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How to make a protostome

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Review

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Abstract.   The origin and radiation of the major metazoan groups can be elucidated by phylogenomic studies, but morphological evolution must be inferred from embryology and morphology of living organisms. According to the trochaea theory, protostomes are derived from a holoplanktonic gastraea with a circumblastoporal ring of downstream-collecting compound cilia (archaeotroch) and a nervous system comprising an apical ganglion and a circumblastoporal nerve ring. The pelago-benthic life cycle evolved through the addition of a benthic adult stage, with lateral blastopore closure creating a tube-shaped gut. The archaeotroch became differentiated as prototroch, metatroch and telotroch in the (trochophora) larva, but was lost in the adult. The apical ganglion was lost in the adult, as in all neuralians. Paired cerebral ganglia developed from the first micromere quartet. The circumblastoporal nerve became differentiated into a pair of ventral nerve cords with loops around mouth (the anterior part of the blastopore) and anus. Almost all new information about morphology and embryology fits the trochaea theory. The predicted presence of a perioral loop of the blastoporal nerve ring has now been demonstrated in two annelids. Alternative 'intercalation theories' propose that planktotrophic larvae evolved many times from direct-developing ancestors, but this finds no support from considerations of adaptation.

Introduction

The name Protostomia was introduced by Grobben (1908) for the group called Zygoneura by Hatschek (1888). Its most conspicuous characteristic (apomorphy) is the paired ventral nerve cord, which is reflected both in Hatschek's name (meaning paired [ventral] nerve) and in alternative names, such as Hypogastrica (Goette 1902), Hyponeuralia (Cuénot 1940) and Gastroneuralia (Ulrich 1951). The monophyly of the group is well supported by characters from adult morphology, embryology and Hox genes (Nielsen 2012) and from phylogenomic analyses and microRNAs (Erwin *et al.* 2011). Also, the 'D-quadrant cleavage' (Nielsen 2012) with the postero-dorsal blastomere of the 4-cell stage giving rise to the germ cells, and in most cases to the mesoderm, seems to be a protostome apomorphy.

Molecular phylogenies can serve as hypotheses for the relationships between clades, but only morphology can give information about evolution from the early ancestors to the living groups.

Several early authors had pointed to the similarities between the 'trochophora' larvae of several main bilaterian groups, and Hatschek (1891) elaborated this in his trochophora theory, which proposed that this larval type was present in the common ancestor of the protostomes (his Zygoneura). The conspicuous ciliary bands were characterised by their positions and functions: prototroch (preoral ciliary ring or trochus: locomotory and feeding), adoral ciliary zone (feeding), metatroch (postoral ciliary ring or cingulum: feeding) and telotroch (perianal ciliary ring: locomotory). The central nervous system of the

trochophore should comprise an apical organ (no distinction between apical and cerebral ganglia) and paired ventral nerves. The origin of the protostomes from a 'protrochula' (resembling a trochaea, see below) was briefly suggested, but the textbook unfortunately remained incomplete.

Goette (1902) was apparently the first to explicitly propose that the protostomian ancestor evolved from a gastraea through anterior–posterior elongation of the blastopore, with the oval or slit-like blastopore representing the ventral side with anterior mouth and posterior anus (Hypogastrica). This idea was further elaborated by Woltereck (1904*b*), and Grobben (1908) proposed a rather similar elongation of the body with the blastopore at the ventral side, but suggested that the anus developed secondarily (Protostomia).

These ideas and newer information have been combined in the trochaea theory (Nielsen 1979; Nielsen 2012). Many papers and textbooks give more or less precise generalisations about ontogeny and morphology of trochophores, so it seems necessary to base the discussion on the primary literature.

The trochaea theory – an update

The fundamental parts of the trochaea theory for the origin of the protostomes, with mouth and anus developing through division of the blastopore through mediolateral blastopore closure and differentiation of the periblastoporal ciliary band (archaeotroch) into prototroch, metatroch and telotroch, were proposed by Nielsen (1979). The theory has gone through several modifications over the years following the growing knowledge about embryology and morphology. The original version of the

theory (Nielsen 1979, Nielsen and Nørrevang 1985) included the deuterostomes, but the revised version (Nielsen 2001, 2012) excludes the deuterostomes, so that only the protostomes are believed to have evolved from the trochaea. The main points of the revised version (Fig. 1) will be recapitulated to facilitate the following discussions.

The protostomian ancestor, trochaea, was a holopelagic, radially symmetrical (or slightly bilateral) gastraea (with only one main axis, the primary or animal–vegetal axis), with the blastopore surrounded by a ring of compound cilia, the archaeotroch (Fig. 1, upper left). This was a downstream-collecting band of compound cilia capturing particles by the catch-up principle (Riisgård *et al*. 2000). This type of ciliary band is usually found on structures with parallel bands with opposing directions of the effective stroke, for example prototroch and metatroch of a trochophore and the two sides of an entoproct tentacle, but a ring-shaped ciliary band, as proposed for the trochaea, is found in *Symbion* (Riisgård *et al*. 2000). Trochaea had a small ciliated sensory organ at the apical pole, the apical ganglion, as seen in most ciliated eumetazoan larvae, with nerves to a ring nerve along the archaeotroch. Captured particles were transported into (and undigested particles out of) the archenteron by separate cilia around the blastopore and in the archenteron. The gastraea type of organisation, seen in the living cnidarians, apparently restricts the life forms to pelagic or sessile.

At the next stage (Fig. 1, left) the adults became benthic, creeping and collecting particles from the bottom by the separate cilia around the blastopore; the archaeotroch, which would have moved the organism away from the substratum, was lost in the adult, but was retained in the planktotrophic larva. A new anterior–posterior axis, forming an angle with the

apical–blastoporal axis, became established, in connection with a preferred creeping direction along the substratum, and the apical sensory organ moved towards the new anterior pole. The directed creeping created a movement of food particles from the anterior part of the blastopore through the archenteron to the posterior part of the blastopore. This 'one-way-traffic' was enhanced by an antero-posterior elongation of the blastopore and, later, by an apposition of the lateral blastopore lips, creating a functionally tube-shaped gut.

The lateral blastopore lips finally fused (Fig. 1, right), and this made differentiation of the various parts of the body possible, probably associated with the elongation of the Hox cluster to comprise the 'protostomian cluster' (laboratory, pb, Hox3, Dfd, Scr, Lox5 and Antp; see Nielsen 2012) collinear with the anterior–posterior axis and the fused blastopore lips. The elongated, creeping body with the tubular gut made way for the evolution of many new life styles, not only detritus feeding, but also the many types of more active feeding, such as 'hunting'. This cleared the way for the occupation of many new ecospaces and made a large radiation possible (Xiao and Laflamme 2009). As seen so often in evolution, the new adult tubular gut soon became established in the larva (through a process aptly called adultation by Jägersten 1972). This resulted in a differentiation of the archaeotroch, of which only the anterior (perioral) part and the posterior (perianal) part were retained. The perioral band retained the particle-collecting function and became enlarged by loop-like lateral expansions, with subsequent differentiation of the anterior part as the prototroch and the posterior part as the metatroch. The blastoporal zone of separate cilia followed the lateral expansion as the adoral ciliary zone between the proto- and metatroch, and its areas along the fused blastopore lips became the gastrotroch, used as a rejection band through the
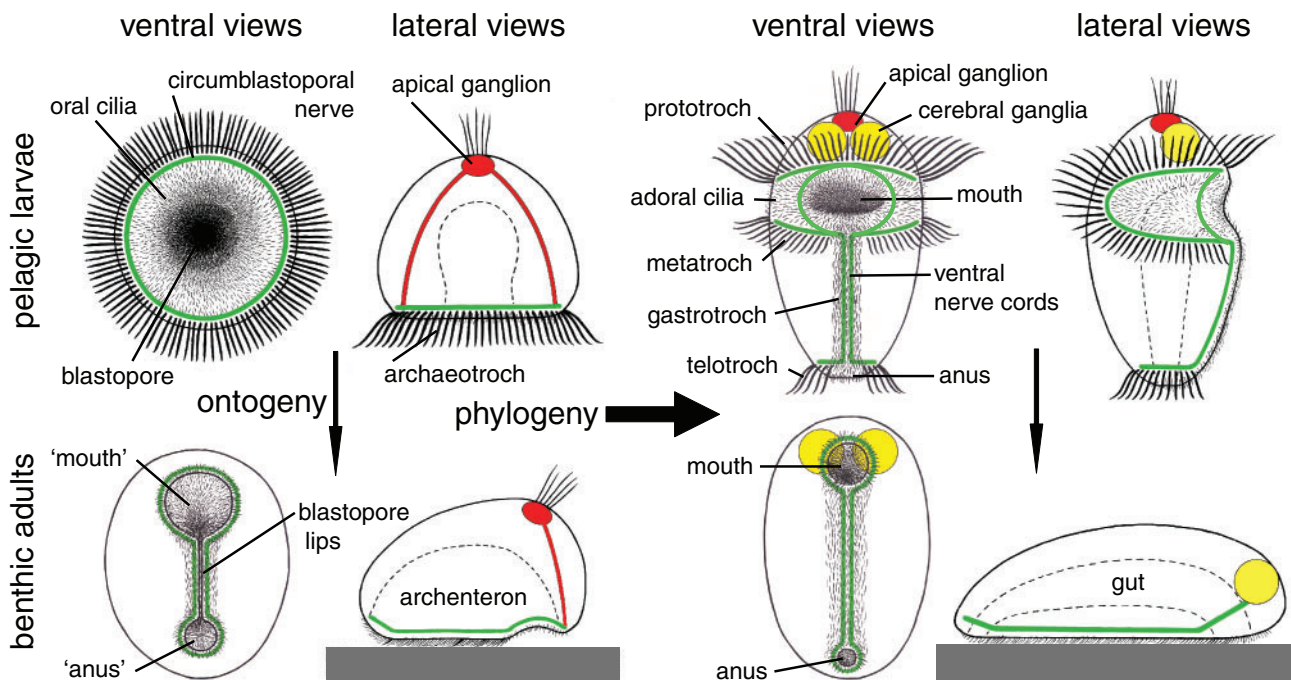

