goldschmidt 1908). These studies provided a foundation to map comparable systems in smaller parasitic and free-living nematodes, e.g., *Ancylostoma* (Looss 1905), *Mermis* (Meissner 1853), *Oxyuris* (Martini 1916), *Cephalobellus* (Chitwood & Chitwood 1933) and *Rhabditis* (Chitwood 1930). The range of taxa, although biased toward representation of class Secernentea and somewhat neglectful of class Adenophorea, nevertheless was sufficiently broad for Chitwood & Chitwood (1950) to suggest that the basic structure of the nervous system is highly conserved across the phylum. This apparent conservation is often presumed as a basis for predicting nervous system patterns, neuron homologies and neuron function in less studied taxa.

The understanding of nervous system structure was revolutionized, beginning in the 1960s, by the application of TEM (Baldwin & Perry 2004). Although nematodes were previously partly defined among Aschelminthes by a lack of cilia (Hyman 1951), and motile cilia are indeed lacking (see section on excretory system), TEM demonstrated that sensory cilia are highly conserved, including for nematodes (Roggen et al. 1966). An irony of this methodology is that although the most complete early studies of the nematode nervous system were constrained by bright field light microscopy to large taxa, including *Ascaris*, the most comprehensive TEM-based overall reconstructions are practically constrained to small nematodes such as *Caenorhabditis elegans* (<1 mm long). This has not precluded, however, the tractability and value of TEM for detailed reconstructions of particular components, such as the synapses of the *Ascaris* system (Stretton et al. 1978).

The first TEM studies of the nematode nervous system focused primarily on the sensory organs of parasites of plants and vertebrates, with little information and integration with overall circuitry (De Grisse 1977, Wright 1980, 1983), but further technical refinement has resulted in complete cell-by-cell reconstruction of the system in the rhabditid, *C. elegans* (Ward et al. 1975, Ware et al. 1975, White et al. 1976, Sulston et al. 1980, White et al. 1986, Hall & Russell 1991). Complementing these TEM reconstructions, advances in photo microscopy and differential interference microscopy provided tools that particularly supported insight into the developmental lineage of the nervous system (Sulston 1976, Sulston & Horvitz 1977, Sulston et al. 1980). To a limited extent, TEM-based serial section reconstructions first developed in *C. elegans* are now being expanded to additional taxa, often with emphasis on innervations of anterior sensory organs (Ashton et al. 1995, Ashton & Schad 1996, Li et al. 2000, 2001, Bumbarger et al. 2007, 2009, Ragsdale et al. 2009). TEM reconstructions generally confirm broad conservation of the basic nervous system structure, and these homologies are being further tested with a range of new tools (Sithigorngul et al. 2010).

1.4.2 General pattern and structure

Prominent in the nematode nervous system is a nerve ring surrounding the anterior half, mid part or the posterior half of the pharynx (Fig. 1.6 A, C). Six papillary cords (two lateral, two subdorsal and two subventral) extend anteriorly from the nerve ring. Extending posterior from the nerve ring is a large ventral nerve cord, a smaller dorsal cord and two pair of sublateral cords (Fig. 1.6 A, B); these are enclosed within basal lamina shared with the epidermis and

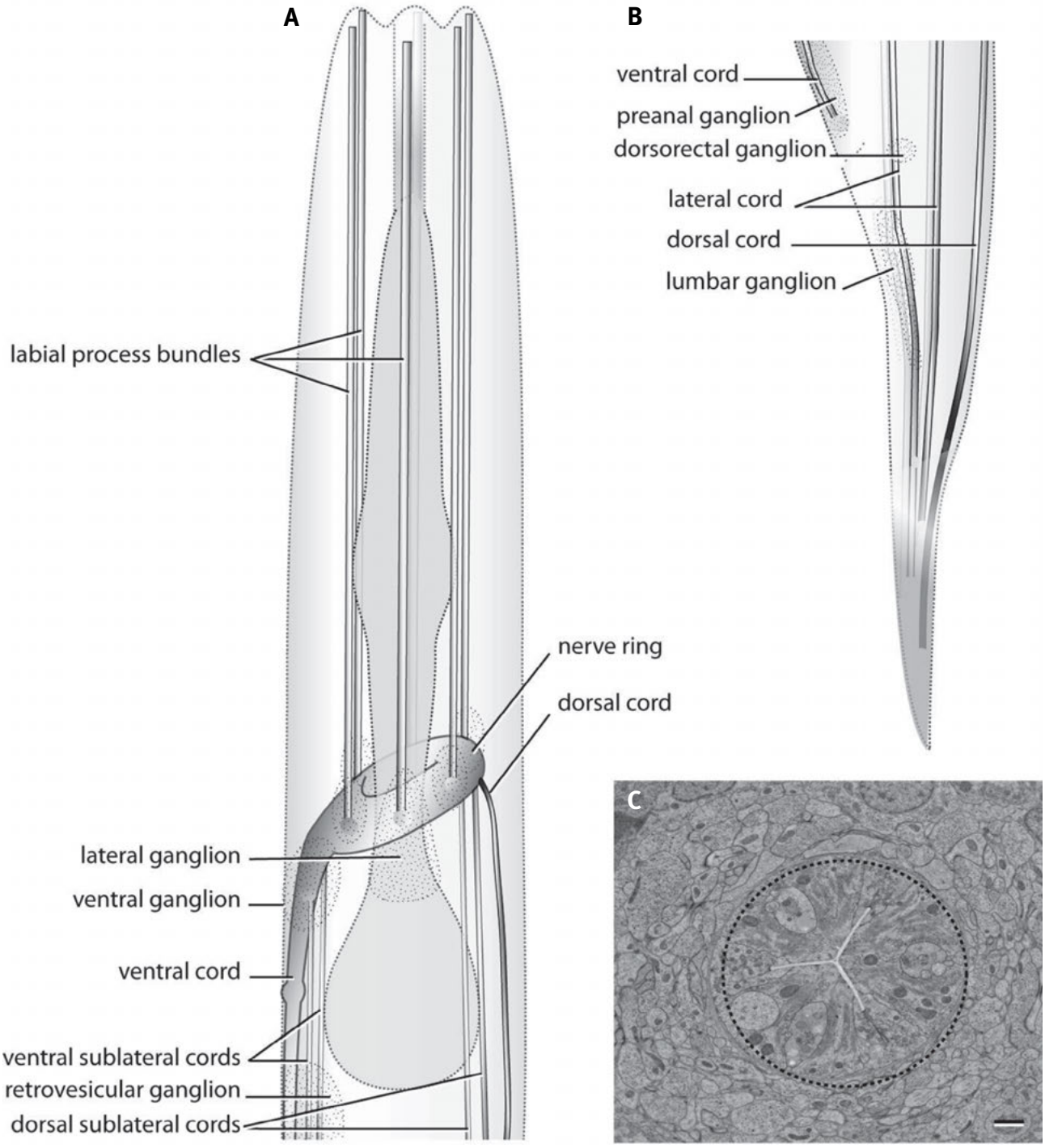

Fig. 1.6: The nematode nervous system. Schematic diagrams are shown unless otherwise indicated. A, Generalization of major components of the nematode nervous system from a lateral view in the anterior region extending to the base of the pharynx; B, Generalization of major components of the nematode nervous system from a lateral view in the posterior region extending from slightly anterior to the anus; C, Transverse TEM section through the nerve ring region of a diplogasterid. Broken lines indicate the boundaries of the pharynx. Scale bars: 1.0 mm. Courtesy of Dan Bumbarger.

isolating the cords from adjacent somatic muscles (Hall & Altun 2008). Posteriorly, the ventral cord terminates in the preanal ganglia. Similarly, dorsal and sublateral cords terminate in the tail region (Fig. 1.6 B). Chitwood & Chitwood (1950) considered that the nematode nervous system is organized to include what they defined as *central* (the nerve ring, ventral cord and associated ganglia) and *peripheral* systems, but with more detailed work, these distinctions seem artificial, such that they are now rarely made. Chitwood & Chitwood (1950) also recognized distinct *sympathetic* systems. Indeed, the highly developed pharyngeal sympathetic nervous system is largely independent, connecting outside the pharynx to the nerve ring by only two neurons in *Ascaris, C. elegans* (Hall & Altun 2008) and perhaps most/all other nematodes. Within the pharynx, the nervous system primarily controlling the pharyngeal muscles and glands consists of a pharyngeal dorsal and two pharyngeal subventral cords. These neurons extend from cell bodies positioned in the basal bulb, and they are internally integrated by lateral connections and commissures in the metacorpus (Hall & Altun 2008). Chitwood & Chitwood (1950) also suggested a rectosympathetic system, but this may not be justified considering that the posterior nervous system, although rich in commissures and ganglia, is well integrated into the overall system.

1.4.3 Basic nerve elements

The nerve cell or neuron, which includes a cell body and one (monopolar), two (bipolar) or more elongate processes (neurites), is the fundamental unit of the nervous system. The cell body (somata or perikaryon) is generally recognized with LM by granular nucleoplasm and with TEM by light-staining cytoplasm, distinctive rough endoplasmic reticulum and a nucleus with dense peripheral chromatin and one or more nucleoli (Hall & Altun 2008). Extending from the cell body, neurites may be expressed as dendrites, that is, receptors specialized to conduct nerve impulses, typically terminating in sensory organs. By contrast, axon neurites are specialized to conduct nerve impulses over a distance and usually form a synapse with another type of cell, such as muscle, or another neuron (interneuron). These synapses may be chemical, characterized by regions filled with minute optically clear vesicles, or they may be electrical, defined by gap junctions making close contact with another cell (Hall & Altun 2008). In part defined by the position and type of synapse, neurons can be functionally defined as motor, sensory or polymodal. Motor neurons are most abundant, including more than one-third of all neurons in *C. elegans*; they form synapses with all somatic muscle cells, as well as some muscles of the alimentary and reproductive tracts. The patterns and connections of these synapses and interconnections on somatic muscles, based on reconstruction in *A. suum*, are particularly congruent with the wave-like contractions typical of nematodes (Stretton et al. 1978, 1985). Beyond motor neurons, sensory neurons and supporting cells are characterized by obvious specializations as discussed in the chapter on nematode sensory organs. The most abundant type of neuron in nematodes is the interneuron; these are characterized by incoming and outgoing synapses and functions, for example, in decisions for motor programs and in integrating processes through converging two or more circuits. Some neurons are polymodal, combining more than one motor, sensory or interneuron function (Hall & Altun 2008).

Among neurite processes from nerve cells, both axons and dendrites are thin (100–200 µm in diameter), but they may have specialized regions or swellings. Dendrites, in particular, may be specialized distal to a ciliary region as flattened sheets or even microvilli (Ward et al. 1975). Contrary to those of vertebrate, dendrites in nematodes are not usually highly branched although some split distally, including two or more ciliary regions.

1.4.4 Organizing components

1.4.4.1 Overview

Nematode neuron cell bodies are generally organized into ganglia, and neurites are often organized into process bundles and commissures. Ganglia are groups of neuron cell bodies that cluster primarily around the nerve ring, along the ventral cord and in the region of the rectum or cloaca (Hall & Altun 2008). Proximity may not be sufficient to define a particular ganglion because cell bodies physically together may nevertheless be isolated by separate basement membranes, and thus they are delimited as separate ganglia (Chalfie & White 1988). Cell bodies within a given ganglion are not necessarily functionally related (Chalfie & White 1988), and generally they do not form synapses with one another (Hall & Altun 2008).

Process bundles, often called cords as in the case of the ventral cord or dorsal cord, are composed of neurites clustered in parallel with groupings that are somewhat consistently ordered and (Hall & Altun 2008) likely to have at least some functional specialization. Neurons of the same functional class may run together and in some cases may contact by gap junctions (Chalfie & White 1988). In nematodes, nerve processes are nonmyelinated, and we have noted that most cords run longitudinal to the

nematode body axis. Commissures are specialized neuron processes that typically connect nerves between different longitudinal cords. In nematodes the largest commissure is the nerve ring (Fig. 1.6 A, C), but additional commissures occur throughout the body including, most notably, within the pharyngeal sympathetic system and tail region.

Details of the pattern of organization of the nerve ring and associated ganglia, cords and commissures require special consideration. Supporting the notion of overall conservation of the nematode nervous system, limited comparisons to date nevertheless suggest only minor differences among taxa.

1.4.4.2 Nerve ring and associated ganglia (Fig. 1.6 A–C)

The nerve ring essentially is a large circumferential commissure that for *C. elegans* includes about 200 neurites (Hall & Altun 2008). Some of these are axons extending from ventral ganglia, and a number of others extend from innervating and regulating the somatic muscles and relatively complex movement of the head region (Hall & Altun 2008). Although there are few studies for a detailed assessment of variation and homology across the phylum, specific divergence in overall shape and configuration of the nerve ring is notable. Some "Adenophorea", for example, reportedly have a "double" nerve ring (Goodey & Hooper 1963), or they diverge in numbers and positions of associated ganglia (Anderson 1966). In *A. lumbricoides,* six separate papillary ganglia are associated with the six anterior cords, but these ganglia are not so clearly defined in other taxa, including in *C. elegans* (Chitwood & Chitwood 1950, Chalfie & White 1988). Posterior to the nerve ring of *A. lumbricoides*, associated ganglia include a small dorsal, two subdorsal, two lateral (subdivided to include amphidial ganglia) and two large ventral ganglia, but these are not completely distinguished in many other taxa (Chitwood & Chitwood 1950), and even within *Ascaris* they may be variously interpreted (e.g., Angstadt et al. 1989). By comparison, in *C. elegans*, only a small dorsal, ventral and two lateral ganglia are clearly defined, in part by delimitation of the partitioning basement membrane (Fig. 1.6 A; Chalfie & White 1988).

1.4.4.3 Ventral and dorsal cords

In *Ascaris,* the ventral cord connects to the nerve ring primarily through the ventral ganglia. From these ganglia, processes converge posterior to the S-E pore at the retrovesicular ganglion from which the cord extends through most of the body length, terminating near the tail in the preanal ganglion (Fig. 1.6). Chitwood & Chitwood (1950) describe the ventral cord as asymmetric with a primary right branch and smaller left component. Although Chitwood & Chitwood (1950) cautiously interpreted some other species to differ by the absence of the left branch, this may have been a limitation of available resolution with bright field light microscopy. Nevertheless, suggesting broad conservation of the feature, the ventral cord is described as double throughout its length, including splitting on either side of the vulva in the dorylaim *Aporcelaimus* (Anderson 1966). In *C. elegans*, the ventral cord extends singly from the ventral ganglion but then, posterior from the retrovesicular ganglion to the preanal ganglion, it separates to include a smaller left branch. The ventral cord is composed primarily of motor neuron axons and interneurons (White et al. 1976), and throughout its length, it is delineating a longitudinal series of ganglia. It includes the synaptic innervation of motor neurons and thus regulates the nematode's typical dorsoventral undulatory movement (Martin et al. 2002, Hall & Altun 2008). In *C. elegans,* additional nerve muscle contacts occur through the sublateral cords and associated ganglia. The dorsal cord, extending posteriorly directly from the nerve ring to near the tail end (Fig. 1.6 A, B), is comprised primarily of motor neuron axons that extend by commissural processes from the ventral cord, but also joining processes from several other neurons in the head and tail (White et al. 1986, Hall & Altun 2008).

1.4.4.4 Commissures

Commissures typically consist of nerve bundles or single processes that pass circumferentially from one longitudinal nerve to another (Hall & Altun 2008). In *C. elegans*, there are more than 40 such commissures involving motor neurons from the ventral cord extending to the dorsal side, and similar commissures between these major cords are also described in *Ascaris* (Chitwood & Chitwood 1950). Additional commissures include those of the head region and those associated with the amphid and deirid sensory systems. In *C. elegans*, the amphid commissure is positioned within the thin layer of epidermal tissue between the body wall cuticle and somatic muscles. It is likely that amphid commissure is homologous with the hemizonid, as recognized in a number of additional smaller nematodes across the phylum (Bird & Bird 1991), and that, additionally, the hemizonion, cephalids and caudalids reflect similar circumferential

clusters of subcuticular axons (Goodey 1951, Hirschmann 1956, Goodey 1959, Hirschmann 1959, Timm 1960, Smith 1974, Baldwin & Hirschmann 1975). Commissures prominent in the tail region especially include pathways connecting preanal ganglia with lumbar and dorsorectal ganglia. Other commissures connect cords; an example is the case of dorsolateral commissures, including some motor neurons that in *C. elegans* ultimately connect to the dorsal cord.

1.4.4.5 Ganglia of the tail region

The nematode tail region is generally highly conserved with respect to the organization of ganglia; minor variations among taxa may include the location or "merging" of ganglia and associated commissures. In females, and specifically the hermaphrodite of *C. elegans*, the preanal ganglion at the base of the ventral nerve, rich in interneurons and motorneurons, is the site of most of the synapses of the tail region (Fig. 1.6 B). Directly dorsal and posterior is the dorsorectal ganglion that includes neurons likely involved in regulating defecation (Hall & Russell 1991). Positioned laterally are the lumbar ganglia from which phasmid neurons extend in both *Ascaris* and *C. elegans* (Fig. 1.6 B). In *Ascaris*, in addition to the ganglia above, a lateral rectal ganglion is defined in conjunction with the rectal commissures (Chitwood & Chitwood 1950).

In males, and specifically those of *C. elegans*, the preanal, lumbar and dorsorectal ganglia are similar to those of the hermaphrodite except that, in each case, they include additional neurons. For example, it is the lumbar ganglia that accommodate the male-specific sensory papillae (i.e., rays) associated with caudal alae (Sulston et al. 1980). In addition, males include a pair of cloacal ganglia positioned right and left between the lumbar ganglia. These contain neurons associated with the spicules including both sensory and motor neurons of the spicules protractor muscles (Sulston et al. 1980).

1.4.4.6 Taxon representation of the nematode nervous system

The nematode nervous system, as primarily interpreted from *Ascaris* and *C. elegans*, suggests a high level of structural conservation. This conservation includes that the total number of neurons, even in homologous structures (e.g., the pharynx), is of a similar order of magnitude in the two taxa (Martin et al. 2002, Hall & Altun 2008). This is surprising considering their divergent biology (vertebrate parasite and microbivore, respectively) and their great discrepancy in size (respectively, ~40 cm versus 1 mm long). The conservation is also remarkable considering the widely separated evolutionary time scale between these taxa (Blaxter et al. 1998). Yet, *Ascaris* and *C. elegans* both share the clade typically defined as class Secernentea (now order Rhabditida, Class Chromadorea). Arguably, the largely unstudied class Enoplea exceeds the Secernentea in biological, morphological and even developmental divergence. Enoplea includes nematodes rich in unique sensory and copulatory receptors, and this raises questions of how such receptors integrate into underlying neuron circuitry and the relevance to nervous system evolution, plasticity and divergence. Perhaps in nematodes, more than in any other phylum, addressing such evolutionary questions is made more tractable by the extraordinary level of understanding of current model systems, especially including *C. elegans*. Understanding the extent of conservation, including beyond Nematoda, is foundational to a frontier of research that includes extrapolating from insight gained from nematode models toward managing human nervous system pathology/neurodegenerative diseases (Locke et al. 2009, Dimitriade & Hart 2010; Ewald & Li 2010, Harrington et al. 2010, Piernaar et al. 2010, Harrington et al. 2011; Kao et al. 2011, Van Ham & Nollen 2011; Zhou et al. 2011).

1.5 Sensory structures

The morphology of nematode sensory structures, also called sense organs or sensilla, is rather well known, especially through the very detailed observations of the model nematode *C. elegans* (Ward et al. 1975, Ware et al. 1975, White et al. 1986). These studies were based on computer-generated reconstructions of serial section TEM. Other authors completed our knowledge through studies on other taxa, such as the animal parasites *Strongyloides* (Ashton et al. 1995), *Haemonchus* (Li et al. 2000, 2001) and *Ancylostoma* (Bhopale et al. 2001), which belong to the Rhabditomorpha (Chromadorea). De Grisse (1977) gathered much information on the plant-parasitic Tylenchomorpha. Later, two major contributions, again based on computerized reconstructions of serial sections, allowed in-depth comparisons with the anterior sensilla of *C. elegans*. In the first study (Bumbarger et al. 2007), the amphid structure of the free-living *Acrobeles complexus*, belonging to Cephalobomorpha, was analyzed in detail, whereas in the second (Ragsdale et al. 2009), the other anterior sensilla of the fungal-feeding

Aphelenchus avenae, belonging to Tylenchomorpha, were reconstructed. Due to the paucity of experimental evidence related to the small size of most free-living nematodes, little direct information is available about the physiology and functionality of sensory structures in general. Therefore, the possible function of a type of sensillum was often deduced from comparisons of its morphology with that of a similar type in other, and mostly larger, invertebrates, especially arthropods. However, the existence of mutants with defective sensilla in *C. elegans*, and the aberrant behavior associated with these mutations on the one hand and laser ablation of particular sensory neurons on the other hand, have nevertheless allowed drawing some conclusions about the function of the sensilla concerned.

Nematode sense organs can be subdivided into two main groups: those in contact with the body wall and those not. The first are called "peripheral" or "cuticular", the second "internal" (Wright 1980).

