REVIEW

Larval nervous systems: true larval and precocious adult

Claus Nielsen*

ABSTRACT

The apical organ of ciliated larvae of cnidarians and bilaterians is a true larval organ that disappears before or at metamorphosis. It appears to be sensory, probably involved in metamorphosis, but knowledge is scant. The ciliated protostome larvae show ganglia/nerve cords that are retained as the adult central nervous system (CNS). Two structures can be recognized, viz. a pair of cerebral ganglia, which form the major part of the adult brain, and a blastoporal (circumblastoporal) nerve cord, which becomes differentiated into a perioral loop, paired or secondarily fused ventral nerve cords and a small perianal loop. The anterior loop becomes part of the brain. This has been well documented through cell-lineage studies in a number of spiralians, and homologies with similar structures in the ecdysozoans are strongly indicated. The deuterostomes are generally difficult to interpret, and the nervous systems of echinoderms and enteropneusts appear completely enigmatic. The ontogeny of the chordate CNS can perhaps be interpreted as a variation of the ontogeny of the blastoporal nerve cord of the protostomes, and this is strongly supported by patterns of gene expression. The presence of 'deuterostomian' blastopore fates both in an annelid and in a mollusk, which are both placed in families with the 'normal' spiralian gastrulation type, and in the chaetognaths demonstrates that the chordate type of gastrulation could easily have evolved from the spiralian type. This indicates that the latest common ancestor of the deuterostomes was very similar to the latest common pelago-benthic ancestor of the protostomes as described by the trochaea theory, and that the neural tube of the chordates is morphologically ventral.

KEY WORDS: Deuterostomy, Metamorphosis, Nervous systems, Dorsal–ventral orientation

Introduction

Studies on ontogeny are very important for our understanding of nervous system evolution and diversification, and new methods have made important contributions to knowledge of the structure and development of larval nervous systems and their relationships to adult nervous systems.

The nervous system has for a long time been considered one of the most conservative animal organ systems and has therefore been given high importance in studies of animal evolution. More than a century ago, Hatschek (Hatschek, 1888) introduced the names Zygoneura (now Protostomia), Ambulacraria and Chordonia (now Chordata) for three major groups of the Bilateria, based on the structure and position of the central nervous system (CNS). The Zygoneura were characterized by a paired longitudinal ventral nerve cord [a cluster of nervous cells including their cell bodies, as opposed to a nerve, which is a bundle of axons (Richter et al., 2010); the ventral nerve cord may

The Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark.

*Author for correspondence (cnielsen@snm.ku.dk)

be specialized into rows of ganglia connected by connectives] and the Chordonia by an unpaired dorsal neural tube. The division of the Bilateria into Protostomia and Deuterostomia (=Cordonia + Ambulacraria) (Grobben, 1908) is still universally accepted and is now supported by numerous phylogenomic studies (Hejnol et al., 2009; Wheeler et al., 2009; Edgecombe et al., 2011). However, the interpretation of the dorsal/ventral orientation of the two groups has been challenged, and it now appears that the two longitudinal nerve cords are homologous (see below).

The topology of the bilaterian part of the animal tree of life is relatively well established, but there is not agreement about the inter-relationships of the basal metazoan groups. In particular, the position of the Ctenophora has recently come into focus. The classical view has been that the Ctenophora together with Cnidaria form the clade Coelenterata, but a number of recent phylogenomic studies (Dunn et al., 2008; Hejnol et al., 2009; Maxwell et al., 2012; Nesnidal et al., 2013) have placed the Ctenophora as the sister group of all the remaining metazoans, and the recent studies of the whole genomes of *Mnemiopsis leidyi* (Ryan et al., 2013) and *Pleurobrachia belcheri* (Moroz et al., 2014) support to this position. This implies either that the nervous systems (and muscles) have been lost in sponges and placozoans or that the nervous systems and muscles of Ctenophora and Neuralia (Cnidaria + Bilateria) have evolved convergently. However, loss of the nervous system appears highly unlikely, and the phylogenomic analyses may suffer from long branch attraction, because the latest common ancestor of the living ctenophores could have been as recent as around the K–T (Cretaceous–Tertiary) boundary (Podar et al., 2001; Jékely et al., 2015). The cydippid stage characteristic of the ctenophore life cycles must be interpreted as a juvenile rather than a larva (Ryan et al., 2013), both because it lacks special larval organs and because it can already carry out sexual reproduction (Martindale, 1987). The 'apical organ' of the mainly holopelagic ctenophores is situated at the apical pole, but its structure is completely different from that of cnidarians and bilaterians (Hernandez-Nicaise, 1991) and it is not considered homologous of the neuralian apical organ (Jager et al., 2011). The discussion of larval nervous systems can therefore be restricted to the nervous systems of Cnidaria and Bilateria, i.e. Neuralia.

Ciliated primary larvae are found in numerous lineages of Porifera, Cnidaria, Spiralia (Lophotrochozoa) and Ambulacraria (Echinodermata + Hemichordata), but are absent in Ecdysozoa and Chordata (except for the non-feeding amphioxus larva). This larval type has been called primary larvae (Jägersten, 1972).

Within the Neuralia, the position of the Acoelomorpha (Acoela, Nemertodermatida and Xenoturbellida) is still debated, with the more traditional view that they are the sister group of the remaining bilaterian groups (Hejnol et al., 2009; Nielsen, 2010; Ryan et al., 2013), or the alternative view that they are the sister group of the Ambulacraria (Philippe et al., 2011). A discussion of their phylogenetic position falls outside the scope of the present paper, and the uncertainty dictates that they will not contribute to the present discussion.

Most recent authors agree that the eumetazoan ancestor was a gastraea, although this is rarely discussed directly (e.g. Knoll and Carroll, 1999; Brusca and Brusca, 2003). Most ciliated larvae have a ciliated sensory organ at the apical pole, and I have called the corresponding ancestor neurogastraea (Nielsen, 2008). Its organization was retained in the larvae when the pelago-benthic life cycle(s) evolved through addition of adult benthic stages; this and alternative theories for the evolution of the pelago-benthic life cycles have been discussed elsewhere (Nielsen, 2013). New nervous centers developed in the benthic adults and gradually became established in the larval stages, through the evolutionary process called adultation (Jägersten, 1972), so that two types of nervous centers can be recognized in the larvae, viz. the exclusively larval apical organ and the larval–adult nervous system.

The following discussions are based on the framework of the trochaea theory (Nielsen, 2012), which appears to be the only theory that explains both the origin of the ciliary bands of the trochophora larva and the morphology of the protostomian CNS in a continuous series of adaptational modifications of existing structures.

The apical organ: the ancestral neuralian brain

The apical organ is a characteristic, ciliated, putative sensory structure in most of the ciliated neuralian larvae. It develops from the most apical blastomeres, as shown in numerous cell-lineage studies (Nielsen, 2004; Nielsen, 2005). The apical group of long cilia works together as a compound cilium (cirrus) in many species (Fig. 1), but other species show a more generally ciliated area. The apical organ consists of a group of columnar or flask-shaped cells usually with one cilium, but in some species with several; it does not fit the narrow definition of a ganglion, because it apparently comprises only sensory cells (Richter et al., 2010). Many spiralians develop lateral (cerebral) ganglia in close apposition to the apical organ, and this compound structure has been called the apical organ in most of the older morphological literature, and this is also seen in some studies on gene expression (Tosches and Arendt, 2013). However, most recent papers use the term apical organ in the restricted sense in accordance with the terminology of Richter et al. (Richter et al., 2010). The homology of apical organs of cnidarians and bilaterians has been questioned because the animal–vegetal axis has the same orientation as the apical–blastoporal axis in the bilaterians, whereas the two axes have opposite directions in the 'coelenterates' (Dunn et al., 2007). However, new observations of gene expression clearly demonstrate that the apical pole of cnidarians and the apical pole of bilaterians are homologous (Sinigaglia et al., 2013; Marlow et al., 2014; Fritzenwanker et al., 2014).

