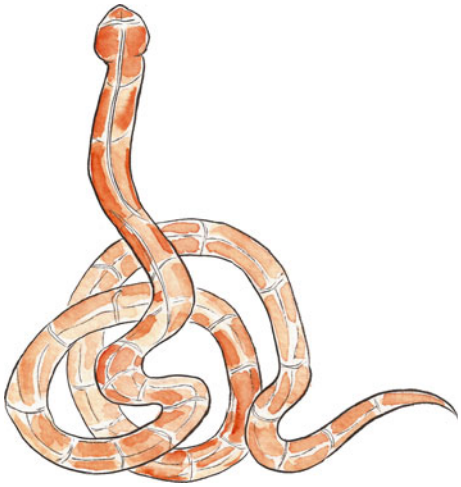
Nemertea

8

Jörn von Döhren



Chapter vignette artwork by Brigitte Baldrian.

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INTRODUCTION

Anatomy and Systematics

Nemertea is a clade of unsegmented, worm-shaped Spiralia comprising about 1.300 described species (Fig. 8.1A–F; Kajihara et al. 2008). The vast majority inhabits marine benthic habitats, but several species are limnic, terrestrial, or marine pelagic. Most species have been described as predators although a number of parasitic, commensalic, and probably even scavengers are known (Gibson 1972). Prey is captured by means of an eversible proboscis that may be armed with one to numerous calcareous stylets in some clades. The proboscis apparatus comprises the proboscis and the rhynchocoel. It represents the apomorphic character that has led to the alternative name Rhynchocoela. The rhynchocoel is a dorsally located, fluid-filled secondary body cavity surrounded by muscle layers housing the proboscis. It opens to the tip of the head via a tube-shaped rhynchodeum (Fig. 8.2A, B). Additional characters that unequivocally qualify Nemertea as monophyletic are the ring-shaped brain surrounding the proboscis insertion instead of the esophagus, a pair of laterally located longitudinal medullary cords, and an endothelialized blood-vascular system. Apart from that nemertean anatomy is marked by characters that are arguably plesiomorphic for Spiralia (Turbeville 2002). These include a largely compact arrangement of the tissue, a medullary cord type organization of the nervous system; a body wall muscle tube comprising minimally two, an outer circular and an inner longitudinal, muscle layers; and one to several paired lateral protonephridia that are not arranged in a segmental fashion. Characters that place Nemertea closer to Trochozoa are a regionalized through-gut with mouth, foregut, midgut, and anus and the presence of glial type cells in the nervous system (Turbeville and Ruppert 1985; Turbeville 1991).

Nemertean ingroup systematics is presently in the consolidation phase with many subclades still being unstable. Traditionally, four higher-ranking taxa have been distinguished: Paleonemertea (Fig. 8.1A, B), Heteronemertea (Fig. 8.1C, D), Hoplonemertea (Fig. 8.1E, F), and Bdellonemertea, the latter comprising only one genus of commensal representatives (*Malacobdella*) (Coe 1943; Gibson 1972). Paleonemertea and Heteronemertea have been classified as Anopla due to their proboscis being uniformly organized and lacking a stylet armature. In Bdellonemertea a stylet armature of the proboscis is also absent, but this has been interpreted as secondary reduction due to the commensalic lifestyle of this group. Hence, Bdellonemertea and Hoplonemertea have been classified as Enopla, characterized by a primarily armed proboscis, the brain being positioned behind the mouth opening and a more intimate connection of the proboscis insertion and the foregut. In Hoplonemertea, two clades have been identified due to their proboscis armature: Monostilifera and Polystilifera. In Monostilifera the proboscis is armed with a single, comparably large stylet in its middle section. In this clade the mouth opens to the ventral face of the rhynchodeum making its distal part a bifunctional rhynchostomodeum. Polystilifera are characterized by a proboscis that is armed in its middle portion with a cushion equipped with multiple, relatively small stylets. The connection of the mouth opening with the rhynchodeum varies between species. Both rhynchodeum and mouth open independently of each other but in nearby positions in many pelagic forms (Pelagica). In most benthic polystiliferan species (Reptantia), a gradual fusion of both openings by sharing a common atrial chamber at the tip of the head is present. Recent molecular analyses and reassessment of morphological data, however, give a different picture putting the traditional classification in jeopardy (von Döhren et al. 2010; Bartolomaeus and von Döhren 2010; Andrade et al. 2012, 2014; Kvist et al. 2014). Of the traditional higher-ranking taxa, only Hoplonemertea and Heteronemertea are recovered (Fig. 8.3). Paleonemertea has been recognized as nonmonophyletic with Hubrechtiidae being more closely related to Heteronemertea than to the remaining paleonemertean clades (Fig. 8.3). The presence of a specialized larva, the pilidium, in both *Hubrechtella dubia* (Hubrechtiidae) and most heteronemertean species leads to them being combined in the clade Pilidiophora (Fig. 8.3).

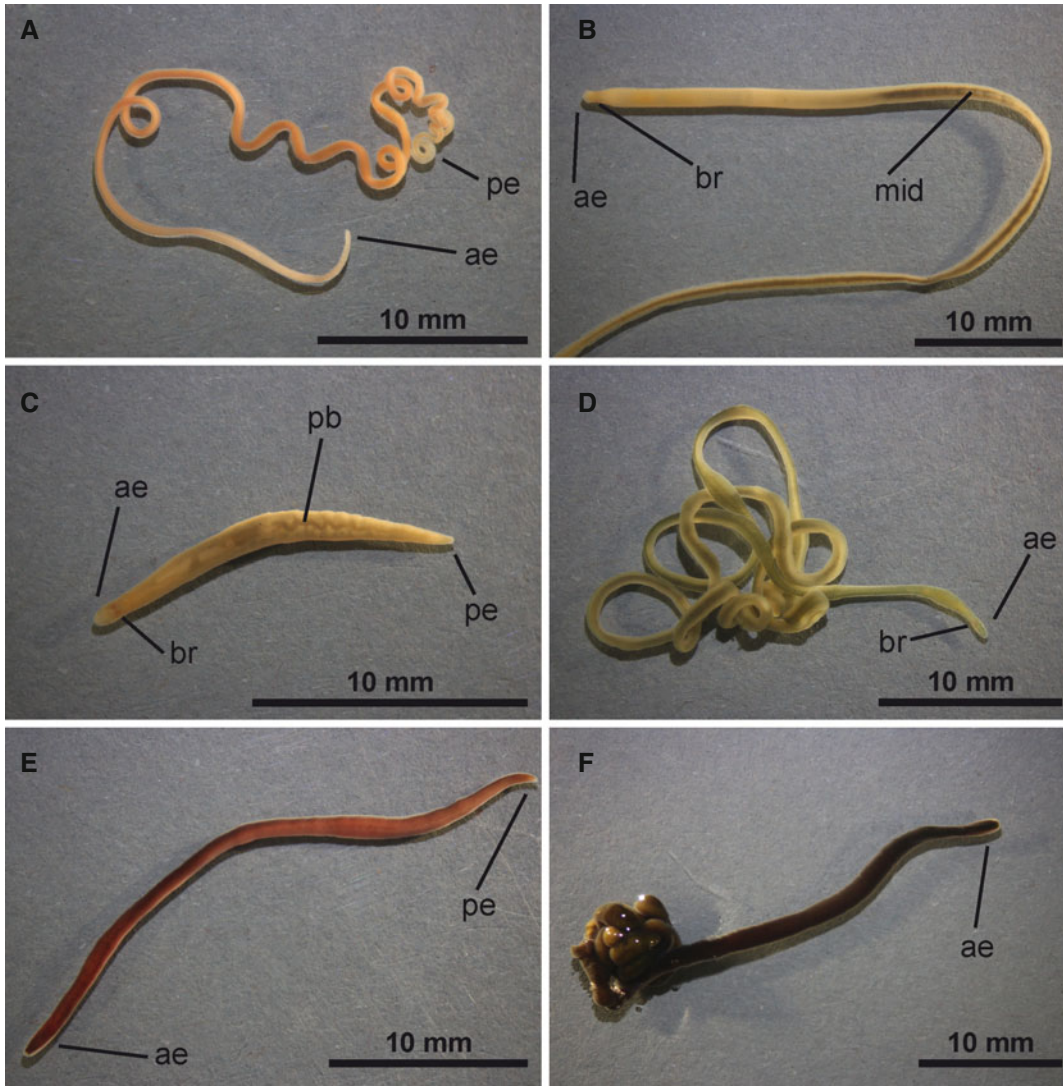

Fig. 8.1 Diversity of nemertean species, living specimens. (**A**) *Procephalothrix oestymnicus* (Cephalothricidae, Paleonemertea), adult. (**B**) *Carinina ochracea* (Carininidae, Paleonemertea), adult. (**C**) *Amphiporus lactifloreus* (Monostilifera, Hoplonemertea), juvenile. (**D**) *Emplectonema gracile* (Monostilifera, Hoplonemertea), adult. (**E**) *Lineus ruber* (Heteronemertea, Pilidiophora), adult. (**F**) *Riseriellus occultus* (Heteronemertea, Pilidiophora), adult. Note: in lightly colored or unpigmented species the brain ring (*br*) is visible through the body wall. *ae* anterior end, *br* brain ring, *mid* midgut region, *pb* proboscis apparatus in rhynchocoel, *pe* posterior end (© Dr. J. von Döhren, All Rights Reserved)

The remaining paleonemertean species have weak support as monophylum based on molecular data (Andrade et al. 2012, 2014; Kvist et al. 2014). Anatomically, Paleonemertea represent the most inhomogeneous taxon within Nemertea with some species having an additional inner circular muscle layer, while others vary with respect to the position of the brain and lateral medullary cords relative to the body wall muscles. In fact, there is no unequivocally apomorphic morphological character that supports paleonemertean monophyly (Fig. 8.3). Within the armed clades (Enopla), traditional taxonomy was not confirmed either. On the one hand, Bdellonemertea proved to be an apparently secondarily reduced member of Monostilifera according to molecular phylogenetic analyses,

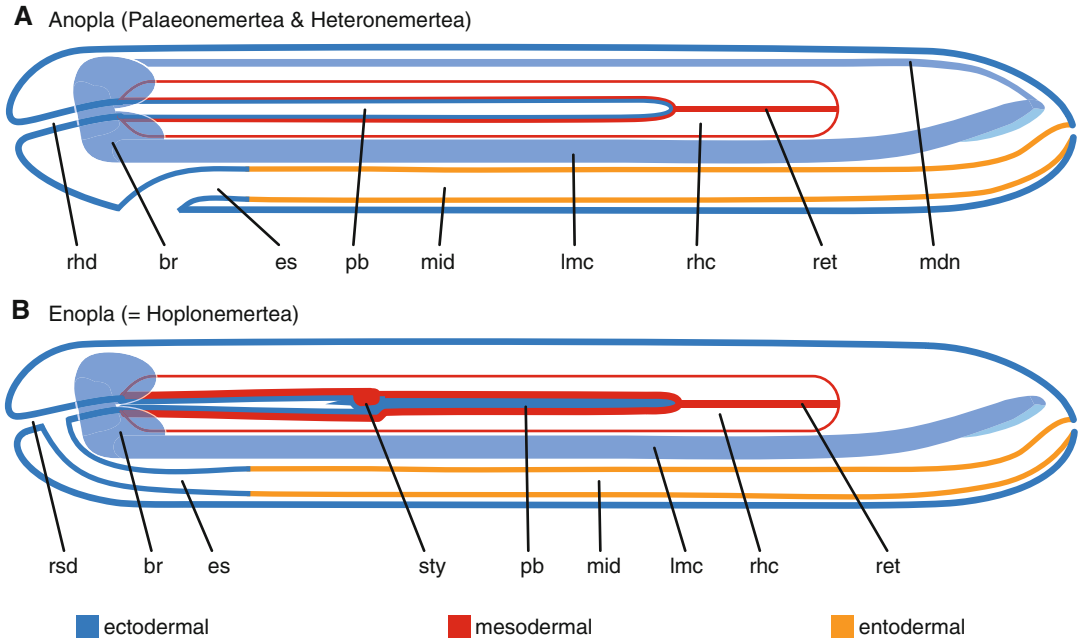

Fig. 8.2 Schematic representation (side view) of the nemertean body plan (**A**) Anoplan organization (Paleo- and Heteronemertea). (**B**) Enoplan organization (Hoplonemertea). Color coding indicates the germ layer that the structure originates from. Note: body wall musculature, nephridia, blood-vascular system, sensory organs, and gland cells have been omitted for clarity. *br* brain, *lmc* lateral medullary nerve cords, *mdn* middorsal nerve, *mid* midgut, *es* esophagus, *pb* proboscis, *ret* retractor muscle of proboscis, *rhc* rhynchocoel, *rhd* rhynchodeum, *rsd* rhynchostomodeum, *sty* stylet apparatus (© Dr. J. von Döhren, All Rights Reserved)

rendering Hoplonemertea and Enopla synonymous, while on the other hand with the same data the monophyly of Polystilifera remains a matter of debate (Thollesson and Norenburg 2003; Sundberg and Strand 2007; Andrade et al. 2012, 2014; Kvist et al. 2014). Currently, there is strong support for a clade Neonemertea comprising Hoplonemertea and Pilidiophora (Figs. 8.1C–F and 8.3; Thollesson and Norenburg 2003; Andrade et al. 2012, 2014; Kvist et al. 2014). All members of this clade share the presence of a median dorsal blood vessel between alimentary canal and rhynchocoel (Gibson 1972).

A number of organs, characteristic of nemerteans, cannot be placed robustly in an evolutionary scenario due to their disparate distribution within Nemertea. Cerebral organs connected to the brain are a feature of many species, although they are quite different in shape and position. While being located behind the brain in tubulanid Paleonemertea, Pilidiophora, and reptant Polystilifera, they are situated far in front of the brain in Monostilifera (Gibson 1972). Lateral sensory organs have a morphology that is reminiscent of cerebral sense organs in paleonemerteans but are located more posteriorly on the sides of the animal in the vicinity of the nephropores. Lateral organs are typical of tubulanid paleonemerteans but have also been recorded from some heteronemertean species (Gibson 1972). Frontal organs that are connected to the almost ubiquitously present cephalic glands and represented by a single epidermal pit in most neonemertean species or by three epidermal pits arranged in a triangular pattern in lineid Heteronemertea might be apomorphic for Neonemertea, although a row of median epidermal pits located in the head region have also been described in the paleonemertean genus *Carinoma* (Gibson 1972). Pigmented eyes in adults are characteristic of neonemertean species as well but show very high variation in size,

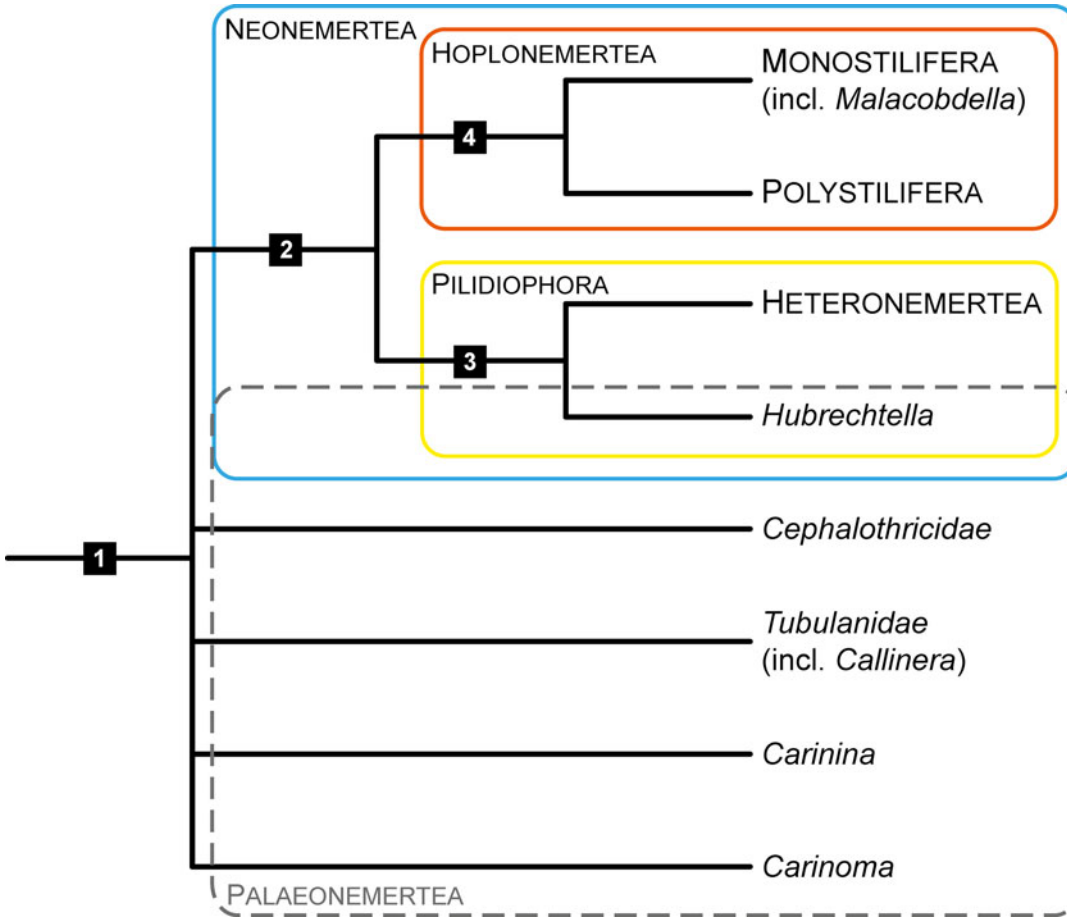

Fig. 8.3 Consensus phylogeny of Nemertea (modified after Andrade et al. 2012, 2014). Numbers indicate apomorphic characters for respective clades. (*1*) Nemertea: dorsal, eversible proboscis housed in fluid-filled rhynchocoel, ring-shaped brain located around the proboscis opening, blood-vascular system lined with endothelium; (*2*) Neonemertea: middorsal blood vessel; (*3*) Pilidiophora: pilidium larva; (*4*) Hoplonemertea: proboscis equipped with stylet apparatus. Note: for the paleonemertean taxa, no apomorphic character exists (© Dr. J. von Döhren, All Rights Reserved)

complexity, and number even between arguably closely related species. Moreover, the eyes in adults have also been described in a few isolated paleonemertean species (Gibson 1972).