1.5.1 Peripheral (cuticular) sensory structures

1.5.1.1 Basic structure (Fig. 1.7 A)

These sensilla are composed of neuronal and non-neuronal elements. The former consists of bipolar neurons in which dendrites end distally in dendritic processes, also called (ciliary) receptors. Originally, the non-neuronal part may have consisted of unspecialized epidermal tissue, as has been reported in some animal parasites (e.g., *Capillaria* and *Trichinella*) or somewhat more specialized "glial" tissue, as found in at least some sensilla of free-living Enoplia (e.g., *Tobrilus, Aporcelaimellus* and *Deontostoma*) and Chromadorida (*Chromadorina*). In an evolutionary more derived state, these non-neuronal elements are elongated cells with a specialized peripheral part and are differentiated into two distinct components, each one specialized for a different function. The two cells were first described in *Ascaris* by Goldschmidt (1903) as "Geleitzelle" and "Stützzelle". Later, they were given other names that resulted in some confusion. Here, we will use the descriptive terminology first proposed by Ward et al. (1975) and now most frequently used: "socket cell" for the anterior cell and "sheath cell" for the posterior. The socket cell is so called because its distal end surrounds the cuticular sensillar canal below the body cuticle as a kind of socket. It seems to fulfill a supporting function, but it also secretes the cuticular lining of the sensillar canal during molting and thus also has an intermittent secretory function. The distal part of the sheath cell surrounds the receptor region of the dendrites and forms an extracellular receptor cavity, which is sealed off from the internal body cavity by tight junctions between the dendrites and the sheath cell. The secretions of the cell fill the receptor cavity and, in a general way, are supposed to mediate receptor specificity and sensitivity by regulating the ionic environment around the receptors. The distal part of the neur(on)al component is called the dendritic or ciliary process. The non-motile cilium is strongly modified and only retains the sensorial capacities of a typical kinocilium. The structure and development of a receptor can be quite variable, but in a general way, one can distinguish, from its entrance into the receptor cavity toward the distal tip, the distal part of the dendrite with a striated rootlet and sometimes with peripheral extensions (microvilli), followed by the basal region, median region and terminal region of the dendritic process. The basal region (also called the "transition zone") consists of a modified basal body that possesses an outer ring of nine doublets in the plesiomorphic condition, whereas in apomorphic forms, a smaller (four to eight) or sometimes a larger (ten) number of doublets has been found. Inside this ring, there may be 0 to 15 singlets. The median region may still contain doublets, but these are usually replaced by single microtubules. When this part of the receptor is inflated (as in some tylenchs), the number of microtubules may be quite large. The terminal (or apical) region of the receptor typically contains only a decreasing number of singlets.

Sensilla that are in open connection with the outer world through pores in the body cuticle are generally considered to be involved in chemoreception, whereas those that do not have such a connection are supposed to be mechanoreptors. However, in many cases, this seems to be an oversimplification of the actual situation.

1.5.1.2 Sense organs at the anterior body end (Fig. 1.8)

There are different terminologies and interpretations of the symmetry, but here we use the terminology first proposed by De Coninck (1942) (see also De Coninck 1965, Coomans 1979, White 1988). Typically, the anterior sensilla consist of two circlets of six labial sensilla each, one circlet of four cephalic sensilla and in addition a pair of lateral amphids. In the first circlet, that of the inner labial sensilla (ILS.), the outer parts are usually papilliform. In the second circlet, that of the outer labial sensilla (OLS.), the apical parts are usually setiform in aquatic nematodes and papilliform in most other species. When six lips are present, each lip bears an inner and an outer

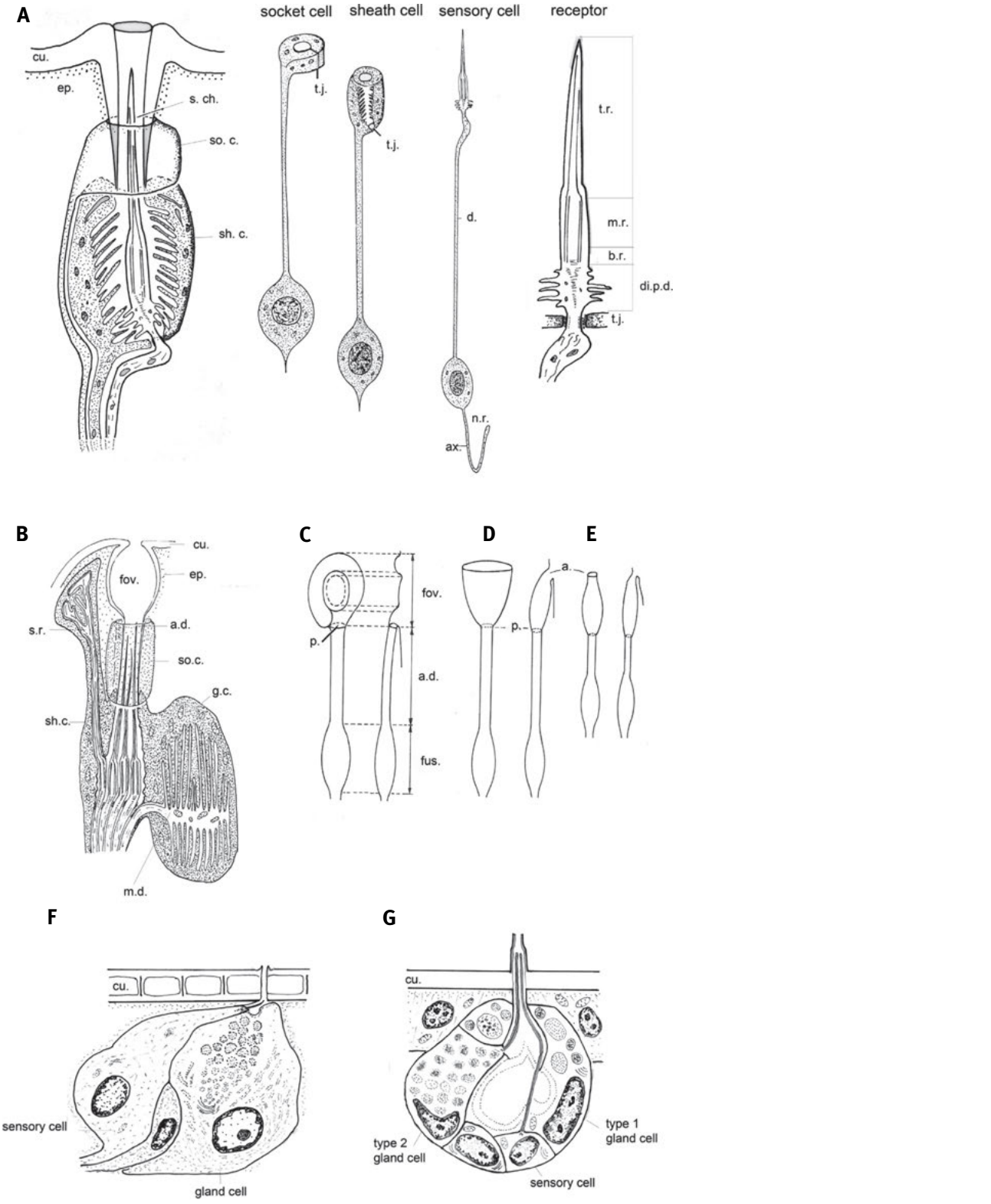

Fig. 1.7: Structure of nematode sensilla. A, Basic structure showing the distal part at left, the three components in the middle and a detail of the ciliated receptor at right; B–E, Amphid, B, Detailed structure of a "secernentean" amphid, C, Amphid with external, spiral fovea, seen from above and in profile, D and E, Amphids with internal fovea, seen from above and in profile. (A–E adapted from Coomans 1979); F and G, Peripheral somatic sense organs; F, in *Chromadorina* (adapted from Lippens 1974) and G, in *Catanema* (adapted from Nebelsick et al. 1992). Abbreviations: a., aperture; a.d., amphid duct; ax., axon; b.r., basal region; cu., cuticle; d., dendrite; di.p.d., distal part of dendrite; ep., epidermis; fov., fovea; fus., fusus; g.c., gland cell; p., porus; s.ch., sensillary channel; sh.c., sheath cell; so.c., socket cell; t.j, tight junction; t.r., terminal region.

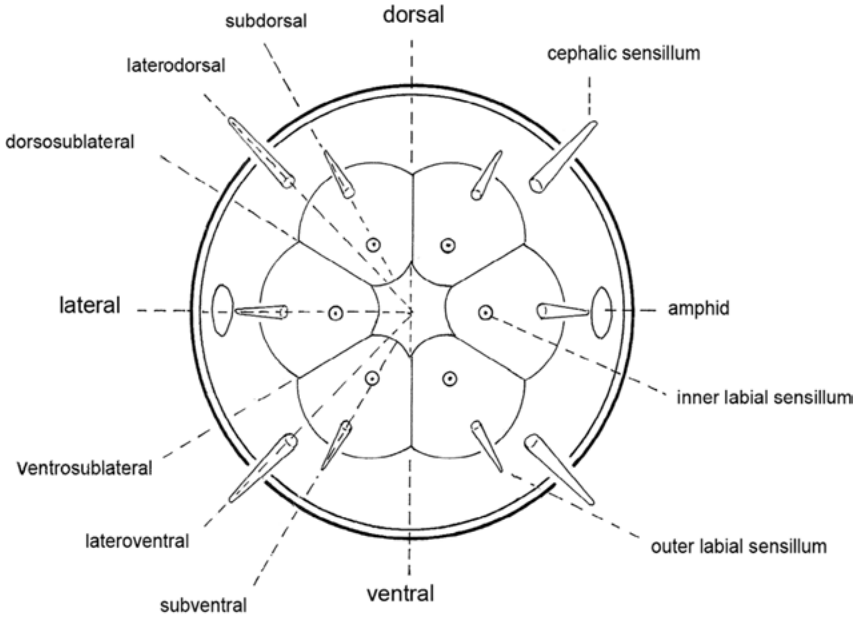

Fig. 1.8: Basic arrangement of the anterior sensilla in nematodes (modified from De Coninck 1942).

labial sensillum on its radial axis; hence, ILS. and OLS are arranged in a hexaradial pattern. This pattern is present also when there are three lips, when there are exceptionally two or when lips are completely fused. The four cephalic sensilla (CS) are bilaterally arranged in the middle of each body quadrant, i.e., in a submedian position. They can be setiform (most aquatic species) or papilliform. Primitively, the CS are located behind the lips as the first in a series of somatic sensilla. In this position, one observes a 6 + 6 + 4 pattern. In apomorphic patterns, one can find 6 + 10 (quite common) or 10 + 6 (e.g., in some Actinolaimidae) arrangements when CS have migrated on the lips. Labial and cephalic sensilla have different origins, as evidenced from (1) the different development of both types in a number of nematodes, e.g., poorly developed LS and prominent CS as in, e.g., *Aphanolaimus*, and the fact that (2) CS contain catecholamines as somatic sensilla do (Sulston et al. 1975, Voronov & Nezlin 1994, Sawin et al. 2000) and are part of a dopaminergic system that senses textural differences, which is important in food gathering, whereas LS lack these substances. The amphids are the largest and most complex sensilla at the anterior body end; their primitive position is lateral and postlabial. They open to the exterior in different ways, varying from a simple pore to elaborate patterns at or below the surface. They are generally considered to be chemoreceptors, but detailed analyses of their ultrastructure have shown that they may have other functions as well. Cholinesterase has been detected in amphids of a number of plant- and animal-parasitic species. The cell bodies (perikarya) of the sensory neurons of all labial and cephalic sensilla occur in the body region anterior to the nerve ring; those of the amphids are located in the lateral ganglia, at or just behind the nerve ring.

That the head region harbors several ciliated sensory processes involved in touch sensitivity is no surprise in view of the morphological information and in vivo observations, but it was fully demonstrated in *C. elegans* (Kaplan & Horvitz 1993). These authors identified three classes of mechanosensory neurons that mediate an avoidance response when the nematode is touched on its anterior end (called the "nose" by the authors). Two types end in the left and right lateral lips, and a bifunctional (chemo- and mechano-sensitive) amphidial receptor acts as the third type. The studies of Bumbarger et al. (2007) and Ragsdale et al. (2009) have convincingly demonstrated that a detailed study of the receptors at the anterior end of the nematode is not only important for comparative morphology and understanding functionality, but also for obtaining insight into phylogeny. From their observations, it appears that, apart from group-specific small differences, there is a high degree of conservation in the anterior sensory organs among immediate and more distantly related outgroups.

The tips of the inner labial sensilla may protrude above the surrounding cuticle as papillae with an apical pore or may not protrude and then are simple pores that may lie around or even partly (the lateral ones only) or completely within the mouth. In some cases, the tips are entirely embedded in the labial cuticle. When a pore is

present, the sensillum may be either bimodal (chemo- and mechanoreceptive) or solely chemoreceptive. When chemoreceptive, the reception probably acts at a short distance, comparable to taste receptors. If a pore is lacking, as in more specialized nematodes, chemoreception is still possible when the labial cuticle above the receptor is permeable for chemical substances. However, most dendritic processes that are embedded in the cuticle show some specialization, e.g., presence of dense material that is related to mechanoreception. The number of receptors per sensillum is one or two in Rhabditida (i.e., secernentean nematodes) and varies from two to several in, e.g., adenophorean nematodes. The structure of the dendritic processes may be similar or dissimilar.

The outer labial sensilla (OLS) are primitively situated on the same radii as the inner labial sensilla (ILS). Their apical parts may protrude above the labial cuticle as papillae or setae in free-living nematodes, especially aquatic ones. In many terrestrial and in most plant and animal parasites, the apical part is a simple pore or is embedded in the labial cuticle. Some specialized plant parasites (e.g., in J2 and males of *Meloidogyne* and *Tylenchulus*) and animal parasites (*Syphacia, Heterakis*) completely lack OLS; in others, only the lateral ones are reduced or absent. The OLS are probably bifunctional in most free-living adenophorean nematodes, but in some animal parasites, such as *Capillaria* and *Trichinella*, they are probably only chemoreceptive (Wright 1974, McLaren 1976). In many secernentean species they seem to be only mechanoreceptive. The number of receptors varies from just one (e.g., in *C. elegans*) and two in other secernentian nematodes and several in adenophorean ones.

The cephalic sensilla are submedian in position. When seti- or papilliform they usually have an apical pore, but when they do not protrude above the surface, a pore may be lacking. Also, here the receptors may be uni- or bifunctional. In secernentean nematodes, there may be one or two receptor(s). When a receptor is embedded in the cuticle and contains electron-dense material, it is considered to be a mechanoreceptor. However, in males of some species (e.g., *C. elegans, Aphelenchoides*) and in a number of others, there is a second receptor that extends to an apical pore. In these cases, the function seems to be unimodal (chemosensitive) or bimodal, with a possible role of mate or host detection through chemoreception. In adenophoreans, there are more (two to three described to date) receptors, and in most cases, they communicate with the exterior through pores and hence seem to be chemoreceptive or bifunctional. Sexual dimorphism in the development of cephalic sensilla also exists in a number of free-living species.

The amphids are the main sensory organs of a nematode (Fig. 1.7 B–E). Their normal position is lateral and postlabial, but in some adenophoreans and in many secernenteans, they have slightly shifted dorsally, whereas in most secernenteans and a few adenophoreans, they have migrated onto the lateral lip region. An amphid consists of a distal part, called a *fovea*, which can be an external excavation in the cephalic cuticle or an invagination of the cuticle that forms a type of pocket connected with the exterior through an aperture (*apertura amphidialis*). This part is completely or partially filled with a gelatinous matrix (*corpus gelatum*) secreted by the sheath cell that is also called the "amphidial gland". The fovea is followed by the amphidial duct (*canalis amphidialis*), and the junction between both is called the duct pore (*porus canalis amphidialis*). The length of the duct is quite variable according to nematode groups; its posterior end enlarges to the next part of the amphid, the fusus (*fusus amphidialis*). This terminology for the different parts of the amphid was proposed by Riemann (1972) and Storch & Riemann (1973), but in earlier and even in recent taxonomical papers, the fovea is often called the "amphid" and the fusus the "sensillary pouch". The fovea, duct and anterior part of the fusus are lined with cuticle. The dendrites enter the amphid at the base of the fusus, the main part of which is formed by the amphidial gland. In many secernentean nematodes, the fovea is very small and may even be absent; in the latter case, the external opening is also the duct pore. In Enoplia and Dorylaimia, the fovea typically forms an invaginated pocket. The most obvious variation in the fovea can be found when it is external, as in the Chromadoria, where it can be circular, oval, mono- or multispiral, elongate, loop-shaped, etc. The fovea may also show sexual dimorphism, being sometimes considerably larger and more complex in males of aquatic species as well as in infective stages of some parasitic species, which is most likely correlated with detection of female pheromones in the first case and with host detection in the second. Exceptionally, sexual dimorphism can also occur in the amphidial gland, e.g., in the genus *Leptosomatum*, in which the amphidial gland in males is extremely long, reaching up to the base of the pharynx or even overlapping the intestine (Bongers 1984). The dendrites that enter the fusus form dendritic processes, which show typically ciliary structures. Their number is much higher than in other sense organs and is also higher in adenophorean nematodes (10–38) than in secernentean ones (3–15). The number of dendrites can be lower as some of them may bear two or more processes (up to 38 processes from four dendrites in *Oncholaimus vesicarius*). Not all

of the processes enter the amphidial duct. Those that do enter the duct (*duct receptors*) are embedded in secretions from the sheath cell. Other processes penetrate more or less into the sheath cell (*sheath receptors*). One of the latter has a very special structure because it has, apart from zero to three ciliary processes, a large number of microvilli (from less than 50 to more than 300) and has therefore been called "multivillous dendrite" or "finger cell". Although direct evidence is scant, the duct receptors are considered to be mostly chemoreceptive, probably detecting chemical substances from a distance and hence comparable to olfactory receptors. This is confirmed by indirect evidence from chemotaxis-defective mutants in *C. elegans* and from laser ablation studies. In *C. elegans*, there are 12 dendrites; one of these is the microvillous dendrite that has been shown to function as a thermosensitive receptor (Perkins et al. 1986). Bumbarger et al. (2009) have compared the amphid sensilla of the cephalobid *A. complexus* with the free-living *C. elegans* and the animal parasites *Strongyloides stercoralis* and *H. contortus*. The authors found that the amphid structure is broadly conserved in number and arrangement of cells, but that details of cell anatomy differ. They proposed some hypotheses of homology based on comparisons between *Acrobeles* and the other Rhabditidae, as well as with Tylenchomorpha. It seems that *C. elegans* and *H. contortus* have lost the terminal part of one of the dendrites, which explains the presence of only 12 dendrites in the amphid of these species versus 13 in *A. complexus* and *S. stercoralis*. Furthermore, the rhabditid species have one ciliate process in the multivillous dendrite, whereas *A. complexus* has two, which is similar to the plant parasite *Meloidogyne incognita* (Baldwin & Hirschmann 1973). This possible synapomorphy is in agreement with the closer relationship of Cephalobomorpha and Tylenchomorpha as established by several molecular evolutionary studies (Bert et al. 2008).

Other functions than those already mentioned, have been proposed for the amphid receptors, such as mediation of sheath cell activity, stretch receptors or vibration detectors, whereas others may be photoreceptors (see below).

1.5.1.3 Peripheral somatic sense organs (Fig. 1.7 F–G)

Primitively these sensilla are numerous and arranged in dorsal, ventral and sublateral rows (four, six or eight) along the body. Dorsally, they are often confined to the anterior neck region. In many aquatic (mainly marine) species, the outer parts are setae that can vary from short to very long. At first sight, it seems likely that these setae are tactile sense organs, but some setae may have an opening at the tip that could indicate chemosensitivity. In other aquatic nematodes, e.g., *Chromadorina* (Lippens 1974) and in many terrestrial ones, the outer parts are pores, and here, the sensilla are considered to be mainly chemosensitive. The presence of several dendritic processes in many somatic sense organs could indicate that they have a bimodal function. The sensory elements are associated with glands that are comparable to sheath cells and often more or less modified epidermal cells comparable to socket cells in other sensilla. A special kind of chemosensitive glandular organ has been detected in the stilbonematid nematode *Catanema* (Nebelsick et al. 1992). According to the authors, it differs in several aspects from other peripheral sensilla in that it comprises two gland cell types, one monociliary sensory cell and one undifferentiated epidermal cell, and lacks specialized supporting cells. It is interesting to note that the secretions of these (numerous) glandular sensory organs may create a microenvironment for the interactions between the bacterial epibionts (typical for this family) and the nematode host. In the posterior region of some dorylaims, a number of the ventrosublateral sensilla have migrated to a subventral position in males, whereas a similar shift can occur near the vulva in females. These somatic sensilla may assist in one way or another in the mating process.

More specialized forms such as some plant- and animal-parasitic adenophoreans and most secernenteans have only a small number of somatic sense organs. Female trichodorids may possess medioventral or subventral pores near the vulva, and most males possess a pair of lateral cervical pores near the base of the stylet and one to four medioventral cervical pores or papillae. A single receptor with an external pore, hence supposedly chemosensitive, was found in *Capillaria* (Wright & Hui 1976); two pairs of post labial papillae have been described in ascarids with supposedly bimodal function (Wright 1980). In free-living and plant-parasitic secernenteans, the somatic sense organs may consist of up to three lateral pairs: *deirids* in the neck region; *postdeirids* around the middle of the body length or halfway between the vulva and anus; and *phasmids*, usually on the tail. Often only the latter are present. In Plectidae, only deirids are found. Deirids may have a papilliform or setiform apical end; their (as far as is known) single dendritic process contains electron dense material and is supposed to act as a mechanoreceptor. It has been suggested that deirids and postdeirids could signal the space available when nematodes penetrate restricted spaces, and if so, they can be considered as somatic sensilla specialized in correlation with a life in narrow cavities. In *C. elegans*, the receptors of deirids and postdeirids are very similar to those of the cephalic sensilla, and together these eight sensilla constitute the dopaminergic system of this nematode. Phasmids are somewhat better known. Chitwood & Chit-

wood (1950) used the names Phasmidia (later Secernentia) and Aphasmidia (later Adenophorea) to distinguish the then two main groups of nematodes, one that (originally) had phasmids and one that lacked them. In most secernenteans, the phasmids lie on the tail and connect with the outside world through a small pore. In males with a bursa, they can be tube-like and situated on the bursal flaps. Occasionally the phasmidial canal is closed by an elongated or a disk-like plug (*scutellum*). In some species, the phasmids have shifted anterior to the level of the anus, and this is even more so with the scutella of some Hoplolaimidae. In a number of secernenteans, the phasmids have disappeared. Little is known about their function, but chemoreception can be deduced from their structure when a pore is present. In mermithids and in free-living *Tricoma* (Desmoscolecida), a phasmid-like structure (phasmata) is present laterally on the ending of the tail. The presence of few somatic sense organs in secernentians does, however, not mean necessarily that chemo- or mechanoreception is limited. Detailed studies on *C. elegans* have shown that mechanoreception is present along the whole body. Several morphologically distinct classes of mechanosensory neurons have been found (Chalfie & Sulston 1981, Chalfie et al. 1985, Wicks & Rankin 1995, Bounoutas & Chalfie 2007).