Cnidaria

The apical organ of cnidarian larvae is well studied, especially that of the sea anemone *Nematostella*, where it consists of a group of monociliated nerve cells (Marlow et al., 2014). The organization of the organ seems rather similar in all groups, but the prominent ciliary cirrus seen in anthozoans (Fig. 1A) is lacking in the medusozoans (Nakanishi et al., 2008).

The apical organ is necessary for settling (Rentzsch et al., 2008). It is lost when the larva settles with cells around the apical pole (Yuan et al., 2008). The nervous system becomes reorganized with degeneration of the larval nerve net and development of a net of new neurons; a brain is lacking both in the polyps and in the medusae (Martina, 2000; Nakanishi et al., 2008).

Protostomia

A slightly twisted cirrus of long apical cilia is seen in many ciliated larvae of annelids, mollusks, nemerteans and phoronids (Nielsen, 1987; McDougall et al., 2006; Maslakova, 2010; Temereva and Wanninger, 2012) (Fig. 1B). There is usually only one cilium per cell, but multiciliate cells are known both from mollusks and from nemerteans (Cantell et al., 1982; Page, 2002). There is much variation in the number and structure of the cells in the organ. A characteristic pattern of eight flask-shaped serotonergic cells, each with one cilium, is found in many species, but the number may increase during development, for example in the phoronid *Phoronopsis* (Temereva and Wanninger, 2012; Temereva and Tsitrin, 2014). Other types of serotonergic cells may also be present (Page, 2002).

Several annelids, mollusks, nemerteans and platyhelminths have been the subject of classical cell-lineage studies (reviewed in Nielsen, 2004; Nielsen, 2005) and the few modern studies have confirmed the earlier results in almost all details (Ackermann et al., 2005; Hejnol et al., 2007; Meyer et al., 2010). The apical organ differentiates from the most apical cells, named $1a^1$–$1d^1$ in the spiral-cleavage terminology. The organ degenerates before or at the time of settling (Dickinson and Croll, 2003), and the cells may undergo apoptosis (Gifondorwa and Leise, 2006). In the gastropod *Crepidula*, the four apical cells degenerate at an early developmental stage, long before the larva hatches from the egg mass (Conklin, 1897). In the pilidium larvae, the apical organ is shed at metamorphosis together with the whole larval body, which in some cases becomes ingested by the juvenile (Maslakova, 2010).

All entoproct larvae have a large apical organ with several types of cells; it disappears at metamorphosis (Nielsen, 1971).

Bryozoan larvae show wide variation in the structure of the apical region. Phylactolaemate 'larvae' show a concentration of
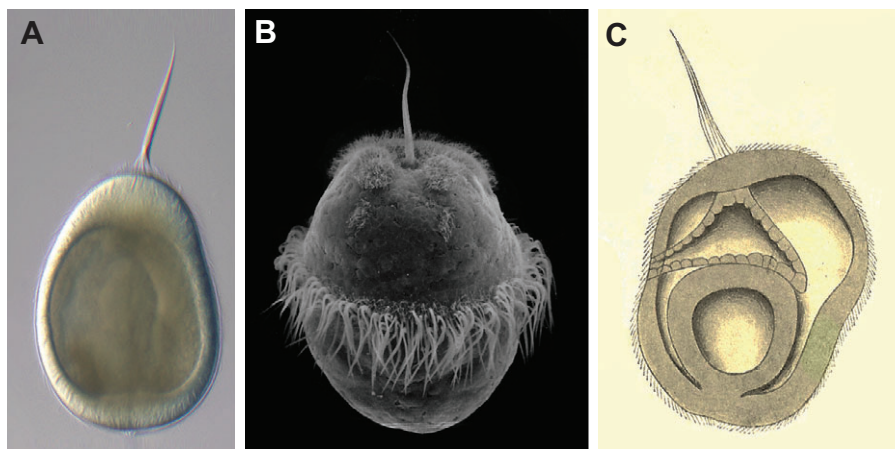

Fig. 1. Apical organs. (A) Light micrograph of a planula larva of the cnidarian *Nematostella vectensis* (courtesy of Dr Uli Technau, University of Vienna). (B) Scanning electron micrograph of a trochophora larva of the polyplacophoran mollusk *Lepidochitona dentiens*. (C) Drawing of an early tornaria larva of the enteropneust *Balanoglossus clavigerus* (Stiasny, 1914).

serotonergic sensory cells at the apical pole, but an apical tuft is not present (Gruhl, 2010); the whole ciliated epithelium including the apical pole is invaginated at metamorphosis and degenerates (Brien, 1953). Nerve cells have not been observed in cyclostome larvae. Eurystome larvae show considerable variation. The planktotrophic cyphonautes larvae have a well-defined apical organ with a central area of neurons connected to a basal nerve plexus surrounded by ring-shaped areas of monociliated and non-ciliated cells and one epitheliomuscular cell. The function of this organ is unknown, but it is connected to the pyriform sensory organ, which is used in testing the substrate for settling (Stricker et al., 1988). All the larval organs degenerate after settling (Atkins, 1955). The lecithotrophic coronate larvae have a much more complicated apical structure, with a radial array of wedge-shaped multiciliated cells (Woollacott and Zimmer, 1971; Reed and Cloney, 1982; Reed et al., 1988). These cells are underlain by a nervous plexus, which sends a prominent nerve to the pyriform organ. A ring-shaped blastema below the ciliated cells surrounds the nerve. At settling, the neural plate is pulled down and disintegrates, and the ciliated cells and the lower blastema develop into the polypide of the first zooid (Reed and Woollacott, 1983; Fuchs et al., 2011). There is no connection between the apical organ and the nervous system of the polypides.

The function of the protostomian apical organ is poorly known, but certain cells in the organ of the gastropod *Phestilla* are necessary for recognition of the right substrate for settling (Hadfield et al., 2000).

Deuterostomia

In the ambulacrarians (hemichordates + echinodermates), ciliated apical organs are found in enteropneusts and in species of all classes of echinoderms, although only some species show the typical, slightly twisted tuft and only in some stages, for example in some enteropneusts (Stiasny, 1914) (Fig. 1C). The various types of larvae show some variation in the structure of the nervous system at the apical pole, with more scattered groups of flask-shaped cells located at the most apical parts of the circumoral ciliary band (neotroch) (Byrne et al., 2007). So the morphology is not very reminiscent of the apical organ in cnidarians and protostomes, but gene expression supports the homology (Yaguchi et al., 2010), and the apical organs always degenerate before or at metamorphosis, both in echinoderms (Byrne et al., 2007) and in enteropneusts (Miyamoto et al., 2010).

The chordates do not have ciliated larvae and an apical organ is not present. The theory for the origin of the chordate CNS proposed by Garstang (Garstang, 1928) and presented in many textbooks shows the apical organ being internalized by the fusion of lateral ciliary bands and becoming situated in the brain. This idea has found some support from ultrastructure and gene expression studies (Lacalli et al., 1994; Tagawa et al., 2000), but it is incompatible with the theory for the evolution of the chordate CNS presented below.