Fig. 1.   The trochaea theory. Modified from Nielsen (2012).

postoral break in the metatroch in the larva and as the locomotory gastrotroch in the adult. The perianal ring (the telotroch) became exclusively locomotory.

The periblastoporal nerve of the trochaea followed the differentiation of the archaeotroch and became organised as a perioral loop, a pair of ventral nerve cords and a perianal loop. In connection with the lateral extension of the perioral band, the perioral nerve ring became split into a branch following prototroch and metatroch and perioesophageal connectives to the ventral nerve cords (Fig. 1, upper right). A pair of new ganglia, the cerebral ganglia, differentiated lateral to the apical ganglion with connections to the oral nerve loop. The apical ganglion, which was the coordinator of the archaeotroch, and later of the proto- and metatroch, lost its function at settling and was lost in the creeping adult where the cerebral ganglia took over as the new brain.

In the following, I will discuss how the old and new information about blastopore fate and cell-lineage of the various epithelial areas with the ciliary bands and of the central nervous systems fits the predictions of the trochaea theory. This is, of course, only possible in the spiralians, because the ecdysozoans lack the spiral cleavage pattern and the ciliated epithelia, but the embryology and morphology of most ecdysozoan nervous systems make some comparisons possible. The most characteristic protostomian apomorphy is the morphology of the central nervous system, with the paired ventral nerve cords, although this cannot be recognised in some of the phyla.

Embryology of spiralian (lophotrochozoan) phyla

The monophyly of Spiralia (Annelida, Sipuncula, Mollusca, Nemertea, Platyhelminthes, Gastrotricha, Gnathostomulida, Micrognathozoa, Rotifera, Entoprocta, Cycliophora, Bryozoa, Phoronida and Brachiopoda) is well supported in almost all molecular analyses. The morphological characters are less strong, because the most conspicuous character(s) are associated with the plesiomorphic retention of the ciliated outer epithelia. A spiral cleavage pattern can be recognised in members of Annelida, Sipuncula, Mollusca, Nemertea, Platyhelminthes, Gnathostomulida and Entoprocta, but it is absent (or the cleavage is unstudied) in the other phyla.

Annelida

Spiral cleavage and highly conserved cell lineages make it possible to follow the ontogeny of the epithelia with the characteristic ciliary bands and of the nervous system both in species with planktotrophic and lecithotrophic larvae and in some cases also in species with direct development (review in Nielsen 2004).

Classical studies on lecithotrophic species using direct observation of cell lineage, such as those on *Amphitrite* (Mead 1897), *Arenicola* (Child 1900) and *Podarke* (Treadwell 1901), and modern studies using blastomere marking, on *Platynereis* (Ackermann *et al.* 2005) and *Capitella* (Meyer *et al.* 2010; Meyer and Seaver 2010), agree in all major points (Figs 2, 3). The only planktotrophic species studied, *Polygordius* (Woltereck 1902, 1904*a*), likewise agrees, although with a modified interpretation of two cells discussed below.

Blastopore fate. Blastopore closure in species with an invagination gastrula has been followed in *Podarke* (Treadwell 1901) and *Polygordius* (Woltereck 1904*a*). The blastopore becomes divided into mouth and anus by lateral fusion of the lateral blastopore lips, with cells of the A–C quadrants surrounding the mouth, whereas cells from the somatoblast (2d) surround the anus. The anus apparently remains open, surrounded by proctodaeum cells in *Podarke*, whereas it closes and a new anus develops in the same region in *Polygordius*. However, many species with lecithotrophic larvae show a drop-shaped to oval blastopore, with an anterior opening that becomes the mouth and a posterior slit-like closure. The mouth is surrounded by cells of the third micromere quartet, for example in *Arenicola* (Child 1900), and the posterior, slit-like closing part of the blastopore is lined by 2d-cells (see below). There is no blastopore closure in species with epibolic gastrulation, but the lineage of *Scoloplos* (Delsman 1916) follows the same general pattern, and the blastomere-marking studies of *Capitella* (Meyer *et al.* 2010) and *Platynereis* (Ackermann *et al.* 2005) show blastomere fates very similar to those of the species with embolic gastrulation, and the position of the nervous ring along the blastopore indicates the closure (see below). Only few exceptions have been described. *Eunice* shows a normal embolic gastrulation, with the blastopore at the posterior pole. In this taxon, the archenteron becomes solid and a large stomodaeum develops near the anterior end of the embryo; a small posterior stomodaeum develops at a late stage (Åkesson 1967).

Epithelial areas and ciliary bands. The cell lineage of the episphere (first micromere quartet) with the prototroch is remarkably conserved, although not all 'prototroch' cells become ciliated. Three tiers of blastomeres may be involved: primary prototroch cells, $1a^2$–$1d^2$, and accessory prototroch cells, $1a^{12}$–$1c^{12}$, from the first micromere quartet, and secondary prototroch cells, $2a^1$–$2c^1$, from the second micromere quartet (Fig. 2). The lack of cilia on the $1d^{12}$ and $2d^1$-cells leaves a dorsal break in the prototroch, but the gap becomes closed by fusion of the posterior ends of the band in most species (Fig. 4).

In the lecithotrophic species, the 2d-cell becomes the somatoblast, which divides profusely, with its descendents moving posteriorly, spreading over the whole hyposphere, except at small oral and anal areas, and finally fusing in the ventral midline, with a small ring with compound cilia, the telotroch, surrounding the anus (Fig. 4). This has been documented in several classical cell-lineage studies, for example of *Amphitrite* (Mead 1897) and *Arenicola* (Child 1900), and in studies using blastomere marking of *Platynereis* (Ackermann *et al.* 2005) and *Capitella* (Meyer *et al.* 2010; Meyer and Seaver 2010) (Fig. 5*A*). Thus, it appears that the lowermost edge of the blastopore rim is situated at the limit between the second and third micromere quartet, and that this line in the later stages can be followed from the secondary trochoblasts, along the fused ventral edges of the somatoblast to the telotroch.