Reproductive Biology

The majority of nemertean species are gonochoristic. Only comparably few (mostly non-marine) hermaphrodites have been described (Friedrich 1979). Free-living nemerteans have the tendency to aggregate during their reproductive season, and there have been anecdotic reports of nuptial dances reminiscent of those of epitokous polychaetes in *Nipponnemertes pulcher* (Berg 1972). In most species, females spawn their eggs freely into the water. Egg masses deposited in mucus sheaths are reported from species living in marine (e.g., *Tetrastemma candidum*, *Antarctonemertes phyllospadicola*), intertidal (e.g., *Lineus ruber*, *Lineus viridis*), freshwater and terrestrial habitats (e.g., *Prostoma jenningsi*, *Apatronemertes albimaculosa*), as well as from species that are living as parasites on or in other animals (e.g., *Carcinonemertes* species on several crustaceans) (Thiel and Junoy 2006). In the monostiliferous hoplonemertean *Amphiporus*

incubator, the female remains in the secreted mucus sheath with the developing offspring apparently providing them with nourishment by means of complete histolysis of its intestinal tract (Joubin 1914).

In general, spermatozoa are released freely into the water as well. In those species that aggregate during reproduction and in mucus spawning species males typically come into close contact with either the eggs or the female to ensure successful fertilization (Bartolomaeus 1984; Thiel and Junoy 2006). In many species viscid mucous secretions around the worms putatively aid in scaling down the space into which eggs and sperm are shed. This behavior has occasionally been termed "pseudocopulation" (e.g., *Carcinonemertes epialti*, *Lineus ruber*, *Lineus viridis*) (Gontcharoff 1951; Bartolomaeus 1984; Roe 1984; Stricker 1986). In *Lineus viridis*, one to several males enter a mucus sheath that is secreted by the female. Into this gelatinous mass, additional mucus layers and the eggs are shed (Gontcharoff 1951; Bartolomaeus 1984). During pseudocopulation in some species (e.g., *Carcinonemertes epialti*), sperm may enter the female gonads through the gonopores, and internal fertilization occurs. Internal fertilization with direct sperm transfer (i.e., true copulation) has been assumed to occur in some pelagic polystiliferans. Several structures have been interpreted as accessory sperm transfer structures such as muscular penes in *Phallonemertes murrayi*, sucker-like attachment organs in *Plotonemertes adhaerens*, and specialized glandular epithelia around the male gonopores in *Balaenanemertes chuni* and some other pelagic polystiliferans (Thiel and Junoy 2006). In species of the monostiliferous hoplonemertean genus *Carcinonemertes*, the efferent ducts of the testes open into a common duct, termed Takakura's duct, that widens into a seminal vesicle which opens into the intestine near the anal opening (Gibson 1972). In some *Carcinonemertes* species, the anal opening is surrounded by a flattened or concave muscular area that is used to transfer sperm from the anus to the female gonopores (Roe 1984). Some accounts of viviparous species from all phylogenetic lineages demonstrate that vivipary is not uncommon in this phylum. In these species internal fertilization has to be expected (Thiel and Junoy 2006).

Egg sizes in nemerteans range from 50 µm in *Carinina* (*Procarinina*) *remanei* (Nawitzki 1931) to 2.5 mm in *Dinonemertes investigatoris* (Coe 1926), with most being between 100 and 300 µm in diameter (Friedrich 1979). With the relatively scarce data at hand, there seems to be no strict correlation of egg size to the various phylogenetic entities, but there is a tendency of paleonemertean eggs being on the smaller side of the spectrum, while larger egg sizes are encountered in hoplonemertean species (e.g., *Pantinonemertes* (*Geonemertes*) *agricola*: 350–450 µm (Coe 1904); *Nipponnemertes pulcher*: 280–340 µm (Berg 1972)). While there is usually only a very thin and delicate chorion surrounding the eggs of anoplan species, the eggs of hoplonemertean species are invested with a chorion (also termed "vitelline envelope") that is thicker and usually set off from the egg membrane by a fluid-filled space of different dimensions (Stricker et al. 2001). In many free-spawning species, a glutinous mucus layer surrounds the egg chorion to attach the eggs to the substrate. In some species this mucus is reported to dissolve in the water soon after the eggs have been shed. In this case the mucus possibly enhances attraction and movement of the sperm to the egg.

Spermatozoa in nemertean species generally comprise a sperm head consisting of an apical acrosomal vesicle, a condensed nucleus, a mitochondrial mass, and diplosomal centrioles. From the distal centriole, a single flagellum with a regular 9×2+2 axoneme emanates. Only in the pelagic polystiliferan *Nectonemertes mirabilis* aflagellate sperm cells together with separate flagella have been observed in the testes (Stricker and Folsom 1997). Morphology of the sperm head is very variable with all components varying in both length and width (Stricker and Folsom 1997; von Döhren and Bartolomaeus 2006; von Döhren et al. 2010). Acrosomal vesicles and mitochondria may be dislocated from the terminal poles of the sperm cell in some species. In most paleonemertean and hoplonemertean species, the single mitochondrion represents the product of the fusion of numerous mitochondria during spermiogenesis. Elongated headed sperm has been hypothesized to be correlated with either internal fertilization (e.g., *Antarctonemertes phyllospadicola*, *Carcinonemertes epialti*, *Cephalothrix*

rufifrons) or the investment of the egg with a tough, resistant vitelline membrane in free-spawning species (e.g., *Cerebratulus lacteus*) (Stricker and Folsom 1997). In some hoplonemertean species, much of the elongation of the sperm head is due to a conspicuously elongated acrosomal vesicle (von Döhren et al. 2010). Interestingly, in these species eggs are invested with both a vitelline envelope and a fairly thick glutinous mucus layer that is resisting degradation well beyond the gastrulation of the embryo inside (e.g., *Paranemertes peregrina*, *Emplectonema gracile*).

In externally fertilizing species, eggs are commonly shed arrested in the prophase of the first meiotic division, i.e., the germinal vesicle is clearly visible. In oviparous species with internal fertilization, this is also the case only in *Carcinonemertes epialti* where the eggs are reported to be shed in early cleavage stages (Stricker et al. 2001). Freely spawned eggs typically show a somewhat compressed morphology due to them having been tightly packed in the ovaries. In some eggs, especially when they are artificially extracted from the female, there is a cytoplasmic protrusion representing the spot where the egg was contacting the ovarian lining during oogenesis (Iwata 1960; Stricker et al. 2001). In contact with seawater, they round up and usually undergo meiotic maturation indicated by germinal vesicle breakdown (GVBD) (Stricker et al. 2001). In some species the cytoplasmic protrusion separates from the egg but remains in its vicinity; in other species it disappears soon after fertilization. Abolishment of prophase I arrest during GVBD in the heteronemertean pilidiophoran *Cerebratulus lacteus* is mediated by intracellular signaling of nitric oxide (NO), cyclic guanosine monophosphate (cGMP), and an atypical protein kinase C (aPKC). Adenosine monophosphate-activated protein kinase (AMPK) blocks GVBD, but it can be resumed by the action of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) signaling. By these alternative pathways, an inactive form of the maturation promoting factor (pre-MPF) is activated. The active maturation promoting factor (MPF) triggers GVBD which is accompanied by a drastic reorganization of the endoplasmic reticulum (ER) of the egg into numerous ER microdomains about 5 μm in diameter, distributed evenly within the cytoplasm (Stricker et al. 2013 and references therein). The egg, arrested in the metaphase of the first meiotic division, has then an eccentrically located nucleus and is ready for fertilization. Fusion of the sperm with the egg induces a sudden cortical calcium flash followed by repetitive calcium oscillations in the egg due to the action of a soluble sperm factor delivered from the sperm to the egg. The first two calcium waves start at the point where the sperm has fused with the egg, while the following waves are elicited from a pacemaker region in the vegetal cortex of the egg opposite of the site of prospective polar body extrusion. Along with the calcium waves, there is a global disassembly of the ER microdomains. The calcium oscillations continue through first polar body formation and cease before the second polar body is extruded (Stricker et al. 2013 and references therein). Polar bodies are situated at the opposite pole of the cytoplasmic protrusion if one is present. This hints at the animal-vegetal axis of the egg being already established in the gonad during oogenesis (Fig. 8.4A; Henry and Martindale 1997). Polar body formation is followed by decondensation of the male pronucleus and subsequent karyogamy (Fig. 8.4B).

EARLY DEVELOPMENT

Diversity of Cleavage in Nemertea

Although exhibiting quite diverse larval types, embryonic development of nemerteans is comparably uniform. In general, cleavage is of the holoblastic, equal (homoquadrant), spiral type (Fig. 8.4C–F). The first cleavage division is meridional, passing through the anterior-posterior plane as indicated by the location of the polar bodies. It results in a pair of equally sized blastomeres. They are initially rounded, with little contact to each other. The embryo soon becomes compact prior to second cleavage division (Friedrich 1979; Henry and Martindale 1997, 1998a; Maslakova et al. 2004a; Maslakova 2010a). In the heteronemertean species *Lineus ruber*, the first cleavage division has been reported to be unequal resulting in a pair of slightly or significantly differently sized blastomeres (Fig. 8.4D; Nusbaum and Oxner 1913). This phenomenon, however, is not

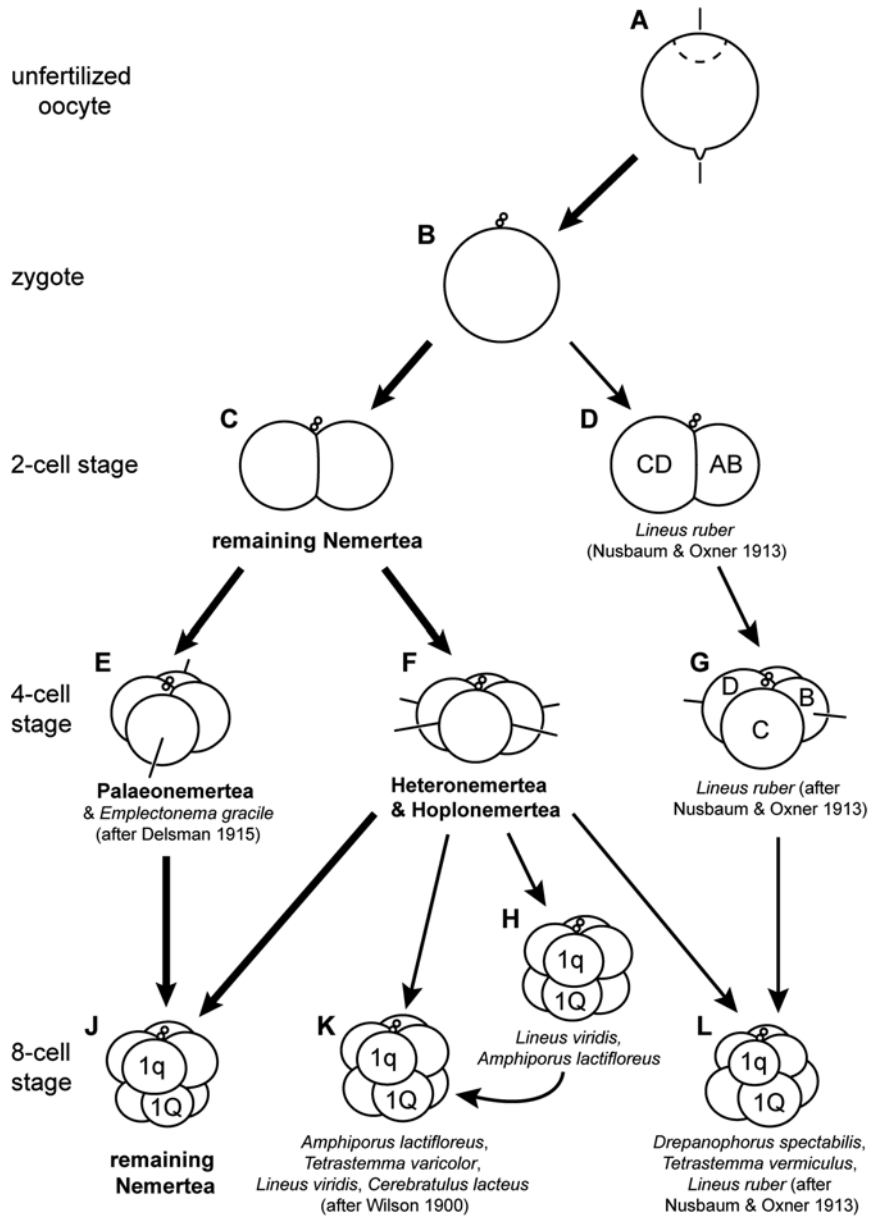

Fig. 8.4 Diversity of the cleavage in Nemertea. *Bold arrows* indicate the most common sequences. (**A**) Unfertilized egg. The animal-vegetal axis is running through the germinal vesicle (*dashed line*), and the cytoplasmic stalk on the opposite vegetal pole of the oocyte. (**B**) Zygote. The animal pole is marked by the polar bodies; the vegetally located cytoplasmic protrusion has been cast off. (**C**) Two-cell stage, equal first cleavage division. (**D**) Two-cell stage, unequal first cleavage division resulting in a smaller *AB* and a larger *CD* blastomere. (**E**) Four-cell stage after second cleavage division with equal sized blastomeres and cross-furrows (see also Fig. 8.5A). One dorsoventral axis is present running through the vegetal cross-furrow blastomeres. (**F**) Four-cell stage after second cleavage division with equal sized blastomeres lacking a cross-furrow. Two alternative dorsoventral axes are present. (**G**) Four-cell stage after second cleavage division with unequal-sized blastomeres (*C* and *D*) and cross-furrows. The dorsoventral axis runs through the larger *D* and the smaller *B* quadrant. (**H**) Eight-cell stage after third cleavage division with equally sized animal (*1q*) and vegetal (*1Q*) blastomeres, showing an initially radial-like arrangement of blastomeres (see also Fig. 8.5B). (**J**) Eight-cell stage after third cleavage division with animal blastomeres ("micromeres," *1q*) being larger than vegetal blastomeres ("macromeres," *1Q*) most common in nemertean species studied to date. (**K**) Eight-cell stage after third cleavage division with animal blastomeres ("micromeres," *1q*) being of the same size as vegetal blastomeres ("macromeres," *1Q*). (**L**) Eight-cell stage after third cleavage division with animal blastomeres ("micromeres," *1q*) being smaller than vegetal blastomeres ("macromeres," *1Q*). Citations in brackets indicate conflicting reports that have been given for the respective species. For further explanations, see text

seen in all embryos of a given clutch and has therefore to be attributed to a certain variability of cleavage in this species. It is unclear to what extend the differing blastomere size influences further development. The plane of the second cleavage division is meridional and perpendicular to the first, dividing the blastomeres into four daughter cells of equal size (Fig. 8.4E, F). In the case of the unequally cleaving embryos of *Lineus ruber*, the second cleavage division results in a four-cell stage in which one pair of blastomeres, the progeny of the smaller two-cell stage blastomere, is smaller than the progeny of the larger two-cell stage blastomere (Fig. 8.4G). This is in contrast to what is observed in, e.g., unequally cleaving annelids or mollusks (see Chapters 7 and 9) in which typically one of the four-cell stage blastomeres, the precursor of the dorsal (D) quadrant, is larger than the other three (Henry and Martindale 1998b). This also speaks for the phenomenon of unequal cleavage in Nemertea not being homologous to the unequal cleavage of Annelida or Mollusca. The four-cell stage blastomeres are initially rounded with little connecting surface to each other but soon move toward each other just like in the two-cell stage. The occurrence of cross-furrows at the four-cell stage has so far been reported from only a few species. The paleonemertean species *Carinoma armandi tremaphoros* and *Procephalothrix oestrymnicus* show distinct cross-furrows (Figs. 8.4E and 8.5A; Maslakova et al. 2004a). In the former species, the cross-furrows are a result of a slightly leotropic (sinistral) second cleavage division with one of the vegetal cross-furrow cells representing the precursor of the future dorsal (D) quadrant. However, it is not clear whether the specification of the D quadrant in this species is mediated by the segregation of cytoplasmic components or by inductive cellular interactions (Maslakova et al. 2004a). In neonemertean species, cross-furrows are reported from *Lineus ruber* (Heteronemertea) and *Emplectonema gracile* (Hoplonemertea), although in the latter species a more recent study does not confirm the existence of a cross-furrow (Fig. 8.4E, G; Delsman 1915; Iwata 1960). In *Lineus ruber* the cross-furrow has been recorded only in four-cell stage embryos with unequal-sized blastomere pairs. It is situated between one of the larger and the opposing smaller blastomere in the majority of examined cases (Fig. 8.4G; Nusbaum and Oxner 1913; Schmidt 1964). It is not completely clear, however, how the cross-furrow is related to the further course of development. The closely related heteronemertean *Cerebratulus lacteus* does not show any sign of cross-furrows at this stage nor do any of the other examined heteronemertean species (Fig. 8.4F; Friedrich 1979; Henry and Martindale 1997, 1998a; Maslakova 2010a). Due to its variability in occurrence, it can be assumed that in *Lineus ruber* the cross-furrow merely represents an intraspecific variation of development that might not have any influence on the determination of the future embryonic quadrants.