From the few detailed studies that exist on somatic peripheral sense organs, it can be concluded that their basic structure is mostly similar to, but simpler and more plesiomorphic than that of the anterior peripheral sensilla.

1.5.1.4 Peripheral genital sense organs (Fig. 1.9 G, H)

In the posterior body region of males, there may be a number of specialized somatic sensilla, called genital supplementary organs, supplements, genital papillae, genital setae, caudal papillae, precloacal-, adcloacal-, postcloacal papillae and bursal rays (or ribs), according to the group studied. Typically, adenophorean species possess midventral, preanal genital sensilla; these are either few in number (sometimes just one) and then often complex in structure. If they are more numerous, they are then usually papilliform. Exceptionally, the midventral row can become a partly staggered, double row. (It should be noted that a few adenophoreans also possess genital papillae in the cervical region). Many of the genital sense organs are associated with glands, which may be very prominent in the case of the complex sensilla mentioned above; these glands can open into protrusible tuboid structures. Apart from a single series of midventral supplements, males of Dorylaimida may have a double precloacal ("preanal") papilla or two separate papillae anterior to the cloacal opening. In trichodorid males, a pair of postcloacal papillae (caudal papillae) is also present. In secernentean species, the genital papillae are pre- as well as postcloacal and usually paired, but a single midventral precloacal papilla occasionally occurs, as in *C. elegans,* for example. In this species, it is located in a shield-like sclerotized cuticular structure, called "hook", and it contains two sensory processes surrounded by socket and sheath cells. Also, a pair of postcloacal sensilla are embedded in a sclerotized cuticle; in these sensilla, the endings of three sensory-motor neurons are also surrounded by socket and sheath cells. When a bursa is present, the genital sensilla may extend into the cuticular flaps as bursal ribs or rays; in *C. elegans*, there is only one non-neuronal cell in these sensilla. In this species, 87 neurons are exclusively found in the tail region of males and determine their sexual behavior, 42 of them have ciliary structures, and many of them are mechanoreceptive (Sulston et al. 1980, Liu & Sternberg 1995, Lints & Hall 2009). In some animal parasites, large ad- and postcloacal papillae occur.

1.5.1.5 Sense organs of the copulatory apparatus and the cloaca (Fig. 1.9 F)

Sensory structures have been observed in the spicules, in the gubernaculum and/or in the cloacal wall of some nematodes. Most information comes from studies on secernentean nematodes, especially *C. elegans* and Tylenchida. Two dendritic processes have been found in each spicule; these can be similar, each with their tips ending near a pore close to the spicule tip, or dissimilar, with a long dendrite ending at a pore near the spicule tip and a shorter dendrite ending about halfway of the spicule. In *Aphelenchoides*, there is a single sensory dendrite, which runs down to the tip of the spicule just beneath its outer side. Because of the fairly exposed track of this spicular dendrite, it was suggested that it is tactile as well as chemoreceptive (Clark & Shepherd 1977). Some information on adenophoreans comes from studies on animal parasites *Capillaria* and *Trichuris* (Wright 1978), plant-parasitic trichodorids (Rodriguez & Bell 1978) and free-living marine nematodes (Hope 1974). In the animal parasites, there are numerous distally located receptors that are connected to the exterior medium through pores. Three sensilla have been found in the cloacal wall of *C. elegans*, an anterior one with two dendrites and two postcloacal ones with three dendrites each (White 1988). In *Aphelenchoides*, there are two lateral projections of the cuticle in the cloacal region that each contain a single dendritic process (Clark & Shepherd 1977). In tylenchs, a cloacal sensillum with a single blind ending receptor occurs at each side on the protrusable posterior lateral wall of the cloacal opening. In some, such as Hoplolaimidae, this part of the cloacal wall forms separate structures called titillae around

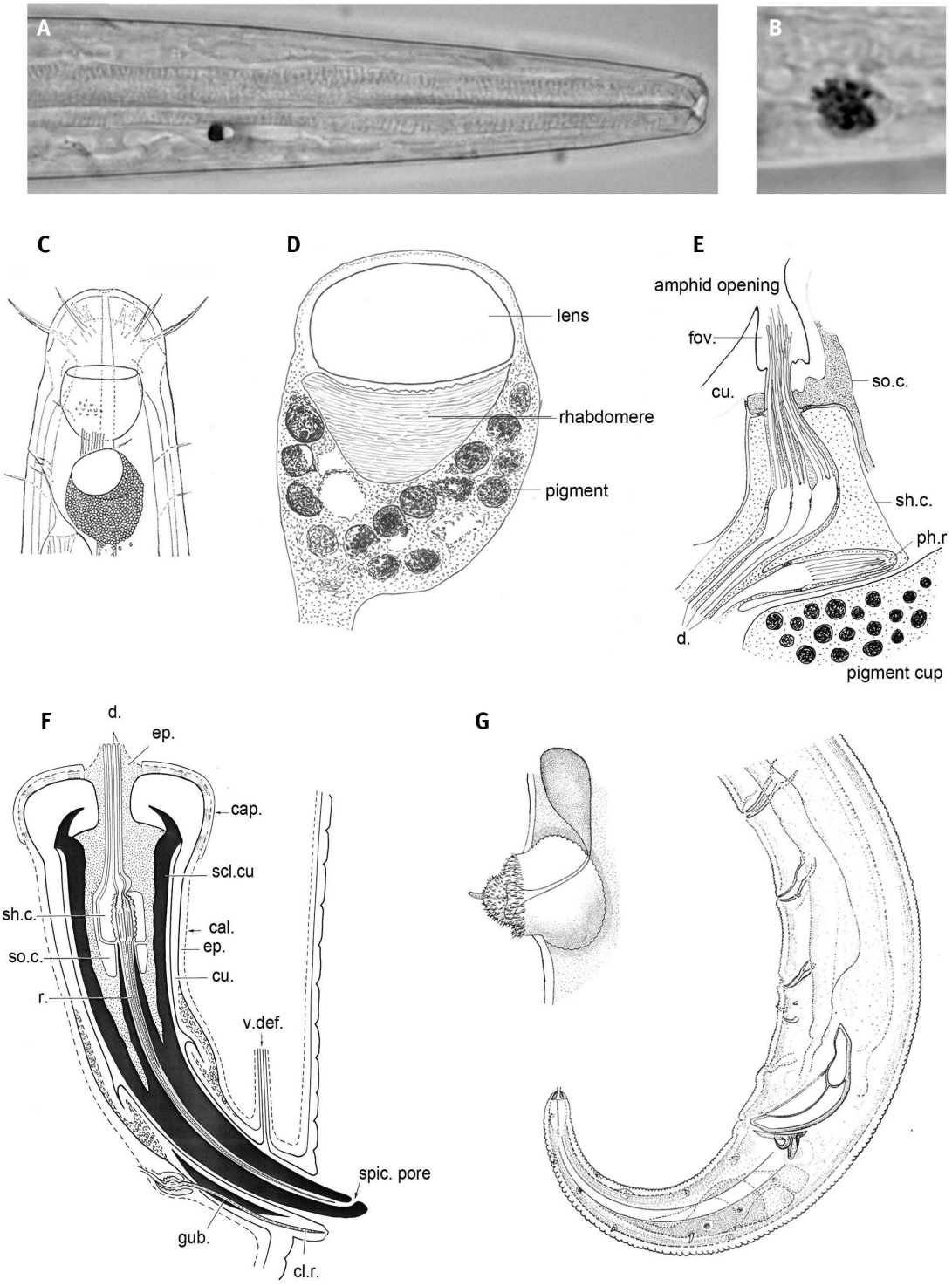

Fig. 1.9: Photoreceptors. A, B and D, *Diplolaimella dievengatensis*. A, Anterior body end showing the position of one of the ocelli; B, Detail of the pigment area; D, Schematic reconstruction based on the ultrastructure of an ocellus. (A and B courtesy of Sophie Derycke, Marine Biology Lab., Ghent University; D adapted from a TEM photograph in Van de Velde & Coomans 1988); C, *Calyptronema acuminatum* male with large ocellus (from *Enchelidium pauli* in Micoletzky 1930); E, *Oncholaimuis vesicarius* ultrastructure of the amphid (adapted from Burr & Burr 1975); F–H, Male genital sense organs, F, Sense organs of the copulatory apparatus (adapted from Coomans & De Grisse 1981); G, Preanal genital sense organ of *Paratobrilus grandipapillatus* (after Brakenhoff 1914); Posterior body end of *Plectus* with tubular preanal sense organs (from Mulk & Coomans 1978). Abbreviations: cal., calamus; cap., capitulum; cl.r., cloacal receptor; cu., cuticle; d., dendrites; ep., epidermis; fov., fovea; gub., gubernaculum; ph.r., photoreceptor; r., receptor; scl.cu., sclerotized cuticle; sh.c., sheath cell; spic., spicula; so.c., socket cell; v.def., vas deferens.

the receptor tips (Coomans & De Grisse 1981). As for other sensilla, those that communicate with the exterior through pores are supposed to be chemosensitive, whereas the others are considered to be mechanoreceptive. The latter could help in locating the vulva, whereas the former may be sensitive for pheromones secreted by the female or may check the interior of the vagina. In this respect, it should be mentioned that laser ablation experiments on *Panagrellus*, in which the bases of the spicules were destroyed, interfered with the location of females (Samoiloff et al. 1974). Observations of live rhabditids have shown that males can detect nearby females with their posterior end, provoking even, backward movements of the males toward the females.

1.5.2 Internal sensory structures

These sensory elements are quite different in shape, complexity, function and location. Their common features, by which they differ from the peripheral sense organs, are that they are not in contact with the body wall cuticle and that they lack a sheath cell and so also a receptor cavity. They may be generally present (or presumed to be so), as those in the pharynx, or they may occur only in certain groups or species, such as metanemes and photoreceptors.

1.5.2.1 Cephalic internal receptors

First detected in the rhaditid *Pelodera* (De Grisse et al. 1974) and then described in more detail in *C. elegans* (Ward et al. 1975, Ware et al. 1975). In the latter species, there seems to be some individual variation; one or two pairs of ciliated neuronal processes and four non-ciliated neurons with sheath-like endings have been identified. In several tylenchids, flattened lamelliform receptors with ciliary rudiments have been found in the subdorsal and subventral sectors of the lip region; they converge in the lateral sectors and are further downward connected with the lateral nerves (Endo & Wergin 1977, De Grisse 1977, De Grisse & Natasasmita 1978, Natasasmita 1979). They have been called "*accessory sensilla*" or "*supplementary nerves*", but neither of these terms seems appropriate. In *C. elegans*, there are five different sensory neurons (inner labial accessory neuron, BAG is a set of two neurons with ciliated endings in the head, with elliptical closed, sheet-like processes near the cilium which envelop a piece of epidermis; endings without socket or sheath cells; FLP is a set of two neurons which have ciliated endings situated immediately dorsal to the lateral inner labial sensilla in the head but have no associated sheath or socket cells; URX is a set of two neurons with cell bodies that are situated subdorsally in the pseudocoelomic cavity just posterior to the ring neuroplile; URY is a set of four neurons with cell bodies situated anterior to the nerve ring, see Ward et al. 1975). These are also present in *A. complexus* (Bumbarger et al. 2007) and *Aphelenchus avenae* (Ragsdale et al. 2009). Internal cephalic receptors have also been detected in the dorylaim *Xiphinema* (Wright & Carter 1979), consisting of a pair in the lateral labial sectors and another pair associated with the amphidial sheath cell; it is as yet not possible to state with which receptors reported in secernenteans they are homologous. Suggestions about the function of these receptors are still largely based upon their structure and comparisons with other invertebrates. Endo & Wergin (1977) and De Grisse & Natasasmita (1978) suggest that they act as mechanoreceptors, and this has been demonstrated at least for FLP in *C. elegans*. An important olfactory role has been assumed for the BAG cells (Bumbarger et al. 2007). The elaborate lamellar extensions of some of these receptors are suggestive of a type of photo-, hygro- or thermoreceptor. There are arguments for each of these possibilities, but they will remain highly speculative until more experimental evidence is available. (BAG is a set of two neurons with ciliated endings in the head, with elliptical closed, sheet-like processes near the cilium which envelop a piece of epidermis; endings without socket or sheath cells; FLP is a set of two neurons which have ciliated endings situated immediately dorsal to the lateral inner labial sensilla in the head but have no associated sheath or socket cells. URX is a set of two neurons with cell bodies that are situated subdorsally in the pseudocoelomic cavity just posterior to the ring neuroplile; URY is a set of four neurons with cell bodies situated anterior to the nerve ring.)

1.5.2.2 Photoreceptors (Figs. 1.9 A–E)

A number of aquatic adenophorean nematodes possess, usually paired, *pigment spots* or *ocelli*. These are located laterally along or partly embedded in the pharynx. In some nematodes, they have shifted to a dorsolateral, subdorsal or dorsal position; in the latter case, they can even become fused. The pigment associated with these structures may be granular or diffuse and is either concentrated or somewhat dispersed. When the pigment spot is accompanied by a hyaline, refractive portion, called a "lens", the structure is called an ocellus. From the available data on the ultrastructure of such ocelli, it seems that the so-called lens is mostly an aggregation of membranes, called a *rhabdomere*. Such a rhabdomere occurs in *Deontostoma californicum* (Siddiqui & Viglierchio 1970), *Araeolaimus elegans, Leptosomatum* sp. and

Chromadorina sp. (Croll et al. 1975). This is one of the two fundamental types of photoreceptors, the other one being the ciliary photoreceptor (Eakin 1968). Only in rare cases has a true lens consisting of amorphous material been detected, e.g., in *Diplolaimella* (Van de Velde & Coomans 1988). The pigment of the eye spot is in fact a shading pigment (mainly melanins) that allows directional sensitivity. It is located in the body cavity (*Araeolaimus*) in a special cell (*Diplolaimella*) or in the wall of the pharynx (*Deontostoma, Chromadorina, Enoplus, Oncholaimus*). The only case so far in which a ciliary photoreceptor has been found in nematodes is in *Oncholaimus vesicarius*, where the special sheath receptors of the amphids are located near and are shaded by a pigmented pharyngeal cell (Burr & Burr 1975). As the pigment spots in *Enoplus communis* are rather similar to those of *Oncholaimus vesicarius*, it is possible that a ciliary receptor is also present. Nematodes without such discrete photoreceptors may nevertheless react to visible light, which suggests the presence of a type of dermal light sense, as has been documented in other invertebrates.

1.5.2.3 Internal receptors of the body wall

In a number of aquatic nematodes belonging to the Enoplida, fine filamentous structures named "*metanemes*" were described by Hope (1965) and Lorenzen (1978 and 1981), and their fine structure was studied by Hope & Gardiner (1982). They occur in the region of the lateral chords and are particularly well developed in some species of *Enoplus, Deontostoma* and *Tobrilus*. Their number varies from 6 to over 100 on each side, depending on species. They may be completely parallel to the longitudinal axis (orthometanemes) or oblique dorsoventral in orientation (loxometanemes). They are composed of an anterior sensory cell with a ciliary process and a posterior secondary sensory neuron; both are connected through a synaptic junction. The ciliary process lies in a ciliary cavity at the top of the sensory cell and has a strongly developed rootlet. Lorenzen considered them to be stretch receptors, but according to Hope & Gardiner (1982), they are proprioceptors. Both studies nevertheless agree that these receptors monitor the bending of the body during locomotion. According to Lorenzen, the metanemes represent an apomorphy for Enoplia as they have not been found outside this taxon. However, these structures have not been observed in a number of groups, and it is at yet not known whether this is due to (independent) secondary loss.

1.5.2.4 Internal receptors of the pseudocoelome

Ciliary neurons exposed to the pseudocoelome have been detected near the pharynx and posterior to the phasmids in *C. elegans*, but little is known about their structure and function.

1.5.2.5 Internal receptors in the pharynx

The nervous system of the pharynx is, as far as is known, well developed and comprises several receptor units. The most detailed information is available for *C. elegans* (Albertson & Thomson 1976). Twelve neurons have free endings just under the cuticle that lines the lumen of the pharynx. Desmosomes attach the neuron tips to adjacent cells. When the shape of the lumen changes due to muscle action, the nerve endings may be deformed and proprioception takes place. Five of these proprioceptors are located behind the stoma, six occur in the median bulb and the last lies in the terminal bulb. In Longidoridae, the nervous system of the pharynx has been studied in *Xiphinema* and *Longidorus* (Robertson 1976, 1979). Also, here there are nerve endings that are closely associated with the cuticular lining of the pharynx. One set lies in the anterior ventral sinus of the odontophore (stylet extension), two sets in the laterodorsal sinuses of the odontophore, two sets in the anterior slender pharynx, two sets in the anterior pharyngeal bulb, one set anterior to the outlets of the ventrosublateral glands and one set at these same outlets. Only the nerve endings in the odontophore contain modified ciliary structures, and it has been suggested that they are chemosensitive despite the fact that there are no pores in the cuticle above them because that cuticle is very thin. In other dorylaims, the presence of small areas with thinner cuticles suggest that such internal receptors are quite common in this group. Structures similar to the five proprioceptors reported for *C. elegans,* which may detect the passage of food and/or the flow of secretions from the pharyngeal glands, have been observed in e.g., the base of the buccal cavity in Diplogasterina (Baldwin et al. 1997, Fürst von Lieven & Sudhaus 2000) and have long been known in Mononchida, where they were called "foramina". It seems probable that the above-mentioned receptors in the odontophore region of dorylaims are homologous with the five proprioceptors in *C. elegans;* and this casts some doubt on their suggested chemoreceptive function. It seems probable that these receptors are far more widespread than hitherto known.

1.6 The digestive system

The digestive system or alimentary canal forms the inner tube of a nematode, which is separated from the outer tube by the body cavity. It consists of three main parts: (1) foregut or stomodeum; (2) midgut or mesenteron; and (3) hindgut or proctodeum. Only the midgut (intestine) has an endodermal origin (derived from the E founder cell); whereas the foregut and the hindgut have a mixed ecto- and mesodermal origin. Some taxa and some developmental stages possess a degenerated alimentary system without a mouth opening, buccal cavity or pharynx and with a rudimentary intestine. In the free-living aquatic genera *Astomonema* (Ott et al. 1993), *Parastomonema* (Kito 1989) and *Rhaptothyreus* (Miljutin et al. 2006), the rudimentary gut contains internal symbiotic sulphur-oxydizing bacteria. Nematode-parasitic stages of insects can show atrophy of the pharynx as, for example, in mermithids (Poinar & Hess 1977) and fergusobiids (Giblin-Davis et al. 2001). The bacterial endosymbiont bacteria *Wolbachia*, which are mutualistic in filariae, being present in the germline, have also been found in somatic tissues (epidermis) and in cells of the intestinal wall (Ferri et al. 2011).

1.6.1 Foregut (stomodeum) (Fig. 1.10)

Comprising the mouth opening, the first part of the mouth cavity (cheilostoma from Gk. *cheilos* = edge and *stoma* = mouth), the second part of the mouth cavity (pharyngostoma from *pharynx* = gullet and *stoma* = mouth), the pharynx proper and the pharyngo-intestinal junction (often called the cardia). The mouth opening and the cheilostome are lined with invaginated body cuticle, and the other parts are lined with pharyngeal cuticle. The latter has a different structure and thickness and resembles only the two outer layers of the body cuticle. The combination of cheilostome and pharyngostome is called the stoma sensu lato, or buccal cavity. The shape and development of the buccal cavity and its cuticular lining, the buccal capsule, vary considerably in relation to differences in feeding type as well as between groups (Wieser 1953) and within a given group (Fürst von Lieven & Sudhaus 2000). Several recent studies have convincingly shown that detailed observations of serial TEM sections of the stomodeum can be used to test evolutionary hypotheses based on molecular data (e.g., Baldwin et al. 1997, Zhang & Baldwin 1999, 2000, 2001, Ragsdale et al. 2008, 2009, 2011).

1.6.2 Cheilostome

Whether the mouth opening, the surrounding lips and the cheilostome are originally hexaradially or triradially symmetric is still a matter of debate. Indeed, a number of primitive Enoplida seem to lack a buccal cavity and true lips, in which case the pharynx is attached directly to the cephalic cuticle and then the mouth opening is triradiate. When the oral opening deepened, the lips that were originated were also triradial, surrounding a likewise triradial cheilostome. However, the labial sense organs are typically arranged in a hexaradial pattern, and this symmetry is also found in the lips and the lining of the cheilostome in many groups of nematodes. Typically the cheilostome is confined to the lip region, but it may extend further posteriorly in derived forms (e.g., in forms with a stylet). This part of the digestive system is formed by the anterior epidermal cells. The cuticular lining of the cheilostome may be more or less sclerotized; occasionally, denticles or larger tooth-like structures may be present.