The planktotrophic larvae have a metatroch of debated origin. Woltereck (1904*a*) interpreted a pair of cells from the third micromere quartet as the precursors of the metatroch. However, their ciliation is continuous with that of their sister
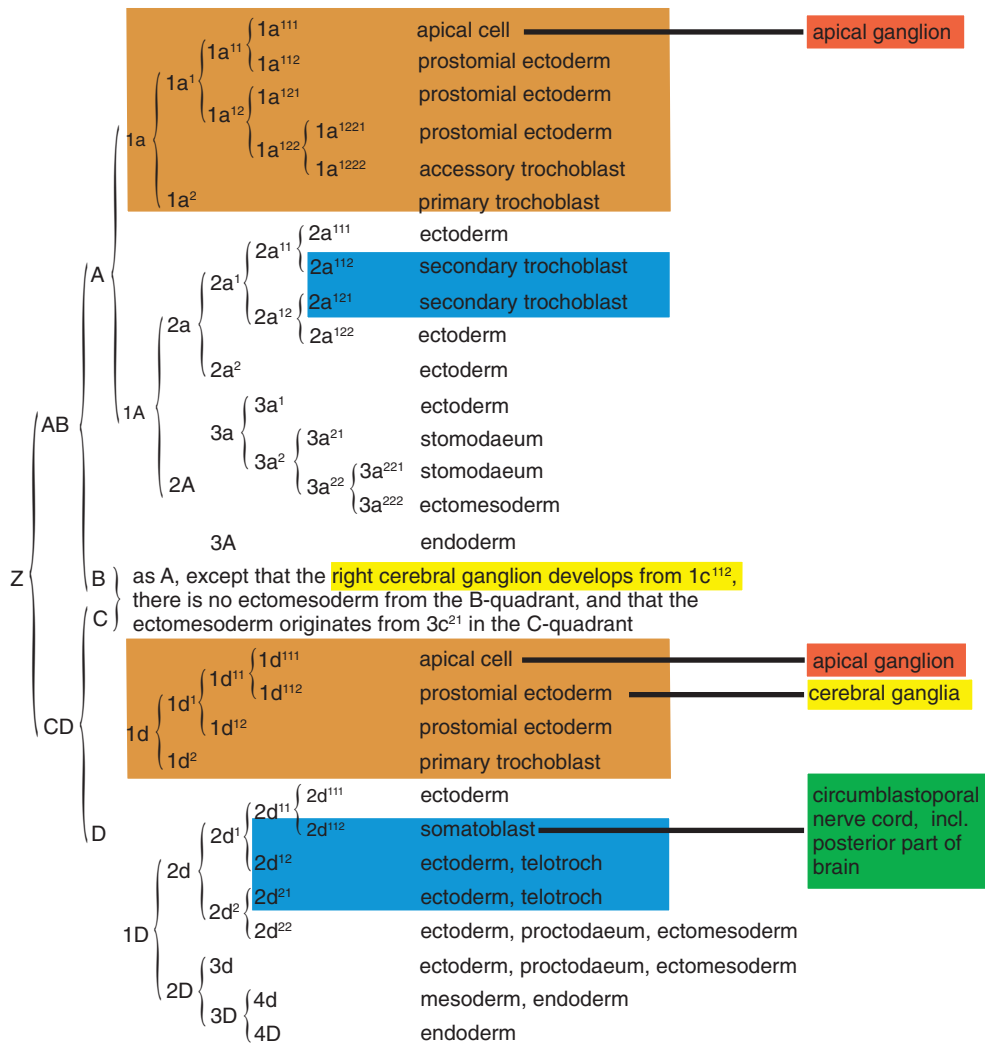
Fig. 2. Cell lineage of a generalised lecithotrophic annelid larva. From Nielsen (2012); based on *Podarke* (Treadwell 1901), *Platynereis* (Ackermann *et al*. 2005) and *Capitella* (Meyer *et al*. 2010). The colours indicate identical areas in Figs 2, 3 and 5*A*.
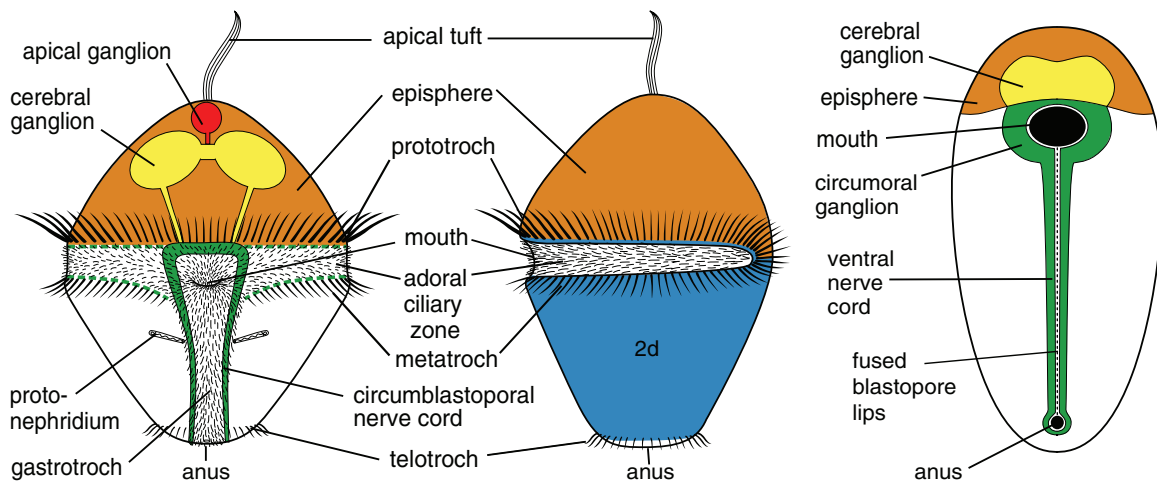

Fig. 3. Development of a generalised annelid with indirect development. Modified from Nielsen (2012). Compare with Figs 2 and 5*A*.
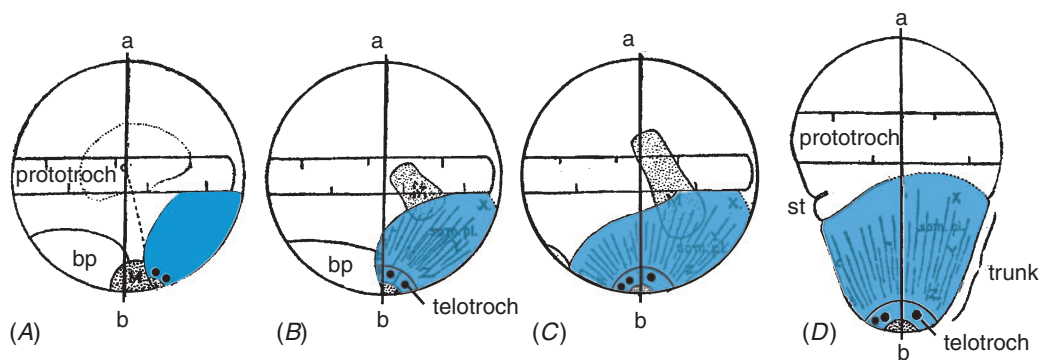
Fig. 4. The growth of the epithelial area originating from the 2d-cell in *Amphitrite*. Note that the prototroch initially has a posterior gap, and that this subsequently becomes closed by fusion of the two ends of the prototroch. The descendants of the 2d-cell (blue) expand posteriorly and then lateroventrally, forming the somatic plate, finally to meet and fuse at the ventral side. The cells of the telotroch are located at the posterior edge of the somatic plate. a-b, apical-blastoporal axis; bp, blastoporal area. Modified from Mead (1897).
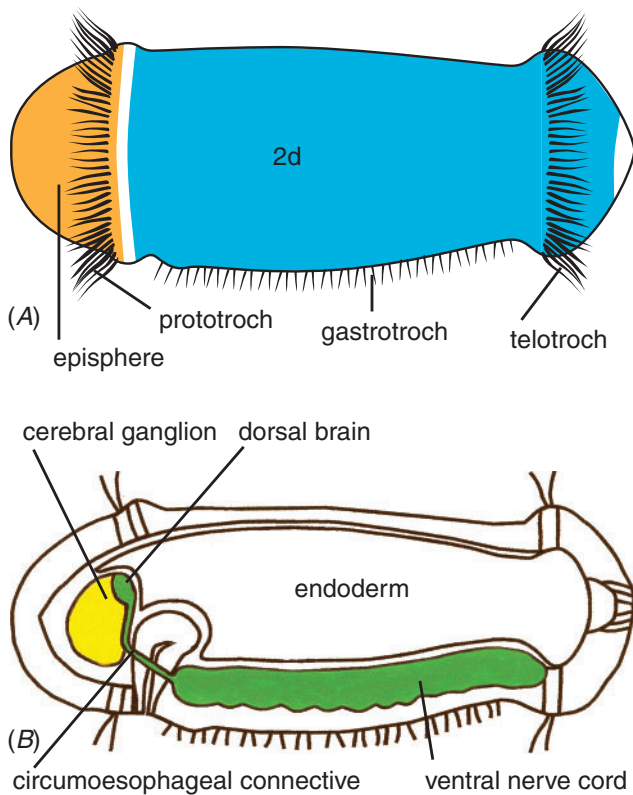

Fig. 5. Diagrams of a stage-6 larva of *Capitella telata*. Based on Meyer *et al.* (2010). (*A*) Epithelial areas; the episphere is brown and the 2d-cell area is blue. (*B*) Central nervous system, the cerebral ganglia are yellow and the circumblastoporal nervous system is green. Compare with Figs 2 and 3.

cells in the cell lineage at the lateral parts of the mouth, and I have interpreted them as precursors of the adoral ciliary zone and, accordingly, a pair of more ventral 2d-cells as the precursors of the metatroch (Nielsen 2004). In my interpretation, the metatroch is formed at the posterior side of the oral loop of the band of secondary trochoblasts around the mouth, at the blastoporal

edge. This inconsistency in interpretation can only be resolved by new studies.

The telotroch develops from cells along the posterior edge of the somatoblast in all species studied, e.g. *Amphitrite* (Mead 1897), *Arenicola* (Child 1900) and *Capitella* (Meyer *et al.* 2010; Meyer and Seaver 2010).

At metamorphosis, the ciliated bands degenerate. The ciliated cells shrink and become resorbed, and their cilia may become cast off or resorbed (Segrove 1941). Types with enlarged epispheres and 'catastrophic' metamorphosis, such as *Polygordius* and *Owenia*, shed the expanded part of the episphere, whereas the most apical part of the episphere with the cerebral ganglia is 'pulled down' to the anterior side of the oesophagus. The reports disagree about the process in both types; some record shedding of the whole structure (Fraipont 1887; Wilson 1932) whereas others record shrinkage and internalisation (Hay-Schmidt 1995; Smart and Von Dassow 2009).