The third cleavage division is synchronous and equatorial. It results in an eight-celled embryo (Fig. 8.4H–L). In most species the third cleavage division is described as clearly dexiotrophic (dextral), but there are some accounts of animal blastomere quartets being positioned initially exactly opposite to their respective vegetal counterparts (Figs. 8.4H and 8.5B), resembling a radial cleavage pattern as found in, e.g., cnidarians (Vol. 1, Chapter 6), ectoprocts (Chapter 11), phoronids (Chapter 10), brachiopods (Chapter 12), or invertebrate deuterostomes (Vol. 6). In *Amphiporus lactifloreus* and *Lineus viridis* (as *Lineus obscurus*), the aligned animal and vegetal blastomeres shift position after segregation to come to lie as if generated by a regular dexiotropic cleavage division (Fig. 8.4K; Barrois 1877). This peculiar behavior might be due to the high yolk content of the large eggs in both of the mentioned species. In *Cephalothrix rufifrons* there is certain variability regarding the angle at which the eight-cell blastomeres are positioned, but subsequent cleavage divisions restore the spiral pattern by being regularly alternating leotropic (sinistral) and dexiotropic (Smith 1935). Regarding the relative sizes of the blastomeres at the eight-cell stage, there is some noteworthy variation among species investigated. In most species the animal eight-cell stage blastomeres (micromeres) are slightly or even considerably larger than cells of the vegetal blastomere quartet (macromeres) (Fig. 8.4J; Friedrich 1979; Henry and

Martindale 1997; Maslakova et al. 2004a), while in *Tetrastemma vermiculus*, *Drepanophorus spectabilis*, and *Lineus ruber*, the relative size differences accord to the general nomenclature, i.e., the animal micromeres are smaller than the vegetal macromeres (Fig. 8.4L; Lebedinsky 1897; Nusbaum and Oxner 1913; Schmidt 1964). Both animal and vegetal blastomere quartets have been reported to be of roughly equal size in *Tetrastemma varicolor* (Hoplonemertea), *Cerebratulus lacteus*, and *Lineus viridis* (Figs. 8.4K and 8.5B; Barrois 1877 for *Lineus viridis* as *Lineus obscurus*; Hoffman 1877; Wilson 1900). In *Cephalothrix rufifrons* there is no externally visible size difference of animal and vegetal blastomeres in the eight-cell stage, but sections reveal that the macromeres are slightly larger, their additional volume projecting interiorly into the blastocoel (Smith 1935). The fourth cleavage division leading to the 16-cell stage embryo is generally reported to be synchronous. The transition to the 16-cell stage in *Lineus ruber* passes through a series of stages in which first the two larger of the animal blastomeres divide in a leotropic manner, followed by the smaller pair. Finally, the macromere quartet (2A-D) divides to accomplish the 16-cell stage. The 32-cell stage in this species is again reached by a transitory stage with the macromeres and the vegetal-most micromere quartet dividing first, followed by the division of the two animal-most micromere quartets (Nusbaum and Oxner 1913). Interestingly, there seems to be a tendency in this species to arrange the micromere quartet daughter cells in a planar rather than a spiral pattern, a phenomenon that has also been described for the equatorial-most blastomeres in *Amphiporus lactifloreus* (Barrois 1877; Nusbaum and Oxner 1913). Relative asynchronies regarding the timing of division of blastomere quartets have been described in a number of other species, e.g., *Carinoma tremaphoros*, *Cerebratulus lacteus*, *Cerebratulus marginatus*, *Emplectonema gracile*, *Malacobdella grossa*, and *Tubulanus nothus* (Wilson 1900; Zeleny 1904; Delsman 1915; Hammarsten 1918; Dawydoff 1928; Maslakova et al. 2004a). However, while in *Lineus ruber* the cleavage divisions of the vegetal pole precede those of the animal pole, the reverse is true in the other exemplified species. Nevertheless, cleavage generally generates four quartets of animal blastomeres along with their respective progeny as well as one quartet of comparably small vegetal blastomeres. The paleonemertean *Tubulanus nothus* represents

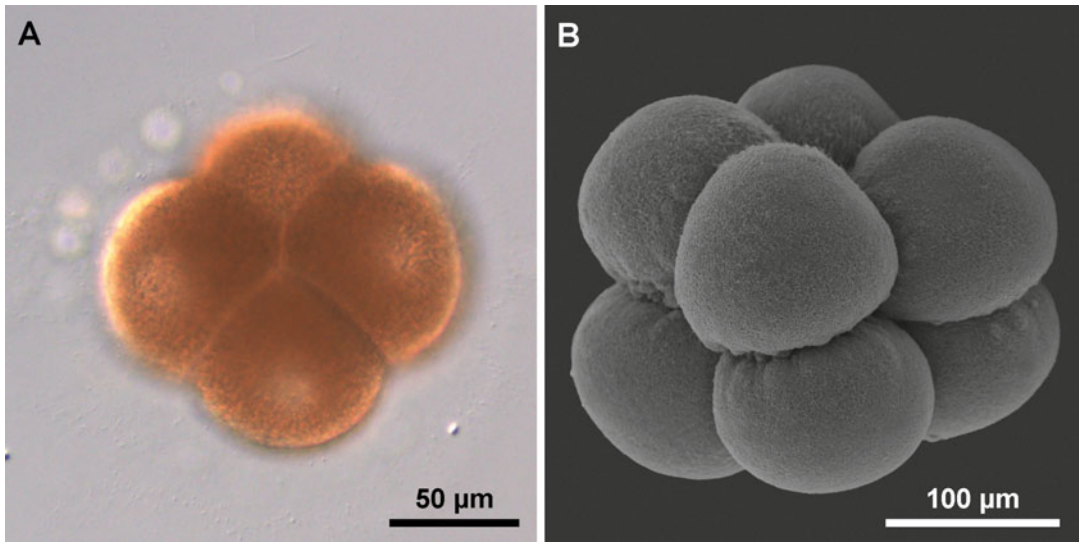

Fig. 8.5 Embryonic stages of Nemertea. (**A**) Four-cell stage of *Procephalothrix oestrymnicus* (Paleonemertea) from vegetal; live specimen; differential interference contrast (*DIC*) light micrograph. Note the prominent cross-furrow. (**B**) Eight-cell stage of *Lineus viridis* (Pilidiophora), from vegetal; scanning electron micrograph. Note the almost radial arrangement of equally sized animal and vegetal blastomeres (© Dr. J. von Döhren, All Rights Reserved)

an exception in that the regular spiral cleavage pattern is largely given up after the fifth cleavage division, resulting in a chaotic cleavage pattern in which neither fourth micromere nor macromere quartets are discernible (Dawydoff 1928).

Determination of the Future Body Axes

Cleavage of nemerteans does not only show the characteristic pattern, but it also complies with the general characteristics exhibited in Spiralia, such as stereotypy and determination. Contributions and capabilities of the embryo to form the future body parts have been most thoroughly studied in the heteronemertean pilidiophoran *Cerebratulus lacteus* (Table 8.1; Henry and Martindale 1997, 1998a). Compared to the spiral cleavage pattern of annelids and mollusks, however, there are some remarkable differences in terms of regulation and contribution of blastomeres to future organ systems in this species (Henry and Martindale 1997 and references therein). The larval anterior-posterior axis is already set up within the ovary and is outlined by the stalk-like process on the vegetal pole and the germinal vesicle situated on the opposite, animal pole of the egg (Fig. 8.4A). During meiotic maturation the polar bodies form near the animal pole. After fertilization the first two cleavage divisions pass through the animal-vegetal axis which thus becomes the anterior-posterior axis of the larva. Experimental alteration of the first cleavage plane by compressing fertilized eggs during first cleavage division results in its decoupling from the larval anterior-posterior and dorsoventral axis, indicating that the cleavage planes in normal development are not the cause of the future larval axes but follow a scaffold of the embryo that is precociously set up in the egg (Henry and Martindale 1995, 1996a). It has been shown that morphogenetic factors are evenly distributed within the egg prior to fertilization and that a progressive restriction due to segregation of these morphogenetic factors along the preformed animal-vegetal axis is executed after fertilization. The determinants seem to be fully segregated at the third cleavage division although vegetal determinants (as exemplified by formation of a larval gut) seem to be already confined to the vegetal pole of the embryo after the second cleavage division, i.e., at the four-cell stage (Zeleny 1904; Yatsu 1909; Hörstadius 1937; Freeman 1978). The acceleration

Table 8.1 Clonal contributions of blastomeres during early cleavage (up to the 64-cell stage) in Nemertea

Taxon	Paleonemertea	Heteronemertea	Hoplonemertea
Blastomere	Contribution		
First quartet	Apical tuft ($1q^1$), ectoderm, 28 "trochoblasts" ($1q^1$, $1q^2$), eye ($1c^1$)	Apical tuft, larval ectoderm, ciliated band, cephalic disks (1a, b), larval nervous system (1c, d)	Apical tuft, ectoderm
Second quartet	12 "trochoblasts," esophagus, primary somatoblast (2d), mesoderm[a]	Larval ectoderm, ciliated band, esophagus, larval nervous system (2a, c, d)	Ectoderm, mesoderm ($2a^{111}$–d^{111})
Third quartet	?	Esophagus, larval muscles (3a, b), larval ectoderm (3c, d), larval nervous system (3c, d)	Ectoderm
Fourth quartet	Mesoderm (3D)[a]	Gut (4a–c), adult mesoderm (4d)	Gut
Macromeres	Gut	Gut	Gut
Species	*Carinoma tremaphoros*	*Cerebratulus lacteus*	*Malacobdella grossa*
References	Maslakova et al. (2004a); [a]*Tubulanus nothus*: Dawydoff (1928)	Henry and Martindale (1998a)	Hammarsten (1918)

[a]Marks data by Dawydoff (1928) for *Tubulanus nothus*

or retardation of this segregation of determinants by stimulation or inhibition of the formation of mitotic asters during cleavage hints at a role the cytoskeleton, especially the microtubules, plays in the segregation process (Freeman 1978; Goldstein and Freeman 1997).

Embryonic Regulative Capacities of Nemertea

Additional remarkable modifications of the stereotypic spiral cleavage are seen in the capacity of the embryo to regulate as well as in the contribution of the blastomeres to the larval body. There is, however, considerable difference regarding regulative capabilities in Pilidiophora as opposed to other embryos. Embryos of *Cerebratulus lacteus* that have been halved at the two-cell stage are capable of forming completely normal but miniature larvae, while embryos dissected at the four-cell stage do not regulate to complete larvae. Cleavage after isolation of blastomeres is resumed in the stereotypic spiral fashion, instead of being reinitiated from a point that is analogous to earlier stages of cleavage (e.g., zygote in the case of isolation at the two-cell stage) (Wilson 1900, 1903; Zeleny 1904; Yatsu 1910; Hörstadius 1937). Embryos of the hoplonemertean *Nemertopsis bivittata* in which blastomeres have been deleted at either the two-cell or the four-cell stage develop into characteristically deficient larvae, indicating that regulation does not take place to such an extent as in *Cerebratulus lacteus* (Martindale and Henry 1995). Compared to other spiralian taxa, the inability to regulate as seen in *Nemertopsis bivittata* seems to be the ancestral state and to be attributed to an early determination of the clonal contributions of blastomeres to the juvenile tissues.

The regulative capacity of Pilidiophora represents a modification of the stereotypic determination in spiral cleavage and can be attributed to the mode of development in which cells remain undifferentiated until forming the juvenile rudiments much later in development. As a consequence of later determination of cell fates, the cells already during cleavage retain a certain ability to compensate for loss of blastomeres. The molecular mechanism underlying this delayed determination is still unknown.

Cell Lineage

Contributions of blastomeres to the larval/adult body plan have most thoroughly been studied in *Cerebratulus lacteus* for which the complete cell lineage is known up to the pilidium state (Table 8.1; Henry and Martindale 1998a). Partial cell lineages are available for the paleonemertean *Carinoma tremaphoros* and the hoplonemerteans *Nemertopsis bivittata* and *Malacobdella grossa* (Hammarsten 1918; Martindale and Henry 1992, 1995; Henry and Martindale 1994; Maslakova et al. 2004a). In all species examined, all blastomeres of the first quartet contribute equally to forming the apical pit with a tuft of long cilia as well as the majority of the larval epidermis. Due to the large size of the first quartet micromeres, the ectodermal domains are positioned in a dorsolateral and ventrolateral orientation, respectively, instead of being clearly dorsal, ventral, and lateral as in other spiralian animals (see Chapters 7 and 9; Henry and Martindale 1997, 1998a, 1999). In four-cell stages of *Cerebratulus lacteus* and *Nemertopsis bivittata* that do not possess cross-furrows, quadrant identities cannot be predicted, while in *Carinoma tremaphoros* the A and C quadrants are formed by animal cross-furrow blastomeres, and the B and D quadrants by vegetal cross-furrow blastomeres (Henry and Martindale 1997, 1998a; Maslakova et al. 2004a). In *Cerebratulus lacteus*, the D quadrant along with the dorsoventral axis is induced by the first quartet micromeres after the third cleavage division. Deletion of all first quartet micromeres results in radialized larvae, while deletion of a single or two adjacent first quartet micromeres leads to the D quadrant being determined in a position where the first quartet macromere had contact with most of the remaining first quartet micromeres during the eight-cell stage (Henry 2002). In *Cerebratulus lacteus* the first quartet micromeres contribute to the apical epidermis down to the circumoral ring of elongated cilia including the outer side of the lateral larval lap-

pets, while in *Carinoma tremaphoros* the first quartet micromeres form the apical epidermis down to the apical row of trochoblast cells (Table 8.1; Henry and Martindale 1998a; Maslakova et al. 2004a, b). The second micromere quartet cells contribute equally to most of the remainder of the epidermis, represented in *Cerebratulus lacteus* by parts of the ciliated band, the inner side of the lappets, and parts of the esophagus (Table 8.1; Henry and Martindale 1998a). In *Carinoma tremaphoros*, contributions of the second micromere quartet are unequal. In this species each of the second micromere quartet cells forms three of the posterior trochoblast cells each and parts of the esophagus. The entire epidermis posterior to the trochoblast ring is formed by the progeny of the dorsal second quartet micromere (2d), the so-called primary somatoblast (Table 8.1; Maslakova et al. 2004a, b). The role of this blastomere is in accord to what is reported from other spiralian taxa. In the hoplonemertean *Nemertopsis bivittata*, there is no somatoblast; all first and second quartet micromeres contribute equally to the ectodermal domains (Martindale and Henry 1995). The band of long cilia in the pilidium has to be regarded as not being homologous to the prototroch of the typical trochophore larva. In the latter, the prototroch is composed by a limited number of cells which originate from the first and second quartet micromeres, while there are a large number of cells in the ciliated band of the pilidium that are additionally contributed by the C and D quadrants of the third quartet micromeres (Maslakova 2010b). Moreover, the ciliated marginal band cells lack the expression of a trochoblast-specific β-tubulin found in other trochophores (van den Biggelaar et al. 1997). Due to the position of Pilidiophora within Nemertea and the absence of prototroch-type long cilia in either hoplonemertean or paleonemertean species, the long cilia that adorn the marginal band of the pilidium thus have to be regarded as newly evolved (Maslakova 2010b).

Contributions of blastomeres after the fifth cleavage division, i.e., the 32-cell stage, are known only from the pilidium larva of *Cerebratulus lacteus* (Henry and Martindale 1998a). As a characteristic of spiralian development, mesoderm is derived from two sources. The so-called ectomesoderm is derived from micromeres of either the second or the third quartet, while the so-called endomesoderm originates from the fourth quartet micromere of the D quadrant, the so-called 4d mesendoblast (Table 8.1; Boyer et al. 1996; Boyer and Henry 1998; Henry and Martindale 1999). In other nemertean species, there have been different accounts on the origin of the mesoderm. While some researchers derive the mesoderm exclusively from ectomesodermal sources such as the second micromere quartet (e.g., Hammarsten 1918 for *Malacobdella grossa*), others claim both ectomesodermal and endomesodermal sources to be present (e.g., second micromere quartet and 3D in *Tubulanus nothus*; see Table 8.1 and Dawydoff 1928). The mesoderm from both a mesendoblast (4d) and a multipolar delamination from the archenteron after gastrulation but without ectodermal contributions has also been described (e.g., Nusbaum and Oxner 1913 for *Lineus ruber*). More recent studies on the cell lineage of the pilidium of *Cerebratulus lacteus* have identified the third quartet micromeres of the A and B quadrant as source of the ectomesoderm forming the array of larval muscles as well as some undifferentiated mesenchymal cells scattered in the blastocoel (Table 8.1; Henry and Martindale 1996b, 1998a). It is known from isolation experiments that blastomeres isolated at the four-cell stage are capable of regulating for mesenchymal tissues in further development. This indicates that inductive interactions from the A and B quadrants inhibit the formation of mesodermal cell types in the progeny of their dorsally located counterparts (i.e., 3c and 3d; Martindale and Henry 1995).