1.6.3 Pharyngostome

The pharyngostome is a specialized anterior part of the pharynx and typically triradially symmetrical, as is the pharynx proper. There are three sectors, one dorsal and two ventrosublateral (often called subventral). The cuticular lining of the cavity, which may be sclerotized, is secreted by a set of six special epidermal cells, the arcade cells, and by the pharyngeal epithelium. In more derived species, the cell bodies of these cells may lie more posteriorly in the anterior body region (e.g., in *C. elegans*, see Albertson & Thomson, 1976). Several subdivisions can be recognized, and these have been given different names (for discussion, see Baldwin & Eddlemann 1995; Baldwin et al. 1997, De Ley et al. 1995, Fürst von Lieven & Sudhaus 2000). To avoid confusion with earlier terminology and for parsimonous reasons, we follow here, except for one detail explained below, the terminology proposed by De Ley et al. (1995) for Rhabditida. Two main regions are distinguished: a gymnostome, secreted by the arcade cells, and a stegosome, secreted by the pharyngeal epithelium. The latter region can be further divided into four subregions: pro-, meso-, meta- and telostegosome (Fig. 1.10 A). To avoid a debate on the use of either oesophastome or pharyngostome, De Ley et al. (1995) preferred to use the term stegostome, but this creates

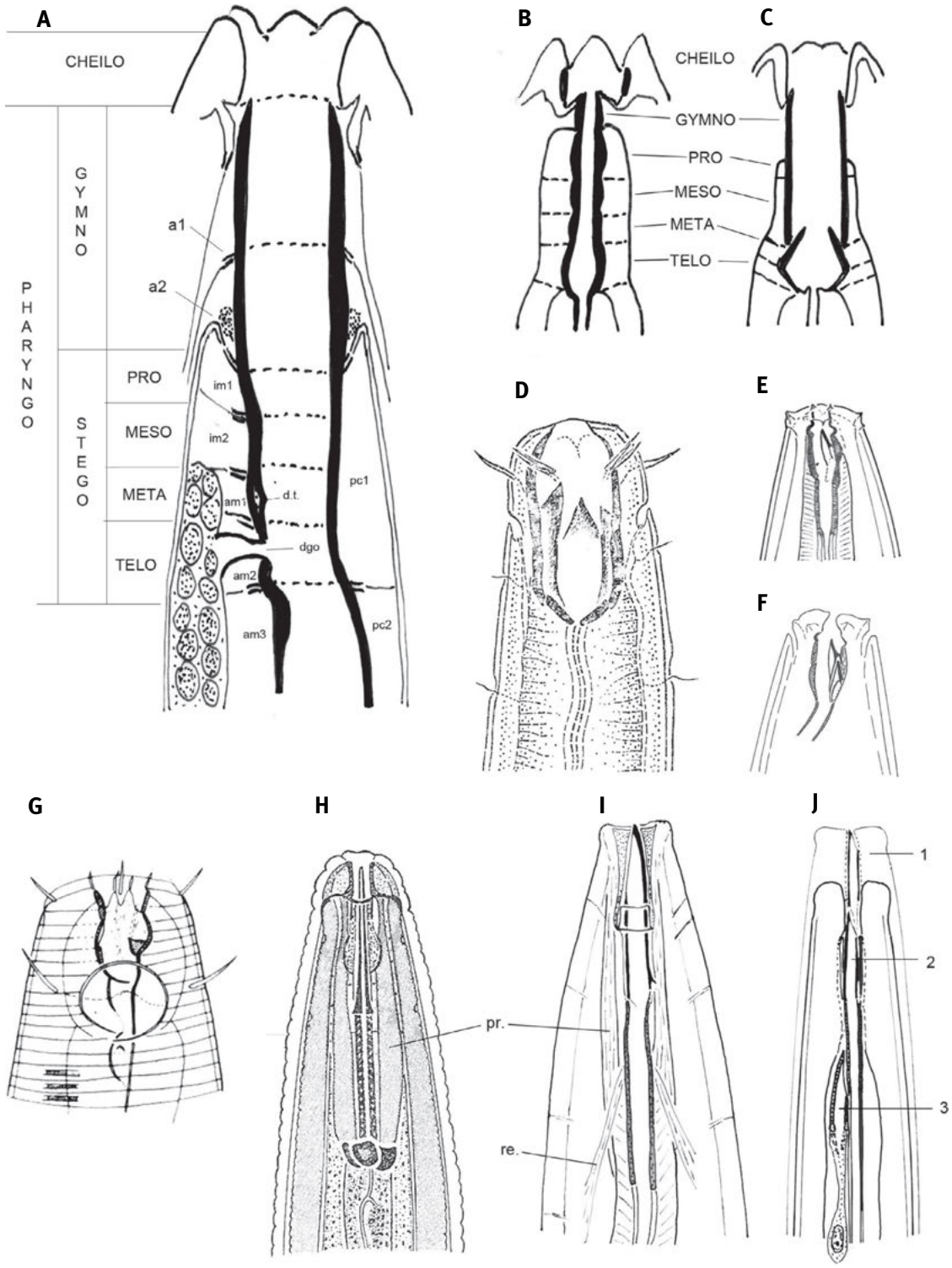

Fig. 1.10: Different stoma structures and feeding types. A–C, Bacteria feeders; A, Stoma structure and terminology of subdivisions in *Panagrellus*; B, Subdivisions of stoma in *Acrobeloides;* C, Subdivions of stoma in *Caenorhabditis* (adapted from De Ley et al. 1995); D and E, Carnivores/omnivores; D, *Oncholaimus* (from Heyns & Coomans 1977); E and F, *Oionchus,* adult and fourth-stage juvenile with a replacement tooth in place behind the functional tooth (from Coomans & Loof 1970); G, *Bolbolaimus*, an epistrate feeder (from Riemann 1966); H and I, Suction feeders; H: *Rotylenchus* (adapted from Coomans 1962); I, *Dorylaimus* (from Coomans & Baqri 1972); J, Diagram of moulting *Labronema,* (1) exuvium with odontostyle of the previous juvenile stage, (2) the functional odontostyle of the new juvenile stage and (3) the formation of the new replacement odontostyle by the odontostyle forming cell (adapted from Grootaert & Coomans 1981). Abbreviations: a., arcade cell; a.m., adradial muscle cell; d.g.o., dorsal gland opening; d.t., dorsal tooth; p.c., perradial (= marginal) cell; pr., protractor muscle; re., retractor muscle. In the different parts of the stoma (see A–C), the ending "stom" is omitted.

confusion as pharyngostome comprises both gymnostome and stegostome. The metastegosome may bear denticles and/or one tooth or three (rarely two) teeth. Teeth are present in many groups of nematodes, especially in predators and in a number of animal parasites. They may be solid or hollow. Two basic types of teeth can be distinguished. In one type, the juvenile stages of the nematode have a replacement tooth behind the functional tooth; in the second type, there is no replacement tooth. The first-stage juveniles of species that possess the first type of tooth (e.g., Ironidae, Dorylaimida and Mononchida) have a functional as well as a replacement tooth, but from the first to the third molt, the old functional tooth is shed and a new tooth is formed behind the new functional tooth. During the last (fourth) molt, the tooth-forming cell loses its secretory activity, the previous tooth is shed and the replacement tooth of the fourth-stage juvenile becomes a functional tooth in the adult (Coomans & De Coninck 1963, Coomans & Lima 1965). In second- to fourth-stage juveniles of dorylaims, the replacement tooth (or odontostyle) migrates backward inside a ventrosublateral sector of the pharynx. In nematodes with the other type of tooth, this is formed in situ during the ongoing molt itself. Teeth may adopt several shapes, such as hooked, curved or elongated, and they may be robust or slender. They may be implanted on a movable or a rigid base. In some taxa, the teeth (e.g., Oncholaimina) or the dorsal tooth (e.g., Diplogasterina and animal parasites as *Necator* and *Haemonchus*) are/is perforated by a gland canal. A specialized needle-like tooth may evolve to a hollow tube (odontostyl) as in predaceous and plant-parasitic dorylaims. This odontostyl (as well as the needle-like tooth from which it evolved) develops in a special, elongated glandular cell in a ventrosublateral sector of the pharynx (Grootaert & Coomans 1981). It is inserted upon a supporting base, called an odontophore, which is a more or less modified part of the pharyngeal wall. Together, both parts form the odontostylet (Coomans & Van der Heiden 1971). In Mononchida, which may possess either one dorsal tooth or three teeth, the replacement tooth is formed in the corresponding sector of the pharynx (Coomans & Lima 1965, Khan & Coomans 1981). Another type of spear originates in situ and is called a stomatostylet; it is found in Tylenchida, Aphelenchidae and Aphelenchoididae. In both cases, the stylet is hollow and used to penetrate the food item (prey or plant cell) and to ingest the liquid or semi-liquid food substance. The forward movements of both types of spear are mediated by protractor muscles, but backward movements are different. In the case of a stomatostylet, there are no retractors, and the backward movement occurs passively, whereas an odontostylet is pulled back by retractor muscles. The formation of both types of stylet is also quite different. According to Endo (1985), the stomatostylet of Tylenchida is derived from myoepithelial cells of the anterior part of the pharynx (the pharyngostome) during each molting process. The stylet cone and the stylet shaft are formed by arcade syncytia, and the stylet knobs are formed by myoepithelial cells just posterior to the arcade syncytia. According to Baldwin et al. (2004), the stylet cone and shaft are homologous with the gymnostom and the stylet knobs with the prostegostom. A similar stomatostylet is found in Aphelenchidae (Ragsdale et al. 2008) and in Aphelenchoididae (Shepherd et al. 1980). Whether this is a matter of common origin or the result of convergent evolution is still a matter of debate. In the case of an odontostylet, the functional odontostyl is shed together with the cuticular lining of the odontophore during molting, as well as the cuticle of the more anterior parts of the buccal cavity and the body cuticle. A new replacement odontostyl is formed and stored in the pharynx. However, the odontophore is newly formed in situ at each molt (Coomans & De Coninck 1963). Other types of stylet do exist, as in some marine nematodes belonging to Camacolaimidae, with a solid dorsal tooth, and some Siphonolaimidae, with an apparently hollow stylet. Furthermore there is a needle-like stylet, called onchiostyle, in the plant ectoparasitic Trichodoridae, which is composed of an elongated tooth (here called onchium) with a closed tip and inserted on a support (onchiophore) formed by a thickening of the dorsal lumen wall of the pharyngostome. The uptake of plant cell content occurs along the stylet. Phenotypic plasticity has been observed in feeding structures (teeth) of bacterivorous *Pristionchus pacificum* in response to an environmental cue (Kionke & Fitch 2010). The form feeding on bacteria possesses a narrow stoma with one rigid dorsal tooth (stenostomatous). When the bacterial food source declines, some juveniles develop into adults having a wide stoma with two movable teeth (eurystomatous) to slice open fungal hyphae and avoid starvation.

1.6.4 Pharynx (Fig. 1.11 A, B)

This triradial part is often called the “esophagus” by nematologists. Although the term pharynx was originally used for vertebrates, it has also been used for the muscular part of the alimentary canal posterior to the buccal cavity in invertebrates. A more glandular part between

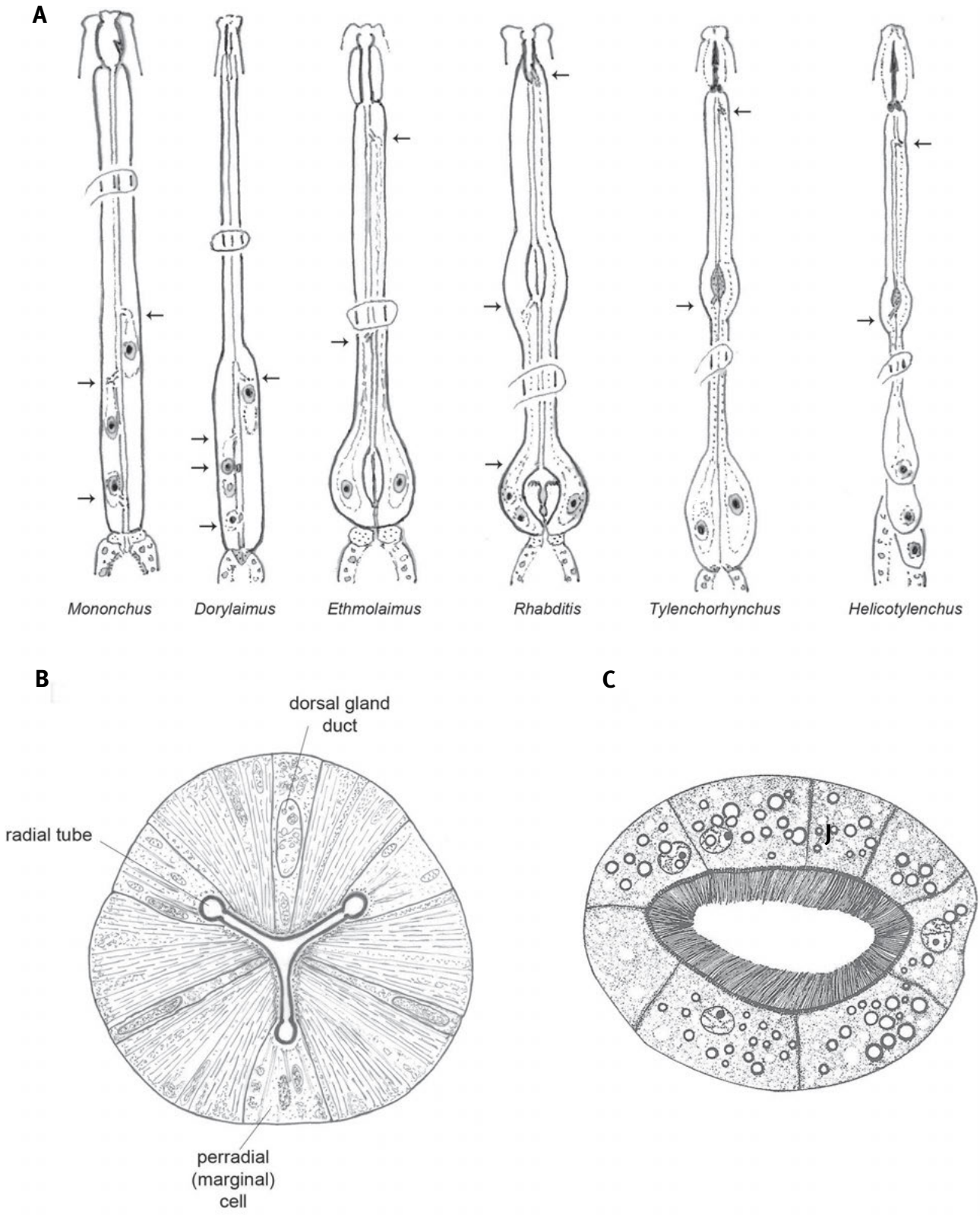

Fig. 1.11: A, Different types of pharynx. The arrow on the right side indicates the position of the dorsal gland opening; arrows on the left side indicate the position of ventrosublateral gland openings; B, Cross section through the procorpus of the *C. elegans* pharynx; C, Cross section through the intestine in *Dorylaimus stagnalis* (from Chitwood & Chitwood 1950) showing microvilli bordering the inner wall.

that muscular part and the intestine, which occurs in a number of invertebrates, is then called esophagus. In other invertebrates, such as nematodes, there is usually no clear separation between an anterior muscular and a posterior glandular part, and this has led to a terminological confusion in nematology, but not so in several other invertebrate groups with a similar constitution. In nematodes, the pharynx is a muscular as well as a glandular organ, but may be partly only epithelial as e.g., the procorpus of tylenchids. It varies in form and function according to the group. The simplest type is that of a muscular cylinder that brings food from the buccal cavity to the intestine. The triradiate lumen is lined with cuticle, which is secreted by the myoepithelial cells. During the molting process, the muscle cells differentiate into secretory cells, indicating their dual function (Grootaert & Coomans 1981). The cuticular lining typically forms three radii, one ventral and two dorsolateral; they may converge distally or may terminate in radial tubes at the apices of the radii, depending on the taxon and the feeding type or on the region within the pharynx. These apical tubes occur, e.g., in Axonolaimoidea, Plectidoidea and Rhabditida; they are confined to the corpus in species that gather their food from liquid media. The food, e.g., bacteria, is retained, and the liquid is evacuated via the tubes back to the buccal cavity. The outer side of the pharynx is covered with an elastic connective tissue membrane (peripharyngeal basal lamina) and sometimes also by longitudinal (arranged parallel or spiral) or circular muscles. Radially arranged muscles, consisting of only one sarcomere in length (Roggen 1973), connect the basal lamina with the cuticle around the lumen. In the resting (non-feeding) condition, the lumen is closed by the hydrostatic pressure in the body cavity. Upon contraction of the radial muscles, the pressure from the body cavity is surpassed and the lumen opens. In this way, a suction can be created for the uptake of food. Food is then moved toward the intestine by means of successive contractions (peristalsis). The triradial form of the lumen is the most advantageous arrangement for optimal functioning (Roggen 1973). The number of cells is usually low, and in several groups cell constancy has been found. In cross section, one can distinguish, per sector, two (ad)radial muscle bands, with a gland duct in their middle (interradially) and a nerve at the periphery and, opposite each radius of the lumen, a perradial (marginal) cell; perradial cells are characterized by junctional complexes and tonofilaments that help to anchor the cuticle lining and provide resistance against contraction of the radial muscles.

Apart from epithelial cells, muscles and nerve cells, the pharynx contains three (one per sector) or five (one in the dorsal sector and two in each of the ventrosublateral sectors) unicellular glands. It is still a matter of debate whether the primitive number is three or five, but there are indications that a reduction from five to three has occurred several times independently. Moreover, the reduction is due either to the disappearance of the anterior pair of ventrosublateral glands or of the second pair. Some or all of these glands may become multinucleate in derived, mainly animal, parasitic forms. In *C. elegans*, the picture is somewhat complicated because the anterior pair of ventrosublateral glands forms a syncytium in the anterior part of the basal bulb and is connected with the dorsal gland; the second pair of ventrosublateral glands occupies the posterior part of the basal bulb (Albertson & Thomson 1976). However, the presence of five-gland nuclei clearly indicates that the presence of syncytia is a secondary condition. Typically, the glands produce digestive enzymes that are mixed in different ways with food and, as the contents of the glands differ, they may produce different types of enzymes. In plant parasites, the secretions from pharyngeal glands may contain a range of cell wall degrading enzymes that enable emigration of the nematode through the plant tissues. In particular, the ventrosublateral glands are highly metabolically active during penetration and migration, but their activity decreases with the establishment of the feeding site induced by the dorsal gland secretions, for example, in cyst nematodes (Vanholme et al. 2004, Davis et al. 2004). The position of the gland outlets varies according to the group of nematodes and is a taxonomically important character. Apart from Mononchida, Dorylaimida and a few smaller groups, the dorsal gland opens anteriorly near the basis of the pharyngostome (e.g., at the meta- and telostegostome boundary), whereas the first pair of ventrosublateral glands opens either anteriorly or further back, depending on the taxon. This is the case in many Enoplida, where the second pair of ventrosublateral glands opens farther backward. The latter outlets are, however, often obscure or even invisible in LM. In Dorylaimida and Mononchida, all five glands open in the posterior half of the pharynx; the outlet of the dorsal gland is usually in the midregion of the pharynx, whereas the ventrosublateral glands open farther back. A more complex pharynx is subdivided in two or three different parts. In the latter, case a corpus, isthmus and terminal bulb (the latter two together are also called “postcorpus”) are distinguished. The corpus acts as a suction pump, and the terminal bulb forces the food into the intestine. Through the division of labor, this type of pharynx can be shorter than a cylindrical type, which is more convenient for small nematodes (Roggen 1970). Species

feeding on bacteria or protists often possess a denticulate or ridged valvular apparatus (grinder) in the terminal bulb. Species consuming liquid food, such as plant-parasitic tylenchs, have a median bulb derived from the posterior part of the corpus (the metacorpus). The median bulb has a thickened cuticular lining (also called valves) and strong musculature, which allows stronger suction. In species that feed on liquid food, part of the digestion may be extracorporeal, due to the injection of secretions into a plant or animal cell. The injection may be mediated by a stylet or a tooth. The pharyngeal gland cells in tylenchs may become so big that they protrude outside the pharyngeal contour as gland lobes. In exceptional cases, as in Mermithidae and Trichuroidea, the pharynx separates from the intestine during development and becomes a series of protein synthesizing gland cells called a stichosome.

1.6.5 Pharyngo-intestinal junction (cardia)

This is the posterior part of the stomodeum. Typically, it is a one-way valve that delivers food to the intestine. In many Enoplia, the pharyngo-intestinal junction is a separately differentiated part between the pharynx proper and the intestine, but in many other groups, the pharyngeal part has invaginated into the intestine and is surrounded by flaps from the anterior-most intestinal cells. In *C. elegans*, the wall of the junction is composed of six cells that lie outside the basal lamina of the pharynx. Originally, the lumen was triradiate with a thin cuticular lining, but in many forms, it became a simple slit lined by membranes from adjacent cells. This is, e.g., the case in several Tylenchida that have been studied in this respect (Geraert 1992). In a number of genera, belonging to unrelated taxa, three unicellular glandular cells with unknown function may be present around the valves in a number of species.

1.6.6 Midgut (mesenteron, intestine) (Fig. 1.11 C)

The intestine is solely derived from endoderm; its wall consists of a single-layered epithelium covered by microvilli toward the lumen. The microvilli may be coated by a more or less developed glycocalyx and by lamellae. The number of cells varies according to the group. An intestine with few cells is called oligocytous (up to 128 cells); one with more cells is called polycytous, i.e., 256–8192 cells (the result of 11–17 cleavages). Above the last number, the term myriocytous is used. In oligocytous intestines, the number of cells in cross section may be only two, as in monhysterids (Van de Velde & Coomans 1989b), or two to four, as in *C. elegans* (White 1988). Possible differentiations include a short ventricular region at the anterior end of the intestine and a prerectum at the posterior end. Occasionally a caecum may occur at either the anterior or the posterior end. In infective stages of some insect parasites (*Steinernema, Heterorhabditis*), symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*, respectively) are present in the intestinal lumen of the ventricular region. When the infective J3 invades the insect host, the bacteria are released in the haemocoel of the insect and kill the host. The nematodes then continue their development in the cadaver. In *Tobrilus gracilis*, commensal flagellates occur in the ventricular region (Coomans, unpublished information). The flagellates have been erroneously considered as ingested food items (Nuβ 1985). The intestine does not possess muscles of its own; however, some specialized somatic muscle cells may connect to the intestinal wall. In Mermithids, the intestine becomes a food storage organ in the parasitic juvenile that stores the necessary nutrients for the non-feeding adult stage. Development of the intestine as a food storage organ is also the case in the sedentary plant parasite *Meloidogyne* (Geraert 1992). The food, partly digested by the enzymes of the pharyngeal glands, is absorbed by the intestinal cells and further digested intracellularly (endocytosis), as established by Van Fleteren (1980) for *C. elegans* and Nuβ (1985) for aquatic nematodes. The latter author also found evidence of membrane digestion by an absorbed bacteriolytic enzyme in *Tobrilus*.