Central nervous system. A small ciliated apical sensory organ, the apical ganglion, develops from the most apical cells, $1a^{111}–1d^{111}$. In some species it comprises a small number of flask-shaped cells (Brinkmann and Wanninger 2009). The apical ganglion is connected to prototroch and metatroch nerves in early *Spirobranchus* trochophores (Lacalli 1984)

A pair of cerebral ganglia differentiates from cells lateral to the apical ganglion. The apical and cerebral ganglia are intimately connected, for example in *Platynereis* (Ackermann *et al.* 2005), but the apical ganglion disintegrates before or at metamorphosis. The cell lineage of the cerebral ganglia has not been followed, but Child (1900) suggested that they should develop from the cells $1c^{112112}$ and $1d^{112112}$ in *Arenicola*. The origin of the ganglia from the cells 1c and 1d has been documented through blastomere marking in *Platynereis* (Ackermann *et al.* 2005) and *Capitella* (Meyer *et al.* 2010), with small contributions from 1a and 1b in *Capitella*. A commissure develops between the two ganglia, which move together, often forming a dumbbell-shaped structure, which may grow to fill the prostomium almost completely. In the direct developing leeches, such as *Helobdella*, the cerebral

(supraoesophageal) ganglia develop from cells of the first micromere quartet (Weisblat *et al.* 1984).

The ventral nerve cords with ganglia differentiate from the somatoblast (2d-cell) along the fusing blastopore lips in all the lecithotrophic species studied, such as *Capitella* (Meyer *et al.* 2010) (Fig. 5*B*) and *Platynereis* (Ackermann *et al.* 2005) and also in the direct-developing leeches (such as *Helobdella*, see Weisblat *et al.* 1984). In *Capitella*, it has been shown that the ventral cords continue around the stomodaeum in a pair of circumesophageal connectives and a small postero-dorsal ganglion in the brain (Meyer *et al.* 2010). A similar loop is indicated in the study of *Platynereis* (Ackermann *et al.* 2005). It could be expected that second micromere quartet cells from the A–C quadrants should form parts of the anterior loop, but this has not been demonstrated. The first cells to differentiate are a pair of pioneer cells at the posterior end of the embryo, originating from the 2d-cell (*Platynereis*: Dorresteijn 1998).

Mollusca

Cell-lineage studies of the early development of several gastropods and a few polyplacophorans, bivalves and scaphopods based on direct observations of cell lineages have been published over the last century (reviewed in Nielsen 2004), and new studies using blastomere marking have updated these reports with information about the internal organs, e.g. the gastropods *Patella* (Dictus and Damen 1997), *Ilyanassa* (Goulding 2009; Render 1997) and *Crepidula* (Hejnol *et al.* 2007; Henry *et al.* 2010), and the polyplacophoran *Chaetopleura* (Henry *et al.* 2004) (Figs 6, 7). Unfortunately,

none of the studies make it possible to follow the movements of the various blastomeres during differentiation. It is clearly demonstrated, for example in *Patella* (Dictus and Damen 1997), that areas of blastomeres move relative to each other in the hyposphere (see Fig. 7*B*), but the lack of information about the intermediate stages makes it difficult to interpret the fate maps and therefore also to make comparisons between different species of molluscs and between molluscs and annelids.

Blastopore fate. Many species with small eggs gastrulate by invagination, but species with large eggs usually show epibolic gastrulation (van den Biggelaar and Dictus 2004). The blastopore is initially situated opposite of the apical pole, but the mouth moves forwards to a position just behind the prototroch. The blastopore becomes the circular mouth in most species, for example in the gastropod *Littorina* (Delsman 1914), but the blastopore is drop-shaped with a posterior slit in a few species, for example in the gastropod *Physa* (Wierzejski 1905) and the polyplacophoran *Stenoplax* (Heath 1899). The blastopore is initially circular in *Physa*, but in connection with the development of the stomodaeum it becomes elongate. The final mouth is surrounded by cells of the A–C quadrants, and the posterior part of the closed blastopore is bordered by 3c- and 3d-cells. A pair of lateral cell groups, apparently from the blastomeres 2a and 2c, extend medially and form the primordium of the foot (van den Biggelaar and Dictus 2004). In *Stenoplax*, the anterior part of the blastopore is lined by cells of all four quadrants, whereas the posterior part is lined only by 3c- and 3d-cells. A puzzling exception is seen in the gastropod *Viviparus*, in which the blastopore becomes the anus (Dautert 1929; Fernando 1931). *Crepidula* initially shows epibolic
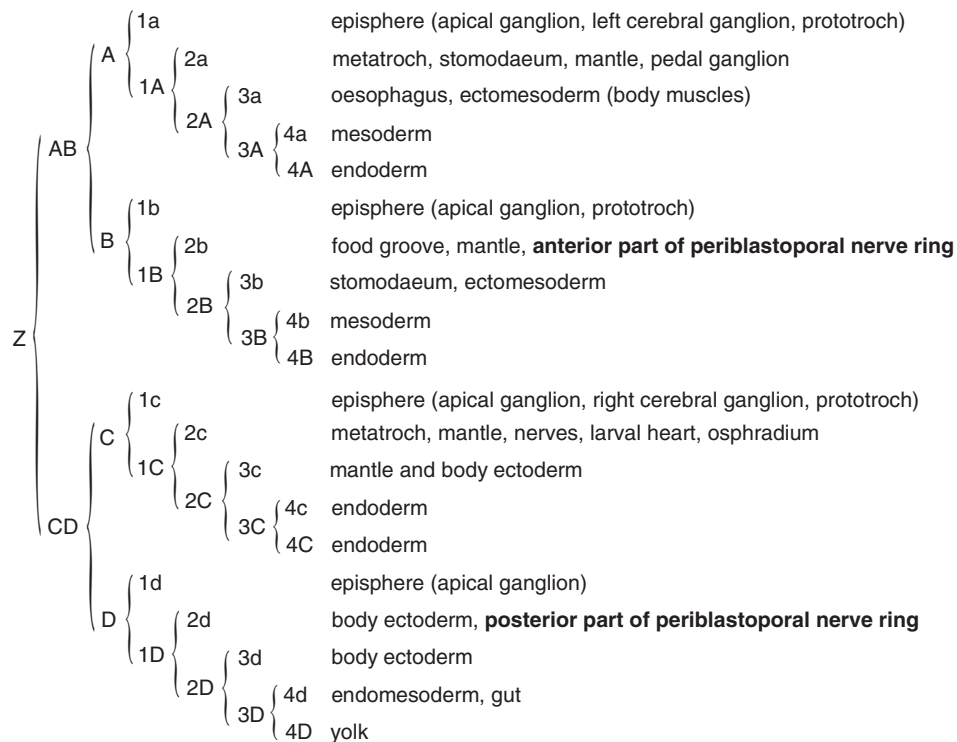

Fig. 6. Cell lineage of *Crepidula fornicata.* Based on Hejnol *et al.* (2007).
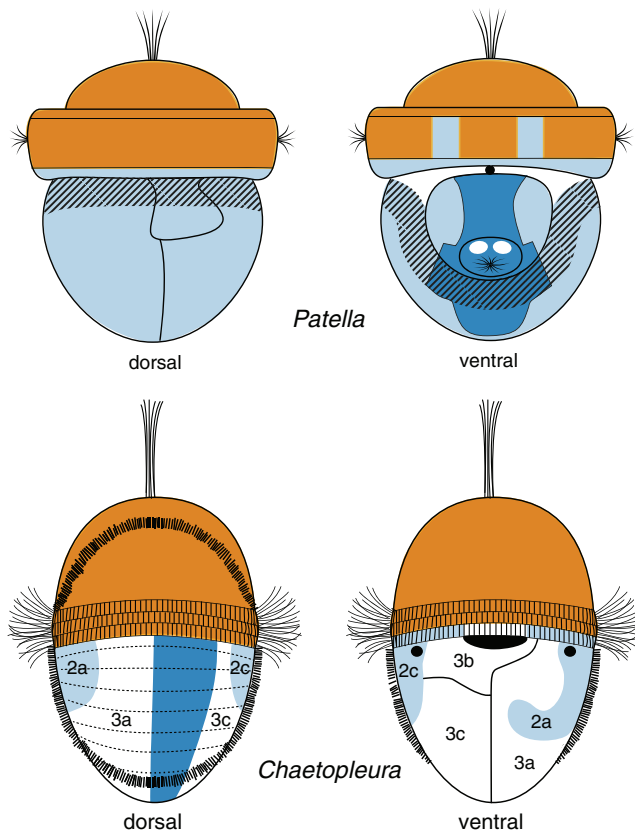
Fig. 7. Epithelial areas of mollusc embryos. Diagrams of a 28-h old embryo of the gastropod *Patella vulgata* in dorsal and ventral views (modified from Dictus and Damen 1997), and diagrams of a larva the polyplacophoran *Chaetopleura apiculata* in dorsal and ventral views (modified from Henry *et al.* 2004). The episphere (cells of the first micromere quartet) is brown, cells of the second micromere quartet are blue, with darker 2d-cells, and cells of the third micromere quartet are white.

gastrulation, but an archenteron is formed by invagination at a later stage, with a narrow, slightly longitudinally elongate mouth (Conklin 1897).