Endomesoderm is formed by the fourth quartet micromere of the D quadrant (Table 8.1). In the pilidium, endomesoderm is represented by a pair of loosely organized mesodermal bandlets situated underneath the epidermis at the junction of the esophagus and stomach and some scattered, undifferentiated mesenchymal cells (Henry and Martindale 1996b, 1998a). The differentiation of adult mesoderm could not be followed but is

assumed to originate from a population of undifferentiated, dormant mesenchyme cells (Henry and Martindale 1997). In paleonemertean and hoplonemertean larvae, assumed cell-cell interactions as operating during the development of the pilidium to specify the dorsoventral axis clearly do not inhibit that all quadrants contribute equally to the muscle layers displayed in the larvae (Iwata 1957; Martindale and Henry 1995). To what extent the musculature is derived from both ectomesodermal and endomesodermal sources in nonpilidial types of development has been a matter of debate and remains to be clarified. The ectodermally derived nervous system of the pilidium of *Cerebratulus lacteus* is formed by progeny of the blastomeres 1c, 1d, 2a, 2c, 2d, 3c, and 3d with all of them contributing to the marginal ciliary neuropil (Table 8.1). The oral and the suboral neuropils are formed without contribution of the first quartet micromeres (Henry and Martindale 1998a). Due to the nervous system generating blastomeres being situated mostly in dorsal positions in the embryo, the authors suggested inductive interactions of other blastomeres specifying neuronal fates of blastomeres in the pilidium as has been shown in D quadrant specification.

There are no data available on the cell lineage of the nervous system in paleonemerteans and hoplonemerteans. The only sensory structures that provenance from blastomeres is known of are the eyes of the monostiliferan hoplonemertean *Nemertopsis bivittata* and the carinomid paleonemertean *Carinoma tremaphoros* (Martindale and Henry 1995; Maslakova et al. 2004b). Although apparently different with respect to both number and ultrastructure (one pair of rhabdomeric eyes in the monostiliferan hoplonemertean *Paranemertes peregrina* versus a single ciliary eye in the carinomid paleonemerteans *Carinoma tremaphoros* and *Carinoma mutabilis*), there seems to be a common pattern in deriving at least one eye from the C quadrant ($1c^1$ in *Carinoma tremaphoros*, right eye in *Nemertopsis bivittata* from so-called RD, i.e., C quadrant; see Martindale and Henry 1995; Henry and Martindale 1997, 1999; Maslakova et al. 2004a). The second eye in *Nemertopsis bivittata* is derived from the oppositely located (i.e. A) quadrant. In the latter species, there seem to be inductive potential located in two adjacent quadrants necessary to induce eye formation (Martindale and Henry 1995; Henry and Martindale 1997). Although there is considerable variation with respect to some blastomere lineages in nemerteans, their mode of cleavage clearly operates within the framework of a spiral cleavage as seen in many other spiralians. In contrast to most other spiralian species, relative blastomere size, inductive interactions, and, to a certain degree, regulation seem to play a prominent role in nemertean development (Henry and Martindale 1999; Henry 2002).

Gastrulation

Accelerated divisions of the progeny of the animal quartets and subsequent flattening of the cells lead to the widening of the blastocoel into which the more columnar vegetal progeny projects to different degrees. This process results in a coeloblastula in nearly all species studied, whereas the shape of the blastula and the dimensions of the blastocoel may vary. The blastula may be domed, but in the majority of species, it is flattened, resulting in an either rather spacious or highly compressed blastocoel (Friedrich 1979). While the blastula is radially symmetric in most species, it attains a somewhat rectangular appearance (having been termed blastosquare) due to some of the progeny of animal blastomere quartets jutting out to the sides in *Cephalothrix rufifrons*, *Malacobdella grossa*, and *Micrura alaskensis* (Hammarsten 1918; Smith 1935; Maslakova 2010a). At this time of development, some species show signs of beginning ciliation, while in other species ciliation only starts when gastrulation sets in. Some species with intracapsular development develop cilia even later after gastrulation (e.g., *Pantinonemertes* (*Geonemertes*) *australiensis*, *Antarctonemertes phyllospadicola*) (Hickman 1963; Maslakova and von Döhren 2009). The apical pole in some species with pelagic stages already shows first rudiments of organs, namely, the apical pit characterized by a group of columnar cells that are housed in a shallow depression. In species with intracapsular development, an apical organ rudiment is

usually absent (Friedrich 1979; Senz and Tröstl 1999). A pair of large, sometimes slightly invaginated cells situated on both sides of the apical pit has been reported in *Procephalothrix simulus*, *Emplectonema gracile*, *Prosorhochmus viviparus*, and *Malacobdella grossa*, already marking the bilateral symmetry of the blastula (Friedrich 1979). The large cells have been interpreted as the first rudiments of the nervous system (Iwata 1960). On the vegetal pole of the embryo, one (*Cerebratulus lacteus*: Coe 1899; Wilson 1903; *Lineus ruber*: Nusbaum and Oxner 1913, but see Schmidt 1964; *Tubulanus nothus*: Dawydoff 1928) or two pairs (*Procephalothrix filiformis*: Iwata 1960; *Tetrastemma vermiculus* and *Drepanophorus spectabilis*: Lebedinsky 1897) of distinct blastomeres, having been interpreted as primary mesoblast cells, are reported. In other species, however, the vegetal-most blastomeres are indistinguishable in size and form (Friedrich 1979).

Gastrulation proceeds by invagination of the blastomeres of the vegetal pole, although epibolic processes caused by the proliferation of the ectodermal components have been indicated to be involved. During gastrulation the shape of the embryo changes to become more domed. Some species, especially hoplonemerteans with very yolky eggs, gastrulate by polar ingression of the vegetal cells (*Malacobdella grossa, Prostoma graecense*, *Argonemertes* (*Geonemertes*) *australiensis*, *Gononemertes australiensis*) (Hammarsten 1918; Reinhardt 1941; Hickman 1963; Egan and Anderson 1979).

LATE DEVELOPMENT

Diversity of Larval Types in Nemertea

Although most nemerteans develop via planktonic stages, their development has traditionally been characterized as direct and indirect development, according to the transformation of the larva to form the juvenile body (Fig. 8.6). While in nemertean, so-called direct development morphological transformations are gradual, resulting in a smooth transition from the pelagic stage to the juvenile, so-called indirect development is characterized by a catastrophic metamorphosis in which the larval epidermis is shed as a whole and usually eaten by the juvenile that has developed inside (Cantell 1967; Maslakova 2010a). Larval types in Pilidiophora are quite diverse, including several morphotypes of pilidia, the Desor larva of *Lineus viridis*, the Schmidt larva of *Lineus ruber*, the Iwata larva of *Micrura akkeshiensis*, and other unspecified pelagic, lecithotrophic larvae of some *Micrura* species (Norenburg and Stricker 2002; Schwartz and Norenburg 2005; Schwartz 2009; Maslakova 2010b). Larvae of hoplonemertean and paleonemertean species have traditionally been classified as so-called planuliform larvae due to their superficial resemblance to the planula larvae of cnidarians (Fig. 8.6A–C). Several hypotheses have been put forward to derive the pilidium from planuliform nemertean or even other, non-nemertean larval types, but the evolution of this aberrant larval form is presently still enigmatic (Iwata 1972; Jägersten 1972; Hiebert et al. 2010).

Recent findings, however, hint at a more complex picture. The planuliform larval type includes the decidula larva that possesses a transitory larval epidermis whose cells are replaced by cells of the definite juvenile epidermis and the hidden trochophore that is characterized by a distorted preoral belt of large cells that have been homologized with the trochoblasts of annelids and mollusks (Maslakova et al. 2004b; Maslakova 2010b). The decidula is typically present in Hoplonemertea but has also been suggested to occur in the tubulanid paleonemertean *Tubulanus punctatus* (Iwata 1960; Maslakova 2010b and references therein). The hidden trochophore has been found in *Carinoma tremaphoros* based on cell lineage studies (Maslakova et al. 2004a, b). The larval type of the remaining paleonemertean species has never been specified. Species with intracapsular development typically show characteristics of the larval type present in their respective phylogenetic lineage (Maslakova and Malakhov 1999; Maslakova and von Döhren 2009; von Döhren 2011). Larvae of hoplo- and paleonemerteans develop from the gastrula by differential growth of one side of the body which becomes the dorsal side of the animal. As a consequence, the blastoporal region moves to

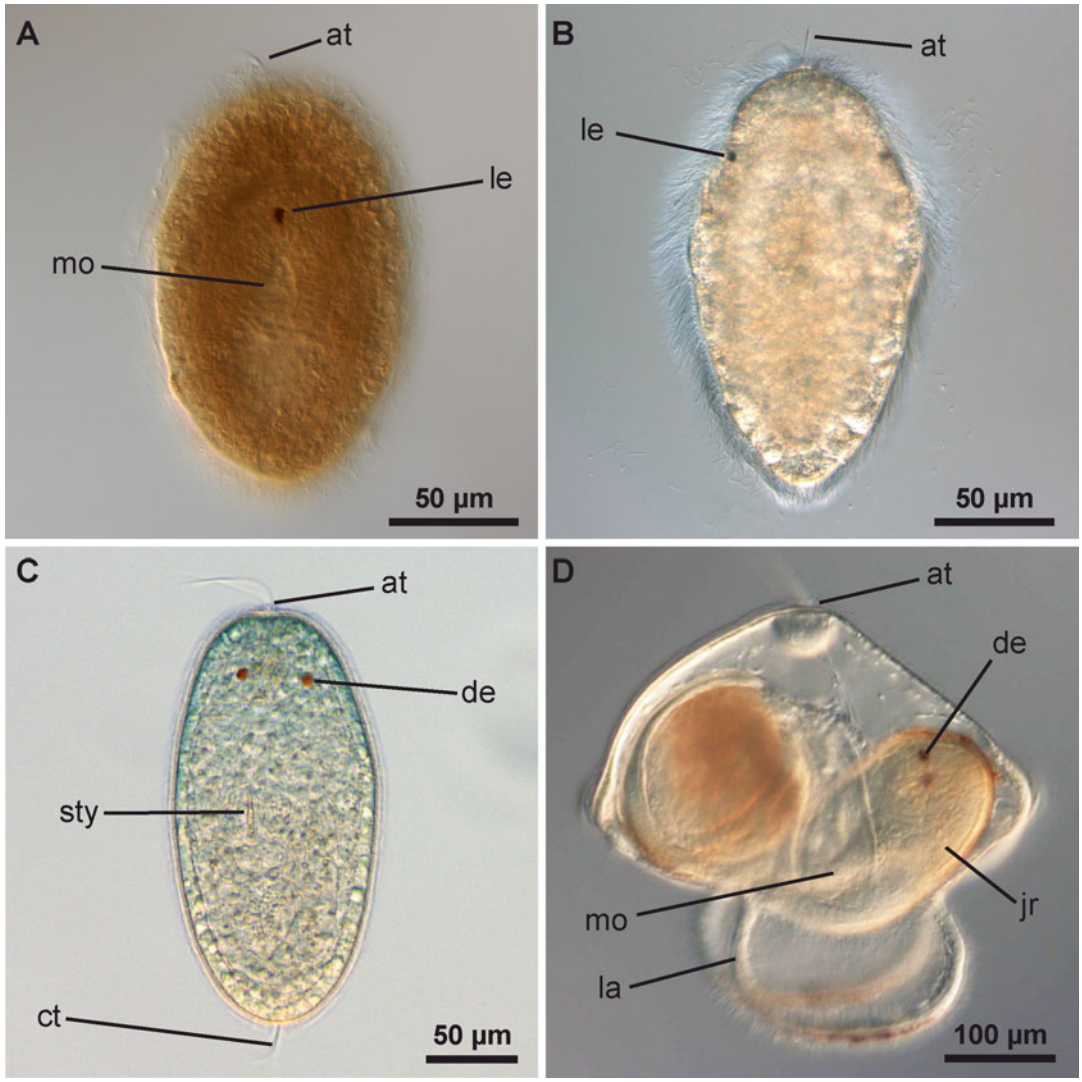

Fig. 8.6 Pelagic larvae of Nemertea. (**A**) Five-day-old larva of *Carinina ochracea* (Paleonemertea), fixed and osmicated specimen¸ differential interference contrast (*DIC*) light micrograph. Note the single median ventral eye. (**B**) Three-day-old larva (at 18 °C) of *Procephalothrix oestrymnicus* (Paleonemertea), live specimen, DIC. Note the pair of dorsal eyes. (**C**) Four-day old larva of *Emplectonema gracile* (Hoplonemertea), live specimen, bright field. Note the first pair of dorsal eyes. The stylet apparatus has already formed inside the larva. (**D**) Six-week-old, advanced pilidium of *Riseriellus occultus* (Pilidiophora), live specimen, DIC. Note the advanced juvenile with eyes inside the pilidium. The imaginal disks are fused; only the dorsal part of the juvenile has yet to form. *at* apical tuft, *ct* caudal tuft, *de* definite (adult) eye, *jr* juvenile rudiment, *la* lateral lappet, *le* larval eye, *mo* mouth opening, *sty* stylet (© Dr. J. von Döhren, All Rights Reserved)

a more frontal position marking the ventral side of the larva, thus changing the angle between the apical and the former vegetal pole of the embryo.

The pilidium was originally described by Müller (1847) as a previously unknown, putatively larval animal from the North Sea. It was consequently given a binomen: pilidium gyrans. Although being aware of the larval nature of the organism, the tradition of binomial nomenclature was adopted by subsequent researchers (e.g., Bürger 1894; Dawydoff 1940; Cantell 1969; Chernyshev et al. 2013). More than half a dozen morphotypes have been described of which the original pilidium gyrans represent the archetype but not necessarily the ancestral state (Figs. 8.6D and 8.7). Apart from different shapes of the lappets, the dome, and the bands of elongated cilia, the pilidial types have been distinguished by the orientation that the juvenile rudiment assumes

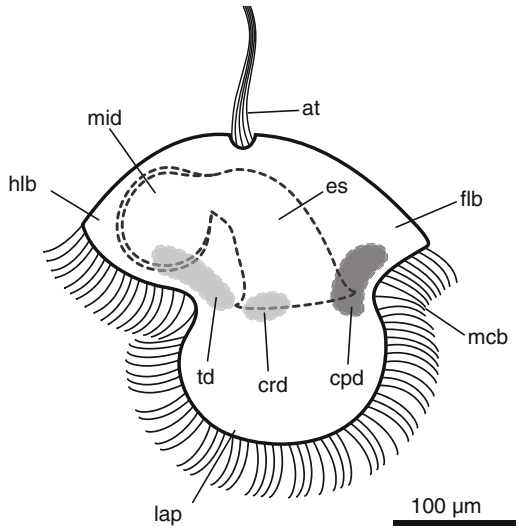

Fig. 8.7 Schematic representation of a young pilidium gyrans (lateral view). The positions of the prospective paired epidermal invaginations forming the juvenile are shown in *shaded areas* (*black*, exumbrellar invagination; *light grey*, subumbrellar invagination). *at* apical tuft, *cpd* cephalic disk rudiment, *crd* cerebral organ disk rudiment, *flb* forelobe of pilidium, *hlb* hindlobe of pilidium, *lap* lateral lappet of pilidium, *mcb* marginal band of elongated cilia, *mid* midgut, *es* esophagus, *td* trunk disk rudiment (© Dr. J. von Döhren, All Rights Reserved)

with respect to the larval apical-vegetal axis. In the archetypical pilidium (e.g., pilidium gyrans), the juvenile anterior-posterior axis assumes a roughly rectangular orientation to the larval apical-vegetal axis as indicated by the larval apical tuft and vegetal mouth opening. There is a hypothetical series to derive the archetypical pilidial forms (pilidium gyrans, pilidium pyramidale) from types in which the larval and juvenile main axes are roughly parallel, such as the pilidium recurvatum (Jägersten 1972). However, more recent findings indicate that the vast diversity of pilidial morphotypes might be a consequence of the uncoupling of morphogenetic processes between the larval stage and the formation of the juvenile (Hiebert and Maslakova 2015).

Comparative Larval Development

Paleo- and Hoplonemertean Larvae

In larvae of paleonemertean species, the blastoporal region differentiates into an ectodermal esophagus and an endodermal, sac-like midgut, both of which are functional. In the majority of hoplonemertean species, the blastopore has been reported to close. A secondary, ectodermal mouth opening along with an esophageal tube is subsequently formed in various ways according to different authors (Friedrich 1979; Maslakova and von Döhren 2009). In some hoplonemertean species, especially those with intracapsular development, no functional mouth opening is formed until much later in development (Salensky 1914; Hammarsten 1918; Hickman 1963; Iwata 1960; Egan and Anderson 1979). The larvae are initially ovoid in shape and gradually elongate with age. While the surface of the larva is covered with cilia of equal length, the apical pole of the larva is equipped with an apical tuft of longer cilia housed in an apical depression, the apical pit. In many species the posterior end of more advanced stage larvae is endowed with another tuft of elongated cilia termed caudal tuft or posterior cirrus (Fig. 8.6C; Iwata 1960; Stricker and Reed 1981; Maslakova and von Döhren 2009). One to several pairs of lateral cirri located anterior of and at the level of the mouth opening have been described for members of the cephalothricids (Iwata 1960). In most larvae a pair of epidermal invaginations on either side of the apical pit is formed which has variously been interpreted as rudiments of the nervous system or the cerebral organs (Lebedinsky 1897; Salensky 1909; Hammarsten 1918; Smith 1935; Iwata 1960; Maslakova and von Döhren 2009; but see Hiebert et al. 2010; Maslakova 2010b).