1.6.7 Hindgut (proctodeum, rectum)

This part is of mixed ecto- and mesodermal origin. It is connected to the intestine by a valve, which is surrounded by a sphincter muscle. Usually it is a short, dorsoventrally flattened tube that leads to the anus in females (exc. *Lauratonema* see reproductive system), whereas in males, it connects with the vas deferens, thus forming a cloaca leading to the cloacal opening. The lumen is lined by cuticle, which is continuous with the body cuticle. Defecation is possible after relaxation of the rectal sphincter followed by a contraction of the anal muscles, which open the anus or the cloaca. Rectal glands are often present, usually three (sometimes six) in females and six in males. The function of these glands is poorly known except where they have unusual specialization. This is the case

in *Meloidogyne*, where the six rectal glands of the female are very large and produce a gelatinous matrix in which the eggs are stored.

1.7 Body cavity or pseudocoel

The body cavity or pseudocoel of nematodes is a primary body cavity that differs from a secondary body cavity or true coelom in that it is not lined by an epithelium of mesodermal origin. The body cavity is lacking in the anterior body region (anterior to nerve ring or most of the neck region). Where it is present, it is lined by the somatic muscles and a basal lamina that covers the epidermal chords. With increasing body size, stronger muscle action and higher body pressures, elaborations of the basal laminae have produced more extensive connective tissues. Pseudocoelomic membranes composed of a basal lamina, sometimes accompanied by fine cell processes, traverse the body cavity in the pharyngeal region (e.g., in *Trichuris*) or connect the anterior and posterior intestine of *Ascaris* to the body wall. Nematode connective tissues likely are types of collagen, and in their more complex form, they serve to reinforce tissues and suspend organs in the body. The body cavity is filled with fluid that bathes the internal organs, serves as a type of circulatory system for nematodes and envelops some large, isolated cells called coelomocytes (= pseudocoelomocytes). Tahseen (2009) provides a review on the biology and possible immune functions of coelomocytes.

1.7.1 Structure and function (Fig. 1.12)

1.7.1.1 Coelomocytes

The number and position of coelomocytes varies according to the species. They were first described as four giant stellate cells in the anterior third of the body in *Parascaris equorum* (Bojanus 1821 in Chitwood & Chitwood 1950), and similar cells (two or four) were later described in other ascarids. Chitwood & Chitwood (1950) observed two coelomocytes near the base of the pharynx and at the anterior end of the ovary or testis, one at the blind end of the anterior gonad and one at three-fourths of the body length from the anterior end in *Rhabditis strongyloides*. Similarly, the *C. elegans* hermaphrodite has six coelomocytes but arranged as three pairs (a right ventral anterior pair, a left ventral posterior pair shortly anterior to the vulva level in dextral- or clockwise-moving animals and, dorsally, one cell on each side) in the body cavity adjacent to the somatic musculature; the male has only five coelomocytes with a single dorsal coelomocyte. Four of these cells are present at hatching, and the additional cell(s) are generated in the J1. In free-living nematodes such as Meyiliinae species, two large coelomocytes are present along the anterior intestine (Decraemer 1982), whereas *Haliplectus muscorum* (Plectidae) possesses four coelomocytes, two large cells located subdorsally in close association with the S-E duct and anterior to the renette cell (located at level of pharynx base) and two additional cells located ventrosublaterally and just posterior to the renette cell (Holovachov et al. 2009).

Their function is only partially known. A phagocytic function was described from large nematodes such as ascarids and strongylids: *A. suum*, for example, endocytose invaging organisms and molecular weight dyes and proteins (Chitwood & Chitwood 1950, Bolla et al. 1972). However, in *C. elegans*, coelomocytes do not seem capable of phagocytosis but rather are considered as scavenger cells that continuously and nonspecifically endocytose fluid from the body cavity. Green fluorescent protein (GFP) secreted into the pseudocoelom from body wall muscles is endocytosed and degraded by coelomocytes. Ablation of coelomocytes results in viable animals that fail to endocytose, a function that is apparently not essential for growth or survival of the animals (Fares & Greenwald 2001). Coelomocytes have an apparently fixed position and rely on movements of the animal and body fluid for accessing foreign material. Active endocytosis in *C. elegans* coelomocytes may represent a primitive immune surveillance function. Other possible functions could be hepatic or secretory or the transport of materials, e.g., from the intestine toward the gonads (e.g., in monhysterids, there are several coelomocytes connected with the reproductive system). Yolk is synthesized in the intestine of adult hermaphrodites of *C. elegans* and is secreted into the body cavity. Low-density lipoprotein complex receptors on the surface of growing oocytes endocytose yolk, which is then stored in vesicles in oocytes (Fares & Grant 2002). An excretory function has also been suggested. In Plectidae, for example, there are coelomocytes connected to the S-E cell. In *Sphaerolaimus*, organelles of the coelomocytes contain urate oxidase (Turpeenniemi & Hyvärinen 1996). In *A. suum*, the pseudocoel fluid contains inorganic and organic ions, lipids, carbohydrates and proteins and also haemoglobin (Lee & Smith 1965).

1.7.1.2 Pseudocoelom

The pseudocoelomic fluid acts as part of the turgor-pressure system (see body cuticle). The hydrostatic pressure in the pseudocoel is high in most nematodes and shows

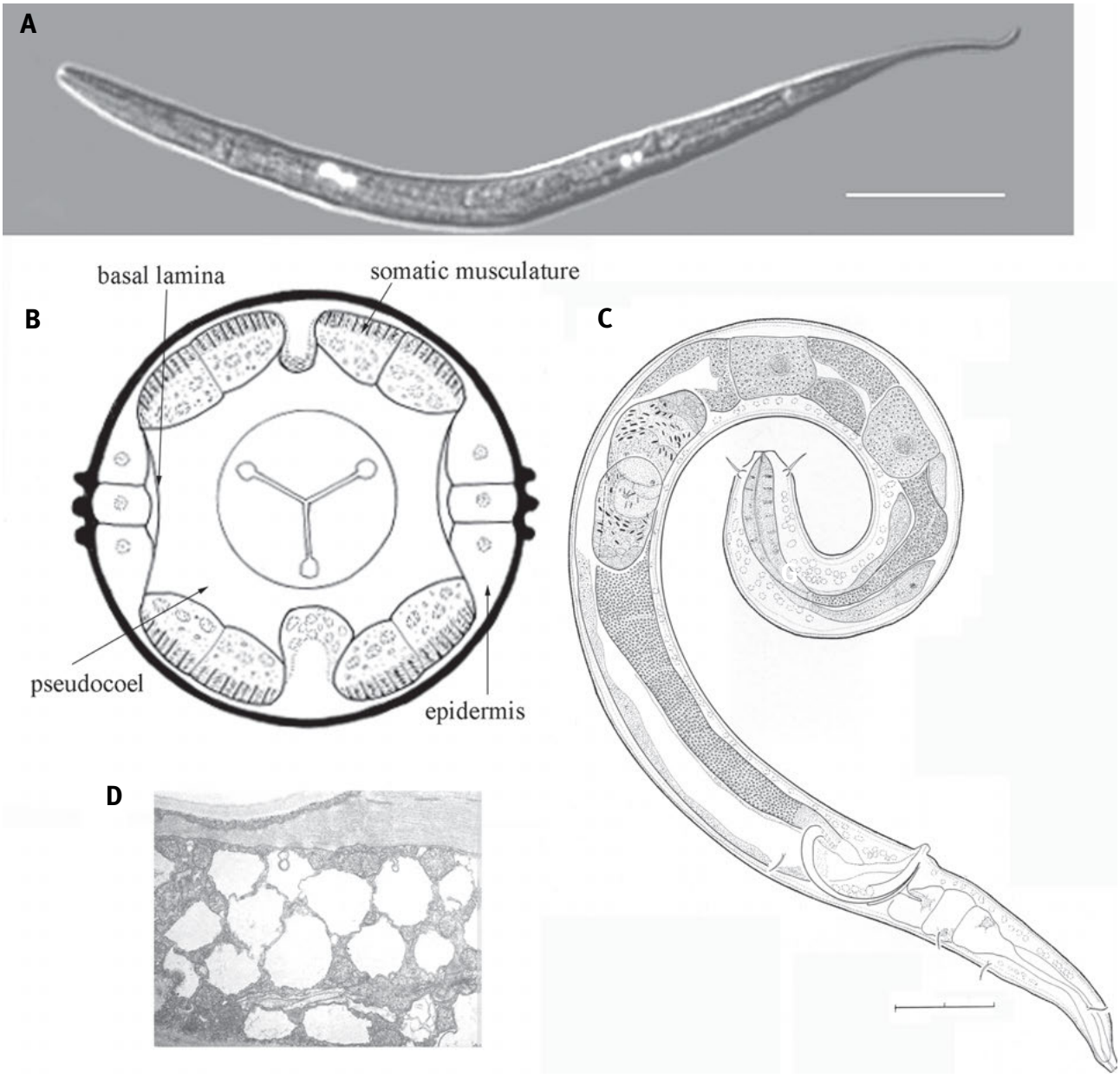

Fig. 1.12: Body cavity (pseudocoel). A, Location of two pairs of pseudocoelomocytes in *Geomonhystera pervaga* (courtesy Q. Tahseen); B, Diagrammatic presentation of a transverse section at the level of the posterior pharynx region; C, *Gerlachius lissus*; D, TEM of the longitudinal section through the lateral epidermal cord at the level of the many vacuoles in the neck region of *Geomonhystera disjuncta* (x 20 000; adapted from Van de Velde & Coomans 1989). Scale bar: A 100 µm; C 20 µm.

rhythmic fluctuations associated with muscle contraction and expansion. Because of the high internal pressure, when nematodes are cut or punctured, their body contents are forcibly extruded. However, in some smaller free-living nematodes (e.g., *Geomonhystera*), this does not occur; large vacuoles in the epidermal chord seem to replace the pseudocoelom. Also, some larger nematodes such as *Oncholaimus* do not explode when cut, apparently because this nematode has weak body muscles. The existence of such a high-pressure system explains (1) why approximately 95% of all known nematodes have a cylindrical body shape, (2) why nematodes have a strong cuticle as antagonist to the high pressure system in the absence of kinocilia (= motile cilia) and the absence of circular body muscles, (3) the absence of kinocilia in the intestine and flagella in sperm that move mainly in an amoeboid way through largely compressed gonoducts and (4) the occurrence of molts, necessary to replace in a short time a cuticle that otherwise becomes too thin and too weak to prevent radial expansion of the body.

The pseudocoelomic fluid also has some osmoregulatory function, but the main osmoregulation is provided by the secrtory-excretory system and, to a lesser extent, by the cuticle. The chemical composition of this fluid in large parasitic nematodes has been found to be a complex mixture of organic and inorganic chemicals buffered by a bicarbonate-phosphate system that maintains a pH close to neutral. Most marine nematodes are isotonic to seawater; soil and freshwater species are hypertonic to their surroundings and animal-parasitic nematodes may be iso-, hypo- or hypertonic to the body fluid of the host. The ability of nematodes to withstand major osmotic changes in their environment probably depends more on the permeability of the cuticle to water than to the membrane-sparing action of organic osmolytes such as glycerol, trichalose, amino acids and urea. Many marine organisms are adapted to a wide range of salinity. Foster (1998) studied osmotic stress tolerance and osmoregulation of intertidal and subtidal nematodes to find answers on how nematodes can overcome salinity fluctuations and to explain their horizontal distribution. He found that upper tidal as well as lower tidal species were able to regulate water content to different extents. In hypotonic solutions, an initial influx and, in hypertonic solutions, an initial loss of water was followed by a gradual recovery to water-content values close to those of nematodes in 100% seawater. However, the efficiency of osmoregulation and the rate was different between the species. Upper tidal species showed the greatest capacity for osmoregulation, a factor that could determine the horizontal distribution of nematode assemblages in littoral habitats. A number of especially freshwater nematodes may harbor crystal-like structures (from 5 to 30 µm in size) of unknown origin or function in their body cavity; some structures contained proteins, sulphides and carbohydrates and could function as storage for waste products.

1.8 Secretory-excretory system (S-E system) (Fig. 1.13)

The nematode S-E system (also called the excretory system) has long been an enigma, and aspects of its structural and functional variability remain poorly understood. Previously, in some species, components were considered to be circulatory vessels or salivary glands; Schneider (1858) was the first to propose the complex of glands, canals and ducts to be an excretory system (Chitwood & Chitwood 1950). The S-E system has been a key feature in defining taxa. Hyman (1951) recognized Nematoda as distinctive from other Aschelminthes by its having an excretory system in which flame bulbs (characterized by motile cilia) or other current-producing mechanisms are absent. Within Nematoda, taxa have been further defined on the basis of the high degree of structural variability of the S-E system. Considering this diversity, Von Linstow (1909) developed a classification with four groups, and one of these groups, Secernentea, was later adopted by Chitwood (1958) in his definition of two nematode classes, Secernentea (with excretory canals) and Adenophorea (without excretory canals, including some groups in which the excretory system seems to be entirely absent). More recently, molecular-based phylogenies support that nematodes with excretory canals define a monophyletic clade (Blaxter et al. 1998, De Ley & Blaxter 2002, 2004). As nematodes thrive in a wide range of parasitic and free-living conditions, varying with species and stages, it is not surprising that excretory systems correspondingly vary in producing secretions specialized to particular life styles and that they might also vary in their role in eliminating soluble wastes, participating in homeostasis and accommodating diverse ionic, osmotic or pH conditions (Thompson & Geary 2002). Indeed, the S-E system of nematodes is structurally highly variable; Chitwood & Chitwood (1950) noted that the only point in common is a ventral pore. Among systems that have been carefully studied, the most complex is represented by *Caenorhabditis elegans* (Rhabditida), which includes four distinct cell types: a canal cell (excretory cell), a fused pair of gland cells, a duct cell and a pore cell (Fig. 1.13; Hall & Altun 2008, Nelson et al. 1983). The excretory canal cell (Fig. 1.13 E), extending almost the entire body length, is the largest cell in *C. elegans*; it is H-shaped with each of the two canals enclosed within the right and left lateral epidermal chords. The excretory canals are linked to the surrounding epidermis by a system of gap junctions, and they are closely associated with several nerve processes (Hedgecock et al. 1987, Hall & Altun 2008). A bridge that includes a single large nucleus connects the two canals. Also within the bridge and anterior to the nucleus is an excretory sinus, lined by a system of small channels, and the sinus is continuous with the lumens of the two canals.

A binucleate A-shaped gland cell develops through the fusion of two other cells (Nelson et al. 1983), and the anterior end of the "A" projects into the nerve ring, where it may have synaptic input. At the transverse connector of the "A", a specialized membrane of the gland cell transitions into a cuticle-lined duct. This transition is near the sinus of the excretory cells, which also opens into the distal end of the same duct (Hall & Alton 2008). A duct cell that, in turn, adheres to a pore cell through which

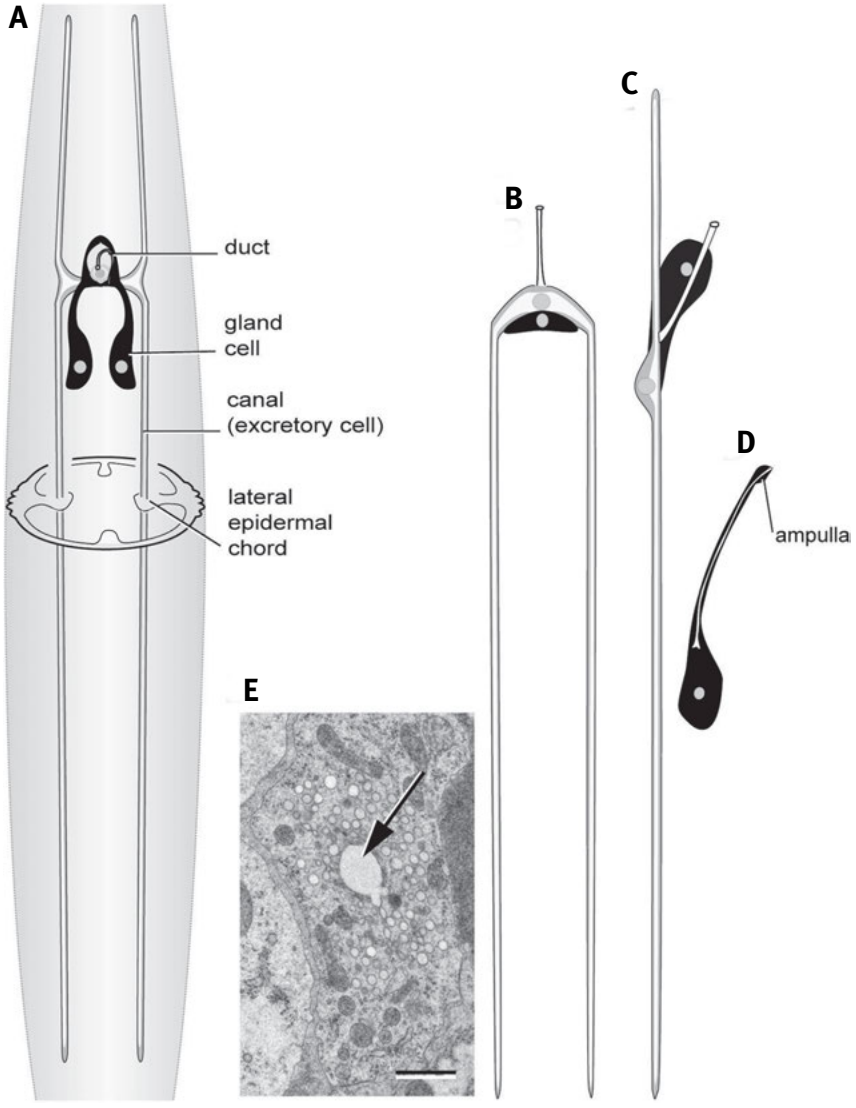

Fig. 1.13: The nematode excretory system. Schematic diagrams are shown unless otherwise indicated. A, Ventral view of a rhabditid in relation to the representation of a transverse section of the lateral epidermal chord; B, Ventral view of a cephalobid; C, Lateral view of a tylenchid; D, Lateral view of an enoplid with only a single excretory gland; E, Transverse TEM section of an excretory canal cell and lumen (arrow) in a diplogasterid; Scale bar: A–D 1.0 mm. A–D are redrawn from Baldwin and Perry (2004); E is courtesy of Dan Bumbarger.

the duct extends and proximally encloses this duct. It is within the pore cell that the cuticle lining of the duct transitions to join the body wall cuticle at the ventral S-E pore opening. The duct cell and pore cell together secrete the cuticle of the duct at each molt, and laser ablation of either precludes development of the cuticle lining (Nelson & Riddle 1984).

Although not studied as extensively as *C. elegans*, other Rhabdtida, including some parasitic strongylids (*Necator, Haemonchus, Stephanurus*), apparently have a similar two-canal, H-shaped system with a pair of large S-E glands (Wharton & Sommerville 1984, Waddell 1968). In many other taxa, including some parasitic ascarids or plant-parasitic tylenchids, the canals anterior to the bridge are reduced (some ascarids), or the system is further reduced by the absence of one of the canals (tylenchids) (Fig. 1.13 B–C; Allen 1960, Chitwood & Chitwood 1950, McLaren 1974, Waddell 1968, Nelson et al. 1983, Wharton & Sommerville 1984).

Particularly in adenophoreans, the S-E system is further reduced, not only by the absence of an excretory cell with canals (e.g., in Trichodoridae, only a ventral pore is present). Often the system essentially consists of a single mononucleate gland cell commonly called a renette (Fig. 1.13 D). The system may be further modified in that the gland cell may have a long unlined neck-like process that only merges with a cuticle-lined duct at the proximal end near the S-E pore (Chitwood & Chitwood 1950, Narang 1970), or there may be more than one gland cell, usually one ventral gland and two additional ventrosublateral or lateral cells (Aboul-Eid 1969, Jairajpuri & Khan 1975, Jensen 1979, De Ley & Coomans 1989, Leduc, personal communication). Near the opening in some species, particularly in free-living former adenophorean taxa, the

cuticularized duct may include valves and a dilated region or ampulla, as well as some supporting cells (Evans & Fisher 1970, Dick & Wright 1973, Turpeenniemi & Hyvärinen 1996). Some species of adenophorean taxa entirely lack an excretory system, but reportedly, for some other groups, the system is only absent in certain stages (Chitwood & Chitwood 1950, Hyman 1951, Bird 1971). Such variability, including phenotypic differences between stages of the same species, may be a starting point for considerations about function. Other variations suggestive of function (see below) include differences among species and stages in the position of the S-E opening. For example, whereas the S-E pore is typically near the level of the nerve ring, in some animal-parasitic strongloids, ascarids, insect-parasitic aphelenchids and free-living monhysterids, it is far anterior within the lip region, and in the plant parasite *Tylenchulus*, it occurs far posterior near the vulva (Lee 1970a,b, Sprent et al. 1983, Maggenti 1962, Coomans & de Waele 1979, Van de Velde & Coomans 1987). In some cases, the position changes with the developmental stage. For example, in second-stage juveniles of the plant parasite *Meloidogyne*, the opening is near the nerve ring, but in adult females, it is much further anterior.

Osmoregulation and liquid waste elimination have long been proposed as key functions for the S-E system; generally, this has been regarded as plausible but not well substantiated in Rhabditida and perhaps doubtful for adenophorean taxa (Bird & Bird 1991). Some evidence of osmoregulation comes from observation of pulsating canals or ducts in response to changes in the environment, including altered (especially hypotonic) osmotic pressure (Weinstein 1952, Waddell 1968, Croll et al. 1972, Narang 1972, Wright 1976, Wright & Newall 1976, 1980, Atkinson & Onwuliri 1981, Nelson & Riddle 1984, Wharton & Sommerville 1984, White 1988), but this pulsation may be limited to only certain stages, as is the case for the dauer (a stage resistant to environmental stress) in *C. elegans* (Nelson et al. 1983). Osmoregulation also may be limited by the particular salts involved, as demonstrated in the free-living marine nematode, *Enoplus,* which apparently regulates in response to NaCl but not when calcium or potassium ions are present (Wright & Newall 1976, 1980). Other tests have suggested osmoregulation by demonstrating the concentration and expelling of injected dyes through excretory ducts (Behrenz 1956). However, more recent evidence of the excretory function comes from *C. elegans*, where laser ablation of the excretory cell, duct cell or pore cell (but not the gland cell) leads to fluid collection within the nematode and death within a few days (Hall & Altun 2008). Similarly, Forrester & Garriga (1997) demonstrated that *C. elegans* mutants defective in canal-associated neurons accumulate excess fluid in the pseudocoelom. Although not specifically implicating the S-E system, Wharton (2010) effectively used an osmometer to measure the remarkable capacity of the free-living microscopic Antarctic nematode *Panagrolaimus davidi* to internally regulate in the face of a wide range of external osmotic concentrations.