Epithelial areas and ciliary bands. The episphere comprises the cells of the first micromere quartet with compound prototroch cilia on primary and accessory trochoblasts in all species studied. Not all species develop cilia on the accessory trochoblasts, and trochoblasts of both types (and of the secondary trochoblasts of the second micromere quartet) may develop cilia that become lost again during the following development (Damen and Dictus 1996). Lecithotrophic larvae usually show a complete prototroch ring formed from all three types of trochoblasts, for example *Patella* (Damen and Dictus 1994) and *Chaetopleura* (Henry *et al.* 2004). *Crepidula* has a prototroch comprising primary and accessory trochoblasts from the first micromere quartet; the secondary trochoblasts apparently have separate cilia and can therefore not be distinguished from other cells of the second micromere quartet (Hejnol *et al.* 2007). Conklin (1897) was uncertain about his interpretation of the cell lineage of the prototroch (the primary trochoblasts, called turret cells or trochoblasts in his cell-lineage table, should only form a small part of the prototroch, the preoral velum), and his interpretation of the prototroch cells as derived from the second and third micromere quartets is not in accordance with modern investigations.

The cell lineage of the metatroch has not been studied, but the study of *Crepidula* by Hejnol *et al.* (2007) shows that the adoral ciliary band (called a food groove) develops from the 2b-cell and the metatroch (called the 2nd ciliary band) from the cells 2a and 2d. Conklin's (1897) drawing of the oral side of an embryo with developing velum shows a postoral row of cells (precursors of the 'postoral velum'), which should be descendents of the $2b^{22}$-cell. This would be in accordance with the results obtained by the blastomere-marking studies, which indicate that the metatroch develops on cells of the second micromere quartet.

The studies on the cell lineages of the body epithelium (hyposphere) of polyplacophorans and gastropods show puzzling differences between the species; compare, for example, the fate maps of *Chaetopleura* (Henry *et al.* 2004) (Fig. 7*C, D*), *Patella* (Dictus and Damen 1997) (Fig. 7*A, B*), *Ilyanassa* (Render 1997) and *Crepidula* (Hejnol *et al.* 2007). The epithelium is mainly derived from the second micromere quartet, but smaller or larger contributions from the third micromere quartet are seen both in *Chaetopleura* and *Crepidula*. In *Patella*, the descendents of the 2d-cell occupy only a small midventral area, but the absence of these cells from the dorsal side remains unexplained. Also, the origin of mantle and shell shows considerable variation between the classes. In *Chaetopleura* (Henry *et al.* 2004), the shells develop from the blastomeres 2d, 3c and 3d, whereas the surrounding perinotum derives from 1a and 1d in addition to the contributions from the second and third micromere quartet. In *Ilyanassa* (Render 1997), *Patella* (Dictus and Damen 1997) and *Crepidula* (Hejnol *et al.* 2007), the mantle fold and the shell field develop from all four quadrants of the second micromere quartet, but only the 2d- and 2c-cells are involved in shell formation in *Ilyanassa* (Cather 1967). In bivalves, such as *Unio* (Lillie 1895), *Dreissena* (Meisenheimer 1901) and *Crassostrea* (Galtsoff 1964), the shell gland initially forms as a large, deep invagination of 2d-cells concomitantly with gastrulation. With so much variation between the few species studied it is difficult to deduce the ancestral pattern, but the contribution from the first micromere quartet to the perinotum of *Chaetopleura* makes its homology with the mantle edge of the conchiferans questionable (see also Kocot *et al.* 2011; Smith *et al.* 2011).

At metamorphosis, all prototroch cells degenerate. In species with a very rapid metamorphosis, the velum is shed, as for example in the gastropod *Polynices* (Page and Pedersen 1998). The pericalymma larva of the solenogaster *Neomenia* infolds the serosa, which subsequently becomes internalised in the head region where the ciliated cells disintegrate (Thompson 1960). The pericalymma larvae of protobranch bivalves such as *Acila* and *Yoldia* shed the serosa, which may become internalised or ingested (Drew 1899; Zardus and Morse 1998).

There is no study of the cell lineage of species with a telotroch.

Central nervous system. A ciliated apical ganglion develops from the most apical cells, $1a^{111}$–$1d^{111}$, often with characteristic flask-shaped cells, e.g. in the polyplacoporan *Ischnochiton* (Voronezhskaya *et al.* 2002), the gastropods *Ilyanassa* and

Crepidula (Dickinson and Croll 2003; Hejnol *et al*. 2007) (see
Fig. 8) and the bivalve *Mytilus* (Voronezhskaya *et al*. 2008). Its
function is poorly studied, but it is involved in settling in some
species (Hadfield *et al*. 2000). It degenerates before or at settling,
and apoptosis of the apical ganglion cells has been demonstrated
in gastropod larvae (Gifondorwa and Leise 2006). In bivalves
with pericalymma larvae, such as *Yoldia* and *Acila*, the apical
ganglion is cast off with the serosa (Drew 1899; Zardus and
Morse 1998).

Paired cerebral ganglia, often with a pair of eyes, develop from
cephalic plates of the episphere lateral to the apical ganglion, in
some types closely apposed to the apical ganglion, for example in
the gastropod *Lunatia* (Page and Parries 2000) and the bivalve
Crassostrea (Ellis and Kempf 2011), but in other types at a
considerable distance, close to the prototroch, for example in
Crepidula (Conklin 1897) and *Ilyanassa* (Rabinowitz and
Lambert 2010). In the gastropod *Lymnaea*, the cephalic plates
(right side: $1b^{1122}$, $1b^{1212}$, $1c^{112}$ and $1c^{121}$; left side: $1b^{1212}$,
$1a^{1122}$, $1a^{121}$ and $1d^{112}$) give rise to the cerebral ganglia with
tentacles and eyes (Verdonk and van den Biggelaar 1983).

The ventral nervous system shows much variation. Two pairs
of longitudinal nerves can usually be recognised, a visceral
(lateral) pair and a pedal pair. The visceral nerves are
connected posteriorly by a suprarectal commissure in many
groups. The nerves are non-ganglionated in most
aplacophorans, polyplacophorans and monoplacophorans, but
several characteristic ganglia are found in the other groups; the
ganglia are quite often fused with each other and with the cerebral
ganglia, so their origin becomes difficult to make out. During
ontogeny, the visceral nervous system develops first, with one
or a pair of posterior pioneer cells (Dickinson and Croll 2003).
It has the shape of an oval, which becomes twisted during
torsion in the gastropods (Dickinson *et al*. 1999; Dickinson
and Croll 2003). Its cell-lineage has only been characterised
in *Crepidula*, where it originates from 2b and 2d (Hejnol *et al*.
2007) (Fig. 8); if the 2d-cell at the posterior loop were a pioneer
cell, this would indicate that the visceral loop is homologous to
the ventral nerve cords of the annelids. The paired nerve cords
are closely apposed or fused in most spiralians, which have a
narrow gastrotroch (or lack the gastrotroch), and their wide
distance in the molluscs may be related to the specialisation of
the gastrotroch area to the wide foot, which has then acquired
the special pedal nerves (compare with the entoproct larva;
Haszprunar and Wanninger 2008).

Nemertea

All studied nemerteans have spiral cleavage with trochoblasts
specialising from the edge of the episphere, i.e. from the first
micromere quartet, indicating the presence of a prototroch
(Maslakova *et al*. 2004; Nielsen 2005). This indicates that
the ancestral nemertean had a trochophore larva and that
a development with a planuliform, in some species
planktotrophic, larva evolved in various 'palaeonemerteans',
whereas a very special larval type, the pilidium, evolved in the
Neonemertea (Nielsen 2012). This larval type is retained in
many species of the Pilidiophora, but apparently highly
derived to become planuliform, both in species within the
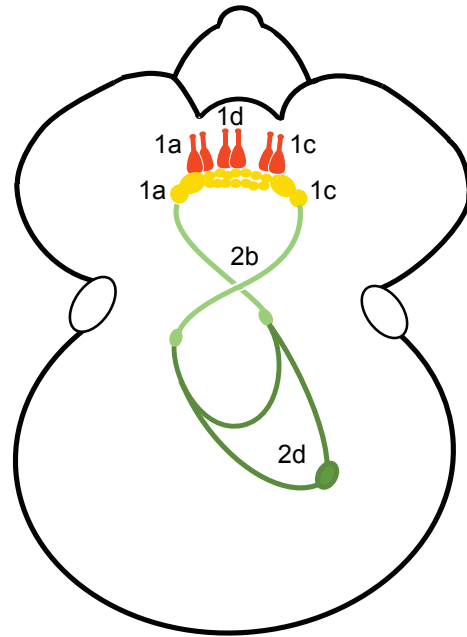Pilidiophora (von Döhren 2011) and in its sister group,

Fig. 8. Diagram of the central nervous system of an embryonic veliger stage
of the gastropods *Ilyanassa/Crepidula*. The flask-shaped cells of the apical
ganglion are red, the cerebral ganglia yellow, and the ventral nerves green.
Modified from Hejnol *et al*. (2007). The cell at the posteriormost loop of the
ventral (parietal) nerve is possibly a pioneer cell (see Dickinson and Croll
2003).