Some larvae of paleonemerteans and most hoplonemertean larvae possess eyes (Fig. 8.6A–C). They are usually simple pigment cup ocelli composed of only a few photoreceptor cells surrounded by one to a few shading pigment cells (von Döhren 2008). While in *Carinoma* species and *Carinina ochracea* there is a single almost median ventral eye anterior to the mouth opening, cephalothricids and most hoplonemertean species possess a pair of eyes situated dorsally, in front of the mouth opening (Fig. 8.6A–C; Hammarsten 1918; Iwata 1960; Maslakova et al. 2004a, b; Maslakova and von Döhren 2009; Hiebert et al. 2010; Maslakova 2010b). In the larva of *Quasitetrastemma* (*Tetrastemma*) *stimpsoni* and *Quasitetrastemma*

(*Tetrastemma*) *nigrifrons*, three pairs of dorsally located eyes have been described (Chernyshev 2008). In paleonemertean larvae, the eyes are epidermal and possess ciliary receptor cells, while hoplonemertean species possess subepidermal eyes equipped with rhabdomeric receptors. Since the adults of those paleonemertean species studied lack eyes, the eyes present in the larvae have to be interpreted as transitory larval organs (von Döhren 2008). In hoplonemerteans, the subepidermal eyes persist to adulthood, increasing in size and number in most species. Only in *Quasitetrastemma* species the one pair of eyes has been hypothesized to be fused to the neighboring pair or completely reduced during development (Chernyshev 2008).

Pilidiophoran Larvae: The Pilidium

The archetypical pilidium develops from the swimming gastrula by growth of the lateral rims of the invaginated vegetal field to form the lateral lappets. A ring of elongated cilia, the marginal ciliary band, is present around the invaginated ciliary field, marking border between the outer (exumbrellar, also: epispheric) and the inner (subumbrellar, also: hypospheric) surfaces of the larva (Fig. 8.7; Friedrich 1979; Maslakova 2010a). In some forms of pilidia, the anterior and posterior parts of the ciliated ring may form additional anterior and posterior lobes in the larva. Denticle-like structures and chromatophores may adorn the ciliated ring. Depending on the species, they may increase in number with age of the larva (Cantell 1969; Norenburg and Stricker 2002; Lacalli 2005). The exumbrellar part, housing the apical pit with a tuft of elongated cilia, expands to form the typical dome shape of the pilidium (Figs. 8.6D and 8.7). The esophagus is formed from ectodermal cell material being dragged in during blastopore invagination (Friedrich 1979). The larval gut is sac-like and located interior of the posterior lobe. The gut is open to the esophagus via a narrow opening with a valve that develops from the blastopore. Along the posterior wall of the esophagus, there is a pair of prominent longitudinal ridges that exhibit elongated cilia. They have been reported to serve as main structures to mediate transport of food particles through the esophagus to the gut (Maslakova 2010a). The larva is composed of two epithelial layers consisting of a single row of multiciliated, large flat cells. In the region of the apical tuft, the marginal ciliary band, and the longitudinal ridges, the cells attain a more columnar shape. Inside the larva, an extensive, comparably loose extracellular matrix with interspersed neuronal and mesodermal cell clusters fills the body.

Both the larval musculature and nervous system are formed early in development, enabling the larva to take up food particles and to locomote. For the most part, nerves and muscles are not taken over into the juvenile organization (Maslakova 2010a), but some neural components are incorporated into the postmetamorphic nervous system (Hindinger et al. 2013). The first structures of the nervous system that differentiate in the pilidium are a prominent ring-shaped neuropil underlying the marginal ciliary band (marginal band neuropil), a ring-shaped neuropil encircling the opening of the esophagus into the gut (oral ring neuropil), as well as a pair of neurite bundles connecting the oral ring neuropil on both sides of the larva with the marginal band neuropil at the posterior base of the lateral lappets. A pair of linear plexuses (circumesophageal plexus) extending from the anterior to the posterior base of the lateral lappets alongside the opening of the esophagus connecting to the marginal band neuropil has been described in some species (Lacalli and West 1985; Hay-Schmidt 1990; Henry and Martindale 1998a; Maslakova 2010a; Zaitseva and Flyachinskaya 2010; Hindinger et al. 2013). The epidermis is underlain by a loosely arranged epidermal plexus that is in contact with the marginal band neuropil and exhibits a prominent pair of monociliated, serotonergic, putatively sensory neurons, laterally next to the apical pit (Maslakova 2010a; Hindinger et al. 2013). The apical pit itself is devoid of neuronal elements (Cantell et al. 1982; Lacalli and West 1985; Chernyshev et al. 2013). From some of the mesodermal cells, the larval musculature forms underneath the epidermis, while others remain in an undifferentiated, almost amoeboid state (Henry and Martindale 1998a; Maslakova 2010a). The most prominent structure

of the larval musculature is a muscle ring situated underneath the marginal ciliary band. The muscle ring branches at the bases of the lateral lappets to send a portion along the rim of the lappets, while the other encircles the esophageal opening. A ring-shaped sphincter muscle underlies the opening between the esophagus and the gut. Underneath the epidermis there is a prominent array of muscles that radiate from the dome into all parts of the larva. By branching they form an oblique muscle network being most prominent in the lateral lappets (Maslakova 2010a). A muscle extending from the apical pit downward (apical muscle or central muscle), branching around the esophagus, and serving to contract the larval apex has been described in most of the larval forms (Bürger 1897–1907; Lacalli 2005; Zaitseva and Flyachinskaya 2010).

The juvenile develops inside the pilidium from a set of epidermal invaginations of the larval envelope, termed imaginal disks. A minimum of three paired, bilaterally located disks are described: the most frontally located cephalic disks, the middle cerebral organ disks, and the trunk disks situated ventral of the gut posterior of the lateral lappets (Fig. 8.7). After invaginating from the larval epidermis, the imaginal disks are pinched off becoming flattened, fluid-filled cavities. While the cells of the proximal wall of the imaginal disk are initially more columnar and differentiate into definite, juvenile epidermis, the distal layer retains its flat, thin structure reminiscent of the larval epidermis. The distal, thin cell layer is referred to as amnion (Friedrich 1979; Maslakova 2010a). In addition to the three pairs of epidermal invaginations, two rudiments have been observed, of which the ectodermal origin has not been unambigouously demonstrated: the anterior-most proboscis rudiment and the posterior-most dorsal rudiment, both of which might not be generally present in all species (Friedrich 1979; Maslakova 2010a). The imaginal disks do not, however, form at the same time, but there is a stereotypic sequence of appearance. The first pair of disks that forms is the cephalic disks. Unlike the remaining imaginal disks, the cephalic disks are invaginated from the exumbrellar epidermis as derivatives of the A and B quadrants of the first quartet micromeres, hence 1a and 1b (Fig. 8.7; Henry and Martindale 1998a; Maslakova 2010a). After some time, the trunk disks are formed, followed by the cerebral organ disks. Both pairs of imaginal disks are unanimously reported to invaginate from the subumbrellar larval epidermis (Fig. 8.7; Friedrich 1979; Maslakova 2010a). Shortly after, an unpaired anterior-most putative proboscis rudiment is observed sitting dorsal of the cephalic disks underneath the larval epidermis. The exact origin of the putative proboscis rudiment is unknown. It has been described as epidermal invagination similar in structure to the paired imaginal disks, as a delamination of the larval epidermis or as a cluster of undifferentiated mesenchymal cells forming underneath the larval epidermis (Bürger 1894; Schmidt 1937; Maslakova 2010a). At the time the cephalic disks fuse with each other, incorporating the putative proboscis rudiment and thus forming the head rudiment, the anterior end of each trunk disk fuses with the posterior rim of the cerebral organ disk of the respective side. During fusion of the proximal cell layer of the imaginal disk, the amnion layers are separated from the proximal layer to fuse to each other, forming a continuous amniotic layer distal to the respective rudiments. In most species an additional, unpaired, posterior-most dorsal rudiment forms (Friedrich 1979; Maslakova 2010a). In contrast to the paired imaginal disks, the dorsal rudiment arises as a delamination from the larval epidermis or a mesodermal cell layer instead of an invagination from the larval epidermis. Although initially single layered, the dorsal rudiment soon becomes double layered, the outer layer forming an amnion as is seen in the imaginal disks that develop as invaginations of the larval epidermis (Bürger 1894; Salensky 1912; Schmidt 1937; Maslakova 2010a). During further development the dorsal rudiment fuses with the dorsoposterior rims of the bilaterally still separate trunk rudiments which themselves fuse posteriorly soon afterwards. As well as during the fusion of the cephalic disks, a continuous amnion layer is formed.

The next stage is accomplished by the fusion of the lateral posterior rims of the head rudiment to the lateral anterior rims of the trunk rudiment

represented by the cerebral organ disks. Thus, a continuous torus-shaped rudiment is formed around the larval esophagus (Maslakova 2010a). The anterior dorsal rim of the trunk rudiment subsequently fuses with the posterior dorsal margin of the head rudiment, completing the formation of the juvenile inside the pilidium. The juvenile is almost completely separated from the larval tissues by the amniotic cavity which is lined by the continuous amnion layer. The only contact of pilidium and juvenile is by the mouth opening that is shared by larva and juvenile. After the development of the juvenile inside the pilidial envelope is completed, the juvenile escapes from its envelope by vigorous movements that rupture the pilidial tissues (Cantell 1967; Maslakova 2010a). This process has been termed catastrophic metamorphosis and is usually a matter of a few minutes. The larval envelope that is still connected to the mouth of the escaping juvenile is usually eaten by the juvenile (Cantell 1967; Maslakova 2010a).

Aberrant Pilidiophoran Larvae

Within Pilidiophora there seems to be an evolutionary tendency toward lecithotrophic stages (Maslakova and Hiebert 2014). The larva of *Micrura akkeshiensis*, named Iwata larva after its discoverer, is a pelagic stage but does not take up food during its development (Iwata 1958). Thus, the larva does not show either lappets or bands of elongated cilia used for food capture in the pilidium. It develops from a comparably flat gastrula that elongates and attains a pyriform shape. The animal pole is marked by an apical pit with an apical tuft of elongated cilia. The opposite vegetal pole is marked by the site of the blastopore. In this type of development, the blastoporal opening is not shifted to either side of the gastrula during further development but remains in its original position. Around the blastopore four larger, mesodermal cells have been observed which proliferate to form a mesodermal mass between the epidermis and the invaginated archenteron.

Apart from an apical tuft and an evenly ciliated epidermis, no distinct larval organs have been reported. Similar to the pilidium, the epidermis covering the larva is a transitory larval envelope in which the juvenile develops. However, instead of developing at an angle to the larval body, the juvenile inside is parallel to the larva but with its head directed toward the side of the blastopore. Furthermore, in the Iwata larva, the number of imaginal disks is restricted to five, a pair of frontal head disks, a pair of posteroventral trunk disks, and an unpaired posterior dorsal disk, all of which form from invaginations of the larval epidermis. An anterior-posterior sequence of their formation is likely (Iwata 1958).

Similar nonfeeding larval types have been described in *Micrura rubramaculosa*, *Micrura verrilli*, and at least two other yet undescribed *Micrura* species; although in the larva of the former species there is an additional equatorial band of elongated cilia present and the larval animal-vegetal axis coincides with the adult anterior-posterior axis (Schwartz and Norenburg 2005; Schwartz 2009). Another nonfeeding larval form possessing an equatorial as well as a posterior band of elongated cilia that have been termed proto- and telotroch analogously to the ciliated bands of the trochophore larva has been described for an undescribed lineid heteronemertean (Maslakova and von Dassow 2012; Maslakova and Hiebert 2014). Unfortunately, there is virtually nothing known on the development in any of the abovementioned larval types.

Although being the first developmental stage described in nemerteans, the Desor larva of *Lineus viridis* might represent one of the most derived larval types. Despite of showing entirely intracapsular development within an egg capsule, it shows distinct characteristics typical of pilidiophoran development, including imaginal disks and the devouring of the larval epidermis during a catastrophic metamorphosis (Friedrich 1979; von Döhren 2011). However, since this larval type is lecithotrophic, no food is taken up until metamorphosis; hence, accessory feeding structures as seen in the pilidium are absent. Following gastrulation a spherical larva is formed that is uniformly ciliated, lacking any apical tuft or band of elongated cilia. Reminiscent of the pilidium larva, three pairs of imaginal disks are formed by invagination along with two rudiments formed by delamination from the larval epidermis (Nusbaum and Oxner 1913; Schmidt

1964). They correspond in position to the imaginal disks and rudiments seen in the pilidium. In contrast to canonical pilidial development, cephalic and trunk disks appear almost simultaneously in the Desor larva, followed by the proboscis rudiment, the cerebral organ disks, and finally the dorsal rudiment (Schmidt 1964). The sequence of fusion only differs from that seen in the pilidium in that the trunk disks first fuse with each other and with the dorsal rudiment before fusing with the cerebral organ disks (Friedrich 1979). At that time, the larva elongates posteriorly, attaining a rhomboid shape.

The larva of the closely related species *Lineus ruber* is smaller but has essentially the same larval morphology (Schmidt 1964). It has been given a different name due to its reported divergent mode of feeding. In contrast to the Desor larvae of *Lineus viridis*, the so-called Schmidt larva of *Lineus ruber* is adelphophagic, feeding on putatively deficiently developing sibling embryos inside the same egg capsule. Hence, differences in timing of the development of the proboscis and the alimentary tract with its associated musculature in later-stage larvae have been reported (Schmidt 1964).

Nemertean Larval Types – Evolutionary Considerations

Including the other nemertean larval types, there is a series of transitions from the paleonemertean larvae to the most derived developmental types, namely, the Iwata and Desor larva (Iwata 1972). This series, however, has to be taken with some reservation. On the one hand, the basally branching, non-heteronemertean pilidiophoran *Hubrechtella dubia* possesses a pilidium of the pilidium auriculatum type which is most similar to the pilidium gyrans type (Cantell 1969), while on the other hand, most pilidial morphotypes cannot be assigned to a certain species or clade, thus rendering phylogenetic inferences needed to set up an evolutionary series impossible. The aberrant pilidium recurvatum type has been assigned to members of the heteronemertean pilidiophoran Baseodiscidae but has also been identified as the larval type of the putatively most basal heteronemertean genus *Riserius* (Cantell 1969; Thollesson and Norenburg 2003; Hiebert et al. 2013a, b).