It is clear from the structure that the glandular portion of the S-E system is secretory, but a single function for the products of these glands has not been deduced and, in fact, evidence continues to grow for a range of products and functions specialized to particular life histories and species. In *C. elegans*, with its large binucleate gland, the function remains unknown and laser ablation of the gland does not result in any obvious defects (Nelson & Riddle 1984, Hall & Altun 2008). It has been suggested that the S-E gland products might be involved in molting (Davey & Kan 1968, Riddle et al. 1981) and although the glands are active in all stages (except the dauer), ablation experiments in *C. elegans* suggest that this is not the case (Singh & Sulston 1978, Nelson et al. 1983, Nelson & Riddle 1984).

Gland products clearly vary among species and stages, and their function appears to include a role in the parasitic phases of penetration, digestion and protection from hostile environment. The S-E glands of the vertebrate parasite *Anisakis* have been implicated in a role in penetration through the secretion of histolytic enzymes (Lee et al. 1973 citing Ruitenberg & Loensersloot 1971), but they also note that a role in host penetration cannot be the only function as the glands are active in more than only the infective stage (Narang 1972, Davey & Sommerville 1974). Other speculation, based primarily on pore position in parasites Anisakinae and *Nipostrongylus* (Lee 1970b, Lee et al. 1973, Hartwich 1974, Gibson 1983) as well as the free-living Monhysterida (Van De Velde & Coomans 1987) suggest a secretory role involving extracorporeal digestion or, in the case of the plant parasite *Tylenchulus*, a protective role for eggs where, in a far posterior position near the vulva, a gelatinous matrix exudes from the pore and in which the eggs become embedded (Maggenti 1962). Beyond extracorporeal digestion, a more specific role of S-E secretions in parasites may be in immuno-suppression, confounding protective reactions from the host (Segura et al. 2007, Anbu & Joshi 2008, Giacomin et al. 2008). For example, Ivermectin, a drug that induces a dramatic drop in circulating microfilariae, was shown to disrupt S-E function in microfilariae of *Brugia malayi*; one hypothesis is that this disruption impacts the role of excretory products in suppressing the host immune system (Moreno et al. 2010). A possible immunosuppressive role of S-E secretions in parasites

and in conjunction with emerging molecular tools holds new promise for research that will lead to a better understanding of the S-E system in these specialized systems, with practical applications in medicine. Research on the specialized S-E systems of parasites may lead the way to unraveling the remarkably varied adaptations to diverse life histories, relative to the broader more conserved aspects of the S-E system that seem to define major taxonomic clades of the phylum.

1.9 Reproductive system and related reproduction

Reproduction in many nematodes is sexual, involving males and females (= gonochorism; Gk. *gonos* = offspring, *chorismos* = separation) and occurs by amphimixis (Gk. *amphi* = both; *mixis* = mingling) or cross-fertilizing. Uniparental reproduction or autotoky has arisen independently in different taxa throughout the phylum. It can occur through automixis (Gk. *auto* = self) or self-fertilization in bisexual individuals that are proterandrous hermaphrodites with one set of gametes (male) maturing before the other; cross-fertilizing hermaphrodites are unknown. Another form of autotoky takes the form of parthenogenesis (Gk. *parthenos* = virgin; *genesis* = descent), where development proceeds without fertilization. Asexual reproduction or parthenogenesis is common in plant-parasitic nematodes, e.g., *Xiphinema index*. Rare sexual reproduction events occur in asexually reproducing species due to environmental stress, e.g., in *X. index,* male production is favored at the overlap of patches of clonal populations, a condition that could create stress (Villate et al. 2010).

1.9.1 Amphimictic reproduction

Amphimictic reproduction implies copulation and internal fertilization. Apart from the genital characters, in general there is often little sexual phenotypic dimorphism (e.g., in amphids, the anterior feeding apparatus of some tylenchs and marine *Enchelidium* spp). However, in some animal- and plant-parasitic species, females are strongly swollen and sac-like, and males are vermiform (e.g., *Verutus*, Tylenchina, criconematids). Hypertrophy of the female reproductive system can lead to extreme sexual dimorphism as in *Sphaerularia bombi* (Röseler 2002). Another striking case of sexual dimorphism occurs in *Trichosomoides crassicauda*, a parasite of the urinary tract in rats, where the male is extremely small and lives in the vagina and uterus of the female.

1.9.2 Female reproductive system (Fig. 1.14)

The basic (= primitive) female reproductive system is didelphic (Gk. *dis* = twice; two uteri), amphidelphic (Gk. *amphi* = both; *delphys* = womb; uteri opposed), with the uteri connected to a single vagina. The vagina opens by a mid-ventral pore, round or slit-like opening, the vulva, located most often at mid-body. Each genital branch consists of a gonad (ovary) and a gonoduct (oviduct and uterus). The terms monogonic/monovarial, digonic/diovarial and pluriovarial refer, respectively, to the presence of one ovary, two ovaries or many ovaries (e.g., 32 tubular ovaries in *Placentonema gigantissima*). The two uteri may lay parallel instead of opposed and be either directed posteriorly (= opisthodelphic) or anteriorly (= prodelphic), with the vulva lying far anteriorly or posteriorly to almost subterminal on the body, respectively. The same terms are used when only one genital branch is present, a condition also indicated as postpudendum (L. *post* = after, L. *pudere* = to be ashamed – pudendum meaning vulva) and antepudendum (L. *ante* = before). Reduction of one of the genital branches is not uncommon and ranges from partial reduction in various degrees, starting with a reduction of one of the ovaries to various degrees of gonoduct reduction in the pseudomonodelphic condition (e.g., in some dorylaims) until complete loss of one branch in the monodelphic condition, as in *Acrobeloides maximus* and a number of dorylaims (Coomans et al. 2001, Bert et al. 2007). Reduction of parts of the reproductive system is often accompanied by a shift of the vulva. The vulva is separated from the anus except in the free-living nematode genus *Lauratonema* (Enoplida), where the vulva lies very close to the anus, forming a type of cloaca (Gourbault & Vincx 1986).

Two types of ovaries can be found: (1) the telogonic ovary, where germ-cell formation occurs at the blind end of the ovary with progressive stage of development along the length of the gonad, and (2) the hologonic ovary, where germ-cell formation occurs throughout the length of the ovary and development is radial across the gonad; hologonic ovaries are restricted to a few parasitic groups including the Trichuroidea, Dioctophymatoidea and Mermithida (e.g., *Benthimermis*). The telogonic ovary consists of three zones: the germinal zone, the growth zone and the ripening zone (= ovarian sac), exhibiting differences in the epithelial cells making up the wall. In some taxa, e.g., *Caenorhabditis, Anguina* (plant-parasite) and ascarids (animal parasites), the germ cells aggregate around a central protoplasmic core or rachis and developing oocytes are connected to the rachis by cytoplasmic bridges. The ovary may be outstretched or reflexed.

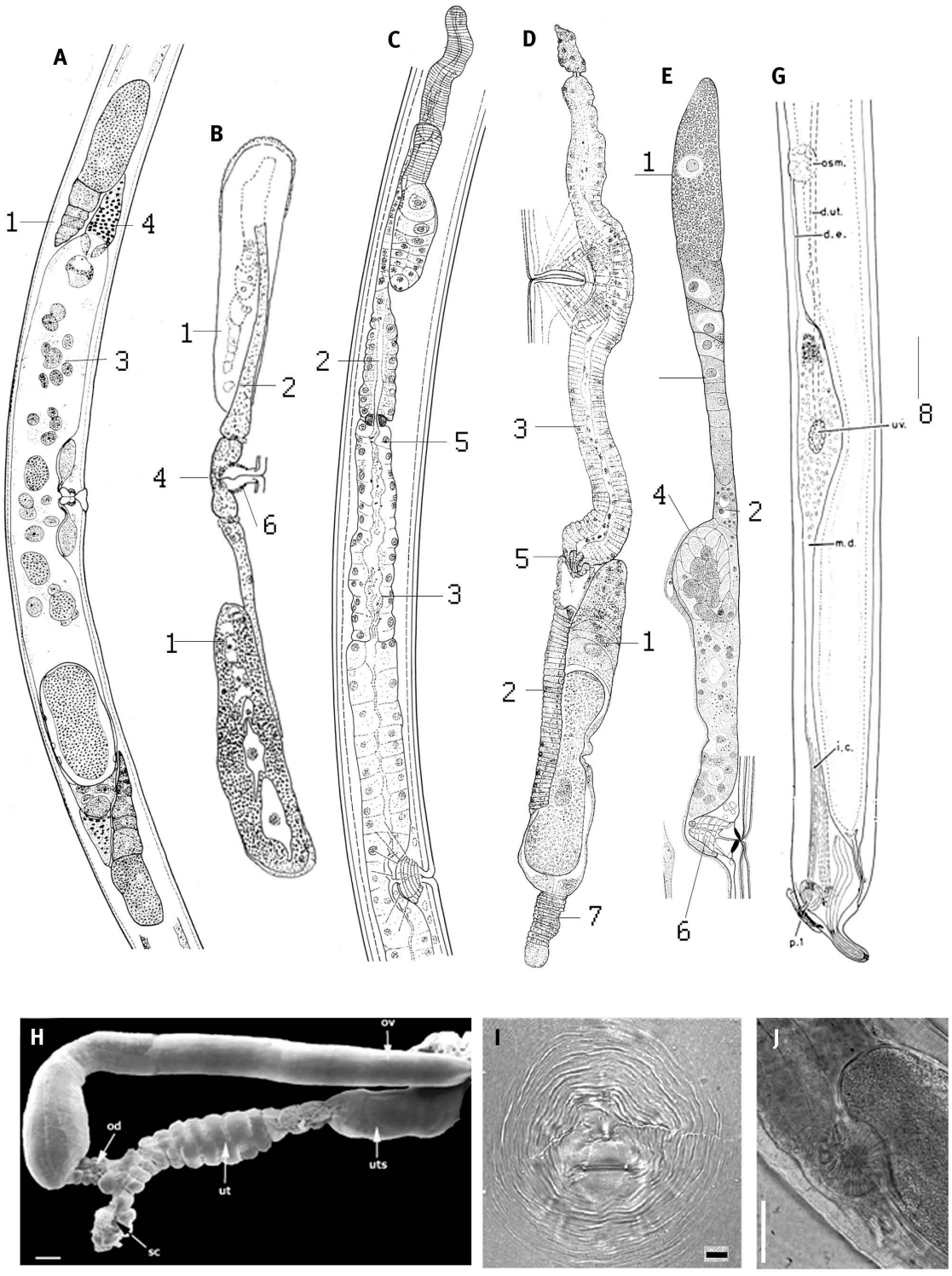

Fig. 1.14: Female reproductive system. A, Didelphic–amphidelphic system with reflexed ovaries (*Anticonema*); B, Didelphic system exhibiting ovaries with symbiontic bacteria (*Xiphinema americanum*); C, Anterior branch of a didelphic system (*Xiphidorus yepesara*); D, Pseudo-monodelphic system (*Xiphinema krugi*); E, Monodelphic system (*Monotrichodorus*); G, Schematic representation of the Demanian system in *Oncholaimus oxyuris* (Oncholaimidae); H, SEM micrograph of the monodelphic female reproductive system in *Acrobeloides maximus* (courtesy of Wim Bert); I, Perineal pattern of *Melodogyne javanica*; J, Detail of the uvette (LM, courtesy of V. Genevois); (1) ovary; (2) oviduct; (3) uterus; (4) ovejector; (5) sphincter muscle; (6) vagina; (7) spermatheca. Abbreviations: Osm., osmosium; d.ut., ductus uterinus; u.v., Uvette; i.c., interstitial channel; m.d., main duct; d.e., ductus entericus; p.t., terminal pore. Scale bar: H–J 10 µm.

This reflection may be of two types: (1) antidromously reflexed where the entire area of the germinal and growth zone is folded alongside the oviduct, and the ova travel from the germinal area in one direction until they reach the fold and then continue in the opposite direction toward the vulva and (2) homodromously reflexed ovaries where the terminal zone and only part of the growth zone are folded over the rest of the genital tract. The ova travel initially in one and the same direction to the fold and then onto the vulva; the folded part is in this case usually long enough for its tip to lie beyond or at the level of the vulva. Homodromously reflexed ovaries are present in several families of the Rhabditida. Lorenzen (1978) provided a phylogenetic assessment of the position of the gonads relative to the intestine. Within free-living nematodes, a variable position of the gonads relative to the intestine was referred to a plesiomorphic condition, whereas a rigidly fixed position suggests holophyly (= apomorphy), e.g., the anterior gonad to the left of the intestine and the posterior gonad to the right of the intestine as interpreted to be a synapomorphy for the Xyalidae (Monhysterida).

The gonoduct comprises a mainly mesodermal part (oviduct and uterus) and a largely ectodermal part (vagina). The oviduct can be uniform or differentiated into a narrow and a wider part (close to the uterus). The narrow part may consist of disc-like cells and collapsed lumen, and the egg is squeezed through by help of somatic muscles (Dorylaimida); the wider part may show some secretory activity and act as a spermatheca (Coomans 1964). Along the gonoduct, there may be one or a pair of sphincter(s) and a *receptaculum seminis*, usually called sperma(to)theca. The uterus may be a simple tube, but is usually more complex. It can be differentiated into several parts, such as a wider part (furthest from the vulva) and a tubular part (closest to the vulva). The wider part is mainly composed of glandular cells (called *crustaformeria* in Tylenchina) and responsible for formation of the outermost egg membrane (Bert et al. 2008). The tubular part has an epithelial wall surrounded by muscle cells, the latter can be more strongly developed in a particular area (e.g., Z-differentiation in some *Xiphinema* spp), in which case the uterus is composed of three parts: a wider part close to the oviduct, a muscular median and a tubular part (closest to the vagina). Part of the uterus often closest to the oviduct may also function as spermatheca. Some species lack a marked spermatheca and have the sperm cells distributed over the uterus. More rarely, sperm are packed in a spermatophore (e.g., the free-living monhysterid *Prorhynchonema* in Gourbault & Renaud-Mornant 1988, parasitic Rhigonematidae in Hunt 2001). Both uteri, at the level of connection with the vagina, can have a common part, acting as an ovejector. Many nematodes have a differentiated muscular ovejector in which muscles squeeze eggs out through the vagina. In *C. elegans*, two hermaphrodite-specific motor-neurons innervate the vulva muscles and are vital to egg laying. A number of other factors can also affect oviposition. For example, in *C. elegans*, an absence of food stops egg laying. Furthermore, in older females of *C. elegans* and other rhabditids (in which elasticity of the cuticle is lost), eggs can no longer be laid; this condition is referred to as "endotokia matricida".

In a monodelphic system, the uterus can also protrude posterior to the vulva as a postuterine sac that may serve as spermatheca. The vagina is internally lined with cuticle, with antagonistically acting muscles at its outer wall: a constriction muscle or the sphincter, and dilator muscles at its junction with the ovejector or uterus. Unlike the other parts of the reproductive tract that are germinal or mesodermal, the vagina is of ectodermal origin. The vagina consists of three subdivisions: *pars proximalis vaginae* for the section with hyaline walls in connection with the uterus, *pars refringens vaginae* for the intermediate section with sclerotized walls and *pars distalis vaginae* for the section with walls continuous with the body cuticle (De Ley et al. 1993). The *pars proximalis* is also referred to as *vagina uterina* and the *pars refringens* and/or *pars distalis* as *vagina vera* in animal parasites. The vulva varies in shape and size, but usually is a transverse slit operated by the vulval dilator muscles; it can also be a longitudinal slit or a round opening. The vulva may show different types of appendages, such as vulval flaps (= modifications of the vulva lips), vulval membranes (= cuticular outgrowths) or epiptygmata (= cuticular protrusions on one or both vulva lips or vaginal wall, e.g., in Tylenchomorpha) (Carta et al. 2009). The vulva morphology is used as a diagnostic feature in, e.g., pinewood nematodes (*Bursaphelenchus*). The vulva may be closed by a copulatory plug (Sarr, Coomans & Luc 1987, Hunt 2001, Decraemer 2011). In *Myolaimus*, a free-living species where males lack spicules (copulatory apparatus), the loose outer cuticle layer of the vulva lips forms a protrusion or vulval sack (Fürst von Lieven et al. 2005). In full-grown females of root-knot nematodes, the terminal perineal (vulva-anus) region shows a characteristic unique cuticular perineal pattern of diagnostic importance for species identification. In cyst nematodes, the surface of cysts shows a pattern of ridges derived from the female cuticle and a thin-walled fenestral area surrounds the vulval cone or perineal area of mature cysts.

The female reproductive system of several genera of the family Oncholaimidae (Enoplida) possesses a most unusual tubular system that connects the uterus by means of a duct to a specialized part of the intestinal wall, the osmosium (Fig. 1.14 G). This ducted system is known as the Demanian system (first described by de Man in 1886). In its most complex form, it consists of a narrow *ductus uterinus* connecting to the uterus by the uvette, an oval multicellular structure with a narrow lumen opening, with a larger main duct (*ductus efferentus principalis*) (Coomans et al. 1988). The latter leads anteriorly to a thin-walled *ductus entericus* ending at the osmosium. Posteriorly, the main duct is connected with the terminal duct(s) (interstitial channels) that open to the outside through terminal pores. The interstitial channel(s), when present, are not pre-existing structures but are built during copulation by the combined action of male ejaculatory and female substances during traumatic insemination when the male injects sperm through the body wall. The Demanian system was thought to act as a sperm storage organ (*receptaculum seminalis*) in which sperm was kept alive with the help of secretions by the osmosium. Other authors believed that the Demanian organ has primarily a secretory function. Its secretion could become fixed around the pre-anal narrowing of the female body, observed, for example, as the brownish elastic girdle in the *Metoncholaimus* species, or it may serve as a sex attractant or protect deposited eggs. In *Oncholaimus*, the Demanian system provides the normal way by which sperm reach the female reproductive system. Further, the Demanian system can help to build up body pressure in species with a low internal pressure by injecting sperm into the female and thus swelling the epidermal tissue of the dorsal and lateral chords of the posterior body region and producing secretions that become associated with the formation of the interstitial channels (Coomans et al. 1988). The increased body pressure is determined to facilitate egg laying. Superfluous sperm pass into the intestine through the osmosium and form an additional food source with the osmosium acting as a safety valve.