The larval–adult CNS

The adult CNS begins to develop already in an early larval (or embryonic) stage in all bilaterians. In protostomes, it consists of a pair of cerebral ganglia and a blastoporal (circumblastoporal) nerve ring, where the anterior part of the ring and the cerebral ganglia together become the adult brain. The deuterostomes are more difficult to interpret, but both embryology and gene expression patterns of the chordate CNS indicate homology with the blastoporal nerve ring of the protostomes.

The recent papers by Tosches and Arendt (Tosches and Arendt, 2013) and Marlow et al. (Marlow et al., 2014) recognize the dual nature of the nervous system of the vertebrates; the cerebral ganglia

are called the apical legacy or apical plate and the blastoporal nerve cord is called the blastoporal legacy or medio-lateral patterning. The 'forebrain' is described as a chimaera. This is in good accordance with the descriptions and interpretations below.

Cnidaria

A concentration of nerve cells is seen around the mouth, but they cannot be characterized as a nerve cord (Koizumi, 2007; Marlow et al., 2009).

Protostomia

Both development and morphology of the adult central nervous system is quite well known in many spiralians (reviews in Nielsen, 2004; Nielsen, 2005), and its evolution has been explained by the trochaea theory (Nielsen, 2012). Typically, it consists of a pair of cerebral ganglia and a blastoporal nerve cord, which differentiates into the paired or fused ventral nerve cord with a perioral and a perianal loop (Fig. 2). This is best seen in annelids (Meyer et al., 2010), but the pattern can clearly be recognized, for example in mollusks (Dickinson and Croll, 2003).

The cerebral ganglia differentiate from two cells of the first micromere quartet in the spiral cleavage, from the cells 1c and 1d in the polychaete *Platynereis* (Ackermann et al., 2005), and from 1a and 1c in the gastropod *Crepidula* (Hejnol et al., 2007). The eyes found in many annelid and mollusk larvae develop from cells of the cerebral ganglia (Conklin, 1897; Ackermann et al., 2005).
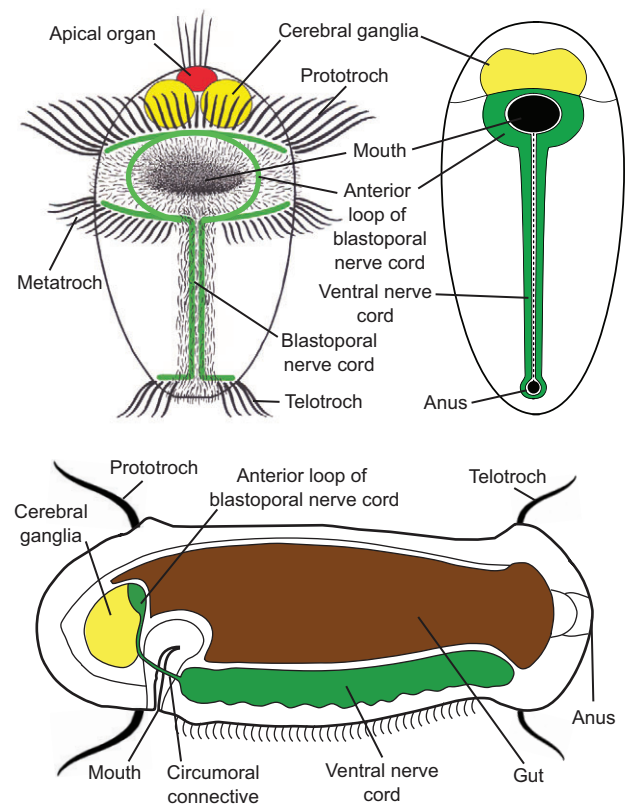

Fig. 2. Central nervous systems (CNSs) in protostomes, exemplified by annelids. The upper two illustrations show the CNS of a trochophora larva and a juvenile, as interpreted by the trochaea theory [modified from Nielsen (Nielsen, 2012)], and the lower illustration shows the CNS of a late lecithotrophic larva of *Capitella* as demonstrated through a cell-lineage study (see Meyer et al., 2010). The shape of the circumblastoporal nerve ring in *Capitella* is exactly as predicted, except that the anal loop is missing; it may have been overlooked, and it is present in many other annelids.
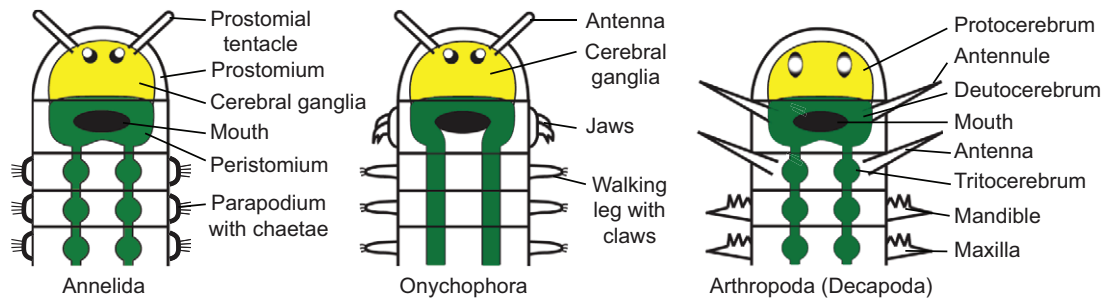
Fig. 3. Comparison of spiralian and ecysozoan CNSs. Nervous systems of annelids, onychophorans and arthropods (exemplified by a crustacean) with the cerebral ganglia in yellow and the blastoporal nervous system in green (cf. Fig. 2). Based on Eriksson et al. (Eriksson et al., 2003), Harzsch (Harzsch, 2004) and Scholtz and Edgecombe (Scholtz and Edgecombe, 2006).

The anterior part of the perioral loop around the mouth, which becomes the posterior part of the brain, has only been demonstrated by the cell lineage in annelids (Ackermann et al., 2005; Meyer et al., 2010; Meyer and Seaver, 2010). In *Capitella*, the right and left ventral nerve cords develop from the blastomeres $2d^{1121}$ and $2d^{1122}$, respectively (Meyer and Seaver, 2010). Observations of gene expression (Tosches and Arendt, 2013) are in full agreement with this interpretation (see below).

The embryological origin of the cerebral ganglia and blastoporal nervous system is difficult to deduce in groups without spiral cleavage. Phoronids and brachiopods seem to lack cerebral ganglia and are usually believed to lack the blastoporal nervous system. However, the large larva of *Phoronopsis harmeri* (Temereva, 2012) and the larva of the brachiopod *Novocrania anomala* (Altenburger and Wanninger, 2010) show paired longitudinal blastoporal nerves with a number of commissures, and this is very similar to the blastoporal nervous system of the annelids.

Entoproct larvae have a paired or unpaired frontal organ/ganglion, which may be modified cerebral ganglia. The organ degenerates after metamorphosis (Nielsen, 1971). Some observations on *Loxosomella* larvae indicate the presence of longitudinal nerves in the foot (Wanninger et al., 2007). Their fate is unknown. Bryozoan larvae lack cerebral ganglia and ventral nerve cords.

Both morphology and gene expression indicate that the cerebral ganglia of the annelids are homologous with the protocerebrum of the arthropods (Harzsch, 2004; Scholtz and Edgecombe, 2006) (Fig. 3). The deutocerebrum, tritocerebrum and the more posterior ganglia and their commissures represent the blastoporal nerve cords with the loop around the mouth.

The few cell-lineage studies of ecdysozoans, such as the shrimp *Sicyonia* (Hertzler, 2002) and the nematodes *Caenorhabditis* and *Romanomermis* (Sulston et al., 1983; Schulze and Schierenberg, 2009), are difficult to relate to those on the spiralians.