An apical organ situated basal to the apical pit present in all nemertean larval types has variously been interpreted as homologous to the apical organ in several trochozoan and lophophorate species, especially in Annelida and Mollusca but also in Brachiopoda (see Chapters 7, 9, and 12 as well as Nielsen 2013 and references therein). In many spiralian larvae, the apical organ is composed of the apical tuft and a usually low number of flask-shaped, serotonergic neurons at its base. These flask-shaped neurons generally degenerate prior to metamorphosis (Nielsen 2013 and references therein). In nemertean species a single flask-shaped serotonergic neuron has been reported only for hoplonemertean species although it does not project directly into the apical pit but reaches the epidermal surface in its vicinity (Fig. 8.8A; Chernyshev and Magarlamov 2010). Moreover, this flask-shaped neuron persists through metamorphosis as part of the brain ring (von Döhren J 2015, unpublished). In paleonemertean and pilidiophoran species, no flask-shaped serotonergic neurons have hitherto been found. In the pilidium, there are two spherical, putatively sensoric serotonergic neurons in the vicinity of the apical pit, while the single apical serotonergic neuron of paleonemertean species has an ovoid shape and does not make contact with the epidermis (Fig. 8.8B). While the ovoid apical serotonergic neuron in Paleonemertea disappears when the brain rudiment starts to form, the apical serotonergic neurons of Pilidiophora are shed at metamorphosis along with the larval envelope. Similar to the situation seen in the larvae of polyclad Platyhelminthes, homology of the serotonergic apical neurons is unclear (Rawlinson 2010). Two evolutionary scenarios seem possible: either the apical serotonergic neurons of Nemertea are homologous to those in Trochozoa, but there have been various substantial transformations in both Paleonemertea and Pilidiophora, or apical serotonergic neurons have evolved multiple times independently in Nemertea. Current hypotheses regarding the phylogeny of Nemertea favor the second

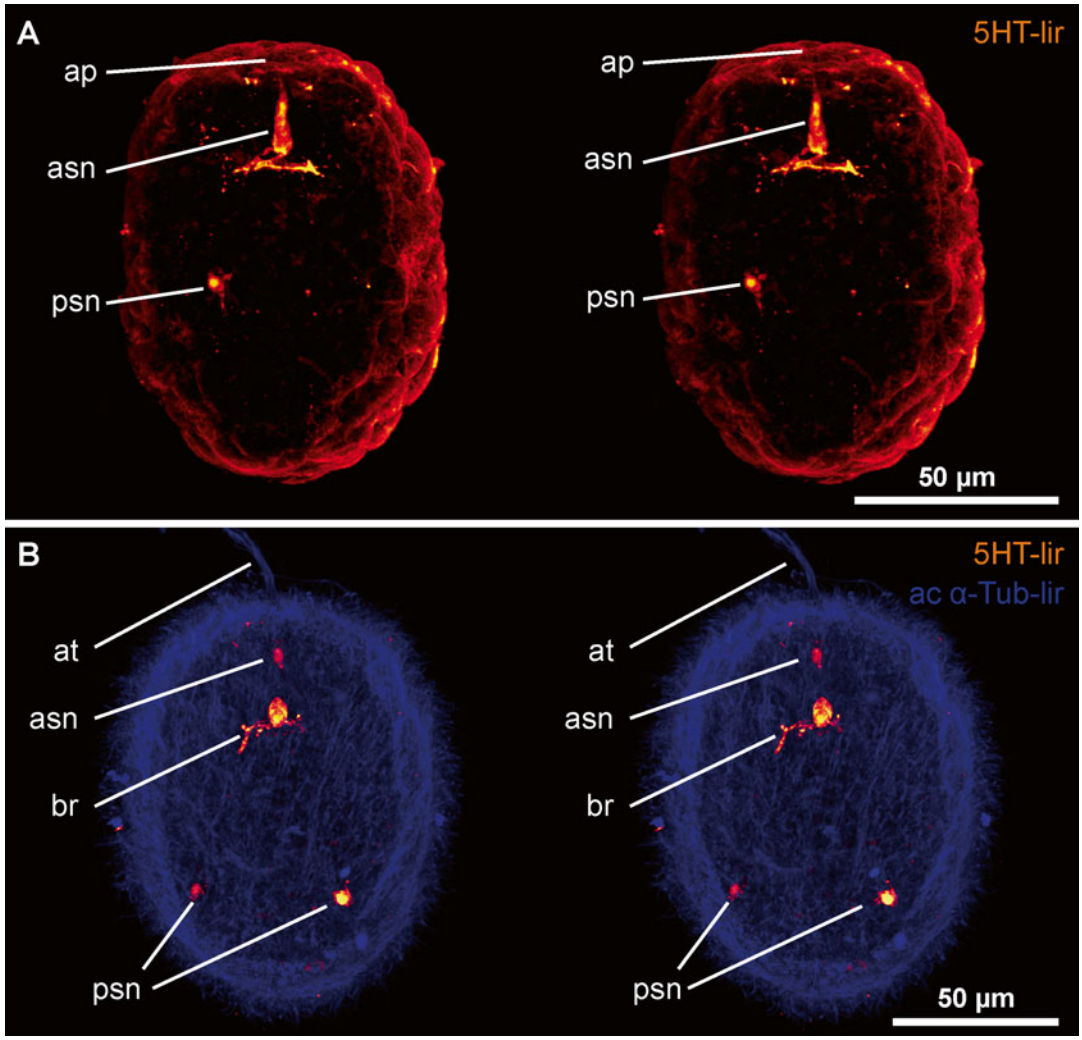

Fig. 8.8 Stereo pair images of confocal microscopy stacks of neural staining of nemertean larvae. (**A**) *Emplectonema gracile (Hoplonemertea)*, one-day-old larva stained with antibodies against serotonin (5HT-lir). Note: the flask-shaped apical serotonergic neuron (*asn*) projects in the vicinity of the apical pit (*ap*). An additional, more posterior serotonergic neuron (*psn*) is visible at this stage. (**B**) *Procephalothrix oestrymnicus (Paleonemertea)*, three-day-old larva (at 12 °C) stained with antibodies against serotonin (5HT-lir) and acetylated α-Tubulin (ac α-Tub-lir). Note: there is no connection of the apical serotonergic neuron (*asn*) to the apical pit that the apical tuft (*at*) emanates from. The rudiment of the brain (*br*) is visible as well as a few more posteriorly located neurons (*psn*) showing 5HT-lir. *ap* apical pit, *asn* apical serotonergic neuron, *at* apical tuft, *br* brain rudiment, *psn* posterior serotonergic neuron (© Dr. J. von Döhren, All Rights Reserved)

alternative scenario as being more parsimonious (Andrade et al. 2012).

Formation of the Adult Body Plan

Data on the formation of the definite adult organization in Nemertea are rather heterogeneous so that generalizations are at best preliminary at this point in time. Comprehensive information is restricted to classical accounts on about a dozen species, most of which are neonemerteans with arguably aberrant, i.e., intracapsular, development (e.g., *Lineus viridis/ruber*: Barrois 1877; Arnold 1898; Hubrecht 1885, 1886; Nusbaum and Oxner 1913; Schmidt 1964; *Prosorhochmus*

viviparus: Salensky 1882–1883, 1909, 1914; *Pantinonemertes* (*Geonemertes*) *agricola*: Coe 1904; *Prostoma graecense*: Reisinger 1926; Reinhardt 1941; *Argonemertes* (*Geonemertes*) *australiensis*: Hickman 1963; *Prosorhochmus adriaticus*: Senz and Tröstl 1999). Organs that are undisputedly of ectodermal origin are the epidermis with the associated cephalic glands, the nervous system and its associated sense organs, the outer (when seen extruded) epithelium of the proboscis, and the rhynchodeum. The esophagus and anus have been reported to be ectodermal derivatives by the majority of authors although there has been disagreement regarding the origin of the esophagus according to older accounts (Barrois 1877; Salensky 1886, 1909, 1912, 1914; Arnold 1898). Of mesodermal origin are the muscular systems, comprising the various body wall muscle layers, the muscle layers and the inner epithelium of the proboscis facing the rhynchocoel, the rhynchocoel epithelium and its underlying muscle layers, as well as the endothelialized blood-vascular system. The intestinal tract exclusive of the esophagus and anus is derived from the endoderm (Fig. 8.2A, B). On the origin and formation of the protonephridia in Nemertea, there has been considerable disagreement as comprehensive data are presently missing.

Epidermis

In Neonemertea there are two generations of epidermal cells that appear sequentially and of which only the second is kept as the definite adult epidermis. The first generation of cells covers the entire body as a larval epidermis in all Pilidiophora. It has been shown to be present in Hoplonemertea according to more recent findings (Maslakova 2010b). While the larval epidermis is kept as intact larval envelope in which the juvenile develops until metamorphosis in Pilidiophora, in Hoplonemertea cells of the larval epidermis are intercalated by definite epidermal cells and later disappear (Maslakova and Malakhov 1999; Maslakova and von Döhren 2009; Maslakova 2010b). In *Pantinonemertes californiensis* larval epidermal cells have been shown to be shed (Hiebert et al. 2010), a mechanism that has also been suspected to occur in the development of *Pantinonemertes* (*Geonemertes*) *agricola* (Coe 1904). Although it has been argued to be homologous to the pilidial larval envelope, the larval epidermis of Hoplonemertea has been given the name "decidula" due to its differing fate (Maslakova 2010b).

A first generation of epidermal cells in paleonemertean species has been hinted at in *Tubulanus punctatus* (Iwata 1960), while in *Carinoma tremaphoros*, there is a population of transitory trochoblast cells forming a distorted preoral belt that has been termed "vestigial prototroch" (Maslakova et al. 2004b). The definite, adult epidermis consists of multiciliated cells, several types of gland cells, basal granular cells, and basal neurites originating from subepidermal neurons constituting a pseudostratified epithelium in paleonemertean and heteronemertean species (Turbeville 1991). In Hoplonemertea, due to the presence of a basal cup cell layer, the pseudostratified appearance of the epidermis is even more pronounced (Norenburg 1985; Turbeville 1991). The heteronemertean dermis is formed late in development, i.e., after metamorphosis, by some gland cells sinking underneath the level of the ciliated epidermis cells. Thus, proximal of the epidermal basal extracellular matrix, a distinct layer of differing extent containing gland cells intermingled with muscle cells and neurons appears to be present at light microscopic resolution (Norenburg 1985).

Nervous System

The nervous system in paleo- and hoplonemertean species seems to have two areas of origin. While the first nervous elements represented by a peripheral plexus are most likely derived from cells that sink in from the apical and the posterior ectoderm of late gastrulae of paleonemertean and hoplonemertean species, the brain and lateral medullary cords have been reported to originate from paired large ectodermal blastomeres bilateral to the apical pit (Salensky 1909, 1914; Hammarsten 1918; Smith 1935; Iwata 1960). The brain primordia sink underneath the epidermis, first forming a pair of narrow epidermal invaginations that soon lose contact to the epidermis and proliferate (Coe 1904; Salensky 1909, 1914; Hammarsten 1918; Smith 1935; Iwata 1960).

The different positions of the brain and lateral medullary cords in different clades have been reported to originate from the depth to which the nervous system rudiments sink in. Remaining in a distal position to mesodermal tissues in *Tubulanus punctatus*, the nervous system rudiments sink in underneath the mesodermal tissues in *Procephalothrix* species and Hoplonemertea (Iwata 1960). From the invaginated cell clusters, the ventral and dorsal brain lobes on either side are formed that are later connected horizontally by the ventral and dorsal commissural tracts (Coe 1904; Salensky 1909, 1914; Hammarsten 1918; Smith 1935; Iwata 1960). Formation of the brain lobes, lateral medullary cords, and ventral commissural tract prior to the dorsal commissural tract has been reported for the monostiliferous hoplonemertean *Prosorhochmus adriaticus* (Senz and Tröstl 1999). In Pilidiophora, the brain and lateral medullary cords are formed independent of the extensive larval nervous system (Salensky 1886, 1912; Maslakova 2010a; Hindinger et al. 2013). The first nervous system rudiment is observable in pilidia as a paired epidermal invagination or delamination on the proximal side of either of the cephalic disks prior to their fusion (Salensky 1886, 1912). With the fusion of the disks, the brain lobes on either side are secondarily connected by formation of both the ventral and later the dorsal commissural tract (Salensky 1912).

In the Desor larva, the brain is formed later in development when the cephalic disks have already fused with each other and with the trunk rudiment. The brain is formed by a uniform, ring-shaped rudiment that later differentiates into the four brain lobes and their connecting commissural tracts. A temporal sequence of development can only be seen by the preceding separation of the ventral commissural tract from the surrounding ectodermal layer (Nusbaum and Oxner 1913). The lateral medullary cords are reported to be outgrowths of the ventral brain lobes in most species. Immunohistochemistry data reveal that the serotonergic neurons of the brain ring start to show immunoreactivity in the ventral lobes and the ventral commissural tract first, while peptidergic (FMRF-like) immunoreactivity is first observed in the dorsal brain lobes and commissural tract. Nervous structures innervating the mouth opening and the esophagus are observable already in larval stages of paleonemertean species. In *Cephalothrix rufifrons* the esophageal nerves have been derived from neuron precursors in the vicinity of the mouth opening, while in *Carinina ochracea* esophageal nerves are putatively outgrowths of the lateral medullary cords (Smith 1935; von Döhren J 2015, unpublished). While paired ectodermal invaginations have been interpreted as the main rudiment of the brain and lateral medullary cords in most species, there have been differing accounts in a few species from all clades.

In the intracapsularly developing species *Argonemertes* (*Geonemertes*) *australiensis* and *Prostoma graecense*, the first nervous system rudiments are represented by a cluster of cells that is not formed by an invagination (Reinhardt 1941; Hickman 1963), while in *Cephalothrix rufifrons* paired groups of cells from the ectoderm in the vicinity of the stomodeum have been suspected to contribute to the ventral brain lobes (Smith 1935). In *Tetrastemma vermiculus* and *Drepanophorus spectabilis*, the brain is reported to originate from a ventrolateral and a dorsolateral pair of epidermal thickenings that disconnect from the epidermis to each form a brain lobe. Connection of the isolated brain compartments is later accomplished by secondarily forming neuroectodermal ridges (Lebedinsky 1897). The same mode of formation has been stated for the lateral medullary cords in the abovementioned species and *Argonemertes* (*Geonemertes*) *australiensis* as well as for the median dorsal nerve of *Drepanophorus spectabilis* (Lebedinsky 1897; Hickman 1963). Likewise, in Pilidiophora, independent rudiments of the dorsal brain lobes from the cephalic disks and the ventral brain lobes as well as the lateral medullary cords from the trunk rudiments have been reported in pilidia by Bürger (1894). The relevance of these rather particular findings, however, has been doubted by several other contemporary authors (Salensky 1912, 1914; Nusbaum and Oxner 1913).

Development of the proboscis nerves or the nerves innervating the sense organs has never been concisely described, but they have been reported to form comparably late during

development in *Prosorhochmus adriaticus* (Senz and Tröstl 1999).

Body Wall Musculature

The body wall muscle layers develop from mesenchymal cells situated between the epidermis and the gut rudiment. In both paleonemertean and hoplonemertean larvae, the sequence of formation of musculature is uniform, the inner longitudinal muscles being differentiated prior to the outer circular layer (Salensky 1914; Iwata 1960). Additional dorsoventral muscles and the musculature of the anterior head region are reported to develop much later in the hoplonemertean species *Prosorhochmus adriaticus* (Senz and Tröstl 1999). There is, however, considerable disagreement over the nature of the mesoderm. In *Tetrastemma vermiculus* and *Drepanophorus spectabilis*, four longitudinal, mesodermal bands originating from four mesendoblasts form all of the body wall and the splanchnic musculature (Lebedinsky 1897). In pilidia, however, there are four somatic rudiments underneath the two cephalic and the two trunk disks, but only one splanchnic mesodermal rudiment surrounding the larval gut formed from initially scattered mesenchymal cells (Salensky 1912).

Classical accounts on the formation of muscle layers in heteronemertean Pilidiophora differ both concerning the origin of muscle layers as well as regarding the sequence of their formation. Hubrecht (1885, 1886) derived the muscle layers in the Desor larva from mesoderm. The first muscle layer to form is the outer longitudinal layer, that is typical of Heteronemertea, followed by the inner circular and longitudinal layers. According to other authors the dermal muscles and the outer longitudinal layer are derived from the proximal layer of the imaginal disks, while the inner circular and longitudinal muscles are derived from the underlying mesenchymal cells (Bürger 1894; Salensky 1912). A similar origin of muscle layers has been reported in the Iwata larva of *Micrura akkeshiensis*, although the sequence of formation of muscle layers is reversed: first, the inner longitudinal and circular layers are formed, while outer longitudinal and dermal muscles have not been seen until 39 days after metamorphosis (Iwata 1958). Recent fluorescent labeling studies have revealed that the sequence of formation of muscle layers is uniform in all species studied. The inner longitudinal body wall muscle layer is formed prior to the outer circular layer. Furthermore, in the Desor larva, the outer longitudinal muscles as well as the dermal muscles develop later during post-larval development. Moreover, these findings argue against an ectodermal origin of the dermal and outer longitudinal muscle layers, since muscle cells of both the dermal and the longitudinal muscle layers are observed prior to the formation of the dermis (von Döhren 2008).

Proboscis Apparatus

Data on the development of the proboscis are only available for neonemertean species (Friedrich 1979; Senz and Tröstl 1999; Maslakova 2010a, b). In paleonemerteans the formation of the proboscis has never been witnessed during development. Therefore, the onset of the formation of the proboscis has been assumed to occur quite late, close to or even after the shift to the benthic lifestyle. While in heteronemertean pilidiophorans the proboscis epithelium is formed from a cone-shaped, proximal process of the fused cephalic disks, it is generally reported to be formed as an epidermal invagination close to the apical organ in hoplonemerteans.

According to classical accounts on pilidia, the cephalic disks fuse and the proboscis is either formed by an invagination of the proximal layer of the head rudiment or by a separate proboscis disk that is incorporated into the head rudiment during fusion of the cephalic disks (Bürger 1894; Salensky 1912; Schmidt 1937). A more recent account shows a different picture (Maslakova 2010a). While the cephalic disks fuse to form the head rudiment by proliferation of their proximal cell layer, the putative proboscis rudiment is represented by a separate cluster of cells underneath the larval epidermis. The arguably mesodermal proboscis rudiment makes its way ventrally to come to lie adjacent to the inner side of the proximal cell layer of the head rudiment. A portion of the proximal layer of the head rudiment invaginates to form a cone-shaped structure that protrudes into the larva so that the putative proboscis rudiment sits on it like a shallow cap, forming a compound proboscis bud. It

has been reported that in the proboscis bud, the cone-shaped structure merely forms the ectodermal components, i.e., the outer epithelium and the gland cells of the proboscis. Its muscular layers, the inner epithelium, as well as the rhynchocoel epithelium and the rhynchocoel muscles originate from the cap-like putative proboscis rudiment by means of schizocoely. The rudiment of the proboscis apparatus elongates on the dorsal side of the esophagus until it reaches the level of the larval gut. It consists of two components. An outer tube-shaped structure, the forming rhynchocoel wall, is clearly separated from an inner, invaginated structure representing the developing proboscis (Maslakova 2010a). After elongation of the proboscis, the dorsal margin of the trunk rudiment extends anteriorly over the larval gut and the proboscis rudiment.