1.9.3 Male reproductive system (Fig. 1.15)

The male reproductive system typically comprises two testes that open into a common gonoduct or *vas deferens*. Such a condition is called diorchic (Gk. *dis* = twice, *orchis* = testis) as opposed to monarchic, where only one testis is present after reduction of the posterior (usually) or anterior testis (free-living *Lauratonema, Poikilolaimus* Fig. 1.15 B). When two testes are present, the posterior one is usually reflexed. The testis is an epithelial tube with germ cells. It is divided in a distal germinal zone and a growing zone (= mitotic zone) filled with spermatogonia and growing spermatocytes, followed by a meiotic zone (not always visible) marking the boundary between spermatocytes and haploid spermatids (Yushin & Malakhov 1998). The process of spermatid formation is called spermatogenesis. In *C. elegans*, the spermatocytes detach from the rachis during spermatogenesis and undergo two meiotic divisions. Spermatids are stored until ejaculation in a *vesicula seminalis*, either a part of each testis or the anterior part of the vas deferens. Activation of sessile spermatids into mature spermatozoa occurs after mating in the female (hermaphrodite) uterus. In a diorchic system, the two spermaducts fuse into a single tube before a sphincter-like constriction of the gonoduct (as in *Enoplus anisospiculus*), followed by a glandular vas deferens. The non-germ or somatic component consists of three tissues: (1) a distal tip cell(s) maintaining the nearby germ cells in a mitotic state; (2) the vesicula seminalis that store spermatids until ejaculation; and (3) the vas deferens, a complex secretory tube composed of several types of cells visible as morphological differences in secretory granules. The anteriormost cells of the vas deferens act as a valve that regulates release of sperm. In spicule-free *Myolaimus*, this valve seals off the spermatids from the vas deferens lumen and functions as a pump during mating; it is composed of different types of cells responsible for the secretion of seminal fluid and a substance involved in the capsule formation of the spermatophore. The posterior part of the vas deferens produces a glue used for initial attachment of the male to the female (Fürst von Lieven et al. 2005). In some taxa, the terminal part of the vas deferens may be differentiated into a strongly muscular ejaculatory duct before it joins the rectum and opens into the cloaca. Ejaculatory glands may be associated with the vas deferens. In many plant-parasitic taxa, sperm are continuously produced, and in others (e.g., Criconematoidea), they are produced in the fourth juvenile stage before the final molt. Most nematodes have numerous small sperm cells, as is usual in animals, but some species possess few large sperm cells. Sperm dimorphism can be observed in free-living species both in diorchic and monorchic males. In *Axonolaimus helgolandicus*, the anterior testis has smaller sperm than the posterior testis and both types are present in females (Riemann 1986). Sperm dimorphism can also be related to differences in the life cycle. *Beddingia siricidicola*, a parasite of woodwasps has a mycetophagous free-living life cycle in which it produces large amoeboid sperm cells and a parasitic life cycle in the insect in which microspermatozoids are formed (Bedding 1986, Yushin

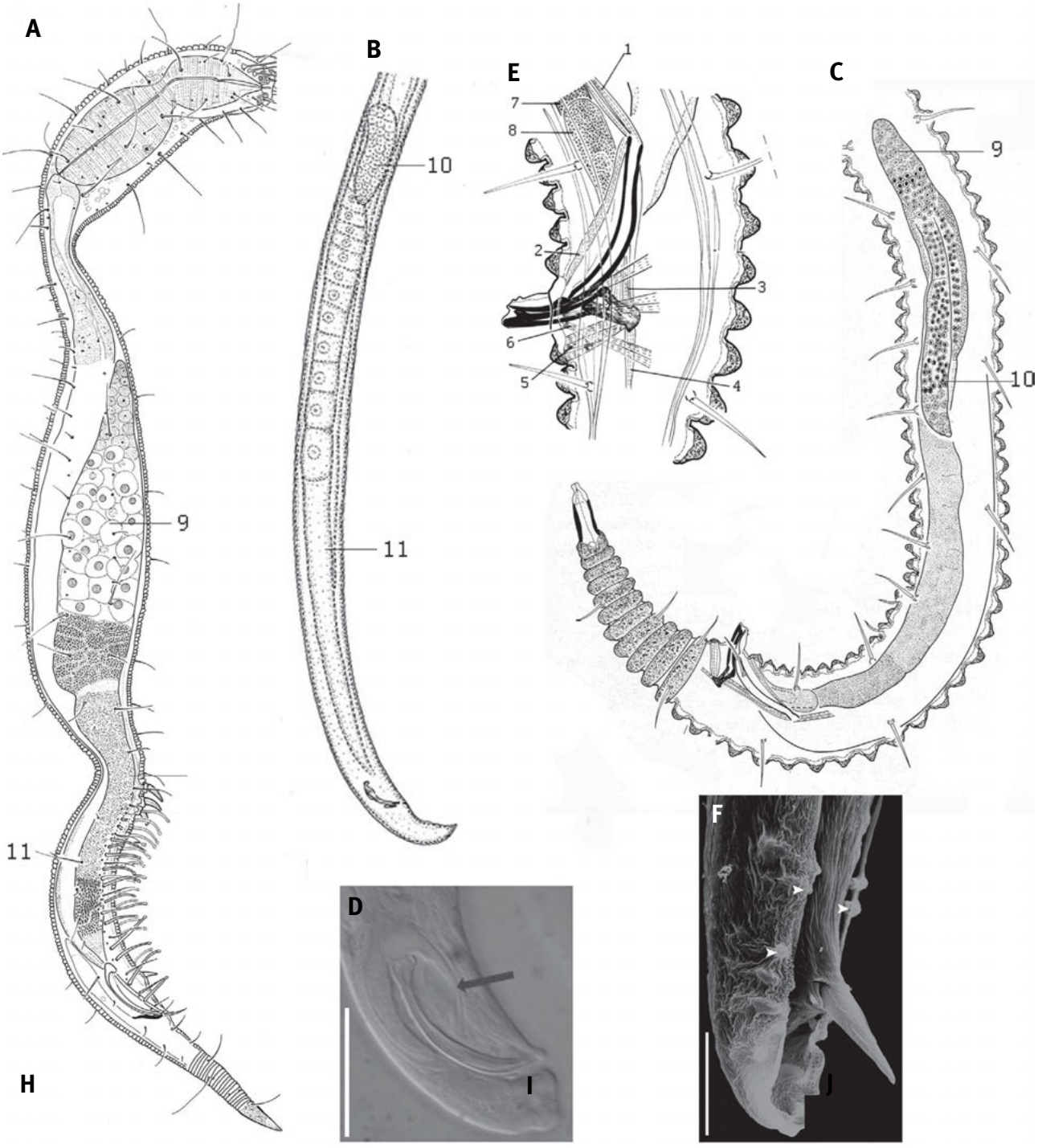

Fig. 1.15: Male reproductive system. A, Monorchic system with outstretched anterior testis (draconematid); B, Monorchic with single posterior testis (*Poikilolaimus*); C, Diorchic reproductive system, posterior testis reflexed (*Tricoma*); D, Transformed protractor muscles (capsule of suspensor muscles) (*Trichodorus similis*); F. Caudal alae with rays (arrowhead) and protruding spicule (*Heterorhabditis*); (1) Spicule retractor muscle; (2), (3) Spicule protractor muscle; (4) Retractor muscles gubernaculum; (5) Protractor muscles gubernaculum; (6) Anal muscles; (7) Vas deferens; (8) Ejaculatory gland; (9) Germinal zone testis; (10) Testis; (11) Vas deferens.

et al. 2007). Males of root-knot nematodes (*Meloidogyne*) normally have one testis, however, many males are sex-reversed females and can be recognized by their possessing two testes. Sex-reversal can be induced epigenetically by unfavorable environmental conditions.

Depending on the period of gonadal development at which sex reversal occurs, the *Meloidogyne* male will have one testis (reversal early- or mid-J2 stage) or two testes (reversal at late-J2 stage) (Papadopoulou & Triantaphyllou 1982).

The copulatory apparatus consists of two cuticularized spicula, rarely fused (*Rhabditis*) or reduced to a single spiculum or absent as, e.g., in the free-living *Myolaimus* (Fürst von Lieven et al. 2005) and the associated gubernaculum; the latter may be absent. In the free-living marine genus *Monoposthia*, the function of spicules has been taken over by the strongly developed gubernaculum. Spicules are formed by gradual invagination of the posterior wall of the spicular pouches that originate from the spicular primordium, specialized cells of the dorsal wall of the cloaca. Each spiculum contains sensilla with one or two dendrites and dendritic processes or receptors enclosed within a channel leading to a pore(s) near the tip of the spiculum. In several taxa, the spicules are differentiated into a marked head (= manubrium or capitulum) offset from a narrower shaft (= calamus) and continuing in a wider blade tapered to a finer distal end; it may show ornamentations such as striae, bristles, a ventral velum or subventral vela (flanges). The shape and size of the spicules vary from species to species and are often of diagnostic value; the overall spicule shape is rather typical for a given group. Usually both spicula are similar in shape, but occasionally they differ in shape and length. The copulatory apparatus functions by means of spicule and gubernaculum protractor and retractor muscles; they allow the spicules to be protruded for copulation and retracted after copulation. In general, the spiculum shape is such that, upon protraction, they form together a kind of tube that keeps the vulva open and through which sperm flows into the female; in other species, the spicules may protrude alternately (plant-parasite *Paratrichodorus*, Trichodoridae). In the Triplonchida, to which the Trichodoridae belong, the spicule protractor muscles form a capsule of suspensor muscles (Coomans 1962b); the latter is not directly attached to the spicules. The sclerotized dorsal and lateral walls of the distal cloaca form the gubernaculum that guides the spicules when protracting or retracting and protects the underlying tissue. The gubernaculum may be a simple trough-shaped structure or it may be complex, with several parts (e.g., corpus = main part, cuneus = central projection in between the spicules, crura = lateral guiding pieces) and possible apophyses. In most Dorylaimida, for example, only the crura remain.

Around the cloacal opening and in the whole caudal area, accessory genital structures may be present, such as genital papillae or setae, suckers, cuticular extensions (caudal alae or bursa), etc., used by the male to locate the female and/or the vulva opening and to hold the female during copulation. The arrangement, shape and number of pre- and postcloacal supplements or genital papillae (Rhabditomorpha) are of taxonomic importance. Paired genital papillae may also be present on the postcloacal lip (= hypoptygma) as in Merliniinae (Tylenchomorpha). Sexes find each other through sex attractants. In species with sedentary females, the latter produce these pheromones, but in freely active species, both sexes may produce attractants. When a male has located a female, it will search for the vulva with its hind part. Males with a bursa occupy a position parallel to the female or under a sharp angle when mating (Sudhaus & Fitch 2001). With the bursa, they encompass the vulva region of the female. Sometimes, a special secretory product provides better adhesion. Males without a bursa encircle the female's vulva region with their posterior end; the rest of the male's body is then usually at a right angle with that of the female. During copulation the spicules are introduced in the vulva-vagina except for traumatic insemination. Exceptionally, sperm is stored in spermatophore(s), resulting not only in special adaptations around the vulva region of the female but also of the spicules in males of *Prorhynchonema warwicki* (Gourbault & Renaud-Mornant 1988).

1.9.4 Autotokous reproduction

Autotokous reproduction or the formation of a progeny by a single individual can occur in two different ways: through self-fertilization in hermaphrodites and through parthenogenesis. The advantage is that a single specimen is sufficient for reproduction and is able to colonize a new locality; it also allows a faster increase of the population. The disadvantages are the same as with inbreeding: loss of genetic variability and adaptive power in the population and accumulation of delirious mutations. In addition, autotokous is only successful in a stable environment at high population levels and when there is strong competition for resources. In self-fertilization, the progeny is limited by the number of sperm and is out-competed by sperm from cross-fertilization.

1.9.5 Reproductive system in hermaphrodites

In hermaphrodites, self-fertilization (automixis) is the rule, but amphimixis may occur from time to time due to the rare presence of males. Hermaphrodites mostly have the habitus of females as in *C. elegans*, hence they

lack a copulatory apparatus. They are androdioecious with syngonic gamete formation, i.e., sperm and oocytes are produced in the same gonad (= ovotestis), whereby sperm is produced first (prot(er)andry) and stored prior to the production of oocytes. Males (at least phenotypically) that give birth to young are very exceptional, though they do occur in nematodes, for example, in the entomoparasitic nematode *Heterogonema ovomasculis* (Van Waerebeke & Remillet 1973). Hermaphroditism has evolved several times from gonochoristic ancestors; reverting to gonochorism is rare (e.g., *Caenorhabditis* in Kionke et al. 2004).

In hermaphrodites such as *C. elegans*, sperm is formed in the fourth-stage juvenile. In total, about 150 mature sperm cells are produced per branch, and production of sperm stops upon molting. The adult hermaphrodite germ cells only produce oocytes throughout their adult lives. *C. elegans* hermaphrodite ovotestes are homodromously reflexed and gametogenesis occurs in the proximal part of the gonad. The distal germ line is a syncytium where oogonia have incomplete borders and are connected to one another via a central canal called the rachis, which terminates near the loop of the gonad (Hirsh et al. 1976). Sperm produced in the fourth juvenile stage are stored in the hermaphrodite spermatheca, near the junction of the oviduct and the uterus. Self-fertilization occurs upon signals from the maturing oocyte and from major sperm protein of the sperm cell (see Chapter 2).

Males appear unnecessary for reproduction, though they may show up sporadically. *C. elegans* for example, possesses two natural sexes: hermaphrodite and male; true females do not occur. Males are able to inseminate the hermaphrodites, in which case their sperm appears to be superior to (out compete) that of the hermaphrodites. Male sperm are larger, crawl faster and are able to physically displace hermaphrodite sperm from the spermatheca, taking precedence in fertilization; however, this comes at a cost, i.e., the larger sperm are produced at a slower rate (LaMunyon & Ward 1999). So we could wonder why selection has maintained the genetic pathway for male development. To assay developmental plasticity, Michael et al. (2003) developed cross-progeny (hermaphrodites with males) and self-progeny tests under varying environmental conditions. The authors found that bacterial growth (food source) affected only sexual development of cross-progeny, resulting in an excess of males, suggesting sexually transformed "hermaphrodite" juveniles. Sexual reproduction increases developmental flexibility of progeny, allowing for better adaptation to changing environments.

1.9.6 Parthenogenesis

Parthenogenesis (Gk. *parthenos* = virgin; *genesis* = descent) is quite common among nematodes and is of the thelyotokous type, i.e., females produce only female offspring (thelyotoky: Gk. *thélys* = female; *tokos* = birth, offspring – as opposed to arrhenotoky: Gk. *arrhén* = male, the parthogenetic production of males.). In some nematodes, e.g., *Aphelenchus avenae*, which normally reproduce by parthenogenesis, rare males may occur through which amphimixis is possible in some populations. Two types of parthenogenesis exist: (1) mitotic, i.e., no pairing of homologous chromosomes during prophase, the oocyte remains diploid or (2) meiotic, in which meiosis occurs, but either the nucleus of the polar body (not split off) fuses with the pronucleus of the oocyte, or there is a doubling of the chromosomes before the first cleavage. Both types may exist, e.g., in the plant-parasitic genus *Meloidogyne*. The female reproductive system has the same main structures as females with amphimictic reproduction, though in many taxa the ovaries are much longer, producing a larger number of ova.

Automixis (parthenogenesis, hermaphroditism) are specializations of reproduction often leading to parasitism. Other examples of specializations are reproduction by pseudogamy or heterogamy. Pseudogamy (Gk. *pseudés* = false; *gamos* = marriage) is intermediate between amphimixis and automixis. In the free-living amphimictic life cycle (e.g., animal parasite *Strongyloides ransomi*), the sperm cell will stimulate metaphase formation of the oocyte during meiosis, but the sperm pronucleus plays no further role thereafter and will degenerate after penetration (Zaffagnini 1973). Heterogamy can occur, e.g., in animal parasites in which an amphimictic generation can alternate with an autotokous one. Often the autotokous generation is then the parasitic one (long ovaries producing a large number of oocytes, fast reproduction in a stable environment), whereas the amphimictic one is the free-living phase (shorter ovaries; more variable and better adapted to a less stable environment).

Parthenogenesis can be induced by bacteria as in the *americanum* lineage of the genus *Xiphinema* (Coomans & Willems 1998, Coomans et al. 2000, Vandekerckhove et al. 2000) (Fig. 1.16 C). This phenomenon is quite exceptional for nematodes, but not uncommon in several insect groups. The endosymbiotic bacterium *Wolbachia* can be found in the female germline (ovaries, uterus) of several Onchocercidae and more rarely in plant-parasitic nematodes (Fig. 1.16 B), e.g., in *Radopholus similis* (Haegeman et al. 2009). In nematodes, *Wolbachia* show a mutualistic

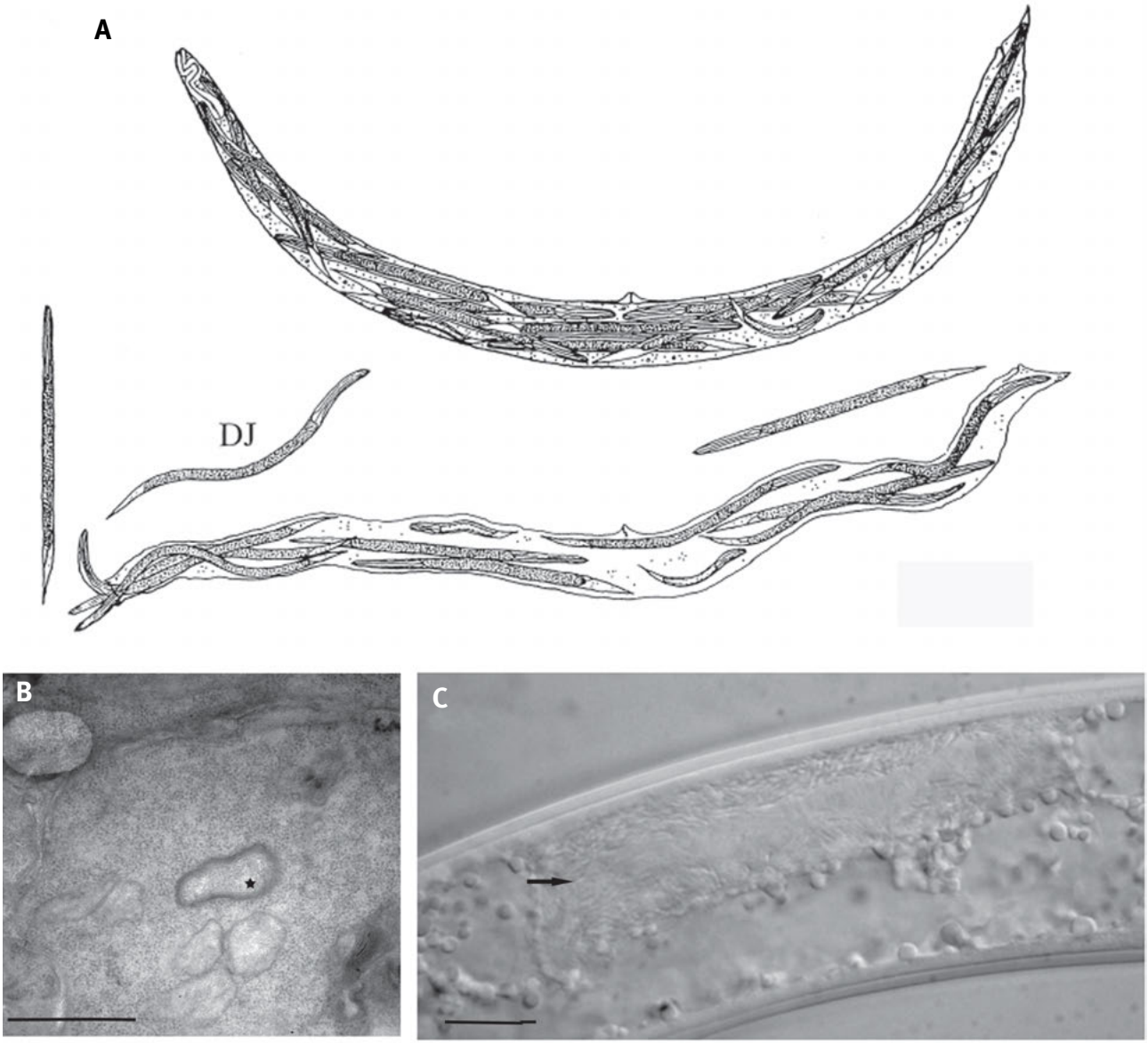

Fig. 1.16: A, Endotokia matricida: death of *Heterorhabditis* hermaphrodite and emigration of dauer juveniles (©Nematology, courtesy Johnigk & Ehlers 1999); B, *Wolbachia* endosymbiotic bacterium (*) in uterus of *Radopholus similis* (courtesy Annelies Haegeman); C, endosymbiotic bacteria in ovary of *Xiphinema americanum*. Scale bars: B 600 nm in B; C 10 µm.

relationship in contrast to their symbiotic lifestyle in most Arthropods.

1.9.7 Intersexes

Intersexes are found in some genera and should not be confused with hermaphrodites because only one set of reproductive organs is functional, the other appearing as a vestige. Female intersexuality occurs with females having secondary male characteristics, such as spicules, bursal muscles, and genital papillae. Male intersexuality occurs when males have secondary female characteristics, such as a vestigial vulva. Intersexuality has been observed in both free-living and parasitic nematodes.

1.10 Gametes-gametogenesis

Gametes develop from primordial germ cells (PGCs) characterized by special germ plasm with large aggregations of mitochondria and conspicuous non-membrane-bound organelles with electron-dense germ granules (= P granules in *C. elegans*). P granules are present in *C. elegans* zygote

P0 and the primordial germ cell P4 and all its descendants (except in mature sperm). PGCs keep their identity and developmental potency of the germ line by delaying the initiation of zygotic transcription that otherwise would promote the somatic cell fate (Seydoux et al. 1996). In *C. elegans*, the primordial germ cell, P4 divides just once to produce Z2 and Z3 and is mitotically inactive during embryogenesis. Transcriptional activity and divisions start in the germ line precursors Z2 and Z3 around the 100-cell stage in *C. elegans* (see embryogenesis). Once the gonad is formed, germ cells begin active proliferation and become oogonia/spermatogonia.

1.10.1 Oogenesis (Fig. 1.18)

Oogenesis is a process in which oogonia differentiate into oocytes. It starts at the tip of the ovary in the germinal zone (mitotic zone or proliferation zone) often during the last juvenile stage (J4) and usually continues during adult life. The germinal zone is usually rather short. A distal cell observed in *C. elegans* (but also present in many other taxa) at the tip of the gonad appears to stimulate mitosis or inhibit meiosis. Ablation of the distal cell leads to the arrest of mitosis and the initiation of meiosis in nearby germ cells (Killian & Hubbard 2005). In *C. elegans*, oogonia develop in a syncytium with 8 to 12 nuclei in cross section and cytoplasm surrounded by an incomplete plasma membrane allowing contact with the cytoplasm of the central rachis (Matova & Cooley 2001). Germ cells move from the mitotic zone into the transition zone (early meiotic prophase I, homolog pairing of chromosomes), progress through the pachytene stage (synapsis of homologs) in the loop region of the ovary in *C. elegans* and enter diplotene (chromosome desynapse and condense) either differentiatng as oocytes or undergoing programmed cell death (apoptosis) (Jaramillo-Lambert et al. 2007). Over half of the female germ cells (mainly syncytial oocytes) in the *C. elegans* hermaphrodite are eliminated through apoptosis. Nematode oocytes in most phyla display less variation than spermatozoa. The nucleus is large; the cytoplasm contains organelles (mitochondria, ribosomes, endoplasmic reticulum) and many cytoplasmic inclusions (Wharton 1979). Oocytes of many species arrest twice during meiosis; the first arrest occurs at prophase I. The penetration of a spermatozoon activates the oocyte to proceed with the meiosis, and sperm signaling promotes gonadal sheath cell contraction that acts in concert to facilitate ovulation. Maturation of the oocyte is visible by the nuclear envelope breakdown and cortical re-arrangement, whereby the oocyte becomes more ovoid in shape. In *C. elegans*, an ephrin receptor protein kinase, *vab-1*, is a receptor for major sperm protein (MSP, the main cytoskeletal element required for the actin-independent amoeboid movement of nematode sperm) and ceh-18 gene, which encodes a POU-class homeoprotein (POU – transcriptional regulators characterized by a highly concerved DNA-binding) expressed in sheath cells (Miller et al. 2001), promotes oocyte maturation and MAPK activation (mitogen-activated protein kinases involved in directing cellular response). POU domain factors are transcriptional regulators characterized by a highly conserved DNA-binding referred to as POU Domain. By using sex-determination mutants of *C. elegans*, Harris et al. (2006) discovered that meiotic maturation and ovulation are coupled by sperm availability through a complex regulatory network involving germline and somatic control. MSP signaling reorganizes oocyte the microtubule cytoskeleton (required for the assembly of a bipolar meiotic spindle) prior to nuclear envelope breakdown and fertilization by affecting their localization and dynamics. At ovulation, contractions of the myoepithelial sheath cells that are in connection with the oocytes through gap-junctions, combined with dilatation of the distal spermatheca connection (by signaling from the oocyte), move the oocyte into the spermatheca. There, the oocyte undergoes fertilization. In *Anguina tritici*, the wheat gall nematode, the oocytes advance to metaphase shortly before or when they enter the spermatheca; the latter usually contain many spermatozoa, and fertilization takes place (Triantaphyllou & Hirschmann 1966). Nematodes are unique in that the maturation hormone is secreted by sperm rather than by the female's somatic tissues. After anaphase I (= separation of homologous chromosomes) and telophase I, the polar body splits off and the secondary oocyte is formed. The second meiotic division soon follows the first one and leads to the second polar body and the ootid (ovum). In the meantime, the male and female pronuclei have formed; they fuse to the zygote nucleus. Cleavage can occur now or later. After fertilization, the outer (vitelline) membrane thickens and becomes part of the eggshell (Fig. 1.17 F, G).