Deuterostomia

Echinoderms and hemichordates have ciliated primary larvae, whereas chordates lack primary larvae (except for the early non-feeding amphioxus larva).

The various types of dipleurula larvae of the echinoderms and the tornaria of the enteropneusts have a perioral ciliary band, the neotroch, which is of a structure and function quite different from those of the protostomian larvae, viz. a band of monociliate cells that capture particles through ciliary reversal (Strathmann, 2007). It disintegrates together with the associated nerves before or at metamorphosis and the nerves are apparently not in any way associated with the adult nervous system. The nervous system of the adult echinoderms consists of an oral nerve ring and five (or more) radial nerve cords. There is nothing like a brain (Heinzeller and Welsch, 2001). The radial nerves of echinoids, asteroids and holothurians develop from invaginations of the ectoderm along the developing radii of the adult rudiment and this resembles the formation of the chordate neural tube (Smith, 1988). The organization of this structure involves a number of genes also active in neurulation, and the radial nerves have been interpreted as homologs of the chordate neural tube (Haag, 2005). However, this must be interpreted as an example of homoplasy (Nielsen and Martinez, 2003; Nielsen, 2006). The tornaria larva has a posterior ring of large, compound cilia, which has often been called a telotroch, but its homology is uncertain (Nielsen and Hay-Schmidt, 2007). There is a nerve along this ciliary band (Lacalli and Gilmour, 2001), and both the ciliary band and the nerve begin to degenerate at metamorphosis. The nervous system of juvenile and adult enteropneusts comprises a dorsal collar cord in the region just behind the mouth, a longitudinal nerve cord extending from the collar cord posteriorly along the length of the body, and nerves around the pharynx to a longitudinal nerve cord along the ventral side. The collar cord develops through a chordate-like neurulation, and a number of the cell types and their distribution resemble those seen in chordates (Kaul and Stach, 2010). Also, gene expression shows strong similarities (Pani et al., 2012), but the gene expression patterns in the dorsal and ventral nerve cords of the body are very similar (Nomaksteinsky et al., 2009). Neither morphology nor gene expression give unambiguous information about the dorsal–ventral orientation (Holland et al., 2013).

The CNS of the chordates develops from the characteristic neural tube, which is specialized in different ways in the three phyla Cephalochordata, Urochordata and Vertebrata.

The dorsal–ventral orientation of the cephalochordates can only be inferred through comparisons with the other chordates, because their ontogeny clearly indicates that their mouth is the modified first left gill opening (Ruppert, 1997; Benito-Gutiérrez and Arendt, 2009). The anterior part of the neural tube differentiates into a brain vesicle, and various types of photoreceptors and other sensory organs are found along its length (Wicht and Lacalli, 2005).

The orientation of most of the adult urochordates is difficult to make out because of their metamorphoses with the rotation of the gut, but the development of certain ascidians, such as *Ciona* and *Clavelina*, shows that the stomodaeum/mouth is situated at the same side as the neural tube, which then ought to be described as ventral (Seeliger and Hartmeyer, 1893-1911; Veeman et al., 2010). The ascidian larvae have a neural tube without nerve cells in the tail and an anterior brain vesicle, in some species with an eye, a statocyte and various other sensory structures (Burighel and Cloney, 1997). The neural tube in the tail is resorbed at settling. In the ascidians,
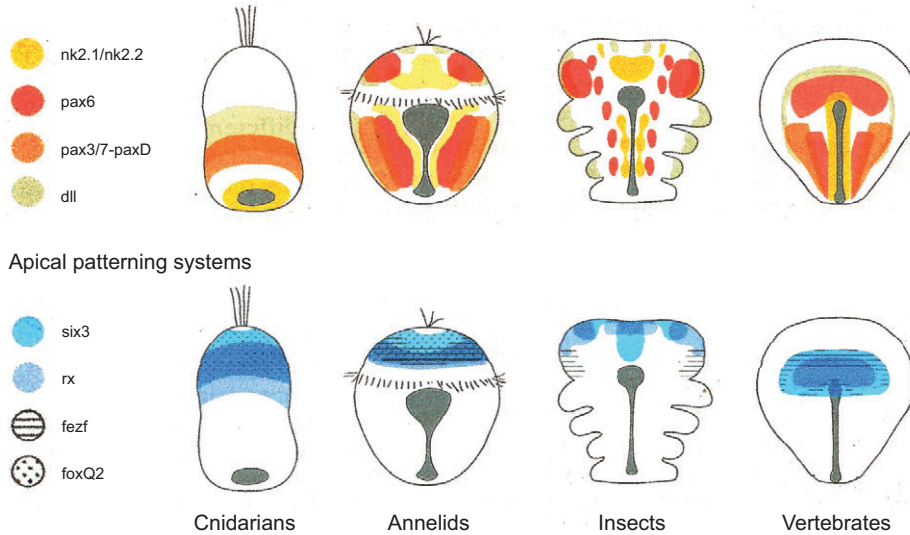
Blastoporal patterning systems

Apical patterning systems

Fig. 4. Regional gene expression systems in cnidarians, annelids, arthropods and vertebrates. None of the genes is expressed at the apical pole. The 'blastoporal patterning system' (especially nk2.1/nk2.2) of the annelid and insect shows strong similarities with the expression in the vertebrate. The 'apical patterning system' in the annelid appears to correspond to the position of the cerebral ganglia at the apical side of the prototroch; homology of the cerebral ganglia of annelids and the protocerebrum of arthropods is supported (see Fig. 3), but homology with the hypothalamus and eyes of vertebrates is questionable. Modified from Tosches and Arendt (Tosches and Arendt, 2013).

the anterior end differentiates into a small brain (Mackie and Burighel, 2005).

The orientation of the vertebrates appears intuitively straightforward, with the mouth on the ventral side (and the CNS on the dorsal side), but more than a century ago Dohrn (Dohrn, 1875) suggested that the central nervous systems of annelids and vertebrates are homologous. Many studies have demonstrated that gene expression exhibits the same pattern in the developing ventral nerve cord of an annelid and the fused neural folds of a vertebrate (De Robertis and Sasai, 1996; Denes et al., 2007; Lowe et al., 2006) indicating homology, and several studies of anatomy and embryology have supported this view, so both CNSs ought to be called ventral (Arendt and Nübler-Jung, 1997; Nielsen, 1999). Comparisons of gene expression in cnidarians, protostomes and vertebrates are in full agreement with this view (Tosches and Arendt, 2013) (Fig. 4).

A fresh view on the classical description of the embryology of the frog *Xenopus* (Hausen and Riebesell, 1991) with the apical pole kept in the same position during development clearly shows how the anterior end of the neural tube extends around the anterior end of the embryo (and the blastoporal/anal area around the posterior end) so that both the mouth and anus become situated on the side called dorsal in the protostomes (Fig. 5).