The hypothetical homology of the nemertean rhynchocoel with secondary body cavities of other coelomate spiralians is said to be supported by the mode of its development (Maslakova 2010b). The hypothesis of a schizocoelous development of the rhynchocoel, however, demands a complete fusion of the ectodermal cone with the mesodermal cap of the proboscis bud. The components of the proboscis rudiment, however, seem to be separated in all stages investigated; schizocoelous processes within the mesodermal component have not been reported (Maslakova 2010a). In the Desor larva, the proboscis epithelium develops from an independent proboscis rudiment, while its musculature, the rhynchocoel and the rhynchocoel muscles, are derived from the mesoderm. It forms a continuous layer around the proboscis to separate into a proximal layer forming the proboscis muscles and the inner proboscis epithelium and an outer layer developing into the rhynchocoel epithelium and the associated muscle layers. In the part where no separation of the layers occurs, the retractor muscle develops (Arnold 1898; Nusbaum and Oxner 1913). Whether this separation is by delamination or schizocoely is not reported. Fluorescent labeling of F-actin reveals that the formation of the proboscis musculature precedes that of the rhynchocoel in the Desor larva of *Lineus viridis* (von Döhren J 2015, unpublished). In the Iwata larva of *Micrura akkeshiensis*, the proboscis rudiment is derived from an invagination of the secondary epidermis, and its formation corresponds to the classical description on the development of the proboscis in the pilidium larva (Iwata 1958).

In hoplonemerteans the proboscis is formed from an epidermal invagination. In *Malacobdella grossa*, *Emplectonema gracile*, *Oerstedia dorsalis*, *Gononemertes australiensis*, *Carcinonemertes epialti*, and *Paranemertes peregrina*, the proboscis rudiment detaches from the epidermis, while in *Drepanophorus spectabilis*, *Tetrastemma vermiculus*, *Prosorhochmus viviparus*, and *Pantinonemertes* (*Geonemertes*) *agricola*, the connection to the epidermis persists (Lebedinsky 1897; Coe 1904; Salensky 1914; Hammarsten 1918; Iwata 1960; Egan and Anderson 1979; Stricker and Reed 1981). In the former group of species (except *Paranemertes peregrina*), the rhynchodeum is formed by an independent invagination located more frontally, in some species underneath the apical pit, while in the latter group the rhynchodeum is differentiated from the anterior part of the epidermal proboscis rudiment invagination. The tripartite organization of the armed hoplonemertean proboscis becomes apparent by a strong stylet bulb with a narrow canal separating an anterior tube shaped from a posterior sacculate portion. In the area of the stylet bulb, the stylet armature is later formed (Bürger 1895; Coe 1904; Stricker and Reed 1981; Stricker and Cloney 1982; Stricker 1985; Senz and Tröstl 1999). While the mesodermal components of the proboscis apparatus are derived from the surrounding mesodermal cells in most accounts by delamination of a mass of mesodermal cells, Lebedinsky (1897) identified a dorsal and a ventral mesodermal strip independent of the somatic mesoderm that form from paired mesoblast cells at the junction of the proboscis invagination in *Drepanophorus spectabilis* and *Tetrastemma vermiculus*. The rudiments become hollow, associate with the proboscis invagination, grow around it, and fuse. The inner wall of the fused mesodermal rudiment forms the proboscis musculature and its inner epithelium, the outer rhynchocoel wall, and the associated muscle layers. The hollow space between the two-layered rudiment represents the rhynchocoel.

Alimentary Canal

The intestinal tract in nemerteans is a one-way through-gut. It comprises the midgut and its derivatives as well as a histologically different foregut. An extensive hindgut connecting the midgut with the anus is absent (Bürger 1895; Gibson 1972). The midgut and its derivatives develop from the embryonic endoderm. In species with gastrulation by invagination, the gut persists in the larva as a hollow cavity, while in species that gastrulate by polar ingression, the midgut rudiment is regularly a solid mass of cells that establish the gastric cavity later during development (Friedrich 1979). In paleonemerteans and hoplonemerteans, the future mouth is moved to the future ventral side by accelerated growth of the dorsal side of the body, while in canonic pilidiophoran development, it remains in its original position (Iwata 1957, 1960, 1985; Friedrich 1979; Maslakova 2010a, b). During and following gastrulation, parts of the ectoderm have regularly been reported to be dragged inside the gastrula with the blastopore to form the ectodermally derived esophagus (Friedrich 1979). In paleonemertean species and Pilidiophora that develop via a pilidium, the blastopore remains open, marking the connection between the esophagus and the blindly ending, sac-like midgut. All or most of the larval intestinal tract is taken over from the larval to the juvenile organization. According to Salensky (1912), the esophagus of the pilidium is divided into a distal and a proximal part by a constrictor muscle in later-stage larvae. During metamorphosis only the proximal part is taken over into the juvenile; the distal part is shed along with the pilidial envelope. In the Iwata larva of *Micrura akkeshiensis*, the blastopore remains open although no food is taken up. The definite mouth opening and esophagus develop from a rudiment composed of multilayered cells located in the dorsal wall of the stomodeum. By elongating in a slight curve, the rudiment becomes the esophagus of the juvenile. The stomodeal part distal of the esophageal rudiment is shed along with the larval epidermis during metamorphosis (Iwata 1958).

During intracapsular development of *Lineus ruber* and *Lineus viridis*, the esophagus development differs markedly. While in the former species the esophagus develops early and retains its functionality, the esophagus in the latter is reported to be separated from the midgut. A definite connection is formed late, i.e., after metamorphosis, by a secondary esophagus from two groups of cells located laterally near the mouth opening (Arnold 1898; Nusbaum and Oxner 1913; Schmidt 1964).

The development of the mouth opening and pharynx in hoplonemerteans is much more diverse and complicated. In general, the blastopore is said to be closed early in development. In some species (e.g., *Emplectonema gracile*, *Oerstedia dorsalis*), the blastopore reopens and a functioning larval gut is formed (Iwata 1960). In other species the larval gut is formed by a secondary invagination of the epidermis, forming the esophagus in a more anterior position than the blastopore (*Drepanophorus spectabilis*, *Tetrastemma vermiculus*, *Malacobdella grossa*, *Paranemertes peregrina*) (Lebedinsky 1897; Hammarsten 1918; Maslakova and von Döhren 2009). In *Tetrastemma vermiculus* and *Drepanophorus spectabilis*, the blastopore remains open even after the secondary esophagus has formed. It has been reported to close later so that the remaining pouch develops into the intestinal cecum present in most hoplonemerteans (Lebedinsky 1897). While the mouth and proboscis pore are separate in most polystiliferan hoplonemerteans, in monostiliferan hoplonemerteans the larval mouth subsequently closes, and the esophagus gains connection with the rhynchodeum forming the rhynchostomodeum that is typical of this clade. In *Prosorhochmus viviparus*, *Prostoma graecence*, *Argonemertes* (*Geonemertes*) *australiensis*, and *Gononemertes australiensis*, neither a reopening nor a secondary esophagus forms, resulting in a midgut that is closed for most of the time of the development (Salensky 1909, 1914; Hickman 1963; Egan and Anderson 1979). A functioning intestinal opening is accomplished by fusion of the midgut with the ventral part of the rhynchodeum.

The fusion of the esophagus with the rhynchodeum is accomplished in various ways. While in *Emplectonema gracile*, *Oerstedia dorsalis*, and *Gononemertes australiensis* the esophagus and the proboscis rudiments gain access to the independently invaginated rhynchodeum, the esophagus

fuses to the distal part of the proboscis rudiment that later becomes the rhynchodeum in *Prosorhochmus viviparus* (Salensky 1909, 1914; Iwata 1960; Egan and Anderson 1979). In *Tetrastemma vermiculus*, *Drepanophorus spectabilis*, and *Pantinonemertes* (*Geonemertes*) *agricola*, the anterior-most part of the proboscis rudiment forms a ventral invagination that fuses with the intestine developing into the adult esophagus (Lebedinsky 1897; Coe 1904). In *Paranemertes peregrina*, the proboscis rudiment gains its connection prior to the closure of the larval mouth opening (Maslakova and von Döhren 2009). In the aberrant commensal *Malacobdella grossa*, there is no true rhynchodeum. The proboscis rudiment connects to the esophagus and the larval mouth is closed. A secondary mouth opening is formed anterior of the larval mouth (Hammarsten 1918). Apart from the disparate development in *Malacobdella grossa*, there is no conceivable reason for the diversity shown in esophagus development within the otherwise relatively uniform clade of Hoplonemertea. It is therefore very likely that the diversity shown in the development of the esophagus and rhynchostomodeum depends rather on the different researchers than on diverging lines of development (Friedrich 1979).

In some hoplonemerteans, an anus is formed early in development by means of a caudal epidermal invagination or ingression (Lebedinsky 1897; Hammarsten 1918; Iwata 1960; Friedrich 1979; Maslakova 2010b). Apparently, in the majority of hoplonemerteans, as well as in Pilidiophora and paleonemertean species studied, the anus is formed much later as the formation of an anal opening has not been reported in these. Initially, the intestinal tract is an undifferentiated tube. The morphological differentiation of the alimentary canal in hoplonemerteans comprising the ectodermal esophagus, stomach, and pyloric tube as well as the mesodermal intestinal ceca and lateral diverticula forms comparatively late (Friedrich 1979; Senz and Tröstl 1999).

Nephridia

Excretory organs have been observed to develop early in paleonemertean larvae of *Carinoma mutabilis* and *Procephalothrix oestrymnicus* as simple protonephridia anterior of the mouth opening (Bartolomaeus et al. 2014). They consist of few (two to three) multiciliated terminal cells constituting the site of ultrafiltration and two to three multiciliated cells that form the nephroduct which modifies the ultrafiltrate. Distally, the nephroduct opens through the epidermis at the level of the mouth opening via a nephropore cell in both species (Bartolomaeus et al. 2014). The protonephridia develop from a subepidermal rudiment underneath the trochoblast cells and are fully formed prior to the degeneration of the so-called vestigial prototroch (Bartolomaeus et al. 2014). In hoplonemertean species protonephridia have been observed in larvae but in a more posterior position behind the mouth opening. Nothing is known about the ultrastructure of the protonephridia in hoplonemertean larvae. Structure, position, and time of development of the protonephridia in paleonemerteans are reminiscent of the "head kidneys" of the trochophore larva, although it is not clear whether the nemertean protonephridia share the same fate as transitory organs that are restricted to the larval organization.

The protonephridia of hoplonemertean larvae correspond in position to the respective organs in the adults. Therefore, it is very probable that the excretory organs in hoplonemerteans represent early developmental stages of the adult organs being elaborated during development to attain the adult morphology. In Pilidiophora, branched protonephridia located slightly anterior of the midgut have been observed as early as 2 weeks after fertilization (von Dassow and Maslakova 2013). In later stages the protonephridia become sandwiched between the intestinal tract and the distally developing juvenile rudiment (Maslakova 2010a). In *Lineus viridis* the first protonephridia were observed in the juvenile after metamorphosis. At this time no trace of the blood-vascular system can be observed. The protonephridia are branched structures with a single terminal cell on the proximal end of each branch. Initially monociliated, the terminal cell becomes soon multiciliated. The nephroduct is intercellular, and the nephropore is formed by four specialized

epidermal cells (Bartolomaeus 1985). Since the first protonephridia formed correspond in structure and position to those seen in the adults, it can be assumed that protonephridia in Pilidiophora are definite persisting adult organs (Bartolomaeus et al. 2014). Although it has been shown that contrary to classical accounts the protonephridia in the pilidium are not derived from bilateral invaginations of the esophagus, their origin as invaginations of the subumbrellar epidermis could not be substantiated either (Hubrecht 1885, 1886; Arnold 1898; Salensky 1912; Maslakova 2010a).

In the Iwata larva of *Micrura akkeshiensis*, the origin of the protonephridia has been identified as a pair of cell groups located in the ventral wall of the stomodeum on the level of the juvenile mouth opening (Iwata 1958). Their further development, however, has not been followed. While the abovementioned data hint at an ectodermal origin of the protonephridia, a mesodermal origin has been hypothesized for the protonephridia in the Desor larva. The rudiment is represented by a cluster of cells which later forms narrow tubular structures situated between the esophagus, the cerebral organs, and the ventral rhynchocoel wall (Nusbaum and Oxner 1913). Further development and opening of the nephridial rudiments to the exterior have not been observed.

Sensory Organs

Sensory organs comprise cerebral sense organs present in many paleonemertean, nearly all pilidiophoran and most hoplonemertean species; frontal organs and eyes, both of which are present in the majority of Neonemertea; and lateral organs that are confined to some tubulanid species (Gibson 1972). Cerebral organs differ in their complexity and position relative to the brain in different taxa. Cerebral organs comprise a ciliated, blind ending canal, running from the epidermis proximally to end in a mass of neuronal tissue that is connected via a nerve to the brain. While they are comparably small, simple canals situated in front of the brain in Monostilifera or behind the brain in *Tubulanus* and *Carinina* species, cerebral organs are large, compound organs comprising ciliated cells, gland cells, and neurons located behind the brain in Pilidiophora and reptant Polystilifera. In the heteronemertean pilidium and the Desor larva, the cerebral organs develop from the cerebral organ disks, a paired rudiment invaginated from the subumbrellar larval ectoderm. They are situated bilaterally between the frontal cephalic disks and the posterior trunk disks and are the last of the invaginated rudiments to form in the respective larvae. The cerebral organ disks first fuse posteriorly with the trunk disks and subsequently with the already fused head rudiment. The invagination of the original disks is retained to later form the cerebral organ canal, while gland cells and neurons form in the proximal portion of the rudiment. The neurons connect to the developing dorsal brain lobes already before the juvenile body has completely formed (Salensky 1912; Nusbaum and Oxner 1913; Maslakova 2010a; Hindinger et al. 2013). Although nothing is known about the formation of cerebral organs in non-heteronemertean Pilidiophora such as *Hubrechtella* species, it can be assumed that the process is similar as described in the heteronemertean pilidium judging from the corresponding larval type and the morphological similarity of the cerebral organs in the respective taxa. In the Iwata larva, the cerebral organs are not derived from paired epidermal larval invaginations but from paired lateral invaginations of the stomodeum. The connection to the stomodeum is soon obliterated, and the cerebral organs open secondarily into the cephalic furrow formed in the cephalic rudiment. A connection to the dorsal brain lobes is accomplished prior to metamorphosis (Iwata 1958). Cerebral organs in larvae of hoplonemertean species have been reported to develop from a pair of narrow invaginations of thickened epidermal areas that can be observed early in development during the larval phase (Lebedinsky 1897; Iwata 1960; Maslakova and von Döhren 2009). In *Paranemertes peregrina* and *Emplectonema gracile*, they are situated bilaterally on both sides of the larva on the level of the mouth opening, while in *Tetrastemma vermiculus* and *Drepanophorus spectabilis*, the epidermal invaginations have been reported to be

located between the dorsal and the ventral brain lobes (Lebedinsky 1897; Iwata 1960; Maslakova and von Döhren 2009). Epidermal invaginations that have initially been interpreted as cerebral organ rudiments in some hoplonemertean species have later been interpreted as invaginations that were hypothesized to be homologous to the imaginal disks of Pilidiophora (Maslakova 2010b). In the direct developing hoplonemertean *Prosorhochmus adriaticus*, the internal nervous and glandular portion of the cerebral organ forms first, while its connection to the exterior via the cerebral organ canal and pore opening in the cephalic furrows is established later in development (Senz and Tröstl 1999). In *Malacobdella grossa* larval cerebral organ rudiments have been suspected although the adult does not possess cerebral organs. The cerebral organ rudiments comprise paired epidermal cell clusters that are situated a little posterior of the mouth opening. After being invaginated they give off some neuronal cells to contribute to the ventral brain lobes. The remainder of the rudiment disappears shortly after (Hammarsten 1918). Although cerebral organs exist in some paleonemertean species, neither their formation nor any rudiments have ever been observed (Iwata 1960). While this appears logical in species lacking cerebral organs, it is somewhat astonishing judging from the fact that cerebral organ rudiments are formed early in other nemertean species. Moreover, recent phylogenetic analyses suggest cerebral organs to be an ancestral character in Nemertea that was reduced repeatedly (Thollesson and Norenburg 2003; Andrade et al. 2012). It would therefore be conceivable to interpret the bilateral apical invaginations that had been attributed as rudiment of the brain in paleonemertean species as a joint rudiment giving rise to not only the brain and lateral medullary cords but also to the cerebral organs. In species that do not possess cerebral organs as adults, e.g., *Procephalothrix simulus*, they are reduced later in development.

In Hoplonemertea and some paleonemertean species, the eyes are formed during the larval phase (Fig. 8.6A–C; Iwata 1960; Stricker and Reed 1981; Martindale and Henry 1995; Norenburg and Stricker 2002; Maslakova et al. 2004b; Chernyshev 2008; Maslakova and von Döhren 2009). But while the eyes in the larva are transitory in most paleonemertean species, they persist and become more in number in Hoplonemertea (Fig. 8.4C). In Heteronemertea pigmented eyes are confined to the juvenile rudiment; no larval type of Pilidiophora is reported to have eyes (Fig. 8.6D; Cantell 1969; Norenburg and Stricker 2002; von Döhren and Bartolomaeus 2007). The eyes in neonemertean species are subepidermal, rhabdomeric eyes (Jespersen and Lützen 1988; von Döhren and Bartolomaeus 2007; von Döhren 2008). In *Lineus viridis* the eyes develop from an unpigmented, subepidermal rudiment comprising a small number of cells of two types. A bundle of rhabdomeric receptor cells is surrounded by undifferentiated, unpigmented corneal progenitor cells. From the latter type of cells, the closed optical cavity is formed that houses the receptor cells. Pigmented cells that form a pigment cup to one side are formed as the eye begins to function. The eye spot enlarges as the number of all cell types increases (von Döhren and Bartolomaeus 2007). Contrary to the statement that the eyes in Heteronemertea form by fragmentation of existing eyes, additional eyes in *Lineus viridis* juveniles are formed de novo in the same manner as described above (Gontcharoff 1960; von Döhren and Bartolomaeus 2007).