Most nematode eggs are morphologically very similar, i.e., ellipsoidal in shape with a transparent shell, except for some animal-parasitic forms (*Mermis nigrescens* eggs with branched byssi at each pole, *Trichuris ovis* eggs with polar plugs) (Bird & Bird 1991). Most nematode eggs are also of similar size irrespective of the size of the adult. However, different-sized eggs may occur in a number of parasitic species that can exist and reproduce either as a free-living form or as a parasitic form (free-living mycetophagous females of *Beddingia siricidicola* have larger eggs than do females of this species, which are parasitic in wood-wasps). The eggshell consists of three layers formed by the egg itself: an external tripartite membrane, a median chitinous layer and an internal lipid layer. Underneath the eggshell

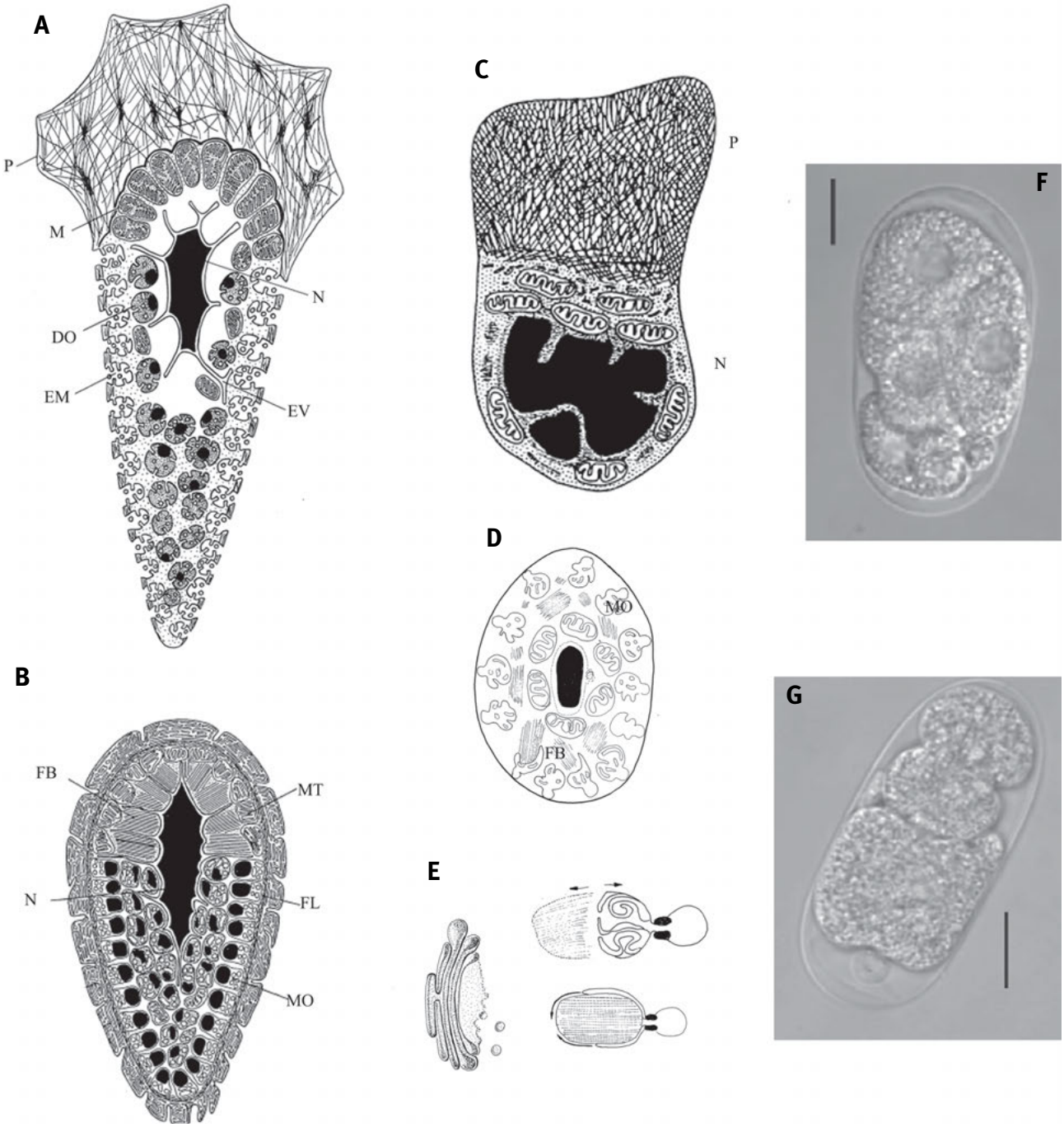

Fig. 1.17: Sperm cells. A, Mature spermatocyte (18 µm) with a nuclear envelope of *Enoplus demani*; B, Immature spermatozoon (25 µm) of *E. anisospiculus* (©Fundamental and Applied Nematology, courtesy Yushin & Malakhov 1994, 1998); C, Mature spermatocyte (4 µm) without MO from the uterus of the chromadorid *Panduripharynx pacifica* (©Nematology, courtesy Yushin & Coomans 2000); D, *Caenorhabditis elegans*, spermatid with MO just below plasma membrane of spermatid and free FB (based on Yushin & Malakhov 2004); E, Golgi and Mo-FB complex formation, respectively, dissotiation of MO and FB. Eggs. F, Six-cell stage of *Acrobeloides thornei*, left lateral view; G, Early four-cell stage of *Procephalus* sp. with division axes of AB and P1 perpendicular to each other (F, G courtesy Dr Sandra Vangestel, Ghent University). Abbreviations: FB, fibrous bodies; MO, membranous organelle; MT, mitochondria; N, nucleus; P, pseudopod. Scale bar: F, G 10 µm.

lies the plasma membrane. The uterus may secrete substances that adhere to the eggshell. This uterine layer(s) may account for a specific ornamentation with spines, warts or polar filaments or consist of systems of pores and spaces (Oxyurida). During its development, the nematode egg is permeable to chemicals initially before the lipid layer of the eggshell is formed during the egg's passage down the uterus, and again when it is broken down before hatching.

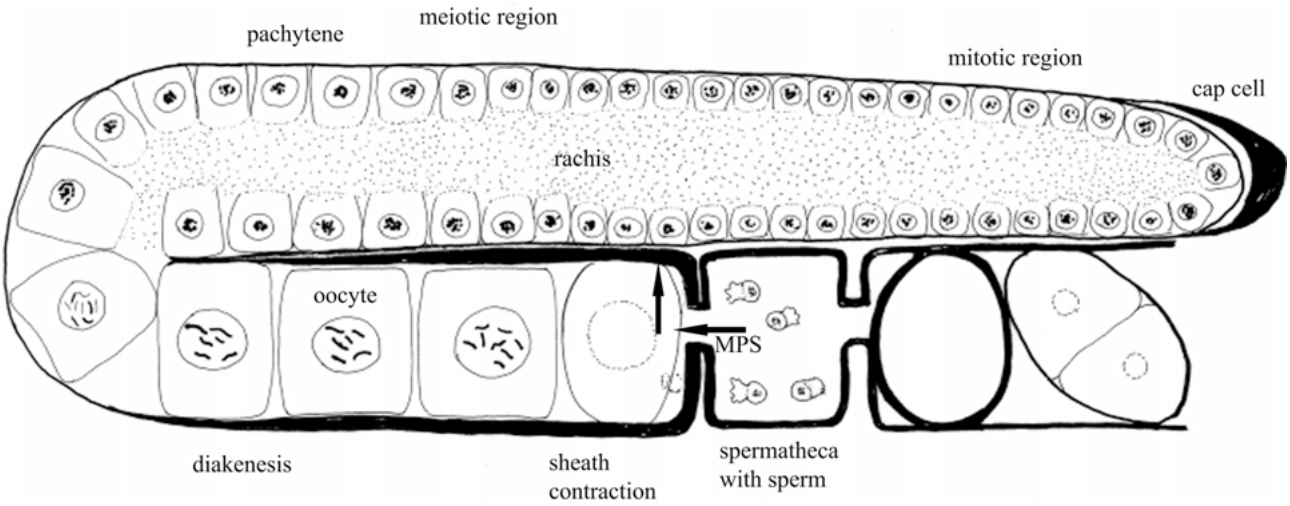

Fig. 1.18: Germ cell development in *C. elegans* hermaphrodite. MPS controls multiple signaling pathways to regulate sheath-cell contraction and promote oocyte maturation and ovulation and spermathecal delation (based on Kuwabara 2003).

Filarial worms are unusual in that they do not produce eggshells with lipid and chitinous layers but instead give rise to a first-stage juvenile in which the surface coat forms a sheath. Movement of the egg down the reproductive tract is induced by contraction of muscles around the uterus and/or by the kneading action of the body wall muscles and intestine. Eggs of most nematode species are laid individually, but in some taxa, additional protection is provided as root-knot nematodes lay their eggs in a gelatinous matrix that hardens and in cyst nematodes the female body forms a cyst with several hundreds eggs upon death. Each egg contains a single juvenile, and the majority of juveniles hatch from eggs laid by the adult female. In some taxa, hatching occurs within the female body, a process known as matricidal hatching or *endotokia matricida* (Fig. 1.16 A). *Endotokia matricida* is rather common in rhabditid nematodes. In *Heterorhabditis*, intra-uterine birth causes maternal death and secures the development of dauer juveniles when the external food supply is reducing. The process in which juveniles hatch within the female uterus and subsequently emerge is known as ovoviviparity. It is a rare phenomenon observed in a few free-living marine, terestrial and parasitic taxa (e.g., *Monhystera disjuncta, Panagrellus, Trichinella* and *Meloidogyne*) and can be induced upon exposure to pollutants (Walker & Tsui 1968, Luc et al. 1979).

1.10.2 Spermatogenesis/spermiogenesis/sperm (Fig. 1.17)

The first observations on spermatogenesis in animals were made on *Parascaris equorum* by the Belgian Edward van Beneden in 1883. Spermatogonia originate at the blind end of the testis from the primordial germinal cell after a number of mitoses (germinal zone). Spermatogonia change shape, enlarge and become more clearly defined in the growth zone; the primary spermatocytes undergo meiosis first leading to secondary spermatocytes and then to spermatids. Spermatids undergo spermiogenesis, resulting in spermatozoa in the male or in the female after copulation. Spermatids are apparently an ideal form for storage, for example, in *C. elegans* males because sperm energy supplies are not depleted by motility, and the round shape facilitates transfer to the hermaphrodite. After transfer into the uterus, sperm needs to quickly become motile. The *swm-1* gene is predicted to encode a secreted serine protease inhibitor with two protease inhibition domains and regulate the activity of two proteases that are natural activation signals for sperm (Singson 2006).

Nematode spermatozoa are characterized by a wide variation in shape (from usually round or oval to elongate) and size. They are *non-flagellate*, possess no cilia, lack an axoneme and acrosome and show an amoeboid motility. *C.elegans* sperm for example, consist of an anterior pseudopod and a posterior main cell body with the nucleus, mitochondria and membranous organelles (MO). These MO are characteristic for many nematode sperm cells. MO are derived from the Golgi bodies and form part of a complex together with paracrystalline fibrous bodies (FB). These fibrous bodies are densely packed parallel filaments of a unique cytoskeletal protein called MSP (Justine & Jamieson 1999). During late spermatogenesis, the complexes of MO and FB dissociate into separate MO and FB in spermatids and immature sperm. In the female uterus, MO join with the plasmalemma of the sperm main cell body and release their contents into the uterus lumen. The FB

are transformed into the cytoskeleton of a newly formed pseudopod or freely surround the nucleus. MO may be absent as in the plant-parasitic tylenchs or reduced as in some free-living chromadorids (Yushin & Zograf 2002). Total absence of FB was described in a variety of enoplids, e.g., *Anticoma possjetica* (Yushin & Malakhov 1999). A full reduction of aberrant organelles (MO and FB) was found, e.g., in the free-living linhomoein *Terschellingia glabricutis* (Yushin 2008) and in the mouse pinworm *Aspicularis tetraptera* (Oxyurida) (Yushin & Malakhov 2004).

Mature spermatozoa lack a nuclear envelope except in Enoplida (Justine 2002). Loss of the nuclear envelope is considered a derived feature; the presence of a nuclear envelope in Enoplida is a primitive character. Enoplids also differ from other nematodes in the development of aberrant organelles because MO and FB do not unite in complexes and develop separately, but their fate is similar to that in other nematodes. Yushin & Malakhov (2004) proposed a scheme of nematode sperm evolution. The plesiomorphic condition of nematode sperm has the nuclear envelope, and separate, asynchronous development of MO and FB. The next step in evolution is the reduction of the nuclear envelope; the third step is the synchronization of the development of FB- and MO-forming complexes (Fig. 1.17 E).

Sperm dimorphism has been observed in males of several species of aquatic nematodes. In *Terschellingia glabricutis* for example, the anterior testis of the diorchic male produces macrospermatozoa (20 µm in diameter), whereas the posterior testis produces microspermatozoa (10 µm) of a simplified structure. Both types of sperm are found in the uterus of fertilized females (Yushin 2008) and in the tylenchid nematode species *Beddingia siricidicola* (see previously). The converse occurs in *Caenorhabditis elegans*, where the sperm of males and hermaphroditic females are morphologically similar.

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2 Reproduction and development in Nematodes

2.1 Introduction

One of the central aims of modern biology is to elucidate the mechanisms leading to the complex structures of organisms. Using the information inherent in the one-dimensional nucleotide sequence of the genome, cleavage divisions of the fertilized egg, with all the associated genetic and epigenetic regulatory steps, generates a specific three-dimensional pattern of differentiated cells. An ensuing series of dynamic processes finally results in a functional organism with many complex structures and phenotypes. Understanding the genetic and molecular basis of embryonic and postembryonic patterning during development has been a central field of research since the 1970s, and nematodes have been at the forefront of this research.

Typical features of many soil nematodes are a transparent body, a fast generation time and passage through four juvenile stages (J1–J4 or L1–L4) before they reach adulthood. Females (or hermaphrodites) can often produce hundreds of transparent eggs (parasitic species many more) with a protective egg envelope that allows development outside the mother. A considerable proportion of free-living nematode species can be easily cultured in the laboratory with bacteria as their food source. A few representatives, notably *Caenorhabditis elegans* and more recently, *Pristionchus pacificus* have been successfully established as valuable model systems where many distinct developmental processes can be correlated to specific gene function.

The taxon Nematoda is considered a very ancient phylum reaching back into the Precambrian (Douzery et al. 2004, Poinar 2011). Estimates for the number of living nematode species range from tens of thousands to several million (Poinar 1983, Lambshead 1993, Meldal et al. 2007). Their successful adaptation to nearly all environmental conditions would predict a high morphological plasticity. The genetic and genomic variance, even among closely related nematodes, is high (Kiontke et al. 2004, Sommer & Streit 2011), and the same is true for individual core developmental pathways; nonetheless, the body plan has remained surprisingly uniform, probably due to the unique construction principle with a single-chamber hydroskeleton. Well-preserved fossils of nematodes from the early Devonian resemble recent basal representatives (Poinar et al. 2008). It is their development that ties together these two disparate phenomena.

Depending on the mode of reproduction (Section 2.2), one or two types of germ cells are produced. With fertilization (or activation) of the oocyte, embryogenesis starts, where processes such as the establishment of polarity, cell fate specification or formation of organs and tissues can be studied (Section 2.4). During postembryonic development (Section 2.5) of free-living nematodes, only a small proportion of the cells continues proliferation (if the findings from the few species studied can be generalized). This results in ontogenetically novel structures not yet present in the young juvenile, like the vulva (required for egg laying), the copulatory organ of the male or the prominent gonad formed from a tiny primordium. For variations in parasites, see Section 2.5.5. Developmental differences on the cellular level based on distinct gene expression patterns lead to animals of female, male or hermaphroditic sex (Section 2.3).

In this chapter, a limited number of examples are given as an introduction to the wide spectrum of questions that have been fruitfully addressed by studying the reproduction and development of nematodes and their evolution.

One of the most productively studied metazoan animal systems is the free-living hermaphroditic nematode *C. elegans*, thanks not only to the long available, detailed description of development from zygote to adulthood (see Sections 2.4 and 2.5), but even more so because of a multitude of techniques now available that allow for experimental interference and thereby permit the generation of deep insights into the molecular basis of cell behavior. Several databases curated and regularly updated by members of the *C. elegans* community are publicly accessible to support scholars interested in research on this system (Lee 2005). The following are examples of such databases:

- *Wormbase (wormbase.org)*: An international consortium of biologists and computer scientists providing the research community with accurate and current information concerning genetics, genomics and biology of *C. elegans* and some close relatives (O'Connell 2005).
- *WormBook (wormbook.org)*: A comprehensive, open-access collection of original, peer-reviewed chapters covering topics related to the biology of *C. elegans* and other nematodes. It also contains *WormMethods*, a collection of protocols for nematode researchers, and the *Worm Breeder's Gazette*, an informal,

non-refereed, biannual newsletter for the interchange of ideas and information related to *C. elegans* and other nematodes.
- *WormAtlas (wormatlas.org)*: A comprehensive image database of behavioral and structural anatomy. It offers a wealth of annotated electron and light microscopical images, cartoons and films that help in identifying cell types and tissues.
- The *Caenorhabditis* Genetics Center (http://www.cbs.umn.edu/CGC) collects, maintains and distributes stocks of wildtype and mutant *C. elegans* plus other selected nematode strains.

The comprehensive knowledge collected by *C. elegans* researchers during the last several decades provides a solid basis for comparative studies exploring the remarkable developmental and functional diversity in the widely ramified phylum Nematoda.

2.2 Reproduction

The nematode phylum exhibits all modes of sexual reproduction known in the animal kingdom, i.e., gonochoristic, hermaphroditic and parthenogenetic. A few species exhibit a mixture of these in parallel, such as *Rhabditis* sp. SB347 (see Section 2.3; Chaudhuri et al. 2011, Shakes et al. 2011). Other species can pass through alternating life cycles, as found in parasites of the genus *Strongyloides* (Nemetschke et al. 2010). However, the majority of nematode species appears to follow a gonochoristic mode of reproduction, usually with equal numbers of males and females. In contrast, asexual reproduction from somatic cells has not been described in nematodes.

Gonochorism, also known as amphimixis, is seen in species living in diverse free-living ecosystems, whereas it is underrepresented in some parasitic groups. It is considered the ancestral mode of reproduction in nematodes with multiple, independent evolutionary transitions toward hermaphroditism and parthenogenesis (Schön et al. 2009; Denver et al. 2011).

Hermaphroditism in nematodes is exclusively of the self-fertilizing type, in contrast to cross-fertilizing hermaphroditism as found in annelids, mollusks and other phyla. Hermaphroditic reproduction of *C. elegans* was one of the key features for selecting this particular *Caenorhabditis* species as a model system because this allows shortcuts with respect to genetic crosses (Brenner 1974). *Caenorhabditis elegans*, like many other hermaphroditic species, still occurs in two sexes. The hermaphrodite is a modified female that forms two types of gametes (see above). Under laboratory conditions, the progeny of hermaphrodites is limited by the number of sperm. Often, hermaphrodites produce an excess of oocytes, which can be fertilized by the sperm of the rare males (about 1:700 in *C. elegans*) that mate with hermaphrodites. Males are known from many hermaphroditic species to occur in small numbers as a result of meiotic non-disjunction and loss of sex chromosomes (Section 2.3).

Hermaphroditism has arisen multiple times in the nematode phylum. Phylogenetic reconstruction suggests that in the genus *Caenorhabditis*, hermaphroditism has evolved independently at least three times and in the genus *Pristionchus*, at least seven times (Denver et al. 2011). In other genera, the situation is less clear as robust molecular phylogenies are lacking. Currently, not a single case is known where two sister species are both hermaphroditic, suggesting that hermaphroditism might represent an evolutionary dead end. Males are not known from all hermaphroditic species, but this might simply be due to the fact that they are so rare that they have been missed, or that environmental conditions to induce males have not been identified. Some species with free-living and parasitic generations have been described as alternating between gonochoristic and hermaphroditic reproduction (see above and Section 2.3).

The third mode of reproduction is parthenogenesis, i.e., reproduction with female gametes only in the absence of sperm and thus without fertilization events. Parthenogenesis appears to represent the second most common mode of reproduction in nematodes (Lee 2001; Denver et al. 2011). Different forms of parthenogenesis can be distinguished by modifications or even the complete absence of meiosis (Mittwoch 1978; Engelstädter 2008). This mode of reproduction has been found in many free-living nematode taxa including Plectidae (clade 6; Fig. 2.1), Panagrolaimidae (clade 10) and Cephalobidae (clade 11) as well as a variety of parasitic species. Prime examples for the latter are the plant-parasitic genera *Longidorus* and *Xiphinema* (clade 2) or *Meloidogyne* (clade 12), where the transition from gonochoristic to parthenogenetic reproduction (or vice versa) can be seen within one genus and sometimes even within one species (Denver et al. 2011).

There are also reports of nematodes where sperm are only required for the initiation of embryogenesis without genetic contribution, a process known as merospermy or pseudogamy (Triantaphyllou & Hirschmann 1964).