The character used for distinguishing Protostomia and Deuterostomia has always been the fate of the blastopore. The protostomian nerve cords typically differentiate along the fusing lateral blastopore lips, or from homologous areas in direct developing species, with the blastopore developing into the mouth, or in more modern interpretation into the mouth + anus (amphistomy). In the deuterostomes, the blastopore becomes the anus (Grobben, 1908). Amphistomy has never been observed in any deuterostome, but it is remarkable that an embryology with the blastopore becoming only the anus has been observed both in the gastropod *Viviparus* (Dautert, 1929) and in the annelid *Eunice* (Åkesson, 1967) (Fig. 6). Both genera are nested within groups with the 'normal' protostomy. Also, the Chaetognatha, which are protostomes, as indicated both by their CNS and by molecular phylogeny (Harzsch and Müller, 2007; Hejnol et al., 2009; Edgecombe et al., 2011; Rieger et al., 2011), show deuterostomy (Doncaster, 1902). This indicates that deuterostomy is in fact a variation of protostomy, with the neural tube of the chordates being a homolog of the ventral nerve cord(s) of the protostomes. This

implies that the mouth (stomodaeum) should develop inside the brain area or be a completely new structure. In the urochordates (ascidians), the stomodaeum is derived from the anterior neuropore, which is situated at the anterior edge of the neural plate (Veeman et al., 2010). In vertebrates (*Xenopus*), the mouth develops from the anterior part of the neural fold, which extends forward in a narrow line and appears to become pinched off from the neural tube (Drysdale and Elinson, 1991). Thus, there is good reason to interpret the mouth of the urochordates and vertebrates as a modified anterior opening of an amphistome blastopore closure.

The question then arises whether a homolog of the protostomian cerebral ganglia could be present in vertebrates. The 'apical legacy' area of the vertebrate brain (Tosches and Arendt, 2013) is situated in the area corresponding to the episphere of the spiralian larva, so it could represent the cerebral ganglia with the eyes of the
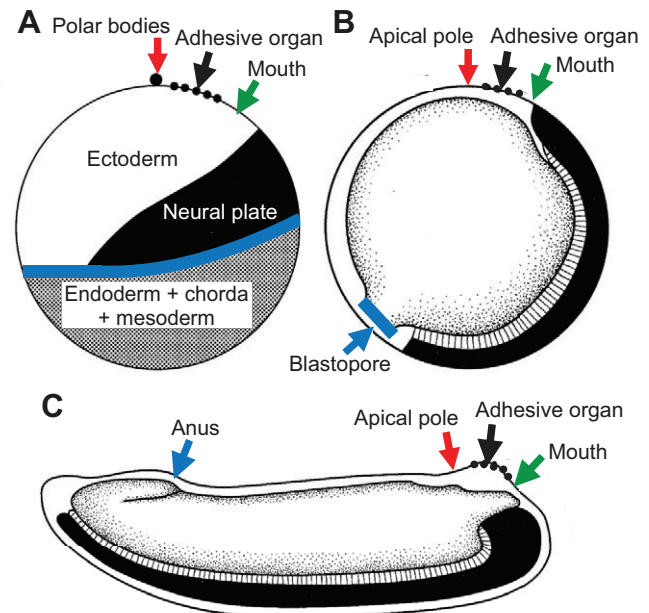

Fig. 5. Development of the frog *Xenopus*. The orientation is determined by the apical pole (red arrow). (A) Fate map. (B) Young embryo. (C) Older embryo. The blastopore rim is blue and the cells of the neural plate developing into the CNS are black. Modified from Nielsen (Nielsen, 1999).
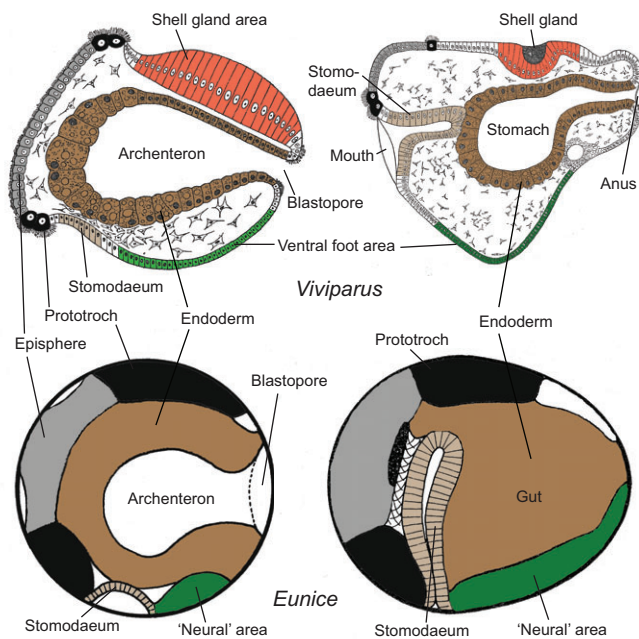
Fig. 6. 'Deuterostomy' in protostomes. Median sections of embryos. In *Viviparus*, the blastopore directly becomes the anus and the whole archenteron becomes the stomach; the stomodaeum develops from an area just behind the prototroch. The ventral foot area is the general area of the blastoporal nervous system, although there are no nerves in the midline. Modified from Otto and Tönniges (Otto and Tönniges, 1906). In *Eunice*, the archenteron becomes solid, but the anus breaks through in the area of the blastopore at a later stage; the stomodaeum develops from an area just behind the prototroch. The ventral nerves develop from the ventral area but there are no nerves in the midline. Modified from Åkesson (Åkesson, 1967).

protostomes, but the vertebrate eyes develop from the apical plate, i.e. in the blastoporal area (Eagleson and Harris, 1990), so this is very questionable.

Conclusions

The CNSs of the neuralians (cnidarians + bilaterians) comprise three main components. (1) The apical organ, which is present in almost all ciliated larvae. It degenerates before or at metamorphosis. It is the ancestral neuralian brain and the only cnidarian 'brain'. (2) A pair of cerebral ganglia that develop in bilaterian larvae/embryos (from the first micromere quartet in the spiralians). They form the main part of the protostomian brain, but it is difficult to identify homologous structures in the vertebrate brain. (3) A blastoporal (circumblastoporal) nerve cord, which is represented by the posterior part of the brain and the ventral nerve cords in the protostomes (developing from the second micromere quartet in the spiralians) and by the neural tube in the chordates (brain plus spinal cord in the vertebrates).

A further conclusion is that the latest common ancestor of the bilaterians resembled the pelago-benthic ancestor of the protostomes as envisaged by the trochaea theory (Fig. 2). The blastopore fate of the deuterostomes could be a specialization of the protostomian lateral blastopore closure (amphistomy), as illustrated by the occurrence of 'deuterostomy' in the annelid *Eunice*, the gastropod *Viviparus* and the chaetognaths. This indicates that the deuterostomes could be an early offshoot from the stem-lineage of the protostomes.

References
Ackermann, C., Dorresteijn, A. and Fischer, A. (2005). Clonal domains in postlarval *Platynereis dumerilii* (Annelida: Polychaeta). *J. Morphol.* **266**, 258-280.

Åkesson, B. (1967). The embryology of the polychaete *Eunice kobiensis*. *Acta Zool.* **48**, 142-192.

Altenburger, A. and Wanninger, A. (2010). Neuromuscular development in *Novocrania anomala*: evidence for the presence of serotonin and a spiralian-like apical organ in lecithotrophic brachiopod larvae. *Evol. Dev.* **12**, 16-24.

Arendt, D. and Nübler-Jung, K. (1997). Dorsal or ventral: similarities in fate maps and gastrulation patterns in annelids, arthropods and chordates. *Mech. Dev.* **61**, 7-21.

Atkins, D. (1955). The cyphonautes larvae of the Plymouth area and the metamorphosis of *Membranipora membranacea* (L.). *J. Mar. Biol. Assoc. UK* **34**, 441-449.

Benito-Gutiérrez, E. and Arendt, D. (2009). CNS evolution: new insight from the mud. *Curr. Biol.* **19**, R640-R642.

Brien, P. (1953). Etude sur les phylactolémates. *Ann. Soc. R. Zool. Belg.* **84**, 301-444.