The frontal sensory organ is typical of Neonemertea being represented by a single protrusible epidermal pit in most species, while comprising three triangularly arranged protrusible pits in lineid heteronemertean species (Gibson 1972). The frontal organ is commonly associated with the head glands that discharge their glandular products through canals opening between the epithelial cells of the frontal organ. In some species the head glands open independently of the frontal organ by numerous smaller ducts to the epidermis (Gibson 1972). According to Lebedinsky (1897), the frontal organ develops from the apical pit of the larva in *Drepanophorus spectabilis* and *Tetrastemma vermiculus*, although this has been doubted by other authors (Bürger 1897–1907; Hammarsten 1918). In *Malacobdella grossa* the

apical pit gives rise only to the cephalic glands (Hammarsten 1918).

Various sensory structures are restricted to certain lineages, such as tactile cirri and statocysts in *Ototyphlonemertes* species; lateral sensory organs in some *Tubulanus*, *Callinera*, and *Micrella* species; or so-called integumentary and subcutaneous organs of pelagic Polystilifera (Coe 1927; Gibson 1972, 1982). There are no data available about the development of these organs.

Blood-Vascular System

The development of the blood-vascular system occurs comparably late in Nemertea after the majority of musculature has already formed. While, according to classical accounts, the blood vessels in Pilidiophora precede metamorphosis, no trace of blood vessels could be found in the postmetamorphic juvenile of *Lineus viridis* according to more recent data (Bürger 1897–1907; Nusbaum and Oxner 1913; Bartolomaeus 1985). There is no account on formation of the blood vessels in paleonemertean species, while in Hoplonemertea data are only available for species with intracapsular development (Salensky 1914; Reinhardt 1941; Turbeville 1986). In classical accounts, the blood vessels have either been derived from gaps remaining or been reopened in the blastocoel or from postulated embryonic coelems (e.g., Bürger 1897–1907; Salensky 1914; Nusbaum and Oxner 1913). The problem in these classical accounts is that the blastocoel was considered to be devoid of matrix or that the matrix within it was later liquefied. This led to the misinterpretation that nemerteans possess extensive primary and/or secondary body cavities. Instead, nemerteans should be considered as largely compact with the exception of the rhynchocoel and the blood vessels that represent secondary body cavities (Gibson 1972; Turbeville and Ruppert 1985; Turbeville 1991). More recent data on two hoplonemertean species suggest that the blood vessels are formed in the parenchymatous tissue underlying the body wall musculature by forming solid bands of cells (Reinhardt 1941; Turbeville 1986). According to light microscopic data in *Prostoma graecense*, the bands of radially arranged cells become hollow by resorbing encased yolk material (Reinhardt 1941). Ultrastructural data on *Prosorhochmus americanus*, however, provide a different picture (Turbeville 1986). The solid bands of the blood vessel rudiments become hollow by a process that is reminiscent of the schizocoelous mode of formation observed in annelid coelomic cavities. In contrast to annelid schizocoely, the cells of the nemertean blood vessels acquire intercellular junctions after the onset of cavitation, whereas the respective intercellular junctions in annelids are formed at the onset of cavitation (Turbeville 1986; Koch et al. 2014). Due to these differences, the question of homology of these laterally located endothelialized, hollow compartments to the secondary body cavities found in Trochozoa can be at best preliminarily answered; more comparative data on the development of the blood vessels in other nemertean taxa are needed to consolidate this hypothesis.

In summary, the development of Nemertea is generally working on the regular spiralian scaffold; although in organs that represent interfaces of two germ layers (e.g., proboscis, foregut, nephridia), there is considerable disagreement over their respective origin and formation. Until more comparative data are collected, concise statements regarding the evolution of organ system development in Nemertea cannot be made.

GENE EXPRESSION

Gene expression studies in development of Nemertea are presently scarce and restricted to the pilidiophoran species *Cerebratulus lacteus*, *Lineus viridis*, *Micrura alaskensis*, and *Ramphogordius* (*Lineus*) *sanguineus*. The data available refer to the expression of *β-catenin*, a trochoblast-specific tubulin-4 gene from the basal gastropod *Patella vulgata*, homeobox-containing genes (Hox genes, as well as *Otx,* and *Cdx*), genes encoding photopigments (*opsins*), and genes of the so-called retinal determination gene network (RDGN) (*Pax-6*, *Six1/2*, *Six3/6*, *Six4/5*, *Dach*) (Loosli et al. 1996; van den Biggelaar et al. 1997; Klerkx 2001; Charpignon 2007; Henry et al. 2008; Döring 2012; Hiebert and Maslakova 2015).

Axis Specification Genes

A transcriptomic assessment of the pilidiophoran *Micrura alaskensis* revealed a single Hox cluster comprising nine Hox genes while only six to seven Hox genes have been identified by genomic screening of the pilidiophoran *Ramphogordius (Lineus) sanguineus* (Kmita-Cunisse et al. 1998; Hiebert and Maslakova 2015). In *Micrura alaskensis (Ramphogordius (Lineus) sanguineus)*, the genes of the Hox gene cluster have been identified as *MaLab (LsHox1)*, *MaPb*, *MaHox3 (LsHox3)*, *MaDfd (LsHox4)*, *MaScr, MaLox5 (LsHox6), MaAntp (LsHox7)*, *MaLox4*, and *MaPost2 (LsHox9).* In *Ramphogordius (Lineus) sanguineus* a seventh putative Hox gene, *LsHox8* that is possibly dislocated from the chromosomal Hox gene region was found using an alternative set of primers (Kmita-Cunisse et al. 1998). Data on the expression of Hox genes during development are only available for *Micrura alaskensis* (Hiebert and Maslakova 2015). Hox gene expression largely complies both temporally and spatially to the canonical bilaterian fashion, albeit with noteworthy exceptions: All pilidial tissues are completely devoid of Hox genes expression during all stages of development. Hox gene expression in *Micrura alaskensis* does not start before the trunk disks are formed. It then proceeds posteriorly. Thus the anterior part of the juvenile comprising the cephalic disks and the cerebral organ disks as well as the later developing head rudiment and the proboscis develop without showing Hox gene expression. The absence of Hox gene expression in the pilidial tissues has been interpreted as an indication of a functional decoupling of the early, larval from the later juvenile development phases (Hiebert and Maslakova 2015). While giving a possible explanation of the diversity of pilidial morphotypes the genetic mechanism for the patterning of the pilidial envelope remains unclear. Preliminary results of inhibition studies hint at an involvement of the *Wnt* and the *fibroblast growth factor* pathways in patterning the early larval shape (Hiebert and Maslakova 2015). Recent findings indicate that *β-catenin*, a downstream component of the Wnt pathway, is both necessary and sufficient to promote endoderm fates in the vegetal cells during cleavage of *Cerebratulus lacteus* (Henry et al. 2008). *β-catenin* is expressed only in the vegetal-most quartet at each cleavage division and is passed on through the cleavage cycles to finally end up in the blastomeres forming the endoderm. Experimental overexpression leads to vegetalized embryos that fail to form typical, anteriorly positioned apical tufts, while blocking of *β-catenin* leads to animalized larvae with supernumerary apical tufts. Animalized larvae gastrulate only to a limited extent. It has been hypothesized that the animal fates of blastomeres represent the default condition which is shifted to vegetal fates under the influence of *β-catenin* (Henry et al. 2008). A trochoblast-specific tubulin gene (*tub4*) was assessed for its expression in the cells that form the marginal band of elongated cilia on the lappets around the pilidium of *Cerebratulus lacteus* (van den Biggelaar et al. 1997; Klerkx 2001). However, no expression signal was recorded in cells bearing elongated cilia, underpinning the hypothesis that the marginal band of cells bearing elongated cilia is not homologous to the prototroch of the trochophore (van den Biggelaar et al. 1997; Maslakova 2010a). An Otx-class and a Cdx-class gene were identified in *Ramphogordius* (*Lineus*) *sanguineus* (*Ls-Otx*, *Ls-Cdx*) (Kmita-Cunisse et al. 1998; Charpignon 2007). A *Cdx* ortholog (*MaCdx*) has also been found in *Micrura alaskensis* (Hiebert and Maslakova 2015). *Ls-Otx* was found to be expressed during the development of postmetamorphic juvenile *Lineus viridis* (Fig. 8.9A; Charpignon 2007). First, it is expressed in the entire brain ring and the anterior-most part of the lateral medullary cords, the cerebral organs and their canals, and additionally, but weaker, in the gut. In older stages there is an *Ls-Otx* expression in the frontal organ and in the frontal organ nerves. Expression in the brain and cerebral organs becomes weaker, although in the cerebral organ canals, *Ls-Otx* is still clearly expressed. At this stage of development, no expression signal is detectable in the lateral medullary cords or the gut (Charpignon 2007). *Ls-Cdx* is strongly expressed in *Lineus viridis* juveniles consistently

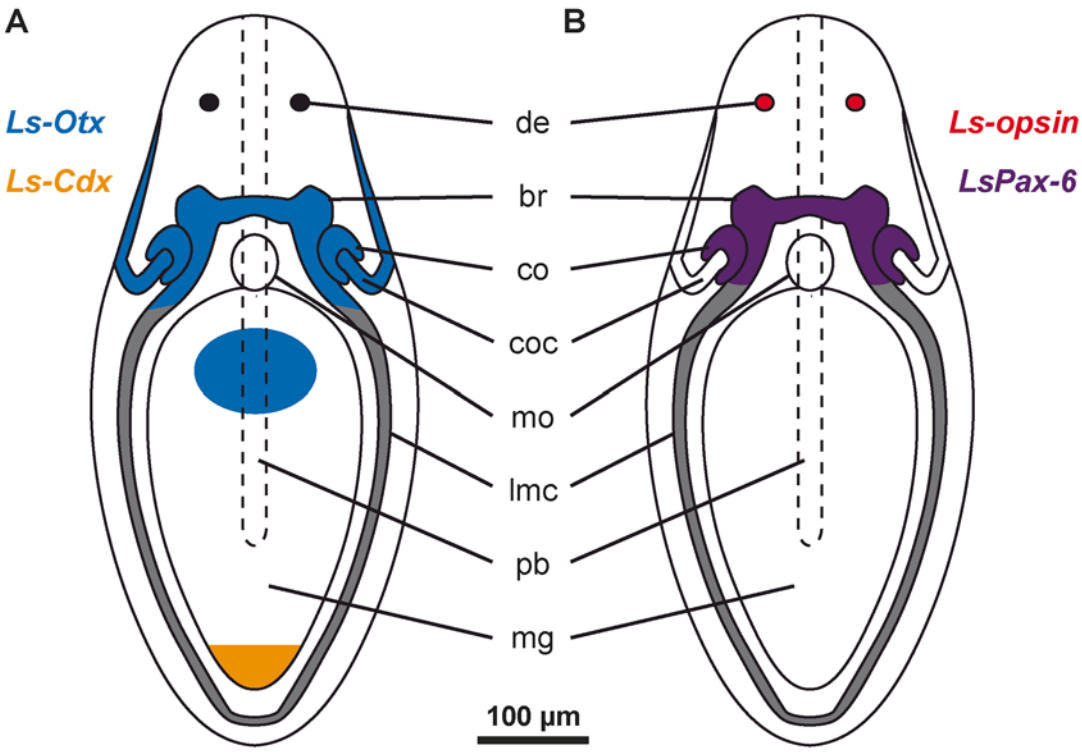

Fig. 8.9 Schematic representation of gene expression of several genes from *Ramphogordius* (*Lineus*) *sanguineus* in postmetamorphic juveniles of *Lineus viridis*. (**A**) Expression of *Ls-Otx* and *Ls-Cdx*. (**B**) Expression of *Ls-opsin* and *LsPax-6*. Note: *LsPax-6* is not expressed in the developing eyes of *Lineus viridis*. *br* brain ring, *co* cerebral organ, *coc* cerebral organ canal, *de* developing eye, *lmc* lateral medullary cord, *mg* midgut, *mo* mouth opening, *pb* proboscis rudiment

throughout development in the posterior-most part of the body. The signal is located in internal tissues, arguably in the developing intestine (Fig. 8.9A; Charpignon 2007). In *Micrura alaskensis MaCdx* is first expressed in the trunk disk being shifted posteriorly during the course of development to be finally located as a ring shaped pattern around the base of the developing caudal cirrus. In this species however, expression of *MaCdx* in the intestinal tissue is absent (Hiebert and Maskakova 2015).

Retinal Determination Gene Network Genes

The most extensive expression data have been gathered on the eye development in *Lineus viridis* (Loosli et al. 1996; Charpignon 2007; Döring 2012). One opsin gene has been isolated from *Ramphogordius* (*Lineus*) *sanguineus*, while two opsin genes have been found in *Lineus viridis* (Charpignon 2007; Döring 2012). The former, *Ls-opsin*, represents an unusual G-protein-coupled-receptor (GPCR) opsin that does not cluster with canonical rhabdomeric-type opsins. Its expression in *Lineus viridis* is restricted to the developing eyes, while expression in adult eyes is absent (Fig. 8.9B; Charpignon 2007). The opsins identified in *Lineus viridis* (*LiVi-ops1*, *LiVi-ops2*) cluster with peropsins and photoisomerases (*LiVi-ops1*) or in a basal position to these together with *opsin5* (*LiVi-ops2*) (Döring 2012). Although a G-protein alpha subunit Q gene (*LiVi-Gq*) was also found in the same species, expression experiments for any of the three genes were unsuccessful (Döring 2012). Genes marking

the canonical neurotransmitters employed by photoreceptor cells of *Platynereis dumerilii*, the vesicular acetylcholine receptor gene in larval eyes, and the vesicular glutamate receptor gene in adult eyes have both been extracted from juveniles of *Lineus viridis* (*LiVi-vacht* and *LiVi-vglut*, respectively). Expression data on these genes in *Lineus viridis* have not been reported (Döring 2012).

Expression of genes of the RDGN during development was studied in *Lineus viridis* and includes the genes *LsPax-6* and *Lv-Six1/2*, *Lv-Six3/6*, *Lv-Six4/5*, and *Dach*, although for the latter an antibody reaction was studied instead of in situ hybridization (Loosli et al. 1996; Charpignon 2007). In *Micrura alaskensis* a *Six3/6* ortholog (*MaSix3/6*) was found to be expressed during early larval development (Hiebert and Maslakova 2015). *LsPax-6* is expressed in *Lineus viridis* during postmetamorphic development in the brain and the cerebral organs, but not in the eye region (Fig. 8.9B; Loosli et al. 1996; Charpignon 2007). Due to differential splicing and a thus changed open reading frame, two isoforms have been hypothesized to exist. One of them is shorter, missing the PST domain. The possible role of this shorter isoform has been suspected to be differential regulation of developmental processes. However, there is no proof of the existence of the theoretical isoform (Charpignon 2007). Of the remaining genes assessed, only *Lv-Six1/2* and the monoclonal *Drosophila melanogaster* antibody against *Dach* show an expression of these genes in the developing eyes of *Lineus viridis*. The former gene is also expressed in the lateral medullary cords near the brain and in the frontal organs. *Lv-Six3/6* also shows expression in the frontal organ, but its strongest expression is observed in the brain lobes. Expression of *MaSix3/6* is restricted to early developmental stages of *Micrura alaskensis*. In the blastosquare stage several cells on one pole of the embryo are labelled while in the feeding pilidium stage some expression signals are detectable near the apical pit along with few additional signals situated in the anterior region of the developing lateral lappets (Hiebert and Maslakova 2015). In *Lineus viridis Lv-Six4/5* is expressed in the posterior region of the developing brain and the cerebral organs but also in two bilateral stripes running from the brain lobes anteriorly. A correspondence with the nerves connecting the eyes with the brain seems likely (Charpignon 2007).

Other Genes

Genes that have been identified in *Ramphogordius* (*Lineus*) *sanguineus* but have not been subjected to expression studies include *Ls-Bmp2/4*, *Ls-Engrailed*, *Ls-Msx*, *LsNK*, *LsPax-2/5/8*, *Ls-Snail*, and *Ls-Twist* (Charpignon 2007). Gene expression of the canonical developmental pathways is very limited both with respect to the gene products involved and to the diversity of developmental trajectories found in Nemertea. Currently, a sound assessment of gene expression networks in Nemertea is impossible.

OPEN QUESTIONS

- How does the high yolk content in Pelagica oocytes influence embryonic cleavage?
- How are the dorsal quadrant and the mesoderm precursor cell specified in Paleonemertea and Hoplonemertea?
- Is there an apical neuronal structure that is homologous to the apical organ of Trochozoa in larvae of Nemertea?
- How did the larval and adult eyes in Nemertea evolve?
- What is the developmental origin of nemertean protonephridia?
- What is the developmental fate of the first formed protonephridia – transitory larval or definite adult organs?
- Where does the adult mesoderm originate from – entirely endomesodermal or with ectodermal components?
- Is there a taxon-specific mode of muscle formation in Nemertea?
- How do the different components of the proboscis and rhynchocoel develop?
- Is there a generalizable mode of foregut formation in Hoplonemertea?

- How does the blood-vascular system develop in Paleonemertea and Pilidiophora?
- How are key developmental regulators such as Hox and ParaHox genes expressed in the various nemertean subclades?

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