Hoplonemertea (Maslakova 2010*b*). The embryology of
representatives of all three types is now well known. The cell
lineage of the planuliform 'palaeonemertean' *Carinoma* has
been studied by Maslakova *et al*. (2004). Development and
metamorphosis of pilidium larvae has been studied for
example in *Micrura* (Maslakova 2010*a*) and the cell lineage of
Cerebratulus (Henry and Martindale 1998). Development
and metamorphosis of several hoplonemerteans has been
studied, and it has been concluded that a transitory epidermis
of the planuliform larva represents the larval tissues of the
pilidium larva (Maslakova and von Döhren 2009; Hiebert
et al. 2010).

Blastopore fate. Several 'palaeonemerteans' and
pilidiophorans develop through a coeloblastula and an
invagination gastrula. The invagination apparently represents
both the archenteron and the stomodaeum with the mouth (for
example in *Cephalothrix*; Smith 1935). The anus develops later
on, and the larvae are usually planktotrophic (Norenburg and
Stricker 2002). In the developing pilidium, the gastrula becomes
bell-shaped, usually with a pair of lateral lappets. In *Cerebratulus
lacteus*, the invagination differentiates into a sac-shaped gut and
a funnel-shaped oesophagus formed by cells of the second
micromere quartet of all four quadrants (Henry and Martindale
1998). The blastopore narrows and becomes the mouth at
metamorphosis, and the anus develops after the liberation of
the juvenile from the larval body (for example in *Micrura*, see
Maslakova 2010*a*). Hoplonemerteans have epibolic gastrulation
and a stomodaeum develops at a late point (for example in
Pantinonemertes; Maslakova *et al*. 2004).

Epithelial areas and ciliary bands. The cleavage of *Carinoma* follows the typical spiral pattern with accessory, primary and secondary prototroch cells, but the ciliation of these cells resembles that of the other cells of the larva (Maslakova *et al.* 2004). However, the prototroch cells are large and clearly distinguishable in light microscopy. They are cleavage arrested and degenerate at metamorphosis. The contributions of episphere and hyposphere cells to the juvenile epidermis have not been followed.

In the Pilidiophora, the cell lineage of the pilidium larva of *Cerebratulus lacteus* (Henry and Martindale 1998) demonstrates the presence of a conspicuous band of long separate cilia from the cells of the first and second micromere quartets at the edge of the episphere. Blastomeres 3c and 3d are also reported to contribute to the ciliary bands, but the blastomere labelling indicates that it could be the postoral band (Nielsen 2012) that is labelled. The cell lineage indicates that the large band is a prototroch, although it does not consist of compound cilia and is only locomotory. The larval epithelium is shed and devoured both in species with the planktotrophic pilidium larva (Maslakova 2010*a*) and species with the lecithotrophic Desor-larva (von Döhren 2011).

The cell lineage of the hoplonemerteans has not been studied.

Nervous system. A conspicuous apical ganglion with a thick apical tuft develops in almost all larvae; it is lost at metamorphosis in all species. In the pilidium larva, a system of fine nerve fibres develops along the ciliary band with connections to an oral ring; these cells originate from the same blastomeres that form the ciliated band (Henry and Martindale 1998). The system is very similar to that observed in the early larva of the annelid *Spirobranchus* (Lacalli 1984). It is not connected with the apical ganglion, and the whole system is lost together with the larval body at metamorphosis.

In species with pilidium larvae, the cerebral ganglia develop as thickenings of the ectoderm of the cephalic discs, i.e. from the episphere (Henry and Martindale 1998; Maslakova 2010*a*) (Fig. 9). The planuliform embryos develop a pair of cerebral ganglia from epidermal thickenings or invaginations of the epithelium lateral to the apical ganglion, for example in the 'palaeonemertean' *Cephalothrix* (Iwata 1960) and in the hoplonemertean *Malacobdella* (Hammarsten 1918). A pair of

anterior epithelial invaginations, which each become divided into two, in the early larvae of *Pantinonemertes*, could be interpreted as early stages of cerebral ganglia, but direct evidence is lacking (Hiebert *et al.* 2010).

The origin of the lateral nerve cords has been much discussed, but the cell lineage study of the pilidium of *Cerebratulus lacteus* demonstrates that blastomeres 2a, 2c and 3d, i.e. cells of the hyposphere, give rise to nervous cells, which probably form the lateral nerve cords. This is in accordance with Maslakova's (2010*a*) suggestion that these nerves differentiate from the trunk discs. Hickman (1963) reported that the lateral nerves differentiate from the lateral embryonic epithelium in the hoplonemertean *Geonemertes*.

Entoprocta

The entoprocts have spiral cleavage and the prototroch develops from the primary and accessory trochoblasts (Marcus 1939; Malakhov 1990). The apical ganglion with a few flask-shaped cells differentiates from apical cells (Wanninger *et al.* 2007; Fuchs and Wanninger 2008). The frontal organ, with eyes in some species, develops from the episphere and may represent the cerebral ganglia (Nielsen 1971). Larvae of the creeping types develop two pairs of longitudinal nerves with few perikarya in the foot, but these nerves seem to be missing in the swimming larvae without the foot (Wanninger *et al.* 2007; Fuchs and Wanninger 2008).

Platyhelminthes

Most platyhelminths have direct development, and a full spiral cleavage and ciliated, planktotrophic larvae are only found in certain polyclads. The very unusual embryology of both macrostomids and neoophorans appears highly derived (review in Nielsen 2005; see also Rawlinson 2010). The discussion will concentrate on the indirect type of development. All platyhelminths lack an anus. This is considered an apomorphy, both because they are deeply rooted in the in the Spiralia and because their embryology shows a very peculiar pattern with programmed cell death of the 3A–C- and 4D-cells, indicating strong reorganisation of the blastoporal area.
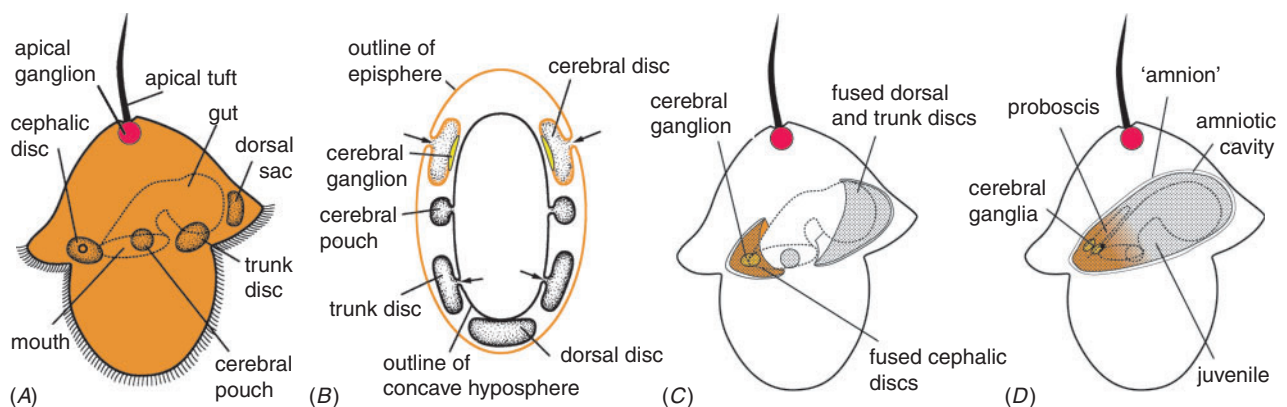

Fig. 9. Development of the cerebral ganglia from the inner epithelium of the cerebral discs in the pilidium larva of *Cerebratulus lacteus*. Nielsen (2012). Fig. (*B*) is a schematic horizontal section passing through the openings of the ectodermal pouches (arrows).

Blastopore fate.   All cells of the third and fourth quartets become internalised through epiboly, but endoderm and endomesoderm develop exclusively from the 4d-cell, whereas the other invaginated cells disintegrate (Boyer *et al*. 1998). The stomodaeum develops from cells of the second micromere quartet.

Epithelial areas and ciliary bands.   The ciliated epithelium of the larva differentiates from the cells of the first micromere quartet (episphere) in all polyclads. In species with planktotrophic larvae, a thick band of longer cilia follows the border between first and second quartets (Boyer *et al*. 1998). This position indicates that the band is a prototroch. It becomes extended onto several lobes, typically four in Goette's larva and eight in Müller's larva, but some species go through a Goette-stadium before reaching the Müller-stage, and even higher numbers of lobes have been described. At metamorphosis, the lobes shrink gradually, the ciliary bands disappear and the body attains the shape of the adult (Ruppert 1978).