Brusca, R. C. and Brusca, G. J. (2003). *Invertebrates*, 2nd edn. Sunderland, MA: Sinauer.

Burighel, P. and Cloney, R. A. (1997). Urochordata: *Ascidia*. In *Microscopic Anatomy of Invertebrates*, Vol. 15 (ed. F. W. Harrison), pp. 221-347. New York, NY: Wiley-Liss.

Byrne, M., Nakajima, Y., Chee, F. C. and Burke, R. D. (2007). Apical organs in echinoderm larvae: insights into larval evolution in the Ambulacraria. *Evol. Dev.* **9**, 432-445.

Cantell, C.-E., Franzén, Å. and Sensenbaugh, T. (1982). Ultrastructure of multiciliated collar cells in the pilidium larva of *Lineus bilineatus* (Nemertini). *Zoomorphology* **101**, 1-15.

Conklin, E. G. (1897). The embryology of *Crepidula*, a contribution to the cell lineage and early development of some marine gasteropods. *J. Morphol.* **13**, 1-226.

Dautert, E. (1929). Die bildung der keimblätter von paludina vivipara. *Zool. Jahrb. Anat.* **50**, 433-496.

De Robertis, E. M. and Sasai, Y. (1996). A common plan for dorsoventral patterning in Bilateria. *Nature* **380**, 37-40.

Denes, A. S., Jékely, G., Steinmetz, P. R. H., Raible, F., Snyman, H., Prud'homme, B., Ferrier, D. E. K., Balavoine, G. and Arendt, D. (2007). Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in bilateria. *Cell* **129**, 277-288.

Dickinson, A. J. G. and Croll, R. P. (2003). Development of the larval nervous system of the gastropod *Ilyanassa obsoleta*. *J. Comp. Neurol.* **466**, 197-218.

Dohrn, A. (1875). *Der Ursprung der Wirbelthiere und das Princip des Funktionswechsels*. Leipzig: Wilhelm Engelmann.

Doncaster, L. (1902). On the development of *Sagitta*; with notes on the anatomy of the adult. *Q. J. Microsc. Sci.* **46**, 351-395.

Drysdale, T. A. and Elinson, R. P. (1991). Development of the *Xenopus laevis* hatching gland and its relationship to surface ectoderm patterning. *Development* **111**, 469-478.

Dunn, E. F., Moy, V. N., Angerer, L. M., Angerer, R. C., Morris, R. L. and Peterson, K. J. (2007). Molecular paleoecology: using gene regulatory analysis to address the origins of complex life cycles in the late Precambrian. *Evol. Dev.* **9**, 10-24.

Dunn, C. W., Hejnol, A., Matus, D. Q., Pang, K., Browne, W. E., Smith, S. A., Seaver, E., Rouse, G. W., Obst, M., Edgecombe, G. D. et al. (2008). Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* **452**, 745-749.

Eagleson, G. W. and Harris, W. A. (1990). Mapping of the presumptive brain regions in the neural plate of *Xenopus laevis*. *J. Neurobiol.* **21**, 427-440.

Edgecombe, G. D., Giribet, G., Dunn, C. W., Hejnol, A., Kristensen, R. M., Neves, R. C., Rouse, G., Worsaae, K. and Sørensen, M. V. (2011). Higher-level metazoan relationships: recent progress and remaining questions. *Org. Divers. Evol.* **11**, 151-172.

Eriksson, B. J., Tait, N. N. and Budd, G. E. (2003). Head development in the onychophoran *Euperipatoides kanangrensis* with particular reference to the central nervous system. *J. Morphol.* **255**, 1-23.

Fritzenwanker, J. H., Gerhart, J., Freeman, R. M., Jr and Lowe, C. J. (2014). The Fox/Forkhead transcription factor family of the hemichordate *Saccoglossus kowalevskii*. *EvoDevo* **5**, 17.

Fuchs, J., Martindale, M. Q. and Hejnol, A. (2011). Gene expression in bryozoan larvae suggest a fundamental importance of pre-patterned blastemic cells in the bryozoan life-cycle. *EvoDevo* **2**, 13.

Garstang, W. (1928). The morphology of the Tunicata, and its bearings on the phylogeny of the Chordata. *Q. J. Microsc. Sci.* **72**, 51-187.

Gifondorwa, D. J. and Leise, E. M. (2006). Programmed cell death in the apical ganglion during larval metamorphosis of the marine mollusc *Ilyanassa obsoleta*. *Biol. Bull.* **210**, 109-120.

Grobben, K. (1908). Die systematische einteilung des tierreichs. *Verh. Zool. Bot. Ges. Wien* **58**, 491-511.

Gruhl, A. (2010). Neuromuscular system of the larva of *Fredericella sultana* (Bryozoa: Phylactolaemata). *Zool. Anz.* **249**, 139-149.

Haag, E. S. (2005). Echinoderm rudiments, rudimentary bilaterians, and the origin of the chordate CNS. *Evol. Dev.* **7**, 280-281.

Hadfield, M. G., Meleshkevitch, E. A. and Boudko, D. Y. (2000). The apical sensory organ of a gastropod veliger is a receptor for settlement cues. *Biol. Bull.* **198**, 67-76.

Harzsch, S. (2004). The tritocerebrum of Euarthropoda: a "non-drosophilocentric" perspective. *Evol. Dev.* **6**, 303-309.

Harzsch, S. and Müller, C. H. G. (2007). A new look at the ventral nerve centre of *Sagitta*: implications for the phylogenetic position of Chaetognatha (arrow worms) and the evolution of the bilaterian nervous system. *Front. Zool.* **4**, 14.

Hatschek, B. (1888). *Lehrbuch der Zoologie*, 1. Lieferung, pp. 1-144. Jena: Gustav Fischer.

Hausen, P. and Riebesell, M. (1991). *The Early Development of Xenopus Laevis*. Berlin: Springer-Verlag.

Heinzeller, T. and Welsch, U. (2001). The echinoderm nervous system and its phylogenetic interpretation. In *Brain Evolution and Cognition* (ed. G. Roth and M. F. Wullimann), pp. 41-75. New York, NY: Wiley.

Hejnol, A., Martindale, M. Q. and Henry, J. Q. (2007). High-resolution fate map of the snail *Crepidula fornicata*: the origins of ciliary bands, nervous system, and muscular elements. *Dev. Biol.* **305**, 63-76.

Hejnol, A., Obst, M., Stamatakis, A., Ott, M., Rouse, G. W., Edgecombe, G. D., Martinez, P., Baguñà, J., Bailly, X., Jondelius, U. et al. (2009). Assessing the root of bilaterian animals with scalable phylogenomic methods. *Proc. Biol. Sci.* **276**, 4261-4270.

Hernandez-Nicaise, M.-L. (1991). Ctenophora. In *Microscopic Anatomy of Invertebrates*, Vol. 2 (ed. F. W. Harrison), pp. 359-418. New York, NY: Wiley-Liss.

Hertzler, P. L. (2002). Development of the mesendoderm in the dendrobranchiate shrimp *Sicyonia ingentis*. *Arthropod Struct. Dev.* **31**, 33-49.

Holland, L. Z., Carvalho, J. E., Escriva, H., Laudet, V., Schubert, M., Shimeld, S. M. and Yu, J.-K. (2013). Evolution of bilaterian central nervous systems: a single origin? *EvoDevo* **4**, 27.

Jager, M., Chiori, R., Alié, A., Dayraud, C., Quéinnec, E. and Manuel, M. (2011). New insights on ctenophore neural anatomy: immunofluorescence study in *Pleurobrachia pileus* (Müller, 1776). *J. Exp. Zool. B* **316**, 171-187.