Central nervous system.   An apical organ with a ciliary tuft and often with a pair of eyes differentiates from cells of the first micromere quartet, but apical and cerebral ganglia have not been distinguished in cell-lineage studies. The early Müller's larvae of *Pseudoceros* were studied by Lacalli (1982, 1983) who observed a small apical ganglion carrying a ciliary tuft and a paired cerebral brain with a pair of eyes. Rawlinson (2010) studied the development of the nervous system of the Müller's larva of *Martigirella* and observed an apical ganglion (apical plate) and paired cerebral ganglia with a commissure (commissural cell bodies and cerebral commissure). Lacalli (1982, 1983) observed paired ventral and lateral nerve cords extending from the cerebral ganglia, but their further development was not studied. Longitudinal nerves with transverse nerve rings form a highly variable pattern in various groups (Reuter and Gustafsson 1995).

Embryology of ecdysozoan phyla

The monophyly of the Ecdysozoa (Panarthropoda + Cycloneuralia) is strongly supported both by morphology (lack of ciliated outer epithelia and moulting of the cuticle) and molecular phylogenetic analyses. The embryology of several arthropods and nematodes has been studied in depth, whereas the other phyla are either unstudied or poorly known.

All ecdysozoans lack the spiral cleavage pattern and ciliated epithelia, but a quadrant-cleavage with a D-cell giving rise to the germ cells has been observed in several groups with holoblastic cleavage (Nielsen 2012).

Arthropoda

The arthropods show many types of cleavage, but total cleavage is found in several groups. The cirripede *Tetraclita rosea* shows a D-cell that is much larger than the A–C-cells (Anderson 1969). The 8-cell stage shows seven smaller cells and a large 1D-cell. The 1d-cell marks the dorsal side of the embryo and the 1a–c-cells the anterior end; a spiral pattern is not obvious. A fate map was constructed for the 33-cell stage, but it was not based on a cell lineage (see below). However, the cell lineages of some shrimps, such as *Sicyonia* (Hertzler 2002), have been constructed.

Blastopore fate.   Gastrulation is epibolic in most species, but some decapod crustaceans show an invagination of cells that develop into midgut and mesoderm with germ cells. This group of cells soon becomes isolated from the ectoderm, at an early stage in *Galathea* (Fioroni 1970) and at an embryonic stage with appendage buds in *Sicyonia* (Hertzler and Clark 1992; Hertzler 2002). Stomodaeum and proctodaeum develop in later stages; in *Sicyonia* the proctodaeum should develop from the area of the closed blastopore.

Epithelial areas.   The lack of precise fate maps makes it difficult to identify the origin of most organs. Anderson (1969)'s study of *Tetraclita rosea* indicates that the ectoderm of the antennal and mandibular segments originate from cells of the 'second micromere quartet'. The post-naupliar ectoderm should be derived from '3d-cells'.

Central nervous system.   There is no apical ganglion, but the central nervous system with paired cerebral ganglia with commissures to the ventral nerve cords is very similar to that of the annelids. However, it is not possible to relate the structures to a blastopore closure (see above).

The paired cerebral ganglia with eyes (protocerebrum) develop as thickenings of the anterior blastoderm from the cells 1a–c in *Tetraclita rosea* (Anderson 1969). The deutocerebrum (with the antennules (or first antennae) in the crustaceans, antennae in the hexapods and the chelicerae in the chelicerates) and the tritocerebrum (with the (second) antennae in the crustaceans, no appendage in the hexapods, and pedipalps in the chelicerates) should develop from the 'second micromere quartet'. The more posterior segmental ganglia develop through differentiation from ventral epithelial areas. New pairs of ganglia are added along the ventral midline from the posterior growth zone in front of the anus (Harzsch 2001). Two pairs of pioneer cells have been found near the anus in *Artemia* and *Gonodactylaceus* (Fischer and Scholtz 2010).

Nematoda

The cell-lineage studies of several species do not show a pattern resembling that of the spiralians, but the 4-cell stage shows the quadrant cleavage with one cell (called the D- or P_2-cell) having a fate different from the other three, giving rise to mesoderm and germ cells, as characteristic of the protostomes (Schulze and Schierenberg 2011).

Blastopore fate.   Nematodes have direct development, and the very thoroughly studied rhabditid *Caenorhabditis* (Sulston *et al*. 1983), with epibolic gastrulation, has usually been taken as representative of the whole phylum. However, the more 'primitive' groups have now been shown to have an unexpected variation in the ontogeny (Schulze and Schierenberg 2011). The dorylaimidan *Romanomermis* shows an invagination gastrula in which the blastopore becomes divided into mouth and anus through lateral blastopore closure (Schulze and Schierenberg 2009). Also the enoplidans *Pontonema* and *Tobrilus* show an early coeloblastula and an invagination of the gut, but the blastopore becomes the mouth (Malakhov 1994; Schierenberg 2005). *Caenorhabditis* and other rhabditids show embolic gastrulation, but longitudinal lateral areas converge and fuse along the ventral midline. The limit between the ectodermal epithelium and the invaginated nerve

cords and mesoderm is formed by a line of six blastomeres on the right and left side, descendants of the blastomeres ABprap and ABplap, respectively. These cells meet in the ventral midline, interdigitate and divide into an epithelial cell and a neuron (in *Caenorhabditis*; Sulston *et al*. 1983). They presumably represent the median part of the lateral blastopore lips. It appears that the blastopore closure shows just the same variation as that observed, for example, in the annelids.

Epithelial areas. The fate maps of nematodes show much variation. Ectoderm (including neurons) develops mainly from the blastomere S_1 (= AB).

Central nervous system. The central nervous system consists of a collar-shaped brain around the pharynx and a midventral nerve cord; lateral and dorsal longitudinal nerves consist of axons without perikarya (White *et al*. 1986). The brain consists of an anterior and a posterior oblique ring of perikarya separated by a ring of neuropil. The cells of the two rings mainly originate from the anterior S_1 (AB) blastomeres in *Caenorhabditis*; both rings show a mixture of lineages, so there is no indication that the rings represent two ganglia (Sulston *et al*. 1983). Most of the neurons originate from S_1 in *Romanomermis* (Schulze and Schierenberg 2009, 2011).

Embryology of Chaetognatha

The morphology and development of the central nervous system of chaetognaths, as well as the molecular studies, clearly demonstrate their protostomian affinities, but their relationships to the two major protostomian clades is undecided. The embryology of the chaetognath *Paraspadella* has been studied by blastomere marking of the 4-cell stage (Shimotori and Goto 2001). The cell lineage does not resemble the spiralian pattern, but one cell of the 4-cell stage, recognisable by the presence of a 'germ granule', gives rise to mesoderm and germ cells, and this resembles the quadrant cleavage characteristic of the protostomes (see Nielsen 2012).

Blastopore fate. Cleavage leads to the formation of a coeloblastula and a wide archenteron develops from an invagination. The blastopore closes and a stomodaeum develops from the 'opposite pole' of the embryo (Burfield 1927). Its exact relationship to the apical pole should be investigated. The embryo elongates and an anal opening is formed at a late stage.

Epithelial areas. The fate of the blastomeres of the 4-cell stage has been documented by Shimotori and Goto (2001). If the blastomeres are named A–D with the cell with the germ granule called D, it was shown that the right and left sides of the head and ventral side of the body were covered by cells of the A- and C-quadrants, respectively, and the dorsal side of the body by B-cells. The D-cells form the internal epidermis and parts of the endoderm and mesoderm, including the germ cells.

Central nervous system. The central nervous system comprises several ganglia in the head region; a paired cerebral ganglion with a ventral nerve loop with oesophageal and vestibular ganglia and a pair of main connectives to a large, elongate ventral ganglion in the trunk region (Rieger *et al*. 2010). The development of the head ganglia has not been studied in detail, but the cerebral ganglion should develop from a dorsal

epithelial thickening at the head (Burfield 1927). The large ventral ganglion differentiates from a pair of lateral epithelial areas that approach each other and form a longitudinal zone of connectives (Doncaster 1902; Rieger *et al*. 2011). This is very similar to the development of the ventral nerve cord(s) of both spiralians and ecdysozoans.

Discussion

The spiralian cell lineage is, on the one hand, highly conserved in major groups (Hejnol 2010; Lambert 2010) but on the other hand, the pattern is absent in several lineages. Spiralian groups without a spiral cleavage pattern, such as bryozoans (ectoprocts), phoronids and brachiopods have not been discussed above, but the striking similarities between fate maps of the groups with spiral cleavage make it possible to infer an ancestral spiralian pattern of development of the main body, including the central nervous system. The ecdysozoans show the 'D-quadrant pattern' in the 4-cell stage, but they do not show any spiral pattern in the following cleavages. However, both development and morphology of the ecdysozoan central nervous systems allow comparisons with the spiralians.