Jägersten, G. (1972). *Evolution of the Metazoan Life Cycle*. London: Academic Press.

Jékely, G., Paps, J. and Nielsen, C. (2015). The phylogenetic position of ctenophores and the origin(s) of nervous systems. *EvoDevo* **6**, 1.

Kaul, S. and Stach, T. (2010). Ontogeny of the collar cord: neurulation in the hemichordate *Saccoglossus kowalevskii*. *J. Morphol.* **271**, 1240-1259.

Knoll, A. H. and Carroll, S. B. (1999). Early animal evolution: emerging views from comparative biology and geology. *Science* **284**, 2129-2137.

Koizumi, O. (2007). Nerve ring of the hypostome in hydra: is it an origin of the central nervous system of bilaterian animals? *Brain Behav. Evol.* **69**, 151-159.

Lacalli, T. C. and Gilmour, T. H. J. (2001). Locomotory and feeding effectors of the tornaria larva of *Balanoglossus biminensis*. *Acta Zool.* **82**, 117-126.

Lacalli, T. C., Holland, N. D. and West, J. E. (1994). Landmarks in the anterior central nervous system of amphioxus larvae. *Philos. Trans. R. Soc. B* **344**, 165-185.

Lowe, C. J., Terasaki, M., Wu, M., Freeman, R. M., Jr, Runft, L., Kwan, K., Haigo, S., Aronowicz, J., Lander, E., Gruber, C. et al. (2006). Dorsoventral patterning in hemichordates: insights into early chordate evolution. *PLoS Biol.* **4**, e291.

Mackie, G. O. and Burighel, P. (2005). The nervous system in adult tunicates: current research directions. *Can. J. Zool.* **83**, 151-183.

Marlow, H. Q., Srivastava, M., Matus, D. Q., Rokhsar, D. and Martindale, M. Q. (2009). Anatomy and development of the nervous system of *Nematostella vectensis*, an anthozoan cnidarian. *Dev. Neurobiol.* **69**, 235-254.

Marlow, H., Tosches, M. A., Tomer, R., Steinmetz, P. R., Lauri, A., Larsson, T. and Arendt, D. (2014). Larval body patterning and apical organs are conserved in animal evolution. *BMC Biol.* **12**, 7.

Martina, V. J. (2000). Reorganization of the nervous system during metamorphosis of a hydrozoan planula. *Invertebr. Biol.* **119**, 243-253.

Martindale, M. Q. (1987). Larval reproduction in the ctenophore *Mnemiopsis mccradyi* (order Lobata). *Mar. Biol.* **94**, 409-414.

Maslakova, S. A. (2010). Development to metamorphosis of the nemertean pilidium larva. *Front. Zool.* **7**, 30.

Maxwell, E. K., Ryan, J. F., Schnitzler, C. E., Browne, W. E. and Baxevanis, A. D. (2012). MicroRNAs and essential components of the microRNA processing machinery are not encoded in the genome of the ctenophore *Mnemiopsis leidyi*. *BMC Genomics* **13**, 714.

McDougall, C., Chen, W.-C., Shimeld, S. M. and Ferrier, D. E. (2006). The development of the larval nervous system, musculature and ciliary bands of *Pomatoceros lamarckii* (Annelida): heterochrony in polychaetes. *Front. Zool.* **3**, 16.

Meyer, N. P. and Seaver, E. C. (2010). Cell lineage and fate map of the primary somatoblast of the polychaete annelid *Capitella teleta*. *Integr. Comp. Biol.* **50**, 756-767.

Meyer, N. P., Boyle, M. J., Martindale, M. Q. and Seaver, E. C. (2010). A comprehensive fate map by intracellular injection of identified blastomeres in the marine polychaete *Capitella teleta*. *EvoDevo* **1**, 8.

Miyamoto, N., Nakajima, Y., Wada, H. and Saito, Y. (2010). Development of the nervous system in the acorn worm *Balanoglossus simodensis*: insights into nervous system evolution. *Evol. Dev.* **12**, 416-424.

Moroz, L. L., Kocot, K. M., Citarella, M. R., Dosung, S., Norekian, T. P., Povolotskaya, I. S., Grigorenko, A. P., Dailey, C., Berezikov, E., Buckley, K. M. et al. (2014). The ctenophore genome and the evolutionary origins of neural systems. *Nature* **510**, 109-114.

Nakanishi, N., Yuan, D., Jacobs, D. K. and Hartenstein, V. (2008). Early development, pattern, and reorganization of the planula nervous system in *Aurelia* (Cnidaria, Scyphozoa). *Dev. Genes Evol.* **218**, 511-524.

Nesnidal, M. P., Helmkampf, M., Bruchhaus, I., El-Matbouli, M. and Hausdorf, B. (2013). Agent of whirling disease meets orphan worm: phylogenomic analyses firmly place Myxozoa in Cnidaria. *PLoS ONE* **8**, e54576.

Nielsen, C. (1971). Entoproct life-cycles and the entoproct/ectoproct relationship. *Ophelia* **9**, 209-341.

Nielsen, C. (1987). Structure and function of metazoan ciliary bands and their phylogenetic significance. *Acta Zool. (Stockh.)* **68**, 205-262.

Nielsen, C. (1999). Origin of the chordate central nervous system – and the origin of chordates. *Dev. Genes Evol.* **209**, 198-205.

Nielsen, C. (2004). Trochophora larvae: cell-lineages, ciliary bands, and body regions. 1. Annelida and Mollusca. *J. Exp. Zool. B* **302**, 35-68.

Nielsen, C. (2005). Trochophora larvae: cell-lineages, ciliary bands and body regions. 2. Other groups and general discussion. *J. Exp. Zool. B* **304**, 401-447.

Nielsen, C. (2006). Homology of echinoderm radial nerve cords and the chordate neural tube. *Evol. Dev.* **8**, 1-2, discussion 3-5.

Nielsen, C. (2008). Six major steps in animal evolution: are we derived sponge larvae? *Evol. Dev.* **10**, 241-257.

Nielsen, C. (2010). After all: *Xenoturbella* is an acoelomorph! *Evol. Dev.* **12**, 241-243.

Nielsen, C. (2012). How to make a protostome. *Invertebr. Syst.* **26**, 25-40.

Nielsen, C. (2013). Life cycle evolution: was the eumetazoan ancestor a holopelagic, planktotrophic gastraea? *BMC Evol. Biol.* **13**, 171.

Nielsen, C. and Hay-Schmidt, A. (2007). Development of the enteropneust *Ptychodera flava*: ciliary bands and nervous system. *J. Morphol.* **268**, 551-570.

Nielsen, C. and Martinez, P. (2003). Patterns of gene expression: homology or homocracy? *Dev. Genes Evol.* **213**, 149-154.

Nomaksteinsky, M., Röttinger, E., Dufour, H. D., Chettouh, Z., Lowe, C. J., Martindale, M. Q. and Brunet, J.-F. (2009). Centralization of the deuterostome nervous system predates chordates. *Curr. Biol.* **19**, 1264-1269.

Otto, H. and Tönniges, C. (1906). Untersuchungen über die Entwicklung von *Paludina vivipara*. *Z. Wiss. Zool.* **80**, 411-514.

Page, L. R. (2002). Comparative structure of the larval apical sensory organ in gastropods and hypotheses about function and developmental evolution. *Invertebr. Reprod. Dev.* **41**, 193-200.

Pani, A. M., Mullarkey, E. E., Aronowicz, J., Assimacopoulos, S., Grove, E. A. and Lowe, C. J. (2012). Ancient deuterostome origins of vertebrate brain signalling centres. *Nature* **483**, 289-294.

Philippe, H., Brinkmann, H., Copley, R. R., Moroz, L. L., Nakano, H., Poustka, A. J., Wallberg, A., Peterson, K. J. and Telford, M. J. (2011). Acoelomorph flatworms are deuterostomes related to *Xenoturbella*. *Nature* **470**, 255-258.

Podar, M., Haddock, S. H. D., Sogin, M. L. and Harbison, G. R. (2001). A molecular phylogenetic framework for the phylum Ctenophora using 18S rRNA genes. *Mol. Phylogenet. Evol.* **21**, 218-230.

Reed, C. G. and Cloney, R. A. (1982). The larval morphology of the marine bryozoan *Bowerbankia gracilis* (Ctenostomata: Vesicularioidea). *Zoomorphology* **100**, 23-54.

Reed, C. G. and Woollacott, R. M. (1983). Mechanisms of rapid morphogenetic movements in the metamorphosis of the bryozoan *Bugula neritina* (Cheilostomata, Cellularioidea): II. The role of dynamic assemblages of microfilaments in the pallial epithelium. *J. Morphol.* **177**, 127-143.

Reed, C. G., Ninos, J. N. and Woollacott, R. M. (1988). Bryozoan larvae as mosaics of multifunctional ciliary fields: ultrastructure of the sensory organs of *Bugula stolonifera* (Cheilostomata: Cellularioidea). *J. Morphol.* **197**, 127-145.

Rentzsch, F., Fritzenwanker, J. H., Scholz, C. B. and Technau, U. (2008). FGF signalling controls formation of the apical sensory organ in the cnidarian *Nematostella vectensis*. *Development* **135**, 1761-1769.

Richter, S., Loesel, R., Purschke, G., Schmidt-Rhaesa, A., Scholtz, G., Stach, T., Vogt, L., Wanninger, A., Brenneis, G., Döring, C. et al. (2010). Invertebrate neurophylogeny: suggested terms and definitions for a neuroanatomical glossary. *Front. Zool.* **7**, 29.

Rieger, V., Perez, Y., Müller, C. H. G., Lacalli, T., Hansson, B. S. and Harzsch, S. (2011). Development of the nervous system in hatchlings of *Spadella cephaloptera* (Chaetognatha), and implications for nervous system evolution in Bilateria. *Dev. Growth Differ.* **53**, 740-759.

Ruppert, E. E. (1997). Cephalochordata (Acrania). In *Microscopic Anatomy of Invertebrates*, Vol. 15 (ed. F. W. Harrison), pp. 349-504. New York, NY: Wiley-Liss.

Ryan, J. F., Pang, K., Schnitzler, C. E., Nguyen, A.-D., Moreland, R. T., Simmons, D. K., Koch, B. J., Francis, W. R., Havlak, P., Smith, S. A. et al.; NISC Comparative Sequencing Program (2013). The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. *Science* **342**, 1242592.

Scholtz, G. and Edgecombe, G. D. (2006). The evolution of arthropod heads: reconciling morphological, developmental and palaeontological evidence. *Dev. Genes Evol.* **216**, 395-415.

Schulze, J. and Schierenberg, E. (2009). Embryogenesis of Romanomermis culicivorax: an alternative way to construct a nematode. *Dev. Biol.* **334**, 10-21.

Seeliger, O. and Hartmeyer, R. (1893-1911). Die appendicularien und ascidien. In *Bronn's Klassen und Ordnungen des Tierreichs, 3. Band (Suppl.), 1. Abt.*, pp. 1-1773. Leipzig: Akademische Verlagsgesellschaft.

Sinigaglia, C., Busengdal, H., Leclère, L., Technau, U. and Rentzsch, F. (2013). The bilaterian head patterning gene six3/6 controls aboral domain development in a cnidarian. *PLoS Biol.* **11**, e1001488.

Smith, A. B. (1988). Fossil evidence for the relationships of extant echinoderm classes and their times of divergence. In *Echinoderm Phylogeny and Evolutionary Biology* (ed. C. R. C. Paul), pp. 85-97. Oxford: Oxford Universiy Press.

Stiasny, G. (1914). Studien über die entwicklung des balanoglossus clavigerus delle chiaje. I. Die entwicklung der tornaria. *Z. Wiss. Zool.* **110**, 36-75.

Strathmann, R. R. (2007). Time and extent of ciliary response to particles in a non-filtering feeding mechanism. *Biol. Bull.* **212**, 93-103.

Stricker, S. A., Reed, C. G. and Zimmer, R. L. (1988). The cyphonautes larva of the marine bryozoan *Membranipora membranacea*. I. General morphology, body wall, and gut. *Can. J. Zool.* **66**, 368-383.

Sulston, J. E., Schierenberg, E., White, J. G. and Thomson, J. N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans. Dev. Biol.* **100**, 64-119.

Tagawa, K., Humphreys, T. and Satoh, N. (2000). T-Brain expression in the apical organ of hemichordate tornaria larvae suggests its evolutionary link to the vertebrate forebrain. *J. Exp. Zool.* **288**, 23-31.

Temereva, E. N. (2012). Ventral nerve cord in Phoronopsis harmeri larvae. *J. Exp. Zool. B* **318**, 26-34.

Temereva, E. N. and Tsitrin, E. B. (2014). Development and organization of the larval nervous system in *Phoronopsis harmeri*: new insights into phoronid phylogeny. *Front. Zool.* **11**, 3.

Temereva, E. and Wanninger, A. (2012). Development of the nervous system in *Phoronopsis harmeri* (Lophotrochozoa, Phoronida) reveals both deuterostome- and trochozoan-like features. *BMC Evol. Biol.* **12**, 121.

Tosches, M. A. and Arendt, D. (2013). The bilaterian forebrain: an evolutionary chimaera. *Curr. Opin. Neurobiol.* **23**, 1080-1089.

Veeman, M. T., Newman-Smith, E., El-Nachef, D. and Smith, W. C. (2010). The ascidian mouth opening is derived from the anterior neuropore: reassessing the mouth/neural tube relationship in chordate evolution. *Dev. Biol.* **344**, 138-149.

Wanninger, A., Fuchs, J. and Haszprunar, G. (2007). Anatomy of the serotonergic nervous system of an entoproct creeping-type larva and its phylogenetic implications. *Invertebr. Biol.* **126**, 268-278.

Wheeler, B. M., Heimberg, A. M., Moy, V. N., Sperling, E. A., Holstein, T. W., Heber, S. and Peterson, K. J. (2009). The deep evolution of metazoan microRNAs. *Evol. Dev.* **11**, 50-68.

Wicht, H. and Lacalli, T. C. (2005). The nervous system of amphioxus: structure, development, and evolutionary significance. *Can. J. Zool.* **83**, 122-150.

Woollacott, R. M. and Zimmer, R. L. (1971). Attachment and metamorphosis of the cheilo-ctenostome bryozoan *Bugula neritina* (linné). *J. Morphol.* **134**, 351-382.

Yaguchi, S., Yaguchi, J., Wei, Z., Shiba, K., Angerer, L. M. and Inaba, K. (2010). ankAT-1 is a novel gene mediating the apical tuft formation in the sea urchin embryo. *Dev. Biol.* **348**, 67-75.

Yuan, D., Nakanishi, N., Jacobs, D. K. and Hartenstein, V. (2008). Embryonic development and metamorphosis of the scyphozoan Aurelia. *Dev. Genes Evol.* **218**, 525-539.