Blastopore fate

Blastopore fate has had a central place in phylogenetic discussions for over a century, but it has unfortunately turned out to be a highly variable character, even within smaller lineages, because it is so much influenced, for example, by the amount of yolk and the related type of development, with embolic to epibolic gastrulation to discoidal cleavage. However, the fate maps of the epithelial areas and the early differentiation of the ventral nervous system along the blastopore rim (from cells of the second micromere quartet in the few spiralians where the lineage has been documented) together give information about the ancestral blastopore closure in many of the groups with derived embryology.

A direct division of a blastopore into mouth and anus by fusion of lateral blastopore lips has only been observed in the annelid *Podarke* and nematode *Romanomermis* (see above).

Epithelial areas and ciliary bands

The episphere consisting of the first quartet micromeres with primary and accessory trochoblasts at the edge (usually at the equator of the young embryos/larvae) is observed in almost all ciliated spiralian larvae. It is especially well documented in annelids and molluscs (Table 1). The trochoblasts carry a prototroch consisting of compound cilia in almost all species. It is the main locomotory organ and is involved in downstream-collecting in the filter-feeding larvae.

The cells of the second micromere quartet cover most of the hyposphere of the larva in many species, and the progeny of the 2d-cell (the somatoblast) spreads over almost the whole body of the larva (and adult), except for the head, in annelids. The lower edge of this cell area marks the blastopore edge and carries the secondary prototroch cells in most of the larvae. The origin of the metatroch is less well documented (Table 1), but could well be the continuation of the row of secondary prototroch cells. The telotroch of the annelid larvae is likewise formed from cells of the blastopore edge.

Table 1.   Contributions of 1st and 2nd micromere quartets to ciliary bands and central nervous system in annelids and molluscs

Entries without a question mark represent observations in accordance with the trochaea theory; those with question marks represent only predictions. See also Figs 2 and 6

Annelida	Mollusca
1st micromere quartet (episphere):	*1st micromere quartet (episphere):*
Prototroch: accessory and primary trochoblasts	Prototroch: accessory and primary trochoblasts
Apical ganglion and cerebral ganglia	Apical ganglion and cerebral ganglia
2nd micromere quartet:	*2nd micromere quartet:*
Prototroch: secondary trochoblasts	Prototroch: secondary trochoblasts
Metatroch (?)	Metatroch
Telotroch	Telotroch (?)
Periblastoporal nerve ring: postero-dorsal brain,	Periblastoporal nerve ring: antero-dorsal and
peripharyngeal connectives, ventral nerve cords,	postero-dorsal loop (2b and 2d)
anal nerve loop (all from 2d)	
Blastopore edge	

The ciliated bands of other spiralian larvae are less well known, but they can, without problems, be interpreted in accordance with the trochaea theory (except in clades without the spiralian cleavage pattern).

The downstream-collecting ciliary system based on the catch-up principle (Riisgård *et al.* 2000) appears to be a spiralian apomorphy, but it may well have been present in the protostomian ancestor and was lost in the ecdysozoans. This is one of the main foundation stones of the trochaea theory. Bryozoans, phoronids and brachiopods show ciliary bands of different structure and function; they do not resemble any other metazoan ciliary bands, and their evolutionary origin has not been explained. The characteristic development, structure and function of the ciliary bands are foundation stones of the trochaea theory.

Central nervous system

The trochaea theory predicts the presence of three main components in the central nervous system: (1) an apical ganglion; (2) a pair of cerebral ganglia from the episphere; and (3) paired ventral nerves with loops around mouth and anus from the blastopore edge (hyposphere).

An apical ganglion is found at the apical pole of ciliated larvae of almost all eumetazoans with a nervous system (Neuralia; Nielsen 2012). Flask-shaped cells are found in the apical ganglion in all spiralian ciliated larvae and may be a spiralian apomorphy (Wanninger 2009; Altenburger *et al.* 2011). The apical ganglion always disintegrates before or at metamorphosis and it is probably an ancient character retained from the radially symmetrical body plan of the earliest neuralians.

Paired cerebral ganglia are apparently a protostomian apomorphy. Their homology with the cerebral ganglia of the vertebrates appears highly questionable, although many studies of gene expression indicate detailed similarities. However, if these structures are indeed homologous, the bilaterian ancestor must have been a complicated organism with a highly differentiated brain, and the origin of such an ancestor has not been discussed. The cerebral ganglia differentiate from the epithelium of the episphere (first micromere quartet) in all the spiralians where a cell lineage has been established (annelids, molluscs, nemerteans, platyhelminths). In some annelids, they

fill the prostomium almost completely. The arthropod brain comprises the protocerebrum with eyes (the ocular region), which is often regarded as homologous to the annelid cerebral brain in the prostomium (Scholtz and Edgecombe 2006). It has not been possible to identify regions in the nematode brain.

The paired ventral nerve cords differentiating from the fusing blastopore lips or homologous areas of the embryo can be recognised in most of the protostomian phyla (Nielsen 2012). This type of development is especially obvious in the annelids where it differentiates from the 2d-cell, and in the nematodes. The loop of nerve cells from the second micromere quartet around the mouth has been observed in the juveniles of the annelids *Capitella* and *Platynereis* (see above), but it has not been searched for in other species. The trochaea theory predicts that the oral loop should be formed of cells of all the four quadrants of the second micromere quartet, but more detailed studies are needed to demonstrate this. The perioral deutocerebrum of the arthropods (in the segment with the antennules in the crustaceans and the antennae in the hexapods) should accordingly be the oral loop of the periblastoporal nerve ring. This interpretation fits well with many gene-expression patterns (Steinmetz *et al.* 2011).

Posterior pioneer cells have been observed in annelids, arthropods and possibly in molluscs.

Both general morphology and ontogeny (especially of the annelids) are in good agreement with the trochaea theory.

Conclusion

It is, of course, never possible to fully corroborate a phylogenetic theory, but if the available observations do not falsify the main points of the theory, and predictions of the theory are actually fulfilled by new observations, the theory must have high credibility.

The trochaea theory proposes that the protostomes evolved from a radially symmetrical (or slightly bilateral) gastraea with a periblastoporal ring of compound cilia (archaeotroch) forming a downstream-collecting system (the trochaea). This ancestor should have added a benthic adult stage to its life cycle, with a tubular gut formed by the lateral blastopore closure. The archaeotroch should have differentiated into proto-, meta- and telotroch, with the downstream-collecting function retained in the proto- and metatroch; the accompanying periblastoporal nerve

should have become a perioral loop, a paired ventral nerve cord (and a perianal loop). The ancestral apical ganglion disappeared in adults, and a pair of cerebral ganglia developed from the episphere (apical to the prototroch).

It appears that the available information on the ontogeny and morphology of the protostomian ciliary bands and central nervous systems is in full agreement with the trochaea theory, both with regard to the development of the structures and their function. The alternative 'intercalation theory' (see for example Sly *et al*. 2003) explains the many types of planktotrophic trochophores as the result of convergent evolution of planktotrophy and the associated ciliary feeding structures by specialisations of a uniformly ciliated non-feeding larva. However, this theory completely fails to discuss the viability of the planuliform ancestor, which was without a gut, and I am not aware of any paper that discusses the adaptational value of the several complicated evolutionary steps implied between a uniformly ciliated planuliform larva and the pelagic, planktotrophic larval types (see also Nielsen 2009, 2012). Cladistic analyses of larval types (see for example Rouse 1999, 2000) are based on the assumption that gain and loss of a complicated structure, such as the downstream-collecting ciliary bands of the trochophores, are equally probable. However, we know of numerous examples of loss of planktotrophy and the associated ciliary bands in small or large lineages. Good examples are found in the nemerteans (see above) and in the echinoids (Wray 1996). There are a few examples of a return to planktotrophy from 'direct development' in gastropods (Reid 1989), but this is simply a regain of function of the velar structures retained in the intracapsular embryos. To my knowledge, there is no well documented example of *de novo* evolution of the ciliary bands of a trochophore. The idea that the metatroch was 'split off' from the prototroch (Hejnol *et al*. 2007; Henry *et al*. 2007) implies that the cilia of the metatroch should have reversed their beat, and that an adoral ciliary zone should have extended from the oral area, but these steps cannot have any adaptive value before the downstream-collection system is fully formed. Thus, this idea appears highly unlikely.

The trochaea theory had predicted that the protostomian brain should consist of the cerebral ganglia from the episphere (first micromere quartet) and the anterior part of the periblastoporal nerve ring along the blastopore lips (from the second micromere quartet), and the presence of a periblastoporal component (from the 2d-cell) has subsequently been observed in the annelids *Platynereis* and *Capitella* (Ackermann *et al*. 2005; Meyer *et al*. 2010).

I can only conclude that, with the present knowledge of morphology and embryology, the trochaea theory is the best-supported theory for the origin and early evolution of the protostomes.

Acknowledgements

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